MEASUREMENT, MATHEMATICS, AND MECHANISMS OF MAMMALIAN GROWTH

A thesis presented in partial fulfilment of
the requirements for the
Degree of Doctor of Philosophy
at Massey University

Ross Graham Clark

1978

VOLUME ONE

Time goes, you say? Ah, no!

Alas, Time stays, we go.

H.A. Dobson

The Paradox of Time, stanza 1.

Abstract of a thesis presented in partial fulfilment
of the requirements of the Degree of Doctor of Philosophy

MEASUREMENT, MATHEMATICS, AND MECHANISMS OF MAMMALIAN GROWTH

by ROSS GRAHAM CLARK

Longitudinal growth experiments using rats, lambs, and heifers were analysed by establishing linear relationships between ages, live weights and body lengths in individual animals. Various analytical methods were investigated. Statistical and biological reasons forced the logarithmic transformation of weights and lengths, a three parameter logarithmic metameter was used if means and standard deviations were correlated on a two parameter logarithmic metameter. Age was transformed to give linear relationships. Changes to the experimental design and analysis of growth experiments were suggested.

Effects were demonstrated in individual animals that were previously only shown for grouped data and the techniques' sensitivity produced novel findings. Rats were ovariectomised at three ages and/or treated with oestrogen and slaughtered at four ages. The rat ovary inhibited growth pre-pubertally, and the response to ovariectomy or oestrogen was negatively related to the pre-treatment growth rate.

Compensatory growth occurred following weaning in rats and following birth in ruminants. Estimated initial weights explained more of the variation in subsequent growth rates than did observed weights. In rats pre-weaning growth lines diverged (compensation being negligible), birth and weaning weights being positively correlated, post-weaning growth rate was strongly negatively correlated with weaning weight. Estimated birth and final weights, and weaning and final weights, were unrelated; compensation being nearly complete.

Two sets of pre-weaning lamb live weights (collected by others) were, for individual animals, linearised. Pre-weaning compensation occurred, as it did in two independent sets of weighings from monozygotic twin heifers (also collected by others). Compensatory growth, between and within sets of twin, occurred rapidly to weaning, then slowed. The efficiency of identical twins for experimentation, using these methods, was shown, as were the disadvantages of using average daily gains.

The linear relationships did not explain all the systematic variation, short- and long-term oscillations in growth rate occurred. Long-term oscillations were related to live weight rather than to age. Neo-natal testosterone treatment of female rats transposed and advanced the pattern of growth. Both sex and strain affected the pattern of growth. The possible use of these techniques in animal breeding was discussed.

The logarithms of lengths and weights, assumed by many biologists to be linearly related (allometry), showed curvilinear relationships.

A technique of carcass analysis was developed and applied. Ovariectomy increased rat body weight and length but did not produce obesity (assayed by percentage composition and by allometry). Oestrogen stimulated fat deposition but inhibited linear growth. Body weight's response to oestrogen was adaptive,bone growth's non-adaptive. Similarly there was a large pre-pubertal sex difference in body length but a small difference in body weight. This separation of the mechanisms controlling bone growth and body weight increase was discussed. Part of the increased size of ovariectomised rats was attributed to increased skin size (and altered composition) and decreased tail length, giving decreased heat loss, and improved energy utilisation for growth.

Body growth occurs in two overlapping phases, of cell hypertrophy and cell hyperplasia, represented by different growth equations, and controlled by different mechanisms. A possible mechanism controlling cell hypertrophy, and directing compensatory growth, based on cartilage growth, would explain some of the effects described. The endocrinology of the mechanism, and oestrogen's interaction with it, were discussed.

ACKNOWLEDGEMENTS

I wish to express grateful appreciation to my chief supervisor, Dr. M.F. Tarttelin, for proposing the general area of research and for his willing assistance given during the course of my work.

I also wish to express my thanks to Professor R.E. Munford, my second supervisor, in whose department this study was carried out, for his encouragement, guidance, and unfailing interest in the various aspects of the work. I am especially indebted to his enthusiastic initiation of my interest in computing and statistics and for his providing financial support for my work. I am also indebted to Dr. R.M. Greenway, my third supervisor, and the other members of the Department of Physiology and Anatomy.

I derived considerable benefit from discussions, on the analysis of growth curves, with Dr. R.W. Purchas of the Sheep Husbandry Department. The readiness with which Dr. M.F. Tarttelin, Ms. Liz Sommerville, Professor R.E. Munford, Professor D.S. Flux, and Dr. L.R. Wallace gave their original data is gratefully acknowledged. I wish to thank the workers, especially Mr. E. Ormsby, of the Small Animal Production Unit for assisting with the care of the experimental animals.

Mrs. F.S. Wicherts patiently typed the majority of the text and helped in many other ways during the work's final preparation. Thanks are due to the staff of the Massey University Printery for reducing and printing the figures.

The staff and workers at the Computer Unit at Massey, especially Mr. Colin Read and Mrs. Nola Simpson, were always willing to assist with my computing. Thanks must also go to Mr. Barry Hitchcock for miraculously maintaining the IBM 1620 computer.

To Dr. M.F. Tarttelin and Professor R.E. Munford, for reading and criticising drafts of the work, and to Ms. Liz Sommerville, Mr. D. Baker-Gabb, and Ms. Gillian Findlay, for proof-reading, acknowledgement is also due.

Finally I wish to thank my friends and flatmates, especially for their perseverance with my nocturnal habits. To Gillian, thankyou.

This work was carried out during the tenure of a University Grants Committee Post-Graduate Scholarship.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE PAGE

PREFACE

This thesis does not begin in the customary manner, with a large review of the relevant literature. The chief reason for this departure from custom being the paucity of literature relevant to the areas covered by Chapters One to Seven. Some parts of this thesis had been, or were about to be, published. Thus the present format reflects an attempt at minimising the amount of re-drafting of this published material. In addition the thesis evolved in a somewhat unusual manner, this also determined its form. The initial scope of the thesis was to investigate the effects of sex steriods on body growth, body composition, and the distribution of body fat in the laboratory rat. But the chief measure of body growth used, live weight, was found to be inadequately analysed in the current literature. This led to an extensive study of the statistical methods that could be applied to live weight data and to the eventual choice of what appears to be the most fruitful line of analysis. The statistical methods developed for analysing rat live weights were also applied to the live weights of domestic animals (sheep and cattle). Since what was, initially, a small part of the thesis grew, into perhaps the most important part, a re-structuring of the work was obviously necessary.

The studies in Chapters One to Seven will be presented in roughly the order that they were completed. This method of presentation does tend to give repetition as the same data is sometimes analysed and re-analysed in separate chapters. Essentially the same statistical techniques are also used and described in more than one chapter. So some parts of the chapters could have been combined but the lines of reasoning may have then become confused. Also a reader interested in the growth of a single species will, under the present format, generally find the information relevant concentrated in one chapter, or section, rather than scattered through the text. Although this lay-out entails some restatement of methods the general reader will note that different statistical problems occur in the various species and data sets and that their solution makes the whole relevant to the particular. The reader, it is hoped, will benefit from seeing the methods described in the order that they were developed, and applied, as the logical progression of the work should become clearer.

CHAPTER ONE

THE LINEARISATION OF BODY-WEIGHT AGE CURVES IN THE LABORATORY RAT: SOME APPLICATIONS AND IMPLICATIONS.

Antipholus of Syracuse .....

Transform me then, and to your power I'll yield.

Comedy of Errors, III, ii.

INTRODUCTION

The present chapter describes the development of a method of analysis for rat body weights, and its application to data collected by the author. Many previous authors' analyses of live weight data have not avoided the many pitfalls and problems inherent to the analysis of such data, or have wasted information, and their invalid,or at best inefficient, statistical techniques have lead to unjustifiable conclusions, and in some cases to plainly wrong conclusions. Thus the techniques developed will be shown to give, in some cases, conclusions that differ from those reached by other workers. Some advantages of the present methods, in terms of the design and analysis of experiments, will also be discussed.

a) METHODOLOGY

In experiments where body weight is studied it is usual to weigh individuals on several occasions. It is also common practice to test for differences between treatments at each of the ages (by 't' or 'F' tests) and to report the effects of treatment at each of the ages. However this method of analysis is not soundly based in statistics or in logic.

R.A. Fisher (1939) noted that successive weighings of individuals are not independent and so to analyse the variation in weight at each age and to combine the data obtained at different ages was 'hopelessly encumbered.' Unfortunately it is still common practice to make separate analyses of successive untransformed measurements on individuals when the measurements are neither independent nor necessarily normally distributed.

An example of the misleading nature of conclusions reached by multiple analyses is as follows. Multiple 't' tests may show the differences between treatments to be increasing with age from age X to age Y, but a significant difference between the means is only reached at Y. One can see that the 't' values are increasing from X to Y but the method of analysis only allows one to say that the

values were different at Y. The regression approach developed herein allows the statistical justification of a much more informative conclusion, that the growth rates (the slopes of the lines) diverged from X.

As most authors analyse rat body weights without transformation it could be inferred that rat body weight is a normally distributed variable. This will be shown to be clearly not the case. However for individual rats a simple transformation of body weight restores normality and the transformation of age then gives a convincingly linear relationship between weight and age. This relationship allows a linear regression, which accounts for over 99% of the variation, so that the difficulties inherent in multiple analyses can be avoided. Some applications and implications of this linear relationship between weight and age, especially concerning the effects of the ovary on body growth, are then discussed in detail.

b) APPLICATIONS

Ovariectomy in adult rats increases body weight, but pre-pubertal ovariectomy has been reported to increase body weight only after puberty (Wade, 1972). Similarly, puberty is said to decrease the rate of body weight gain in the female rat (Wade, 1976). As the techniques used to justify these conclusions were those criticised by Fisher (1939), the present study was designed to re-investigate the influences of puberty on body weight gain in female rats.

Monteiro and Falconer (1966) showed that at weaning there was a greater variation in the live weight of rodents (presumed to be due to maternal influences) than at later ages, which suggested a form of compensatory growth occurred post-weaning. As a similar compensatory growth may occur following ovariectomy the effects of the pre-treatment rate of growth and the ultimate body weight, on post-treatment growth, were investigated following ovariectomy and oestrogen treatment.

Data will be presented to test the hypothesis that body weight is controlled to preserve a predetermined, but modifiable, ultimate body weight.

MATERIALS AND METHODS

Female Sprague-Dawley rats, originally of the Simonsen strain, were bred at Massey University. Litters were adjusted soon after birth to 10 pups per female. Animals were randomly assigned to treatment and slaughter groups. Bilateral ovariectomy, with full sham surgery for controls, was carried out by a conventional flank technique using hypothermia for the newborn rats (day 2) and ether anaesthesia for the older rats (weeks 4 & 7). Rats were weaned at 3 weeks of age and randomly distributed amongst 10 large colony cages housed in a light and temperature controlled room (14h light, lights off at 7.00pm; temperature $24^0C \pm 1^0$). A pelleted rat diet and tap water were offered *ad libitum*.

A group of rats which were ovariectomised at week 7, were injected daily with oestradiol benzoate (EB, 2µg/day in arachis oil, total volume 0.05ml) subcutaneously in the nape of the neck, from 10 weeks of age, for 2 weeks or 5 weeks before slaughter: a control group was injected daily with arachis oil (see Table 8.1, page 183).

Completeness of ovariectomy was ascertained at slaughter (at 7, 9, 12 and 15 weeks) by visual inspection and by analysis of uterine weights.

Rats were weighed weekly (to the nearest gram) until slaughter and examined daily for vaginal opening.

BIOMETRICAL CONSIDERATIONS

The lognormal distribution of body weight (Bliss, 1967) was verified in the present experiment. Figure 1.1a shows that the untransformed mean body weight was, for OvX W4 animals, strongly correlated with the standard deviation (r=+0.973). However, after the transformation of the data to logarithms mean and standard deviation were unrelated (r=+0.086). The regressions were calculated after weighting each standard deviation with its degrees of freedom. Chapters 2 and 6 further discuss the normalisation of rat body weight measurements, especially Fig. 2.4 page 35 and footnote on the facing page.

As stated in the introduction to this chapter the commonly used technique of applying an analysis of variance to the live weights at each age can be dismissed as being statistically unsound. The method of increments, or calculating the average daily gain, is discussed in Chapter 10 where it is also shown to be a statistically questionable method of analysis. Thus a regression approach to the analysis was investigated.

Following the transformation of body weight to logarithms, unweighted polynomial curvilinear regressions were fitted to the mean weekly treatment group body weights by sequential addition of powers of higher order. However this method of statistical analysis was inappropriate for several reasons:

1. Sequential slaughter, necessary in the design of the experiment, resulted in the earlier weighings having a greater influence on the fitted curves than later weighings.

2. Data from 4 until 15 weeks of age was adequately explained by linear, quadratic and cubic terms whereas data from 3 until 7 weeks was explained by a linear equation. Such discordant equations are difficult to compare.

3. Differences could not be shown between the coefficients of the polynomial equations for different groups whereas analysis of variance showed clear differences between these body weights at slaughter.

Although polynomial functions have convenient mathematical properties their biological interpretation is obviously difficult. Furthermore, the coefficients of the powers of time are statistically dependent, so that subsequent statistical analyses are difficult from both the technical and interpretative viewpoints. Similar conclusions were reached, and discussed, by van't Hof *et al.*(1976).

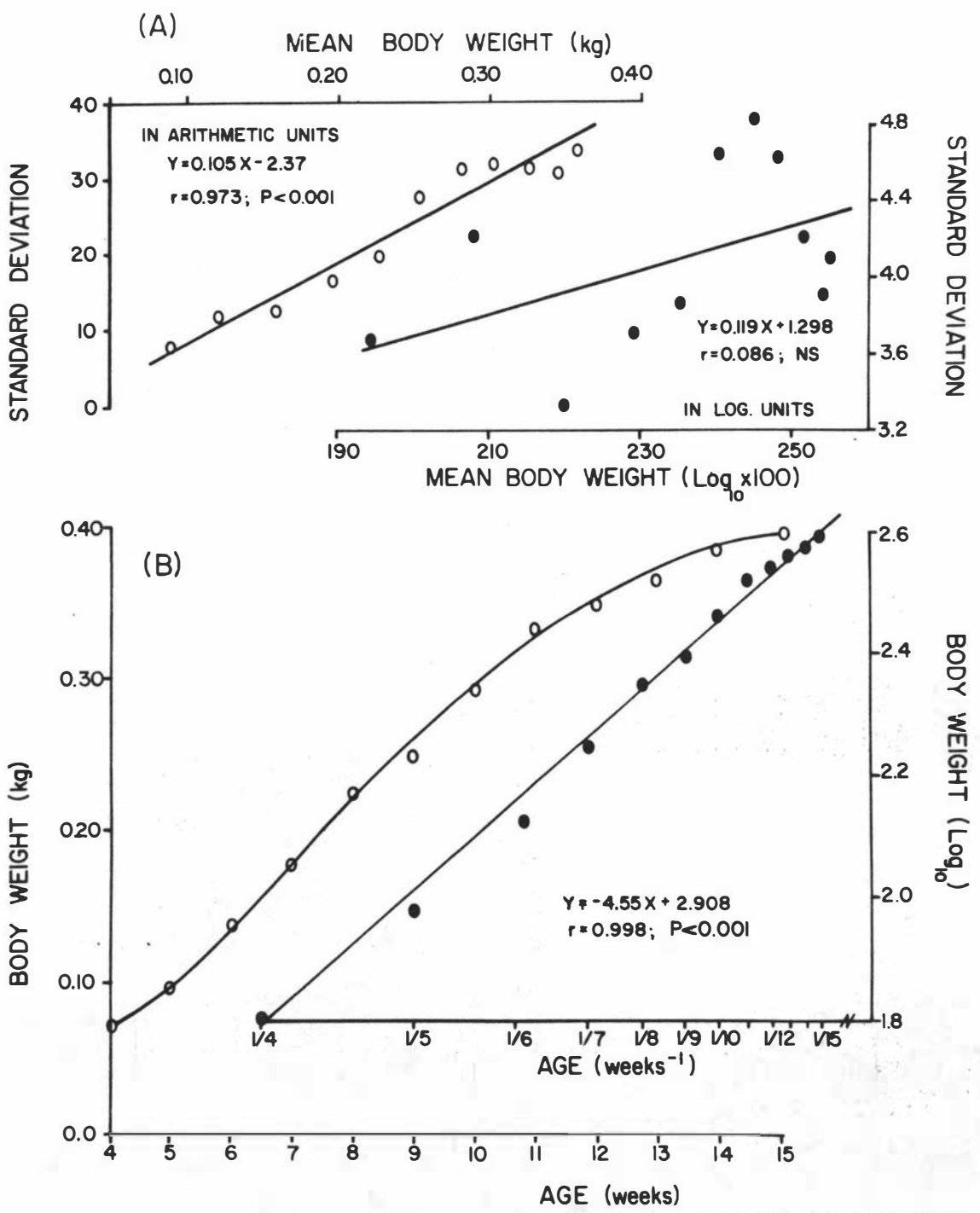
These problems were resolved by the use of two techniques that had not, to the author's knowledge, been previously combined in the one analysis.

FIGURE 1.1a

THE RELATIONSHIPS BETWEEN MEANS AND STANDARD DEVIATIONS FOR BODY WEIGHTS, ON ARITHMETIC AND LOGARITHMIC METAMETERS, FOR FEMALE RATS OVARIECTOMISED AT 4 WEEKS AND WEIGHED UNTIL 15 WEEKS OF AGE. *

FIGURE 1.1b

PLOTS OF BODY WEIGHT AGAINST AGE FOR ONE FEMALE RAT ON UNTRANSFORMED AND TRANSFORMED (LOG BODY WEIGHT vs THE RECIPROCAL OF AGE) METAMETERS.



* see Figure 2.4 page 35

The first innovation was the use of a linearising equation, initially described by Zucker & Zucker in a series of publications (1941, a and b ; 1942) and recently described by Bliss (1970), where the logarithm of body weight is plotted against the reciprocal of age, from birth, in weeks. Linear regression follows. The independent variable (age) is assumed free from error so the effects on variance of transforming age to the reciprocal can be ignored.

The second divergence from common practice follows from the idea that the growth of an individual animal (the individual curve) is in many ways more informative than the mean curve from a group of animals (the mass curve). This important point is discussed in detail in Chapter 6. The first statistician to implement this idea, in animal experimentation, appears to have been Wishart (1939). Instead of fitting a line to the mean weights for each treatment group Wishart fitted lines to each individual animal's live weights. A statistical advantage of fitting lines to individuals, and then combining the data for a group of animals, is that the within animal variation is separated from the between animal variation in the combined analysis.

Therefore, by a combination of Wishart's method and the Zuckers' equation, a line was fitted to each individual rat's weekly body weights. Linearity was statistically confirmed when sequentially fitted powers of higher order gave no greater explanation of the data than did the linear equation; correlation coefficients below r= 0.99 were uncommon. Slopes and constants for individual animals were weighted by their degrees of freedom. Estimates were analysed as data, as was recommended by Fisher (1939). Groups were compared by analysis of variance and mean squares were subdivided to give comparisons for individual degrees of freedom. Weighting using the inverse of the variance was considered but as the slopes and constants were estimated for descriptive rather than for comparative reasons weighting

based upon variances was considered unjustified. Although the more conservative weighting, by degrees of freedom, was adopted an unweighted analysis of variance indicated essentially the same differences between the treatment groups. Also, for intuitive reasons, one would choose weighting by degrees of freedom. For example, if the variances of two estimates of a parameter (slopes or constants) were equal, but one estimate was based upon 3 and the other upon 10 measurements, one would still tend to place more confidence in the estimate based upon 10 measurements. In Chapter 6 the residuals about the fitted lines are shown to show a highly significant degree of serial correlation. This finding adds further weight to the above arguments for the use of the more conservative weighting. The above method of comparing the treatment groups is conservative and was, upon reflection, found to be somewhat crude; Chapter 10 describes and illustrates the more refined techniques discussed by Bliss (1967, Chapter 13).

An example of the successful linearisation obtained by the log-reciprocal equation is illustrated in Fig 1.1b.

The calculated linear equation,

$$\log \text{body weight} = b \,/\, \text{age} + \log A$$

yields two parameters,

a) The slope (b) called by Zucker and Zucker (1942) a growth intensity factor, which will herein be called the rate of body weight gain.

b) the constant (log A) termed the inherent size factor by Zucker and Zucker, but is called the ultimate body weight in this work.

Body weights were transformed to 100 times the logarithm to the base 10 for computation.

For the present purposes the methods of statistical analysis developed in this thesis are considered most appropriate. This does not imply that other methods of analysis are invalid in other situations.

TABLE 1.1a

Weighted treatment means and standard errors for the slopes (rates of body weight gain) and constants (ultimate body weights) of the linear equations relating log body weight to the reciprocal of age.

Treatment	DF	Slopes(±SE)	Constants (±SE)
Pre-Ovx	13	-379.03 ± 9.13	267.95 ± 2.45
OvX	13	-494.31 ± 16.52	285.64 ± 2.11
EB-OvX	13	-264.08 ± 10.22	261.83 ± 10.73
			+ LOG_{10} x 100

Weighted paired analysis of variance of the above data.

SOURCE OF VARIATION	DF	Slopes (MS)		Constants (MS)	
		Pre-Ovx vs. OvX	Pre-OvX vs. EB-OvX	Pre-OvX vs OvX	Pre-Ovx vs EB-OvX
PAIRS	13	2575.0	2799.4	92.98	106.73**
TREATMENTS	1	89493.5***	77743.3***	2192.4***	166.92*
ERROR	13	2594.0	1612.8	64.93	26.59

* P < 0.05, ** P < 0.01, *** P < 0.001

(MS) mean square

FIGURES 1.2a, b, c, & d.

PLOTS OF LOG BODY WEIGHT AGAINST THE RECIPROCAL OF AGE FOR RATS:

1.2a - SHAM OPERATED AND OVARIECTOMISED AT WEEK 4,

1.2b - SHAM OPERATED AND OVARIECTOMISED AT WEEK 7,

(dashed line - extrapolated pre-ovariectomy growth line)

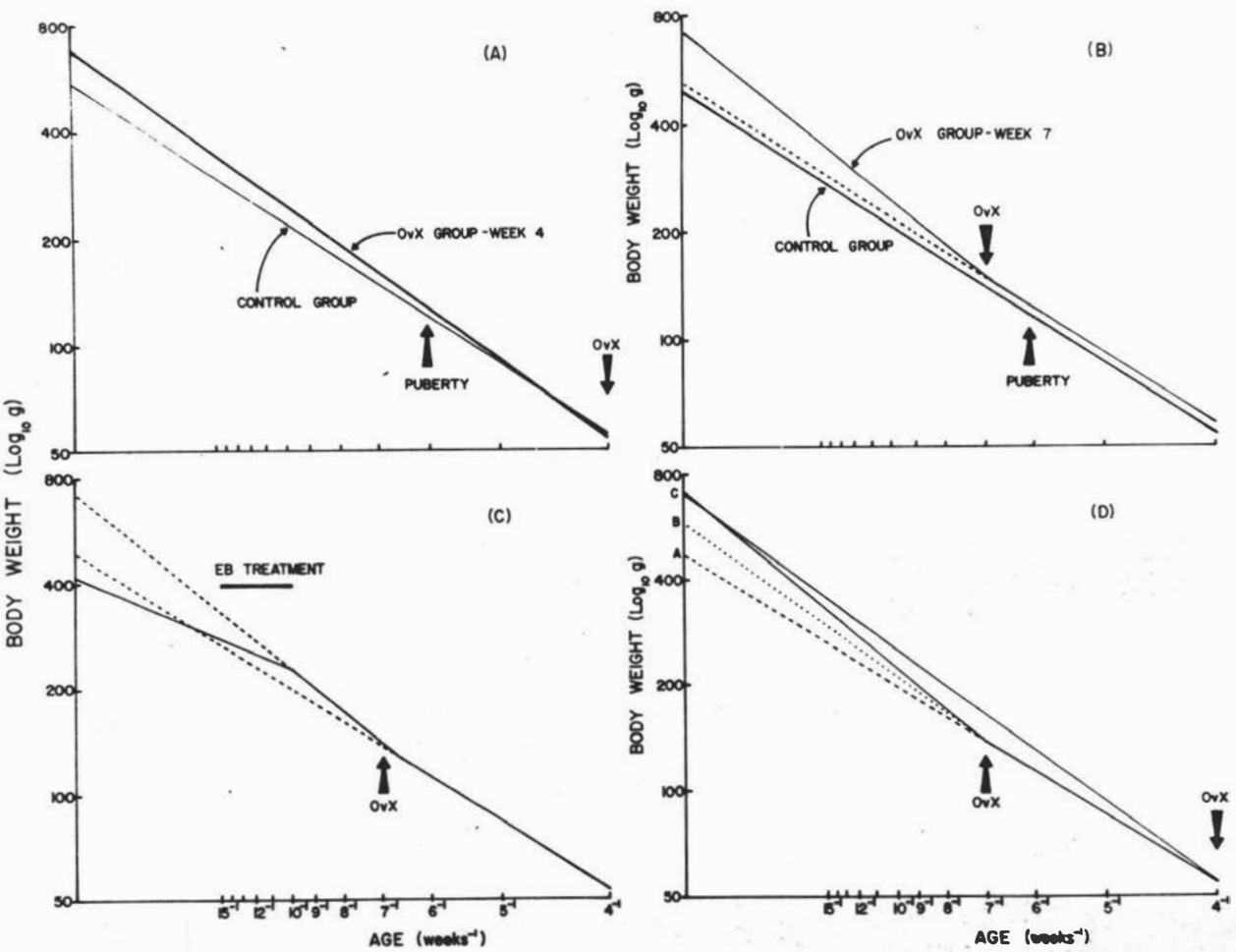
1.2c - OvX W7 RATS, AND THOSE GIVEN EB FROM WEEK 10,

(dashed lines- extrapolations of pre-treatment growth lines)

1.2d - OVARIECTOMISED RATS OF FIGURES 1.2a & 1.2b,

(dashed line - extrapolated pre-ovariectomy growth line)

(dotted line - growth line of OvX W7 rats if their growth rate had equalled that for OvX W4 rats)



Although the ultimate body weight is hypothetical, being the weight at infinite age, Zucker *et al* (1941) have shown that body weight increases linearly as a function of age up to 70 weeks. The weight at such an age, when expressed as a reciprocal, closely approaches the ultimate weight so it is reasonable to extrapolate beyond the period measured in the present study. This point is further discussed in Chapter 6, and is investigated using unpublished data in Chapter 10.

RESULTS AND DISCUSSION

The results and discussion are presented under 5 headings:

1. Body Weight
2. Puberty and growth
3. Ovariectomy, ultimate weight and rate of body weight gain
4. Weaning weight, weight gain and ultimate weight
5. General discussion

1. BODY WEIGHT

The analysis of the body weight data is summarised in Table 1.1 (a & b) and illustrated in Figure 1.2. The advantage of the linearising equation when comparing groups of widely different body weights can best be seen in Table 1.1a where slopes and constants are compared before ovariectomy, after ovariectomy and following EB treatment. The condition of linearity clearly maximises the information available from a limited range of values of the independent variable.

If both pre-treatment and post-treatment measurements are made on individuals then linearity allows within animal comparisons, giving increased precision (Table 1.1a). For example, pre-ovariectomy and EB treated rats had ultimate weights which could not be distinguished

TABLE 1.1b

Weighted treatments means and standard errors for the slopes and constants of the linear equations relating log body weight to the reciprocal of age.

Treatment	No	Slope ± SE	Constant ± SE
Sham D2	15	-395.87 ± 4.29	271.63 ± 0.82
Sham W4	12	-394.72 ± 2.93	273.61 ± 0.75
Sham W7	15	-387.85 ± 3.37	269.97 ± 0.52
OvX D2	31	-452.58 ± 2.53	279.64 ± 0.52
OvX W4	33	-438.17 ± 2.68	283.25 ± 0.54
OvX W7	20	-482.56 ± 6.0	286.85 ± 0.88

Analysis of variance of the above data and comparisons, by subdivision of the between treatment variation, for single degrees of freedom

Source of variation	d.f	Slope (MS)	Constant (MS)
Treatment	5	130029.8***	4483.6***
1. Sham D2 vs. Sham W7	1	2830.9+	99.7+
2. Sham W4 vs. Sham (D2+W7)	1	281.9	312.4*
3. OvX D2 vs. OvX W7	1	48801.6***	2841.2***
4. OvX W4 vs. OvX (D2+W7)	1	92516.2***	0.1
5. Shams vs. OvX *	1	554062.7***	17625.7***
Error	121	1387.3	47.1

+ $0.05 > P < 0.2$, * $P < 0.05$, *** $P < 0.001$
(MS) mean square

* Oil injected and control animals did not differ; they were therefore combined.

using confidence intervals based on the group means and standard errors. In the weighted paired analysis of variance, 'pairs' contributed 72.5 percent of the total variation and the difference between pairs was significant ($P<0.01$). The basis of this difference between pairs is discussed in detail in section 3. The removal of this large component of the variation from the within group variation enabled the demonstration of a significant reduction in ultimate body weight by EB.

Ovariectomy resulted in an increased rate of gain and an increased ultimate body weight compared to controls (Table 1.1b, and Fig 1.2). Oestrogen treatment reduced both the rate of gain and the ultimate body weight (Fig 1.2c). These results support the hypothesis that the effect of ovariectomy on body weight is due to the removal of oestrogen. As EB reduced the ultimate body weight, compared with that predicted from the pre-ovariectomy growth curve (dashed projection on Fig 1.2c), it would seem that 2 µg EB/day is supraphysiological for the rat. Since EB lowered the ultimate body weight below that predicted before ovariectomy I cannot support Wade's (1976) conclusion that oestrogen treatment will not depress body weight below that of an untreated female rat.

Despite random allocation of rats to treatment groups there was a significant difference in ultimate body weight between control groups (Table 1.1b) although the magnitude of this difference was small. Week 4 controls had a higher ultimate body weight than the day 2 and week 7 controls. No explanation for this difference can be offered.

Ultimate body weight increased linearly with age at ovariectomy (Table 1.1b, comparison 3, OvX D2 v OvX W7) with the quadratic effect being non-significant. Both the linear (comparison 3) and the quadratic (comparison 4, OvX W 4 v OvX D2 + OvX W7) effects of age at ovariectomy on rate of gain were significant.

If, irrespective of age at ovariectomy, the post-ovariectomy rate of gain tended to be invariable then the post-ovariectomy ultimate weight should decline with increased age at ovariectomy i.e. in Fig 1.2d from (c) to (b). But ultimate weight did not decrease with increased age at ovariectomy as the post-ovariectomy rate of gain did vary with age at ovariectomy.

If ultimate weight tended to be invariable then the rate of gain should increase with increased age at ovariectomy. This is confirmed and illustrated by Fig 1.2d where OvX W7 rats have a higher rate of gain than OvX W4 rats, giving a similar ultimate body weight. OvX D2 rats had a higher rate of gain than OvX W4 animals but a lower ultimate body weight, implying a lower weaning weight. This could be due to the stress of neonatal surgery reducing the weaning weight, causing compensatory growth post-weaning, and giving the higher than predicted rate of gain.

Oestrogen treatment reduced the ultimate weight below that predicted in the same animals before ovariectomy. But the reduction in the rate of gain was more dramatic (Fig. 1.2c).

These results support the hypothesis that rats will grow towards a predetermined ultimate body weight rather than at a predetermined rate of body weight gain.

2. PUBERTY AND GROWTH

The point of inflexion near puberty in the body weight-age curve of female rodents is claimed to indicate that the rate of growth slows at puberty (Brody, 1945; Kennedy, 1969; Wade, 1972). Furthermore, Kennedy (1969) quotes Monteiro & Falconer (1966) who state that the growth curve of the mouse can be divided, at its inflexion, into an initial exponential phase and a second asymptotic phase. However, after describing the two distinct phases Monteiro & Falconer fitted only one equation, the logistic, to the mean weekly litter weights from birth to 8 weeks. From their data a plot is neither symmetrical about the age of puberty nor the calculated point of inflexion. No tests of goodness of fit of the logistic curve to their data were given but they noted that the point of inflexion was not precisely determinable.

An inflexion may be apparent in the body weight-age curve, but the specific growth curve, which is approximated by the logarithm of body weight plotted as a function of age, has no inflexion at puberty as the specific growth rate progressively decreases with increased age. Minot (1908) stated that tissues progressively lose their power to

grow and he concluded that the point of inflexion in the body weight-age curve was of no particular significance. The specific growth curve has the advantage of simplicity and its use is dictated by the need to transform body weight to logarithms to allow the use of statistical procedures based on the normal distribution (see Fig 1.1b; Medawar, 1945; Reeve & Huxley, 1945).

It is clear from Figures 1.2a and 1.2b that control animals showed no change in specific growth rate at puberty (vaginal opening occurred at 43 ± 2 days). Figure 1.2a also shows that the increase in specific growth rate started at the time of ovariectomy rather than at puberty. Wade and Zucker (1970) dealt with untransformed body weights and found that the induction of precocious puberty did not change growth rates.

If puberty did affect the rate of gain, and thus the ultimate body weight, rats slaughtered at week 7 would be expected to have higher projected ultimate body weights than those slaughtered at later ages. But age at slaughter did not affect the rates of gain or the ultimate body weights of ovariectomised or intact rats. This supports both the linearity of the transformation and the absence of a major effect of puberty on body weight.

These results support the conclusion that the ovary inhibits body weight increase before puberty and probably before 4 weeks of age.

3. OVARIECTOMY, ULTIMATE WEIGHT AND RATE OF BODY WEIGHT GAIN

It has been proposed on the basis of the effects of oestrogen or ovariectomy on body weight in groups of animals of different hormonal status that oestrogen will only reduce body weight in 'overweight' groups; similarly, ovariectomy, is said to have no effect on body weight in groups that are already overweight (Wade, 1972, 1976). Regression techniques allowed these hypotheses to be tested for individual rats rather than groups.

FIGURES 1.3a & b.

FIGURE 1.3a

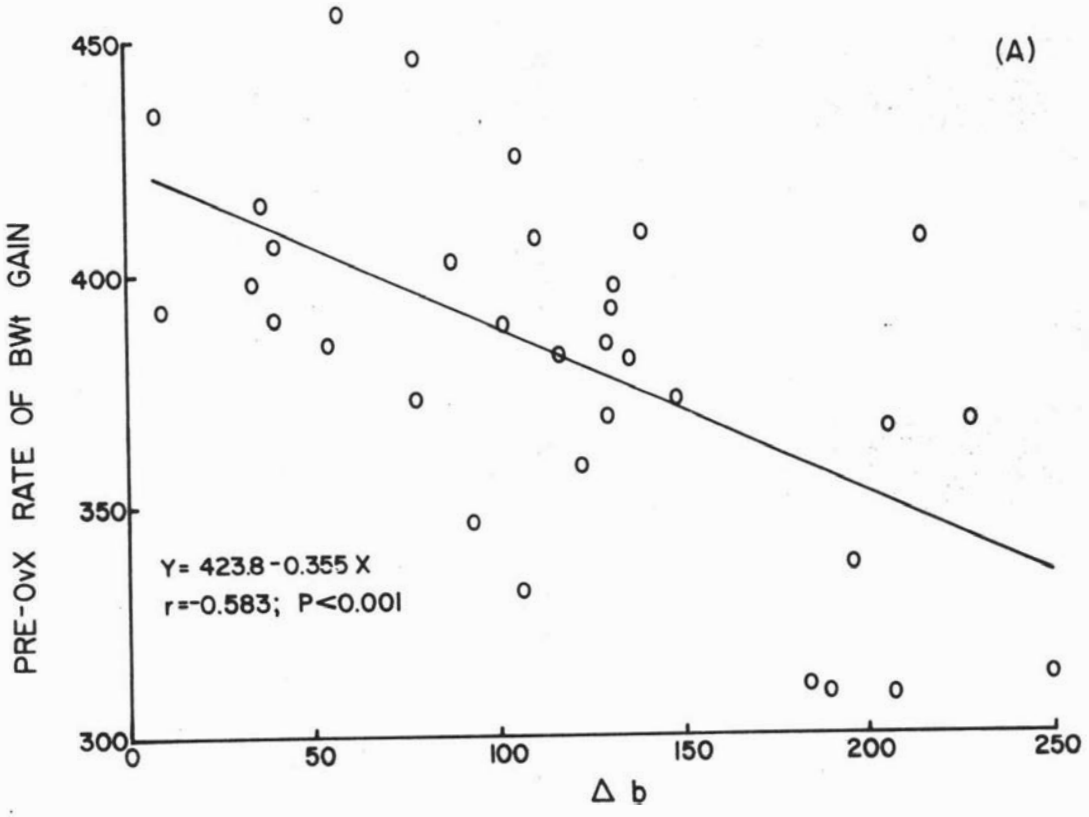
THE PRE-OVARIECTOMY RATE OF BODY WEIGHT GAIN

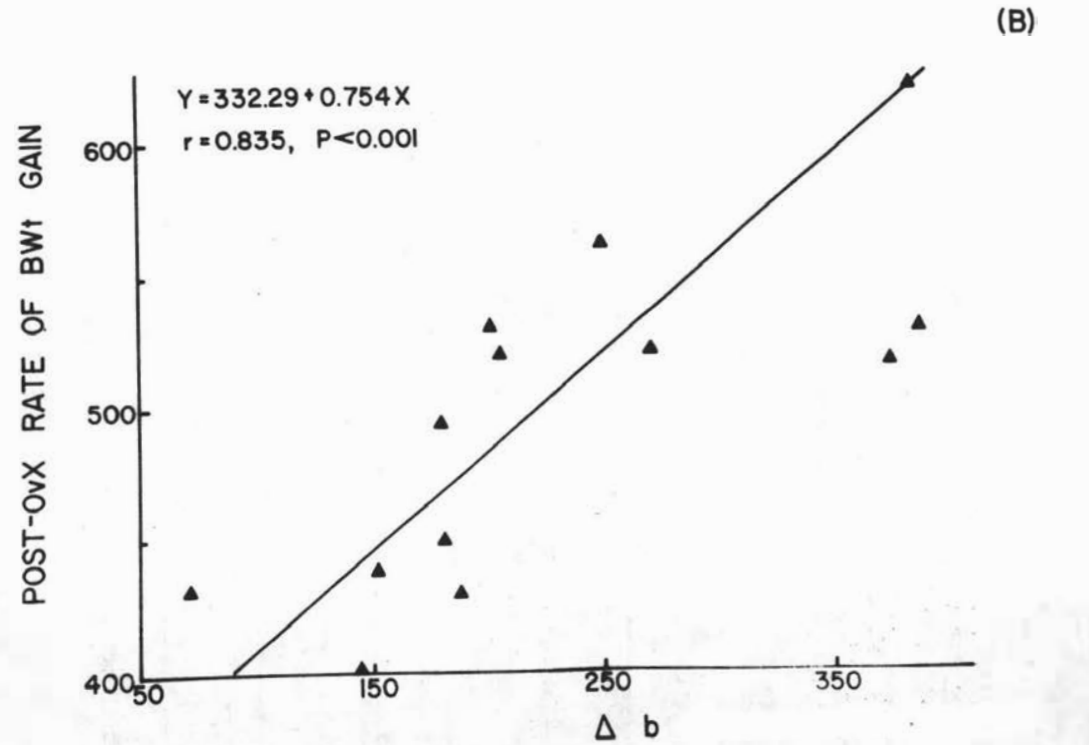
vs THE CHANGE IN RATE OF BODY WEIGHT GAIN FOLLOWING OVARIECTOMY AT WEEK 7.

FIGURE 1.3b

THE RATES OF BODY WEIGHT GAIN OF OVARIECTOMISED RATS

vs THE CHANGE IN THEIR RATES OF BODY WEIGHT GAIN FOLLOWING EB TREATMENT.





Estimates of ultimate body weight and rate of gain were available prior to ovariectomy, following ovariectomy (W7), and after EB treatment (Table 1.1a). There was a non-significant correlation between the pre-ovariectomy and post-ovariectomy ultimate body weights ($r = -0.0941$). However the correlation between the change in ultimate body weight following ovariectomy and the pre-ovariectomy ultimate body weight was significant ($r = -0.479$, $P<0.002$) as was the correlation between the change in rate of gain following ovariectomy and the pre-ovariectomy rate of gain ($r = -0.583$, $P<0.001$), Fig. 1.3a. These significant correlations suggest that a process similar to compensatory growth occurs following ovariectomy as rats of a lower predicted pre-ovariectomy ultimate body weight tend to have a greater post-ovariectomy increase in rate of gain than rats of higher pre-ovariectomy ultimate body weight. The lack of correlation between the pre-ovariectomy and post-ovariectomy ultimate body weights suggests that there is an optimal ceiling size towards which, environment permitting, rats grow.

As the rate of gain following ovariectomy was influenced by the pre-ovariectomy rate of gain (Fig 1.3a), it would be expected that the decrease in rate of gain following EB treatment would be influenced by the pre-treatment rate of gain. This hypothesis was convincingly upheld (Fig 1.3.b) when the correlation between the change in rate of gain and the pre-treatment rate of gain was positive and highly significant ($r = +0.835$, $P<0.001$). As discussed previously, for the untreated rats, the correlation between the change in ultimate body weight and the pre-treatment ultimate body weight was significant ($r = +0.913$, $P<0.001$) but the relationship between pre-treatment and post-treatment ultimate body weights ($r = +0.189$) was non-significant.*

Nilson *et al* (1935) and Palmer *et al* (1946) selected a strain of rats of high food conversion efficiency and high body weight, whose body weight response to growth hormone (a stimulatory treatment) and to thyroid hormone (an inhibitory treatment) was compared with a strain of rats selected for low body weight and low food conversion efficiency. The high body weight strain was less responsive to growth hormone and more responsive to thyroid hormone than the low body weight strain. In the present study individual rats rather than strains were compared but inherently larger rats were less responsive to stimulatory

* Each of these correlations contains an element of a part-whole relationship. However in each case this relationship would generate a correlation which would be opposite in sign to that actually found in the sample correlation.

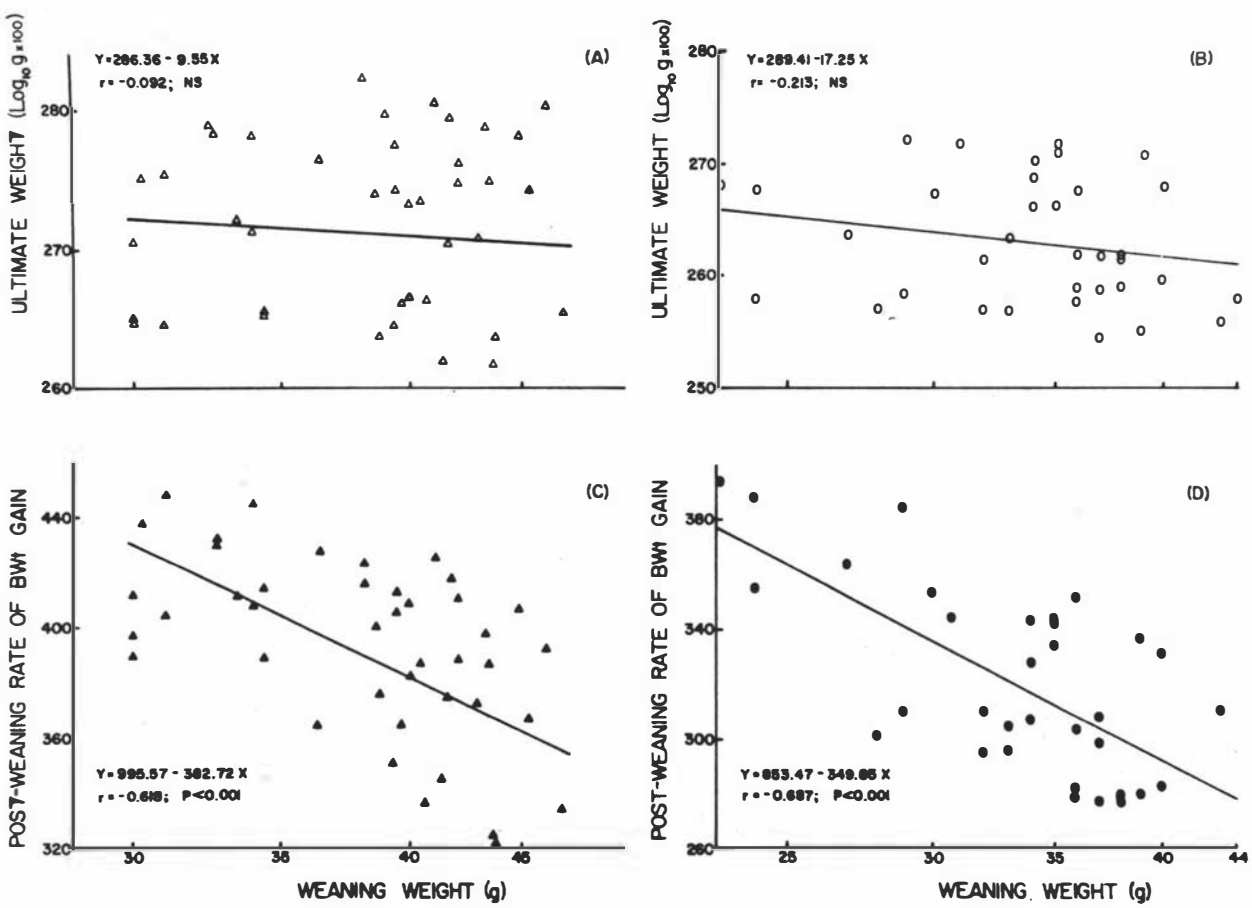
FIGURES 1.4a, b, c, & d

PLOTS OF SLOPES AND CONSTANTS, FROM THE LINEAR EQUATIONS RELATING LOG BODY WEIGHT TO THE RECIPROCAL OF AGE, AGAINST WEANING WEIGHTS.

1.4a & 1.4b - ULTIMATE WEIGHT vs LOG WEANING WEIGHT.
1.4c & 1.4d - RATE OF BODY WEIGHT GAIN vs LOG WEANING WEIGHT.

1.4b & 1.4d - INTACT FEMALE RATS OF TARTTELIN et al. (1975), U.C.L.A. COLONY.

1.4a & 1.4c - INTACT FEMALE RATS FROM THE PRESENT STUDY.



treatment (ovariectomy) and more responsive to an inhibitory treatment (EB) than inherently smaller rats.

4. WEANING WEIGHT, WEIGHT GAIN AND ULTIMATE WEIGHT

The equation of Zucker & Zucker (1942) linearized the body weight-age curve from week 4 onwards, with the week 3 body weight (the weaning weight) being excluded as an outlying value. This exclusion allowed the weaning weight to be a variable independent of the regression statistics.

Data from the present study is illustrated in Figs. 1.4a & c. Data derived from a study (Tarttelin, Shryne & Gorski, 1975) in which rats, having a larger range of weaning weights, were weighed from weaning until 18 weeks of age is shown in Figs 1.4b & d. In both studies, correlations between weaning weights and ultimate weights were non-significant, but significant correlations were seen between weaning weight and rate of gain (Fig 1.4c, $r = -0.618$; Fig 1.4d, $r = -0.687$). This could be interpreted to mean that potential differences in ultimate weight due to differences in weaning weight were abolished by compensating differences in the post-weaning rate of gain. About 40 percent of the variation in rate of gain was associated with weaning weight differences. 'Catch-up growth' occurs following an experimentally reduced rate of gain, but the above compensatory growth follows differences in body weight at weaning between rats due to their pre-weaning environment. Monteiro & Falconer (1966) concluded that compensatory growth occurs in the mouse only until puberty. The above results indicate that, in the rat, compensatory growth starts well before puberty, is not altered by puberty and continues throughout active growth. Widdowson & Kennedy (1962) reached similar conclusions from groups of rats rather than individuals.

In the present study weaning weight influenced rate of gain but not ultimate weight. This implies that rate of gain is variable to give a predetermined ultimate body weight. McCance & Widdowson (1974) agree that rate of gain post-weaning is determined by pre-weaning rate of gain, but they assume that a rat can somehow monitor this pre-weaning rate of gain, or size, which is an unnecessary assumption if ultimate

body weight is predetermined.

Rats can be selected for a predetermined adult body weight (Robinson, 1965). If ultimate body weight is genetically determined then weaning weight need not be detected because post-weaning growth, towards a predetermined weight, will be inversely related to weaning weight and will produce compensatory growth.

GENERAL DISCUSSION

The linear regression of log body weight on the reciprocal of age provides a method of describing body weight-age changes which is both convenient and statistically valid. The method permits within-animal comparisons where several weekly weights are obtained from individuals of known age before and after treatment.

Zucker & Zucker (1942) showed that their equation linearised the body weight curves of rats undergoing successive pregnancies. The present study shows that the post-ovariectomy body weights of individuals can also be linearised. The fact that control, pregnant, and ovariectomised rats all follow a similar growth curve, explained by an equation with only two parameters, indicates the precise and orderly control exerted on body weight increase and the robustness of the linearising technique.

In both the present study and that of Zucker & Zucker (1942), the weaning weights did not lie on the regression line describing body weights at later ages. It is clear that there is a post-weaning change in specific growth rate. Zucker *et al* (1941) showed that the logarithm of body weight is linearly related to the logarithm of conception age, from birth to 6 weeks of age, and the reciprocal of age from 4 weeks of age onwards. The two phases overlap, which does not support the concept of an inflexion or sudden change in growth rate at puberty. Clearly the change in growth rate occurs gradually and is initiated before puberty.

Wade (1976, p.237) concludes that, '...ovarian hormones do not restrain eating or body weight prepubertally.' He quoted Grunt (1964)

and Wade & Zucker (1970) in support of his statement. In the study by Grunt (1964) the first post-operative weighing was at 60 days of age, clearly a post-pubertal age, when the ovariectomised rats were heavier than controls. It is difficult to understand how conclusions about pre-pubertal growth can be drawn from this investigation. Wade & Zucker (1970) sequentially tested for differences between the untransformed body weights of ovariectomised and control rats by multiple Mann-Whitney U tests. This nonparametric testing is conservative in that it does not use all the information available and is less likely to detect real differences (second order error).

The present study shows that the ovary does restrain body weight before puberty. The high circulating level of oestradiol in pre-pubertal female rats is reduced by ovariectomy (Rabii & Ganong, 1976) so ovariectomy, possibly by the same mechanism postulated for mature rats, stimulates weight gain. The finding that compensatory growth starts from weaning, rather than puberty, and continues unaltered through puberty and into adulthood, supports the hypothesis that mechanisms controlling adult body weight are functional before puberty.

Growth recovery following weaning, post-ovariectomy growth and post-oestrogen growth all have common elements. All three effects involve compensatory growth and thus the removal, or partial removal, of differences in ultimate body weight between individual rats. Weaning removes the 'maternal' effects and equalize conditions between individuals. Ovariectomy removes differences between individuals in their oestrogen production, and treatment with a supraphysiological dose of oestrogen would similarly remove differences in response between individuals. The response to equalisation in these three situations, and following ovariectomy at different ages, is that the rate of gain is altered to equalise the ultimate body weight of individuals.

It has been proposed by Wade (1976) that the degree of body weight response to ovariectomy or oestrogen treatment is governed by whether or not a rat is obese or 'overweight'. However, the present results show that the body weight response is dependent upon the growth rate and thus the ultimate bo dy weight, rather than the body composition at the time of treatment.

That individuals, strains, and breeds may differ in their response to body weight stimulants or depressants should be considered in situations where substances are being tested for effects on body weight. For example, female rats grow at a slower rate than male rats so females may be more sensitive to body weight stimulants and less responsive to inhibitory substances than males.

CHAPTER TWO

LAMB LIVE WEIGHTS:

NORMALISATION, LINEARISATION, AND COMPENSATORY GROWTH

INTRODUCTION

A common practical and experimental use for body weighings (live weights) is to measure growth or performance in the agriculturally important domestic animals. In the calculation of statistics from live weight data the distribution of the original untransformed weighings is usually assumed to be normal. The use of the average daily gain follows from this assumption. For rat body weights a lognormal distribution was shown to be a superior explanation of the variation about the means. Despite the common use made of live weight measurements the literature concerning the normalisation of live weights in domestic animals is surprisingly sparse. The linearisation of live weight-age growth curves in these species has likewise been the subject of little recent published research. The advantages of linearisation have been discussed, for the laboratory rat, in Chapter 1. The linearisation of the growth curves of domestic animals should bestow similar advantages.

For these reasons the live weight measurements collected from sheep by Wallace (1948) were re-analysed to study the distribution and linearisation of live weights, and to test for the occurrence of compensatory growth in a species other than the rat. The results also proved valuable in an unexpected way as they caused a reappraisal of similar work in the rat which had hitherto been considered completed.

MATERIALS AND METHODS

The data that is analysed in this chapter is held in the Massey University Library as appendices to Wallace's published thesis.

The original experiment studied the effects of differing planes of nutrition during pregnancy and lactation on the growth and development of lambs. The lambs (9 singles, 11 twins, 2 twins where only one partner survived, and a set of triplets) were the offspring of Border-Leicester x Cheviot ewes mated to a single Suffolk ram and were the result of two years breeding. The lambs were fed variable amounts of supplementary rations. In the present analysis the effects

of nutrition are used, rather than analysed, since the varying nutrition gave the lambs a large range of birth weights.

For the investigation of compensatory growth the offspring of one ewe in one season (lambs L.48 and L.49) have been excluded from the analysis since Wallace noted that this ewe refused food and that she declined in weight from 104 lbs to 68 lbs during the early part of lactation. The adverse effect of this can be seen in the growth curves of these two lambs (Fig 2.2.).

The live weights of the lambs were recorded, in pounds, from birth to 16 weeks of age (weaning). Two values recorded in Wallace's appendices were clearly mis-recorded or wrongly transcribed. These were,

L.6 weighed at 35 days of age, weight given as 14.9 analysed as 24.9

L.34 weighed at 63 days of age, weight given as 29.2 analysed as 39.2

For further details on the experimental conditions, results, and the conclusions reached by Wallace, the original work and its appendices should be consulted.

BIOMETRICAL CONSIDERATIONS

A) NORMALISATION

Figure 2.1 shows the mean weekly live weights and their standard deviations on three scales (metameters),

a) the original arithmetic metameter,

b) for the data transformed to logarithms to the base 10, and

c) after the addition of a constant term, to give a constant origin, for the data transformed to logarithms to the base 10.

On the arithmetic scale standard deviation increased linearly with the mean ($r = +0.9912$). This confirms previous findings for rat live weight. Following the transformation of the data to logarithms mean and standard deviation were, unexpectedly, still strongly correlated ((b) in Fig 2.1). But the correlation was negative ($r = -0.9462$), standard deviation decreasing with increased live weight. As can be seen from (b) in Fig 2.1 this correlation was removed by the introduction of a third parameter as suggested by Bliss (1967). Since both the

FIGURES 2.1A & 2.1B
RELATIONSHIPS BETWEEN MEANS AND STANDARD DEVIATIONS, ON ARITHMETIC TWO, AND THREE PARAMETER LOG METAMETERS, FOR BORDER LEICESTER - CHEVIOT x SUFFOLK LAMBS, WEIGHED WEEKLY FROM BIRTH TO WEANING, DATA OF WALLACE (1948), THESIS APPENDICES PP. 56-67.

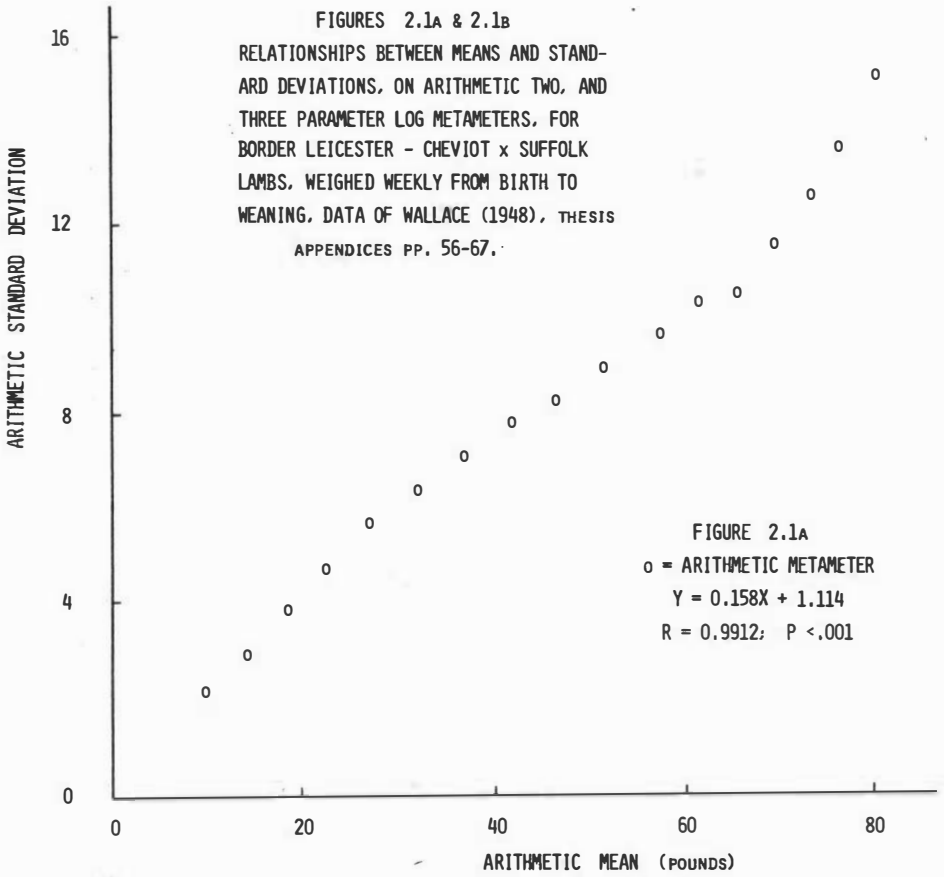


FIGURE 2.1A
o = ARITHMETIC METAMETER
Y = 0.158X + 1.114
R = 0.9912; P <.001

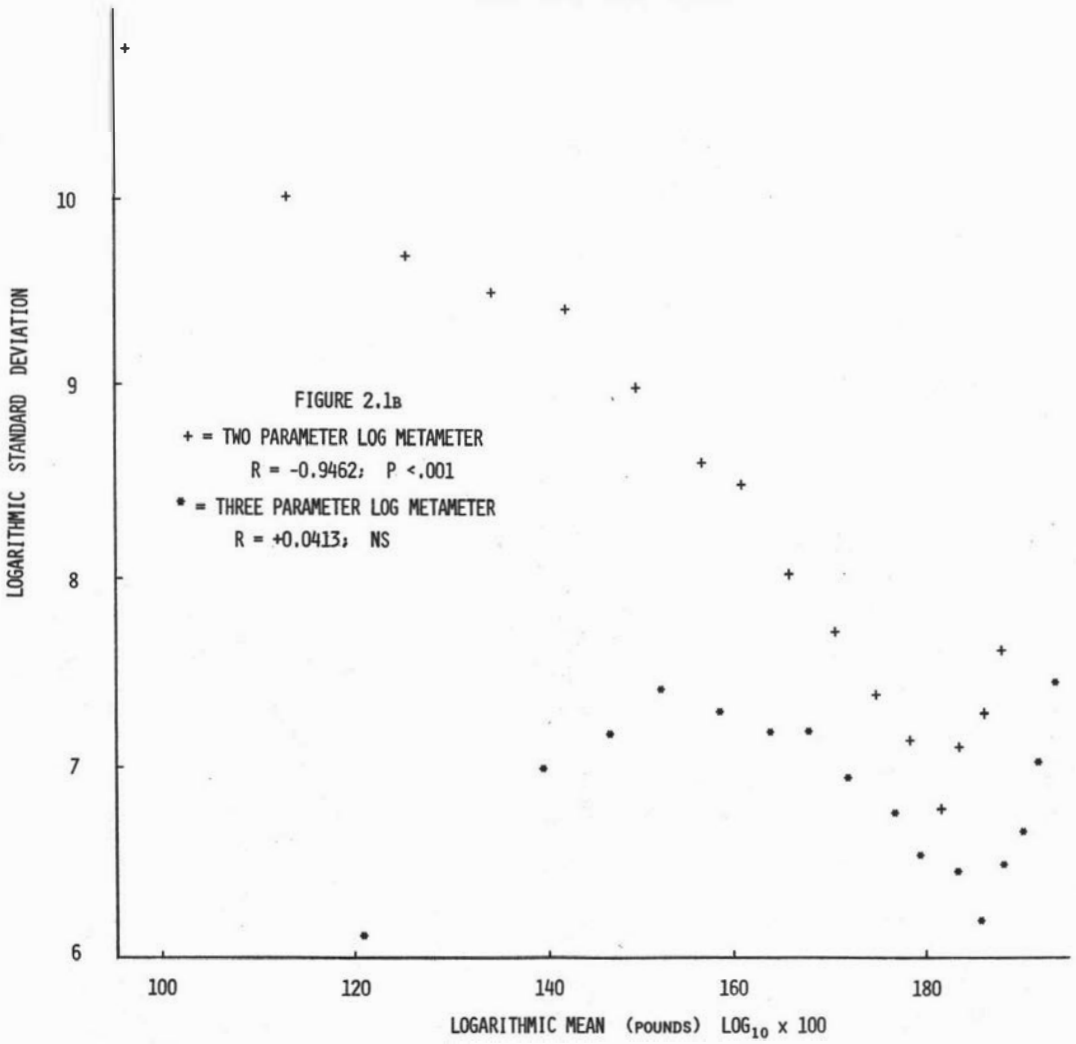


FIGURE 2.1B
+ = TWO PARAMETER LOG METAMETER
R = -0.9462; P <.001
* = THREE PARAMETER LOG METAMETER
R = +0.0413; NS

constant and the slope of the arithmetic plot differed from zero a common origin for the log transformation should be calculated. This is obtained by dividing the constant by the slope (i.e. 1.114/0.158 = 7), from the arithmetic plot, and adding the resultant (7) to all the original measurements before taking logarithms. The transformation (termed the three parameter lognormal) removed the significant correlation between mean and standard deviation shown on the arithmetic scale. All the regressions were unweighted as roughly equal numbers of measurements were available at all ages.

B) LINEARISATION OF THE PRE-WEANING WEIGHT-AGE CURVES OF INDIVIDUAL LAMBS

For an individual rat a plot of the reciprocal of age against the logarithm of live weight resulted in a linear relationship from 4 weeks of age onwards. The pre-weaning growth of a lamb represents a different stage of growth to post-weaning rat growth and thus would be expected to follow a different pattern explained by a different equation. So different linearising transformations would be expected in the lamb.

For individual lambs the original arithmetic scales for weight, the dependent variable, and age, the independent variable, gave a reasonable linear fit. However, a priori, and on the results of plotting means and standard deviations, live weights were transformed to logarithms and regressed against arithmetic age. This also resulted in a good fit. However the best fit was obtained by transforming age to its log, a double log-log plot resulting in correlation coefficients that in 31 out of the 36 individuals were above 0.99. Equations and correlation coefficients are given in the appendices (Appendix: Table 2.1). To avoid the logarithm of zero 1.0 was added to all ages, making birth equal to 1.0, before taking logarithms.

In Chapter 7 it will be shown that the residuals (the differences between the observed and expected values) about the log-log plot are systematic, suggesting that the log-log plot is not the best, or complete, description of the growth in live weight of the lamb.

For the young rat a log-log plot was found by Zucker *et al* (1941a) to give the best fit to live weights obtained from birth to 6 weeks of

FIGURES 2.2A & 2.2B
THE LINEAR RELATIONSHIPS BETWEEN LOG LIVE WEIGHT AND LOG AGE (WEEKS + 1.0) FOR A SINGLE, A PAIR OF TWINS AND A SET OF TRIPLET LAMBS, DATA OF WALLACE (1948).

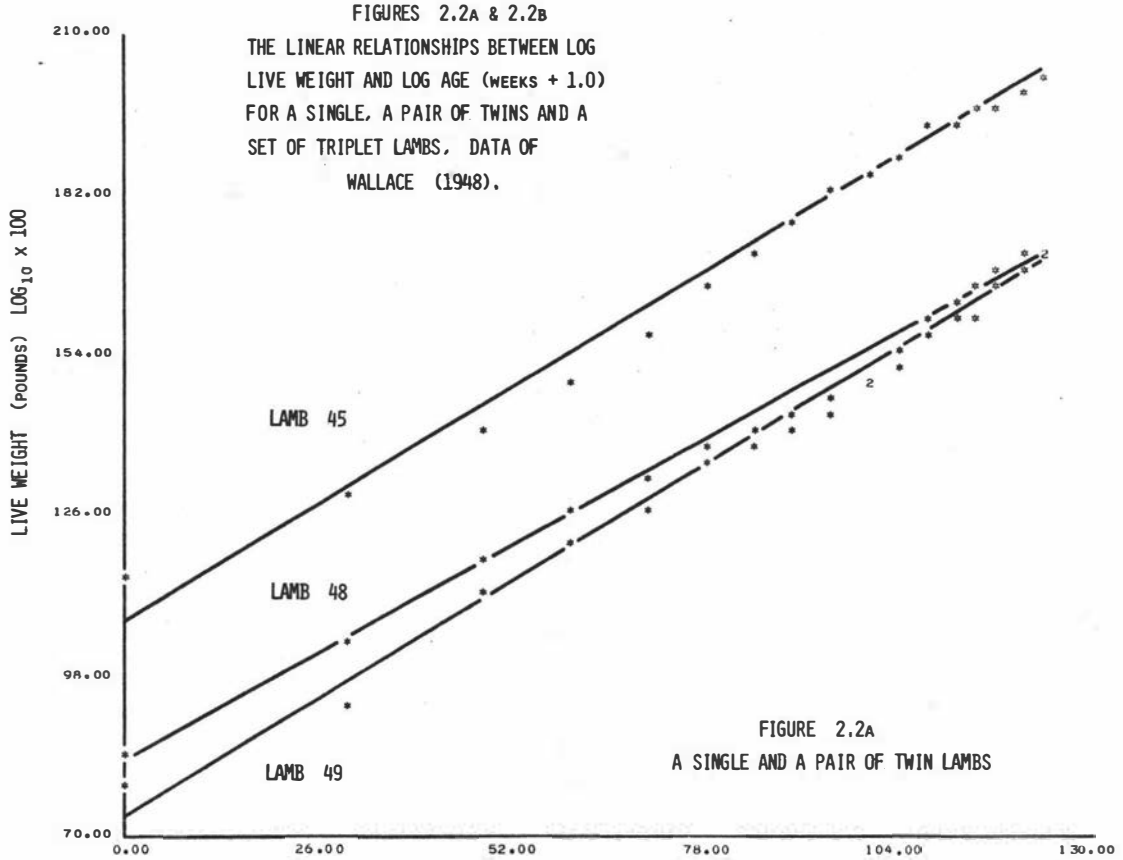


FIGURE 2.2A
A SINGLE AND A PAIR OF TWIN LAMBS

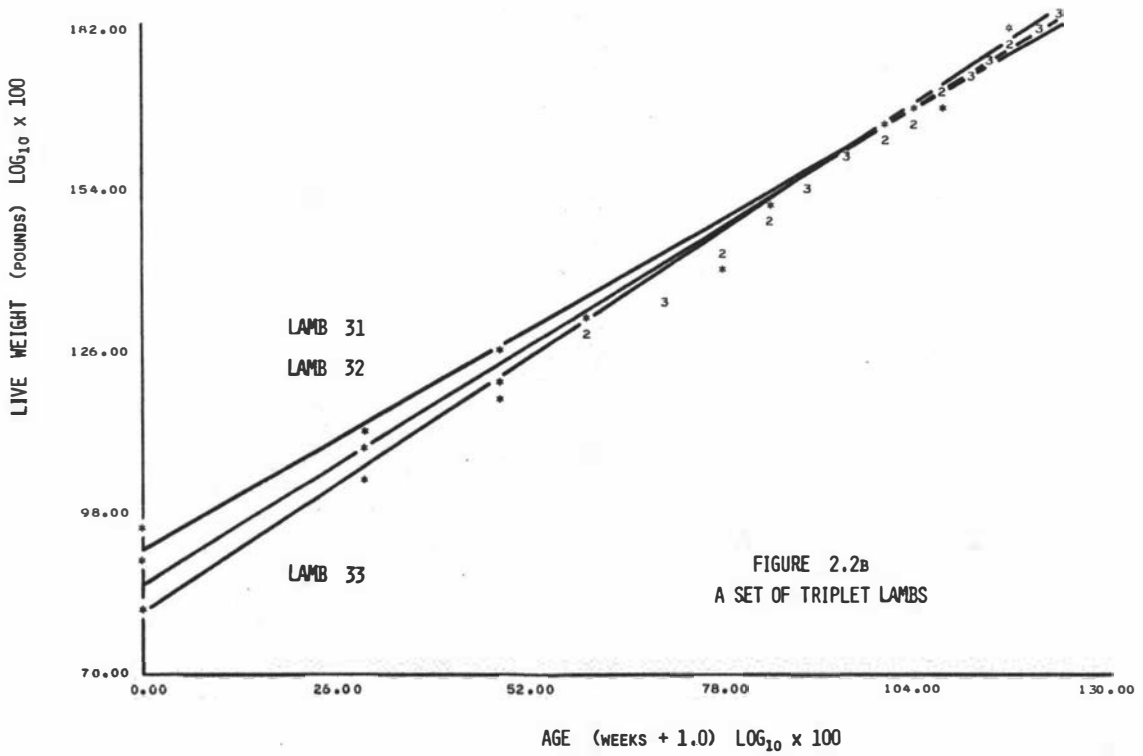


FIGURE 2.2B
A SET OF TRIPLET LAMBS

age. However in the rat zero time was taken as conception while for the lamb birth has been taken as zero time. But it is surprising that in such different species post-natal to weaning live weight increase should be best explained by a similar equation.

Examples of the successful linearisation of the weight-age curves for single lambs, twins, and triplets are illustrated in Fig 2.2.

The closest approximation to the normal distribution followed the transformation of live weights to a three parameter lognormal metameter, so live weights transformed to this scale were also regressed against log age. The three parameter transformation gave similar correlation coefficients to those obtained from the two parameter transformation. The slopes, constants, and correlation coefficients are given in the Appendix (Table 2.1). However the two parameter transformation is both simpler to apply, and to comprehend, and in the present case it was used in preference to the more complex three parameter transformation. Some other reasons for using the two parameter transformation are discussed in Chapters 6 and 7.

Post-weaning weighings were available from several individuals to which the two parameter log-log plot again seemed to give a good fit.

C) BIRTH WEIGHT OR CONSTANT FOR DEMONSTRATING COMPENSATORY GROWTH?

In Chapter 1, following customary practice, the recorded weaning weight was used to ascertain the degree of compensatory growth in the rat. However as the constant is the calculated birth weight in the log-log equation the birth weight is easily available in the lamb (the constant in the rat's log-reciprocal equation being at infinite age). So the constant was regressed against the rate of weight gain (the slope). The result was, initially, somewhat surprising.

It can be seen from Figs 2.3(a) and 2.3.(b), that for individual lambs the correlation between rate of gain and the measured or observed birth weight was -0.6785 while that between rate of weight gain and the constant (the calculated or expected birth weight) was higher at -0.8129. The variation in birth weight accounting for 46% and 66% of the variation in rate of weight gain respectively. The advantage

FIGURES 2.3A & 2.3B

THE LINEAR RELATIONSHIPS BETWEEN THE SLOPES, FROM THE LINEAR EQUATIONS RELATING LOG LIVE WEIGHT TO LOG AGE, AND THE MEASURED AND CALCULATED BIRTH WEIGHTS, LAMBS OF WALLACE (1948).

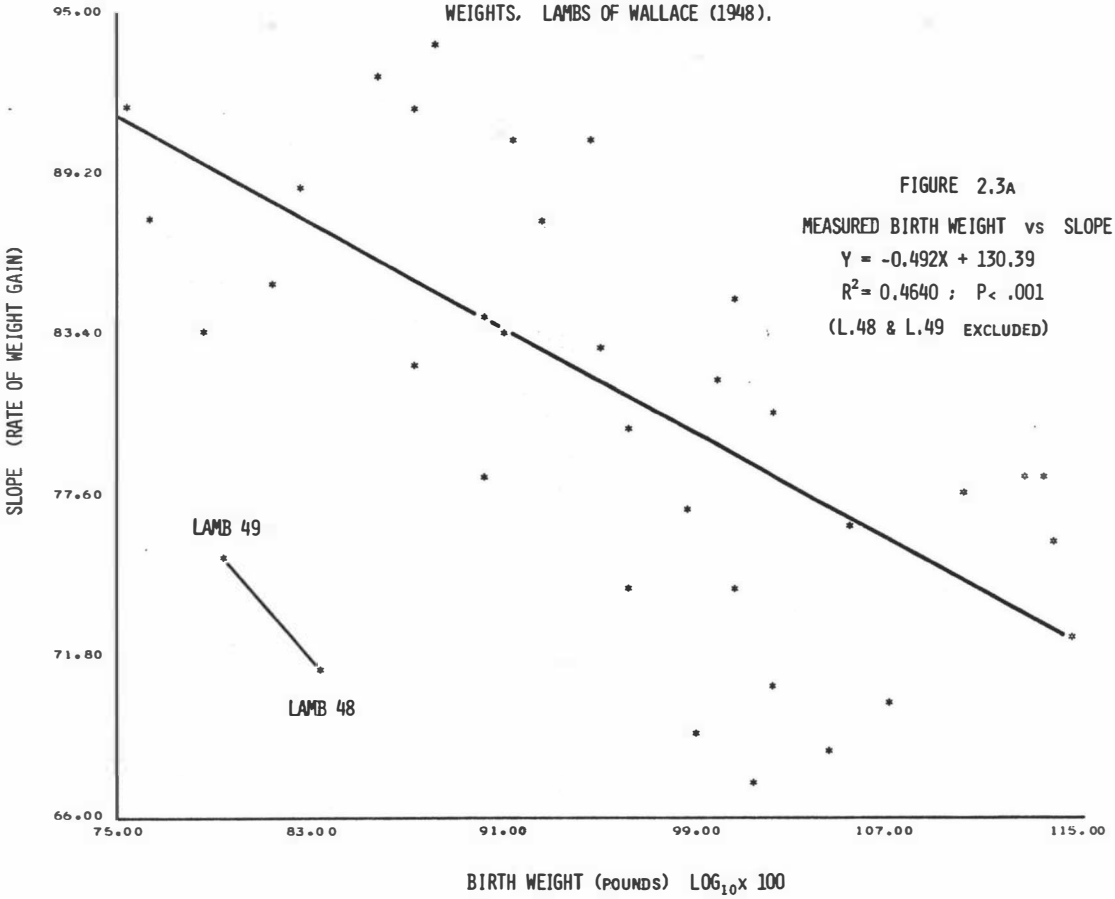


FIGURE 2.3A

MEASURED BIRTH WEIGHT vs SLOPE

$Y = -0.492X + 130.39$

$R^2 = 0.4640$; $P < .001$

(L.48 & L.49 EXCLUDED)

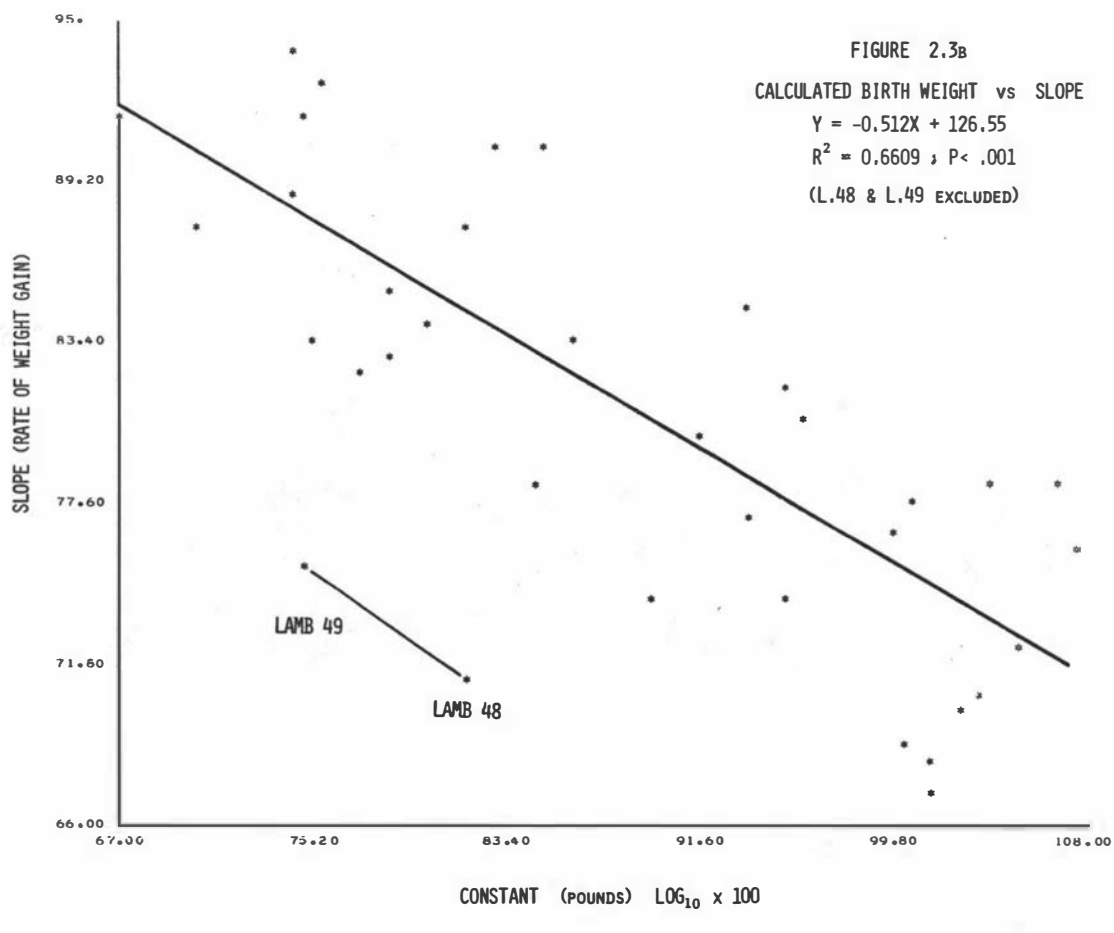


FIGURE 2.3B

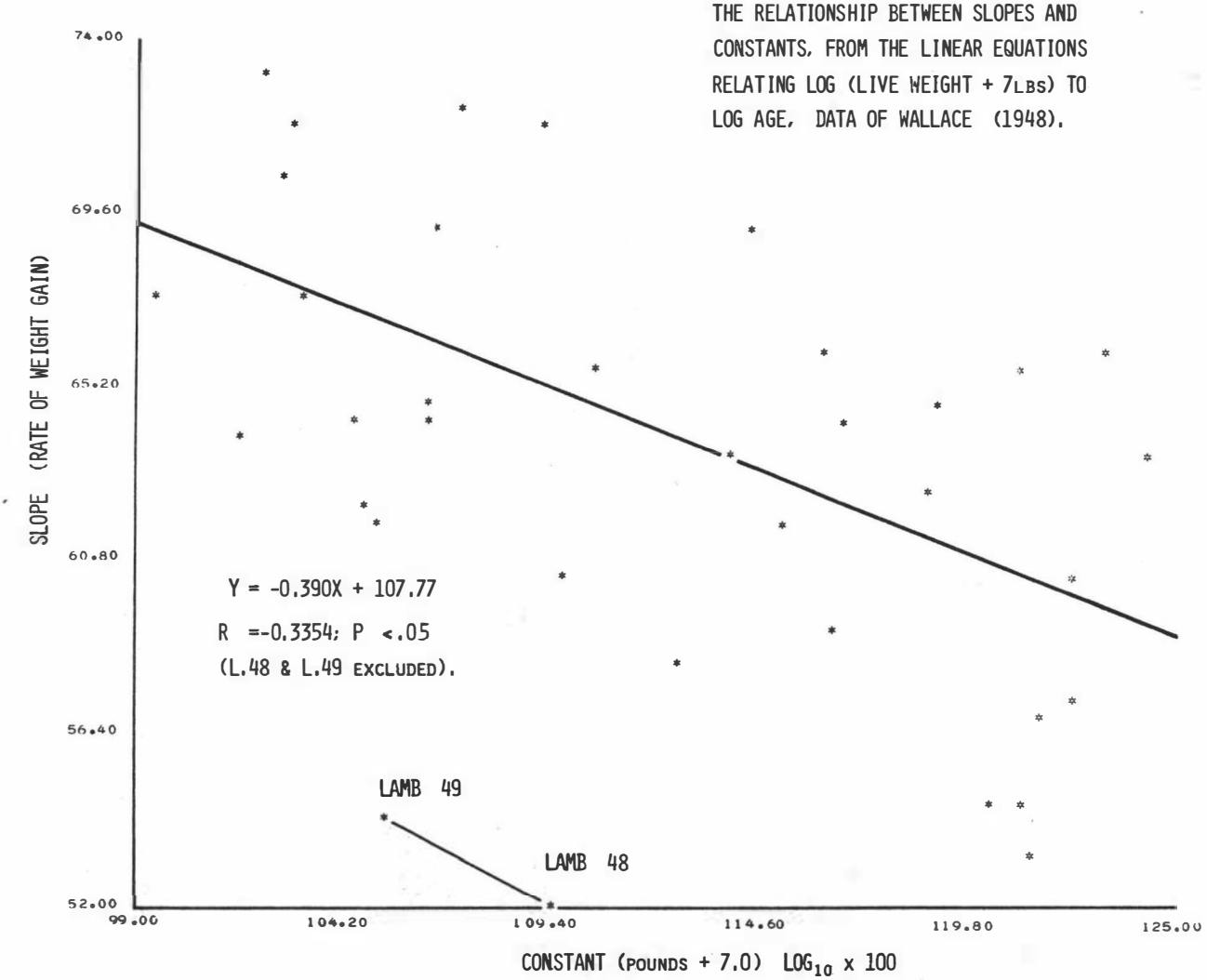
CALCULATED BIRTH WEIGHT vs SLOPE

$Y = -0.512X + 126.55$

$R^2 = 0.6609$; $P < .001$

(L.48 & L.49 EXCLUDED)

FIGURE 2.3c
THE RELATIONSHIP BETWEEN SLOPES AND CONSTANTS, FROM THE LINEAR EQUATIONS RELATING LOG (LIVE WEIGHT + 7LBS) TO LOG AGE, DATA OF WALLACE (1948).



of using the calculated constant, rather than the recorded birth weight, is obvious.

RESULTS AND DISCUSSIONS

Although an arithmetic plot showed standard deviation increasing with live weight the two parameter lognormal plot showed the within group variation to be decreasing with increasing live weight. This systematic heterogeneity of the variation was suspected to be due to a non-random effect, compensatory growth, causing differences between animals to be reduced, on the log scale, with increased live weight. Although the three parameter lognormal distribution was theoretically the superior transformation to achieve normality, in the present case it was possible that the addition of an empirical constant would mask compensatory growth.

To discover if pre-weaning compensatory growth was occurring, and what effect the three parameter transformation had on the detection of compensatory growth, constants and rates of gain calculated on both two and three parameter scales, were regressed. The data (slopes and constants) is given in the Appendix (Table 2.1) and illustrated in the text (Figs 2.3.b and 2.3.c).

On the two and three-parameter scales compensatory growth was indicated. Apparently the planes of nutrition the ewes were subject to during lactation, although widely divergent, still allowed sufficient nutrition for all lambs (singles, twins, and triplets) to display compensatory growth. That compensatory growth occurs following birth suggests that the experimentally imposed nutritional stress had a greater influence on lamb growth during pregnancy than it did during lactation.

The three parameter transformation gave a much reduced correlation between birth weight and rate of weight gain compared to the two-parameter case. This was because the addition of 7.0 to the smaller birth weights doubled them, while the larger birth weights were only increased by half, with later weighings being increased by progressively smaller amounts. This caused, on a logarithmic scale, the standard deviation of the initial weights to be reduced while those of the larger weights remained comparatively unaffected. The

three-parameter transformation seems to act, in the present example, by partially removing the effect of birth weight on the standard deviation. The three parameter transformation is useful, unless one is concerned with the sources of the between animal variability.

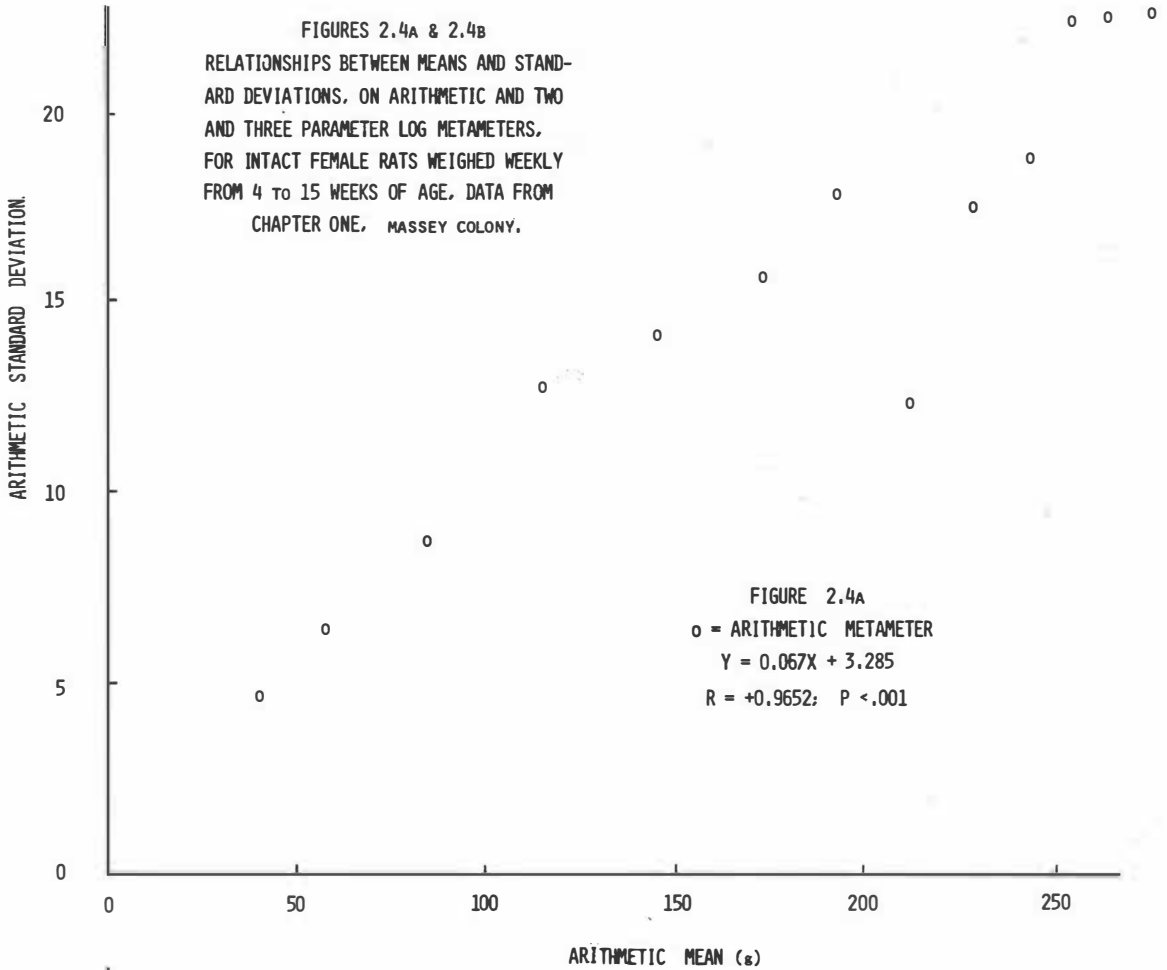
The three parameter lognormal distribution is undoubtedly a valuable statistical tool in situations where variance problems are present, but it should be used with caution. For example; it could be erroneously concluded from Figure 2.3c that only 10% of the variation in rate of weight gain is associated with birth weight, simply because the three-parameter transformation largely equalised the birth weights. Conversely, if the desired result was to compare groups of animals, with the effect of the initial weights removed, then the three-parameter lognormal transformation may be advantageous as a simple, but rough, alternative to, or aid in, covariance analysis. For example, the variation in rate of weight gain due to initial weight, although not removed, was reduced from about 66% to about 10% by the three-parameter transformation.

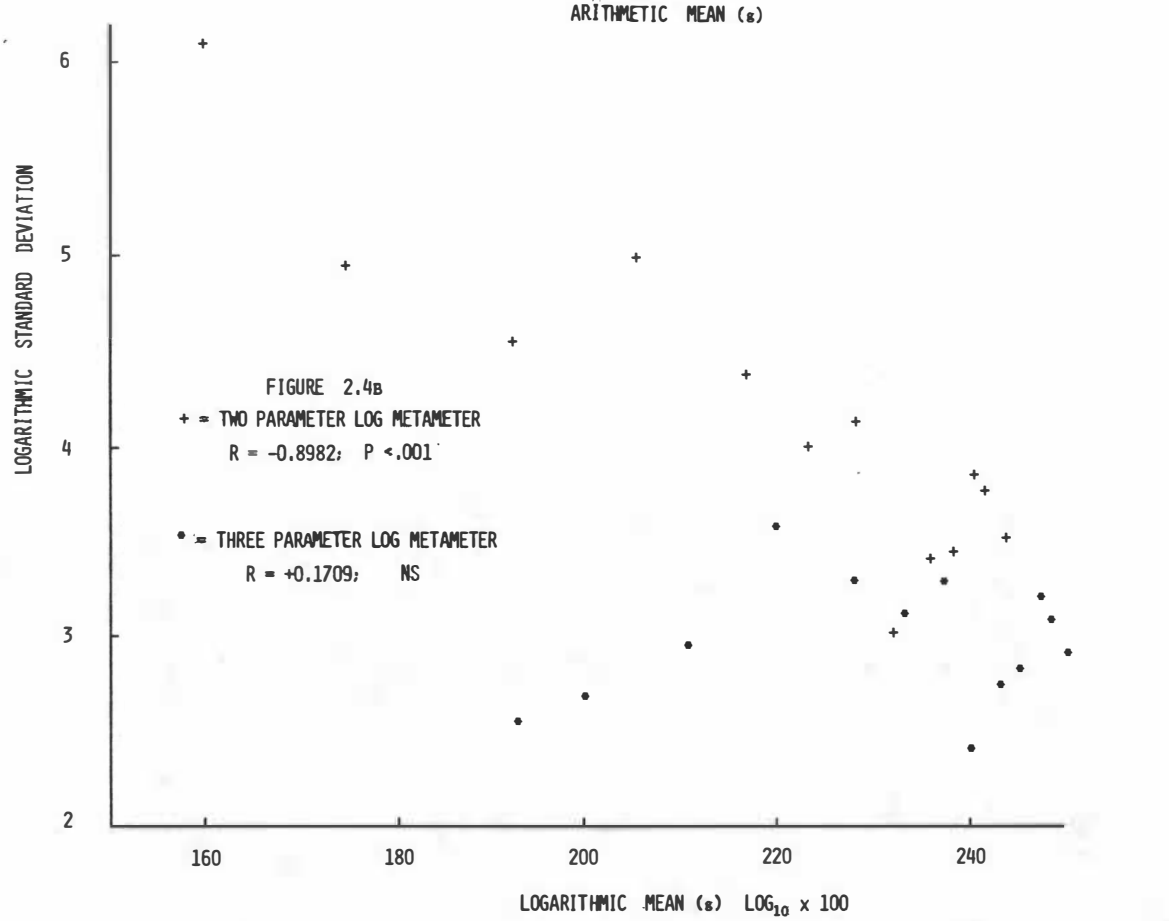
It was previously concluded, for rats ovariectomised at four weeks of age, that the transformation of body weight to a two-parameter lognormal metameter removed the significant correlation between mean and standard deviation shown on the arithmetic scale (Chapter 1, Fig 1.1.a). However post weaning compensatory growth was demonstrated in a different treatment group, the intact controls. Here a negative correlation between mean and standard deviation, on the two-parameter log scale, would be expected if the findings from the sheep data applied equally to the rat.

As predicted the intact control rat data did show a strong negative correlation between the mean weekly weights and their standard deviations (Fig 2.4, $r = -0.8982$). By adding 45.0 to all the original meansurements, before taking logs, this correlation was removed (Fig 2.4b, $r = +.1709$), So the compensatory growth of intact rats was reflected in a decreased standard deviation with increased mean live weight.

In the case of both rat and ovine live weight measurements compensatory growth may cause the within group variation to be heterogeneous, but it is probable that the two-parameter lognormal metameter is the underlying normalising distribution of the random

FIGURES 2.4A & 2.4B
RELATIONSHIPS BETWEEN MEANS AND STANDARD DEVIATIONS, ON ARITHMETIC AND TWO AND THREE PARAMETER LOG METAMETERS, FOR INTACT FEMALE RATS WEIGHED WEEKLY FROM 4 TO 15 WEEKS OF AGE, DATA FROM CHAPTER ONE, MASSEY COLONY.





variation (or at least that part not dependent upon birth weight). The present regressions of weight on age used individual animal's weekly weights, rather than the weekly group means. However the finding that the three-parameter lognormal metameter normalised the mean live weights is definitely germane when group means are to be compared or regressed. Although the three-parameter lognormal metameter has been applied to human body weights (see Aitchison & Brown, 1966; Bliss 1967) the transformation seems to have been overlooked in the analysis of the live weights of other animals.

This chapter demonstrates that the techniques developed, and exploited, for rat live weight data can be applied to the live weights of sheep. It seems that for sheep live weights the demonstration of normality and linearisation by logarithmic transformations has not previously been explicitly described. The sensitivity of these techniques is highlighted by the calculated birth weight, or constant, accounting for 20% more of the variation in rate of weight gain than the recorded birth weight. Both biological and statistical explanations for this strengthened relationship are possible. Birth weights obtained from wet membrane-covered newborn animals may be greater than weights obtained from the dry animal a few hours later. So if all the birth weights are not obtained immediately following birth a large amount of variation could be generated. But Fig 2.1 shows no indication that the variation in birth weight was greater than that predicted from the overall relationship between mean and standard deviation. But, statistically, the predicted birth weight is derived from all 17 weighings and would be expected to provide a better estimate than a single recorded weight.

CHAPTER THREE

LAMB LIVE WEIGHTS AND COVARIANCE;

AN EXPERIMENT ANALYSED

INTRODUCTION

It was shown in previous chapters that compensatory growth in lambs, before weaning, and in rats, following weaning, could be demonstrated using linearised weight-age growth curves for estimating rate of weight gain. This result could be of practical significance in the analysis of some growth experiments by allowing a more precise analysis, and subdivision, of the between group variation. No attempt was made to apply these findings to Wallace's data, due to the availability of data of a more immediate interest and to the low numbers of animals in Wallace's experiment.

In longitudinal growth experiments the initial weights (i.e. birth weights, weaning weights, or simply the first weights) largely determine the subsequent growth rates. So the experimenter has from the initial weights some knowledge as to the probable subsequent growth rates of the animals. To take advantage of this prior knowledge, when the animals are being allotted to treatment groups, the matching of groups or pairs is sometimes employed, frequently solely on the basis of one initial weighing. However in many situations a limited number of animals or subjects are available, all of which are to be included in the experiment, so matching would be far from exact. Another objection to matching solely on one initial weight is that, although there may be a strong relationship between initial weight and growth rate, individuals of the same initial weights can, subsequently, have very different growth rates. A fundamental statistical objection is that such matching involves the non-random allocation of individuals to treatments which introduces potential sources of bias to the experiment. Billewicz (1965) concluded that 'There is little doubt that in the statistical sense matching wastes information, ...' and he found his computer simulations to '...show that matching improves the precision of the experiment but that for quantitative response variables the improvement is smaller than that which can be achieved by covariance analysis'.

The data used in this chapter was collected by Dr M.F. Tarttelin and Prof R.E. Munford during the Spring and Summer of 1974-75 at Ripley Rise on a Massey University farm. The experiment attempted to

FIGURES 3.1A & 3.1B

RELATIONSHIPS BETWEEN MEANS AND STANDARD DEVIATIONS, ON ARITHMETIC, TWO, AND THREE PARAMETER LOG METAMETERS, FOR LAMBS WEIGHED WEEKLY UNTIL WEANING, DATA OF TARTTELIN & MUNFORD (1974-5).

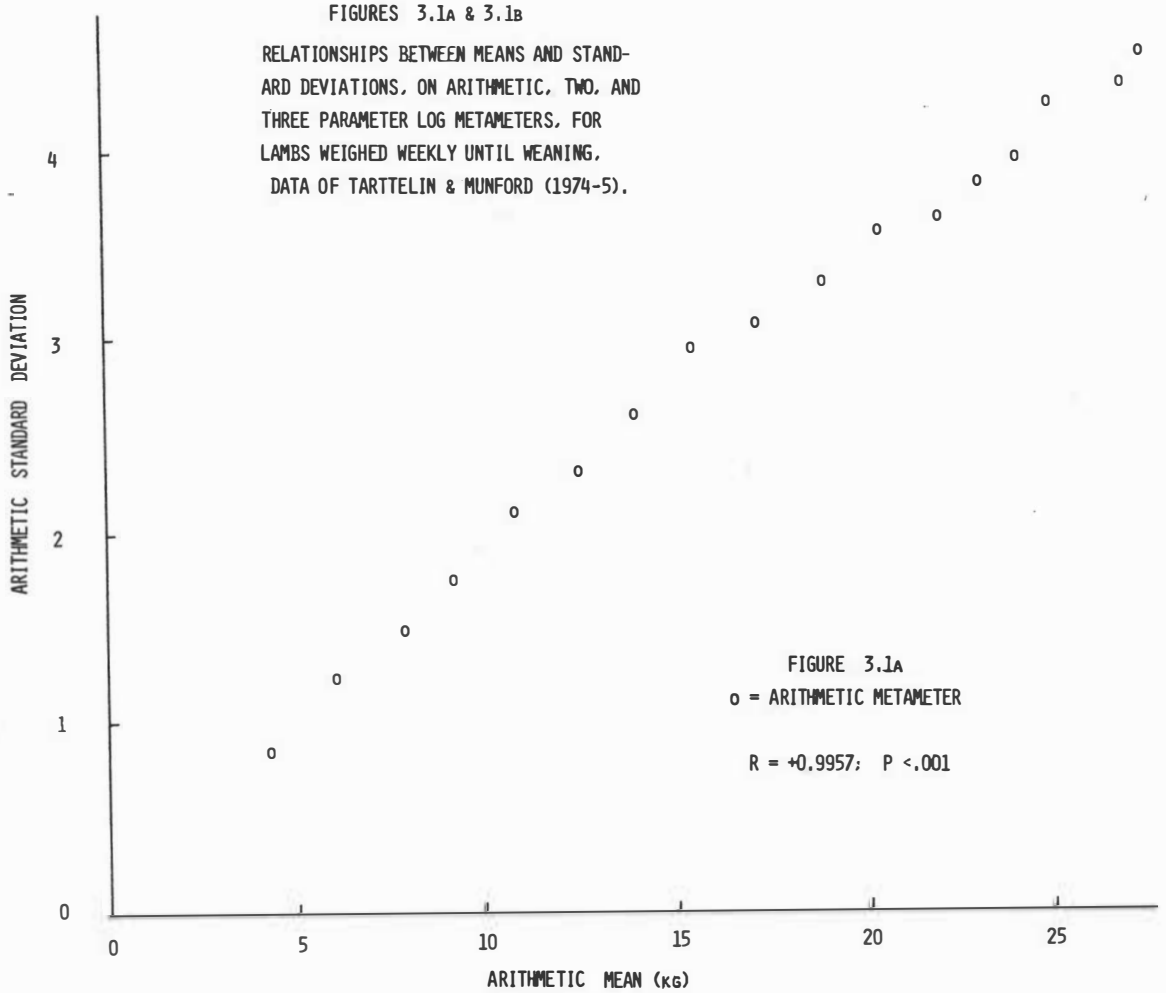


FIGURE 3.1A
o = ARITHMETIC METAMETER

R = +0.9957; P <.001

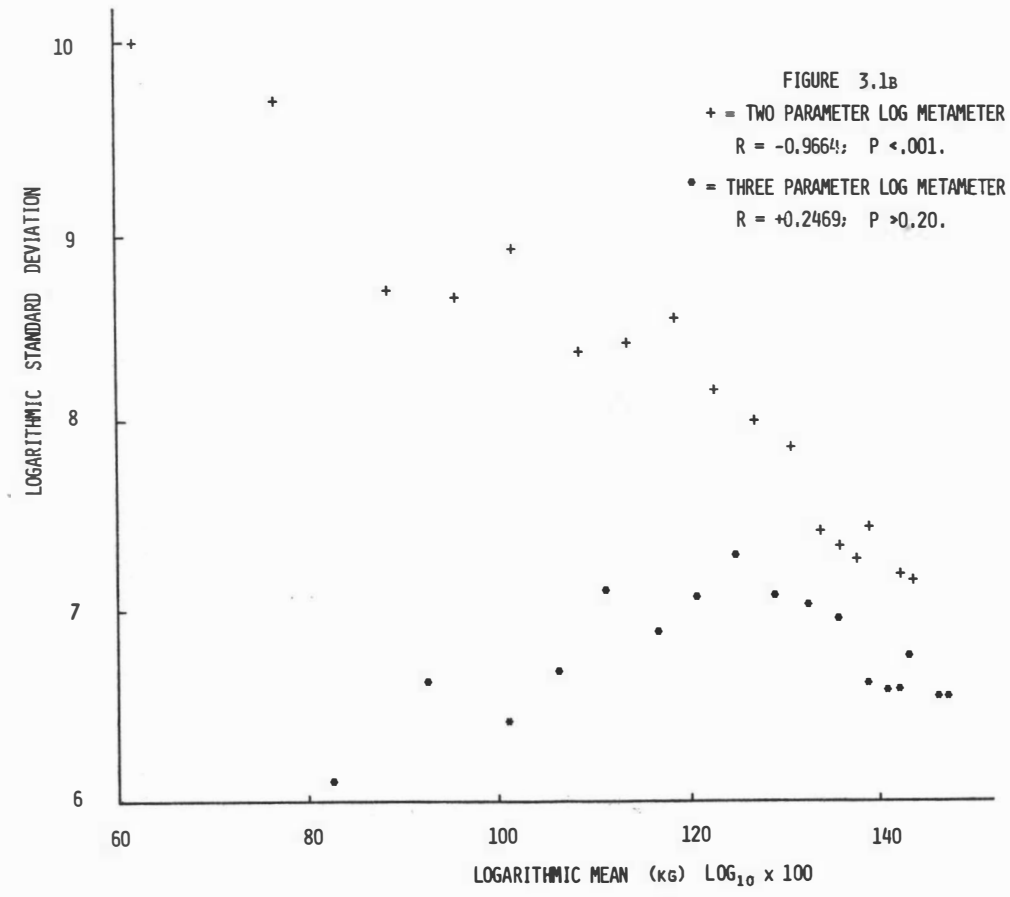


FIGURE 3.1B
+ = TWO PARAMETER LOG METAMETER
R = -0.9664; P <.001.

• = THREE PARAMETER LOG METAMETER
R = +0.2469; P >0.20.

produce 'androgenised' lambs by treating the pregnant ewes with testosterone propionate (TP). The lambs were weighed weekly (in kilograms) from birth and weaned at 16 weeks of age.

A conventional method of analysis, multiple one way analyses of variance of the untransformed weights at each of the 17 weighings, indicated that male lambs were heavier than females; no statistically significant effects of TP treatment on live weight could be shown. But in Chapter 1 this method of analysis was shown to be statistically unsound and inefficient. So using the methods described in Chapters 1 & 2, and some extensions of them, the data was re-analysed.

Using an analysis of covariance, as recommended by Billewicz, the model to be investigated was; if the effect of birth weight was removed would TP treatment, sex, or birth status influence the slope of the pre-weaning growth curves.

BIOMETRICAL CONSIDERATIONS

Figure 3.1 illustrates the mean weekly live weights and their standard deviations, for all seventeen weighings from the sixty one lambs, on three metameters;

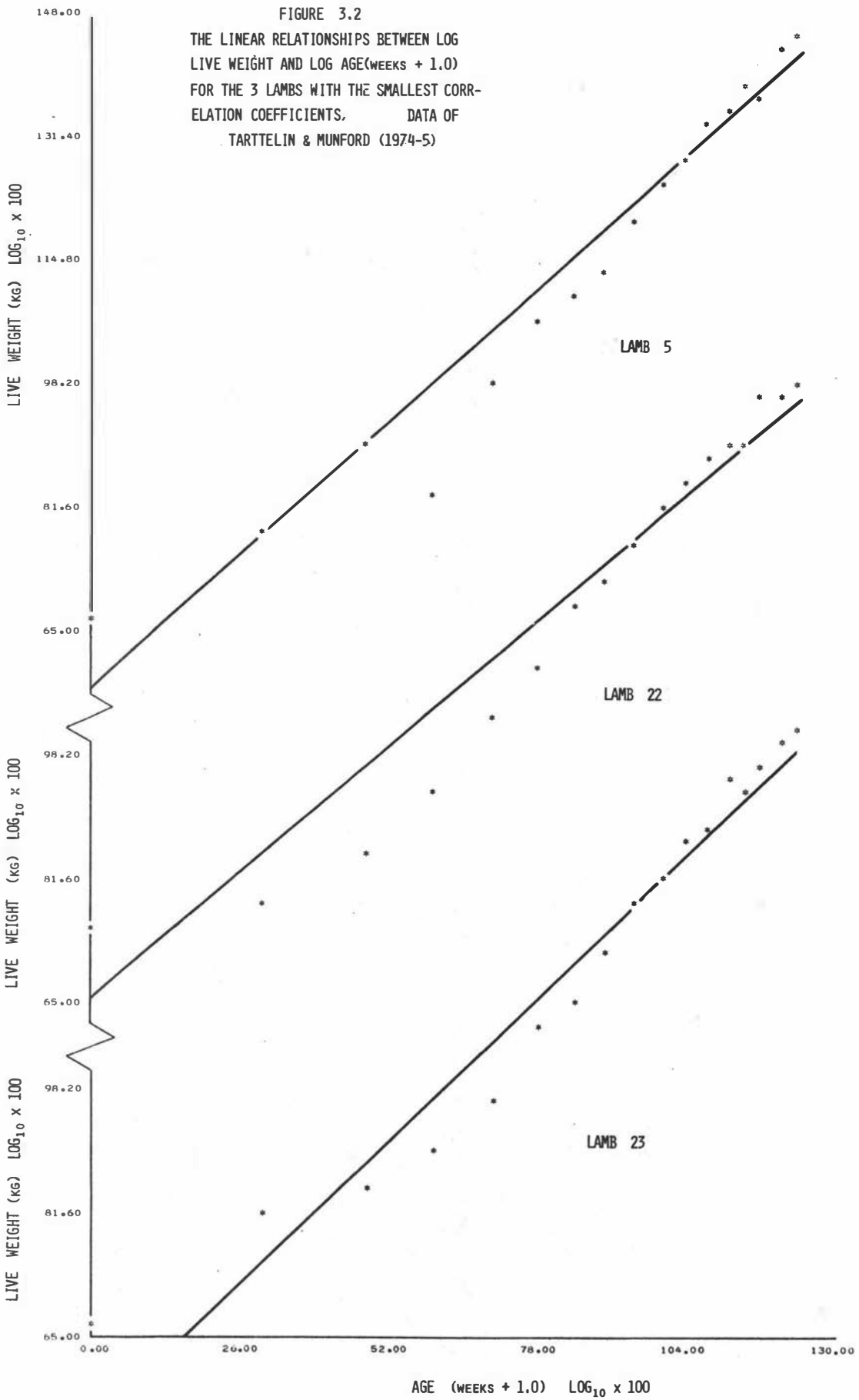
a) the measured arithmetic scale

b) as logarithms to the base 10 (two parameter lognormal)

and c) on a three parameter lognormal scale.

The results in Figure 3.1 can be seen to be comparable to those calculated from the data of Wallace (Chapter 2 Fig. 2.1). On the arithmetic scale the standard deviation increased with the mean, the relationship again being convincingly linear ($r = +0.9957$). Following the transformation of the data to logarithms the variation about the means decreased with increased mean live weight ($r = -0.9664$), confirming the results in Chapter 2. The addition of a constant calculated from the arithmetic plot (constant/slope = calculated constant; 0.4/0.15 = 2.5) followed by the taking of logarithms (giving a three parameter logarithmic transformation) removed the previously statistically significant correlation between means and

FIGURE 3.2
THE LINEAR RELATIONSHIPS BETWEEN LOG LIVE WEIGHT AND LOG AGE(WEEKS + 1.0) FOR THE 3 LAMBS WITH THE SMALLEST CORRELATION COEFFICIENTS, DATA OF TARTTELIN & MUNFORD (1974-5)



standard deviations (r= ±0.2469).

The techniques and transformations used in Chapter 2, for the data of Wallace (1948), were applied. Namely, slopes and constants for individual animals were computed, by regressing 100 times log live weight and log age. To avoid the logarithm of zero 1.0 was added to all the ages before taking logarithms. Slopes, constant, and correlation coefficients are given for the males and females in Table 3.1 of the Appendices. It was noted in Chapter 2 that the three parameter logarithmic transformation could be of use where groups of animals are being compared. Thus live weight (log (live weight + 2.5)) was regressed against log age. The slopes, constants and correlation coefficients for the individual animals are also shown, in Appendix Table 3.2.

The three animals with the lowest correlation coefficients (calculated with live weight transformed to a two parameter logarithmic metameter) all showed an early retardation of live weight increase followed by compensatory growth (Fig 3.2). This result deserves illustration as a low correlation coefficient could have been due to the imposed treatment altering the overall relationship between live weight and age giving a systematic pattern of departures from linearity, rather than the explicable episodic deviations displayed. Furthermore Fig 3.2 shows the recorded birth weights to be greater than the constants (i.e. when x=0). It could be argued that birth weights should be excluded from the regressions. Birth weights are probably more representative of the pre-natal population of weights, which are subject to very different influences than post-natal weights, and may follow a different relationship to time.

It was shown in a previous chapter that the calculated birth weight (the constant) accounted for a much greater percentage of the variation in rate of gain than did the recorded birth weight. So the analysis of covariance used the constant as the covariate and the slope (rate of gain) as the response. Unfortunately sub-class numbers were unbalanced making a three way analysis of covariance impossible, using the computer programmes available. Methods for such an analysis have however been formulated (Grossman and Gall, 1968). So slopes and constants were analysed by a one way analysis of covariance.

RESULTS AND DISCUSSION

Tables 3.1 and 3.2 of the Appendices show that the correlations between log live weight and log age were greater than 0.990 for 45 of the 61 lambs. This result is slightly inferior to that obtained from the data of Wallace (Appendices, Table 2.1). This was as expected as the pasture grazing of the Massey lambs and ewes was uncontrolled compared to the indoor housing and controlled nutrition of Wallace's animals. But the high correlations obtained were actually better than anticipated as near drought conditions prevailed during the summer of 1974-75. These results support the wide applicability of the linearising technique since the lambs, both male and female (and androgenised), of different breeds, in different countries, under very different nutritional regimes, and being singles, twins, and triplets, all grew in live weight in general accordance with a single relationship to time. This result is especially important because if these factors or treatments had affected the shape of the growth curves, to cause significant departures from linearity, linearisation would be of limited value for the analysis of between group variation; the fitting of different relationships to different groups making comparisons impossible. On the other hand the ability to detect departures from linearity is also a valuable tool as it allows outlying points, or individuals, to be identified.

The full analysis of covariance of the slopes and constants, calculated following a two parameter logarithmic transformation of live weight, is shown in Table 3.1.* A one way analysis of variance for slope and constant (Table 3.1) indicated, at the 5% level of probability, no significant overall differences for either slope or constant, although the 10% level was exceeded. So there was no conclusive evidence that the covariate, or the response, was influenced by treatment, sex, or birth status. The strong relationship between slope and constant, assumed a priori, was demonstrated by the statistically significant and negative average within group correlation ($r=-0.8325$). The regressions within the treatment groups were not significantly different although a difference is suggested by the 10% level of significance being exceeded. After fitting the regression

* Analyses of variance programme written by Dr. F. Cockrem adapted by Prof. R.E. Munford.

TABLE 3.1

ANALYSES OF VARIANCE AND COVARIANCE, FOR THE SLOPES (Y) AND CONSTANTS (X) FROM THE LINEAR EQUATIONS RELATING LOG LIVE WEIGHT (ON A TWO PARAMETER LOG METAMETER) TO LOG AGE. LAMBS OF TARTTELIN AND MUNFORD (1974 - 75).
M = MALE, F = FEMALE, TP = TP TREATED, C = CONTROL, S = SINGLE, TW = TWIN, Cst = CASTRATE.

TREATMENT	X MEAN	Y MEAN	ADJ.Y MEAN	± SEM	CORRELATION
F S TP	60.36	67.02	69.43	1.059	-.8337
F TW TP	53.43	70.43	69.03	1.152	-.8849
F S C	45.71	75.68	70.05	2.051	-.5460
F TW c	53.62	65.86	64.57	1.407	-.5819
M S TP	61.57	73.55	76.61	1.123	-.9372
M TW TP	55.01	63.39	62.86	1.983	-.8570
M S C	61.23	72.11	75.00	1.427	-.4287
M TW C	52.94	70.66	69.00	1.543	-.9397
M TW Cst	49.73	70.52	67.10	1.255	-.9340

AVERAGE WITHIN GROUP REGRESSION, CORRELATION = -.8325
TOTAL ESTIMATES, IGNORING GROUPS, CORRELATION = -.6405

MEAN SQUARE FOR EACH VARIABLE

SOURCE	DF	SLOPES	CONSTANTS
TOTAL	60	42.450	99.614
BETWEEN	8	73.348	182.422
WITHIN	52	37.696	86.874
F RATIO		1.945	2.099

ANALYSIS OF WITHIN GROUP VARIANCE OF Y

SOURCE OF VARIATION	DF	SUMS OF SQUARES	MEAN SQUARE	F RATIO
DUE TO AV. REGR.	1	1358.666	1358.666	115.188
DEV. FROM AV. REGR.	51	601.553	11.795	
BETWN. IND. GRP. REGRS.	8	165.864	20.733	2.046
DEV. FROM IND. REGRS.	43	435.689	10.132	

ANALYSIS OF VARIANCE OF Y, AFTER FITTING REGRESSION ON X

SOURCE OF VARIATION	DF	MEAN SQUARE	F RATIO
TOTAL	59	25.455	
BETWEEN GROUPS	8	112.543	9.541
WITHIN GROUPS	51	11.794	

ORIGINAL MEAN SQUARE = 37.69 PERCENT REDUCTION OF ERROR = 68.711

TABLE 3.2

ANALYSES OF VARIANCE AND COVARIANCE, FOR THE SLOPES (Y) AND CONSTANTS (X) FROM THE LINEAR EQUATIONS RELATING LOG LIVE WEIGHT (ON A THREE PARAMETER LOG METAMETER) TO LOG AGE. LAMBS OF TARTTELIN AND MUNFORD (1974-5).

(symbols for treatment as in TABLE 3.1).

TREATMENT	X MEAN	Y MEAN	ADJ.Y MEAN	± SEM	CORRELATION
F S TP	78.61	54.50	55.95	0.937	-.6871
F TW TP	73.94	56.15	55.52	1.012	-.7052
F S C	68.98	59.10	56.28	1.796	-.3292
F TW C	72.66	53.74	52.54	1.248	-.5956
M S TP	78.82	61.19	62.73	0.983	-.8817
M TW TP	75.20	50.08	50.02	1.747	-.8551
M S C	78.50	59.92	61.31	1.252	-.1354
M TW C	73.01	56.89	55.86	1.362	-.9077
M TW Cst	71.68	55.33	53.69	1.096	-.7877

AVERAGE WITHIN GROUP REGRESSION, CORRELATION = -.6860
TOTAL ESTIMATES, IGNORING GROUPS, CORRELATION = -.3807

MEAN SQUARE FOR EACH VARIABLE

SOURCE	DF	SLOPES	CONSTANTS
TOTAL	60	23.641	45.354
BETWEEN	8	67.012	76.857
WITHIN	52	16.969	40.507
F RATIO		3.949	1.897

ANALYSIS OF THE WITHIN GROUP VARIANCE OF Y

SOURCE OF VARIATION	DF	SUMS OF SQUARES	MEAN SQUARE	F RATIO
DUE TO AV. REGR.	1	415.355	415.355	45.354
DEV. FROM AV. REGR.	51	467.056	9.157	
BETWN. IND. GRP. REGRS.	8	89.587	11.198	1.275
DEV. FROM. IND. REGRS.	43	377.469	8.778	

ANALYSIS OF VARIANCE OF Y, AFTER FITTING REGRESSION ON X

SOURCE OF VARIATION	DF	MEAN SQUARE	F RATIO
TOTAL	59	20.556	
BETWEEN GROUPS	8	93.223	10.179
WITHIN GROUPS	51	9.157	

ORIGINAL ERROR MEAN SQUARE = 16.96 PERCENTAGE REDUCTION OF ERROR = 46.034

the within group variation for rate of gain was reduced by 68.7% giving a highly significant difference between groups. Follow-up comparisons between the adjusted means showed TP treatment to have significantly ($P<.05$) increased the growth rate of female twin lambs.

Although one would expect female lambs to show a greater response to androgenisation than male lambs, if androgenisation bestows a masculine pattern of growth upon the female, one could explain the female's greater response in another way. It may be recalled, from the results presented in Chapter 1, that a growth stimulus seems to give a greater response in a slowly growing animal; a rat's response to ovariectomy was negatively related to its pre-ovariectomy growth rate. Thus as male lambs grew faster than females they would not be expected to show as large a response to TP treatment; TP treatment had no significant effect in males. Similarly single lambs grew faster than twins; singles showed a damped response to TP.

The analysis of covariance of Table 3.2 evaluates the slopes and constants (Appendices, Table 3.2) that were calculated following the transformation of live weight to a three parameter lognormal metameter. When compared to the analysis of covariance of Table 3.1 several features of interest and differences between the analyses, are apparent. The analysis of variance of Table 3.2 shows that a highly significant difference in slope was present before the effect of birth weight was removed. This demonstrates the use of the three parameter transformation as a rough alternative to an analysis of covariance. As shown in the last chapter this effect is due to the three parameter transformation resulting in less of the variation in rate of weight gain being explained by the calculated birth weight (Table 3.1, $r = -0.8325$; Table 3.2, $r= -0.6861$). But the 'F' value for slope following covariance was increased for the three parameter case, albeit by a small amount (Table 3.1, $F= 9.54$; Table 3.2; $F= 10.18$).

The correlations between the comparably calculated slopes and constants obtained from the data of Wallace ($r= -0.8129$) and that for the Massey lambs ($r=-0.8325$) are almost indistinguishable.

This similarity suggests that pre-weaning compensatory growth (as defined in Chapter 10) may be inherent to the growth of lambs.

The advantages of linearisation can be clearly seen from the analysis of this experiment as linearisation allows more informative statements to be made about the overall growth of animals and of comparisons between animals. For example, multiple one way analysis of variance, besides being statistically questionable, will only indicate that at a certain age a difference in weight occurred. Linearisation provides for more precise, and valuable, conclusions to be reached; for example, 'animals were growing at different growth rates' or 'animals differed in size but grew at similar rates'. In addition the ability to distinguish the significantly increased growth rate of androgenized female twin lambs illustrates the increased sensitivity of linearising techniques, following covariance analysis, compared to the more traditional methods of analysing longitudinal growth experiments.

Since R.A. Fisher first described the analysis of covariance it has been shown to be valuable in many situations. But the use of covariance in longitudinal growth studies is not a common practice. Work by Bailey *et al* (1958) is frequently quoted as evidence that covariance bestows little or no advantage in the analysis of short term growth experiments. However Bailey *et al*. used the recorded initial weight of their dairy heifers as the covariate. Although they calculated a linear regression of weight on age no use was made of the increased precision of the regression to estimate an initial weight to act as the covariate. It is possible that, as in the present example, an estimated initial weight would explain more of the variation in growth rate than the recorded weight. In longer experiments a reduction in error variance has been shown by the use of covariance for cattle (Kincaid *et al*. 1945) and pigs (Wishart 1939; Ashton *et al*. 1955) using live weight gain in a previous period and initial live weight, respectively, as the covariate. In these situations a much greater explanation of differences in rate of weight gain would probably result if an estimated initial weight, or the slope of the regression line, was the covariate.

In the present chapter some advantages resulted from the use of the three parameter lognormal transformation. In the next chapter a situation where definite advantage accrued will be described.

CHAPTER FOUR

THE REGRESSION OF LIVE WEIGHT AND AGE IN CATTLE

I Monozygous twin heifers at Ruakura

INTRODUCTION

Chapters one to three have shown that the analysis of the growth curves of rats and sheep is facilitated by the use of transformations of weight and age. The application of these techniques to the growth of cattle will be investigated by re-analysing the live weight data collected at Ruakura and published by Hancock (1951).

It was stated by Hancock (p.17) that

"Especially under New Zealand pastoral conditions growth rates are highly irregular...." and that "The accurate measurement of body size and growth of large herbivorous animals, and especially ruminants, is difficult because of uncontrollable variations due to ruminal, intestinal and bladder contents. Another difficulty is due to the fact that individual animals do not grow at a constant rate at any stage." Hancock proposed to "...overcome the inaccuracies of measurements by using averages of repeated observations to determine 'true' size at any given time".

Hancock assayed the efficiency of using monozygous twins in growth trials and found, using running averages, that the fluctuations of the variances between and within sets of twins caused estimates of the twin efficiency to vary widely. Twin efficiency for the absolute rate of live weight gain varied between 4 and 21, depending on the number of periods included in the calculations. This large variability could indicate that the method of using running averages did not give reliable estimates of the rate of weight gain. This high variability has even led to doubts, perhaps ill-founded, as to the usefulness of monozygous twins in growth trials (Smith, 1973).

The linearisation of the weight-age growth curves of individual heifers would question Hancock's statement that heifers do not grow at a constant rate and also his use of running averages rather than the original live weights. The variations in live weight of individual ruminants may not be wholly 'uncontrollable', as Hancock described them, but may change in a wave-like manner (Baker and Guilbert, 1942). This intriguing possibility, which is further discussed in Chapter 7, would also count against the use of running averages.

So, using the Ruakura data published by Hancock, the linearisation of the growth curves of individual heifers was attempted. Using the estimated slopes and constants twin efficiencies were then calculated using the mean squares within and between sets of twins obtained from an analysis of covariance.

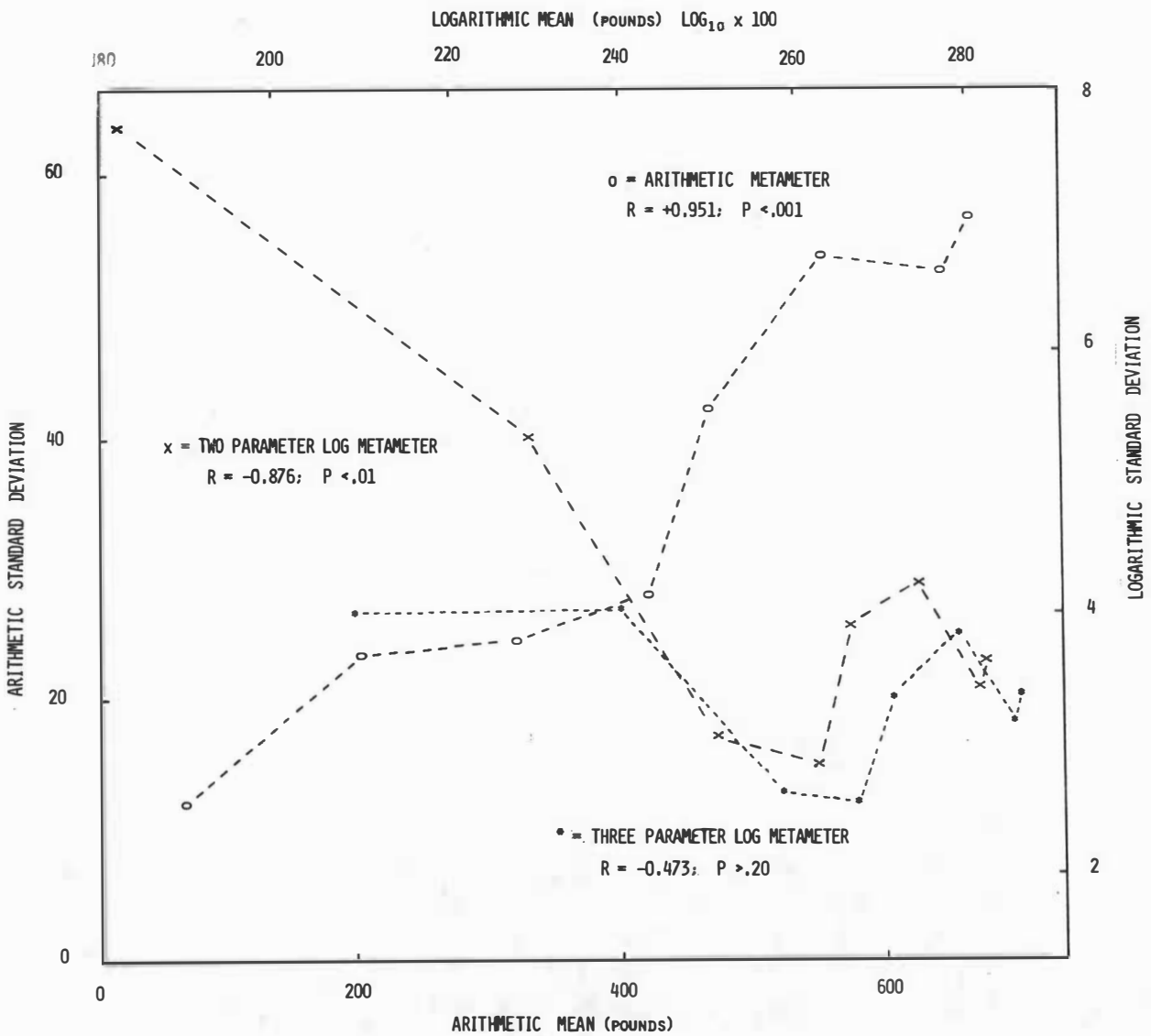
It will be shown, using the techniques developed herein, that birth and weaning weights are greatly influenced by the environment while the mature weight is largely pre-determined or genetically controlled. Thus birth weights largely determine logarithmic growth rates due to compensatory growth. Although these are not original findings the techniques developed in this work do allow a clear and simple demonstration of these effects. The relevance of these findings to the methods used for the selection, for breeding, of meat producing animals is also discussed. Taylor (1968) recognised that selecting animals for high average daily gains leads to increases in mature weight. Therefore he developed a criteria of selection which he believed would bend growth curves (or leave animals that mature early in life). Thus the idea that growth curves can be bent by selection will be critically examined.

THE DATA

The identical twin heifers, born in the winter of 1944, were taken to Ruakura at 7-10 days of age and fed on milk until weaning (at 16-18 weeks of age). Following weaning they were given pasture grazing supplemented with hay and silage. (For further details see Hancock 1951).

The live weight measurements (in pounds) published by Hancock were the individual live weights from twenty heifers (ten sets of twins) but consisted of the moving averages of consecutive weekly measurements centred at 4, 16, 28, 40, 52, 64, 76 and 88 weeks ofage.

FIGURE 4.1
RELATIONSHIPS BETWEEN MEANS AND STANDARD DEVIATIONS FOR LIVE WEIGHTS AT 8 AGES, ON ARITHMETIC AND TWO AND THREE PARAMETER LOG METAMETERS, FOR RUAKURA MONOZYGOTIC TWIN HEIFERS. DATA OF HANCOCK (1951).



BIOMETRICAL CONSIDERATIONS

The statistical techniques employed were those previously applied to both rat and sheep live weights. For the live weights on both arithmetic and logarithmic metameters, means and standard deviations were calculated at each of the eight ages, and then regressed. Figure 4.1 shows that on an arithmetic scale mean and standard deviation were strongly positively correlated ($r = +0.951$), while on a two parameter logarithmic scale mean and standard deviation were negatively related ($r = -0.876$). To achieve normality the three parameter lognormal metameter was employed by adding a constant ($4.385/.0777 \simeq 60$) to all the live weights before taking logarithms. Following a three parameter logarithmic transformation means and standard deviations were statistically unrelated (Fig 4.1, $r = -0.473$; $P > .20$), confirming the results from other species in previous chapters.

So to attain both normality and homogeneity of the variation live weight was transformed to logarithms. Apart from the knowledge gained in previous chapters there was little basis, a priori, for the selection of a transformation of age that would achieve linearisation. However as log age linearised the growth curves of lambs, a related species, log age was the initial and seemingly successful choice. Therefore each animal's live weights were transformed to both two and three parameter log scales, multiplied by 100, and regressed against log age. The resultant slopes, constants, and correlation coefficients for both scales are given in the Appendices (Table 4.3). The relationships between weight and age for two sets of twins are illustrated in Fig. 4.2(a) for the two parameter transformation, and for the same animals in Fig. 4.2(b) for the three parameter transformation of live weight. On both scales the correlation coefficients were comparable to those previously obtained from rats and sheep. Possible systematic departures from linearity are evident from the average weights obtained at 4 and 16 weeks of age. It was noted in sheep (Chapters 2 and 3) that the birth weight was consistently above the weight calculated from the regression; in the Ruakura heifers the average weight at 4 weeks was also above the

FIGURES 4.2A & 4.2B
RELATIONSHIPS BETWEEN LOG AGE AND LOG LIVE WEIGHT, ON TWO AND THREE PARAMETER LOG METAMETERS, FOR TWO SETS OF MONOZYGOTIC TWIN HEIFERS WEIGHED FROM 4 TO 88 WEEKS OF AGE, DATA OF HANCOCK (1951).

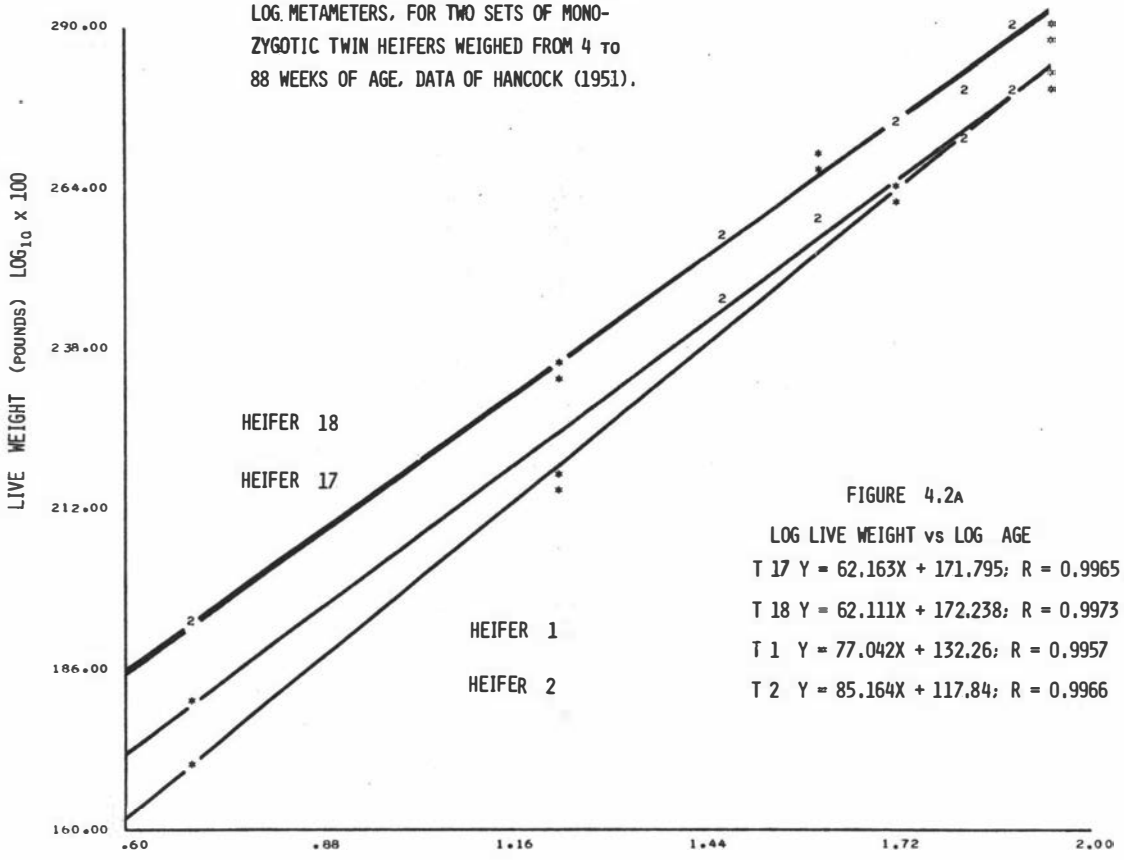


FIGURE 4.2A
LOG LIVE WEIGHT vs LOG AGE
T 17 Y = 62.163X + 171.795; R = 0.9965
T 18 Y = 62.111X + 172.238; R = 0.9973
T 1 Y = 77.042X + 132.26; R = 0.9957
T 2 Y = 85.164X + 117.84; R = 0.9966

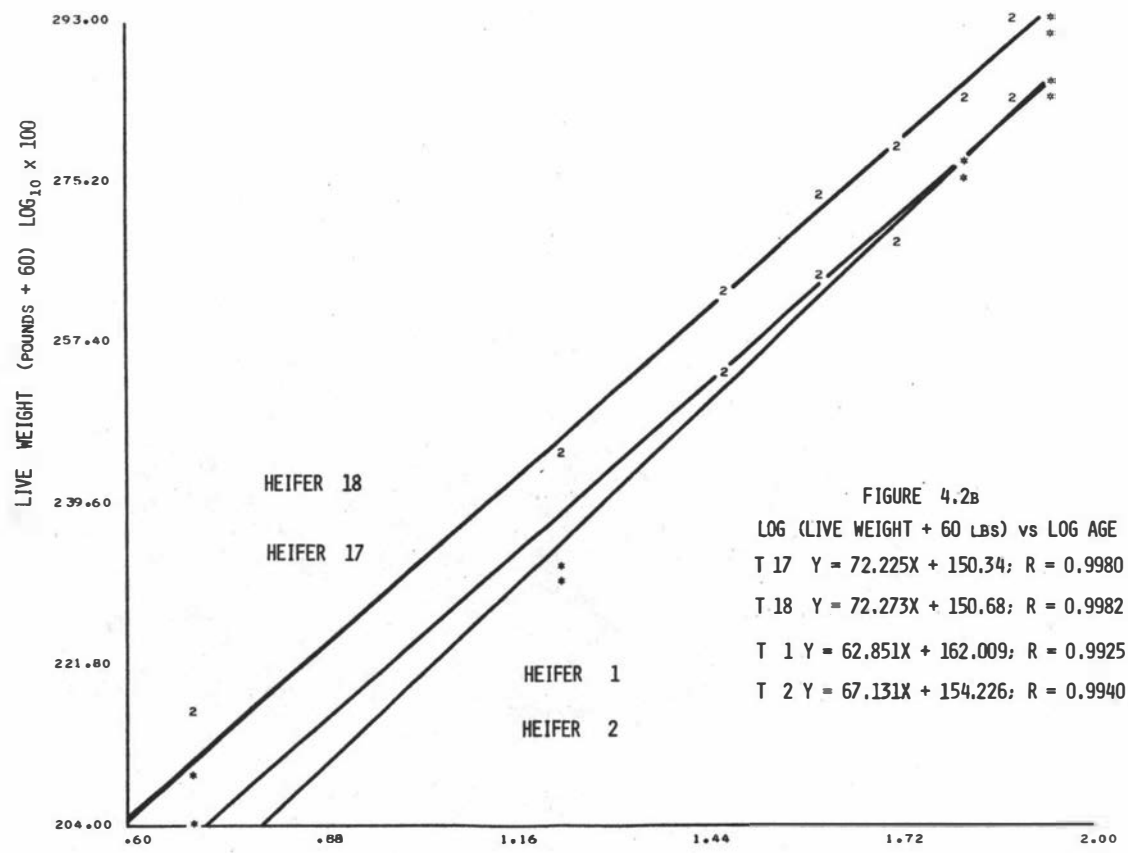


FIGURE 4.2B
LOG (LIVE WEIGHT + 60 LBS) vs LOG AGE
T 17 Y = 72.225X + 150.34; R = 0.9980
T 18 Y = 72.273X + 150.68; R = 0.9982
T 1 Y = 62.851X + 162.009; R = 0.9925
T 2 Y = 67.131X + 154.226; R = 0.9940

line. Animals were weaned at 16 weeks to 18 weeks; the average live weight from this period is below the calculated value.

To obtain estimates of the twin efficiency slopes and constants were subjected to analysis of covariance with the slope (rate of gain) the variate and the constant as the covariate.

RESULTS AND DISCUSSION

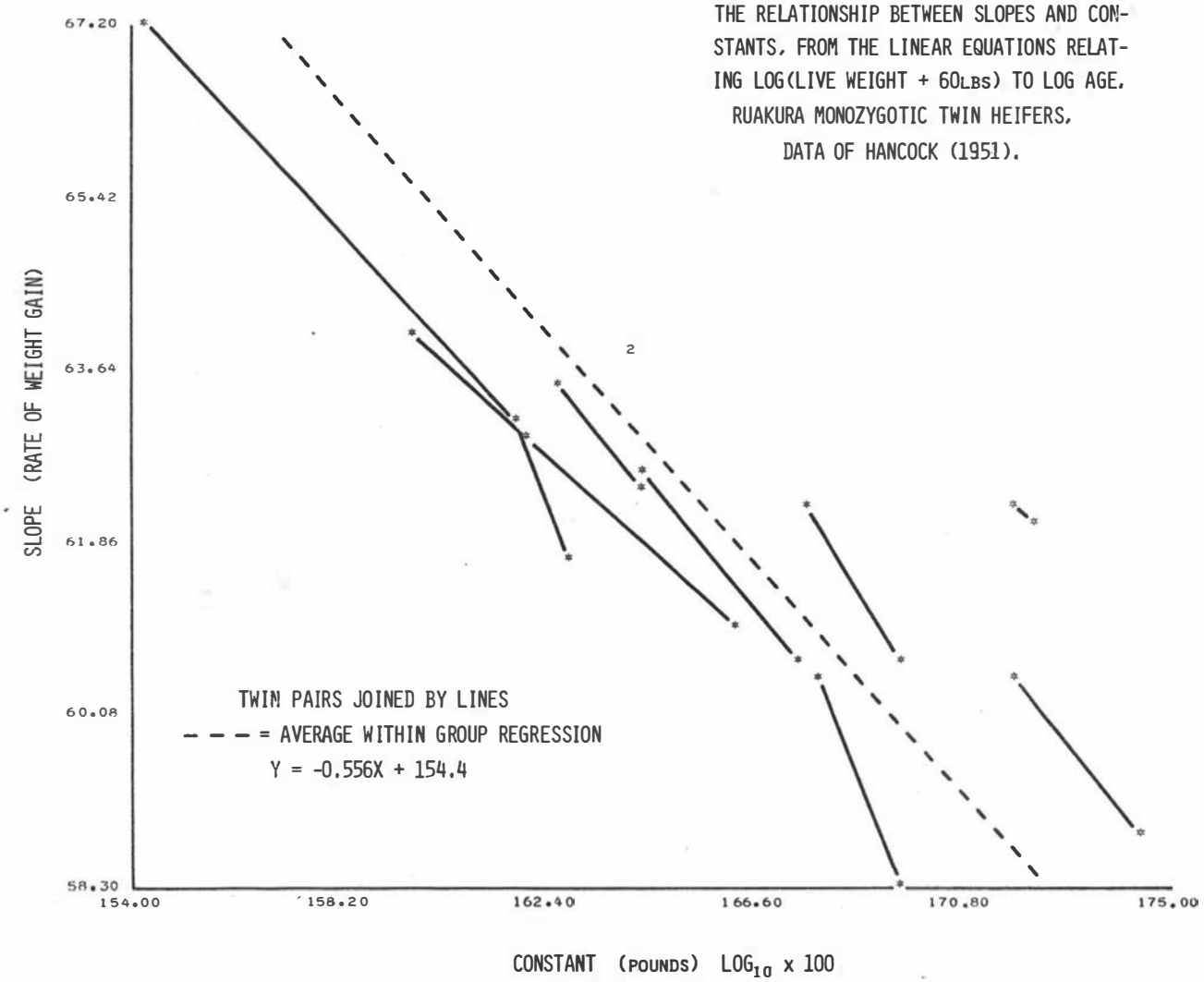
COMPENSATORY GROWTH

The arithmetic means and standard deviations were positively correlated but following the transformation of live weight to a two parameter logarithmic metameter means and standard deviations were negatively correlated. This result is contrary to what Hughes (1976, p.116), in a review of the literature concerning live weight measurement in cattle, recently stated,

"The size of the experimental error (standard deviation) has been found to increase somewhat with the age of cattle..... Weight by itself does not appear to increase the size of the error....."

But the present results are consistent with the author's view of the multiplicative nature of live weight growth (see Chapter 10), agree with similar findings in other species, and suggest that post-natal compensatory growth also occurs in heifers. Figure 4.3 illustrates the strong negative relationship ($r = -0.9667$) between slope and constant, on an average within group basis, for the three parameter case. This result is also indicative of compensatory growth. In lambs the correlation between the rates of gain and the constants was about 0.80 while the comparable correlation, for the two parameter case, (ignorning groups) in monozygotic heifers was about 0.96 (Appendix Table 4.1). The high correlation for the monozygotic twins indicates that environmental factors control birth weight which in turn largely controls the subsequent rate of weight gain. These results also suggest that mature body size is heritable while growth rate is largely determined by the birth weight. Compensatory growth was demonstrated in another way by

FIGURE 4.3
THE RELATIONSHIP BETWEEN SLOPES AND CONSTANTS, FROM THE LINEAR EQUATIONS RELATING LOG(LIVE WEIGHT + 60LBS) TO LOG AGE. RUAKURA MONOZYGOTIC TWIN HEIFERS, DATA OF HANCOCK (1951).



Knapp & Clark (1947) when they showed a steady rise in the hereditary control over bovine weight gain, from 10 to 54 to 84 percent, in three successive 84 day periods from weaning. Donald (1960), using one-egg twins, two-egg twins, full sibs, half sibs, and unrelated individuals, estimated the genetic and environmental components of the variation in untransformed live weights and concluded that "....about weaning time environmental variation reaches its maximum." Donald's interpretation differs from that of the present study as the transformed live weight age curves of individuals showed a convergence, from birth, and thus a steady decrease in variation, which was also shown by the logarithmic plot of means and standard deviations. This difference in interpretation could be explained by Donald using the weaning weight where an additional component of variation, that associated with individual responses to weaning, could have inflated the variation at this age. But overall a steady decline in the environmental influences could be occurring with only a transitory rise at weaning. (See Chapter 5 wherein this point is further studied). The majority of evidence led Dickerson (1954), in his review of the literature,to state that "....mature size is affected more than early growth by the individuals' genotype and less by maternal and other environmental factors".

However the results obtained by Donald illustrate the poor estimate of heritability obtained by using only the weaning weight to select for body size. Weaning and birth weight have been advocated as criteria for selecting for mature body size. The above results, and their discussion, indicate why estimates of heritability for weaning weight are low, averaging 0.27 (Nicoll, 1975) or 0.30 (Preston & Willis, 1970), compared to those obtained at later ages. For example Preston & Willis's review gives 0.52 as the estimate for post-weaning rate of live weight gain and 0.70 for final weights.

Within a colony of laboratory rats genetic variation in ultimate weight would, on a logarithmic scale, seem to be small compared to the environmentally controlled variations in weaning weights. Thus the high correlations between the weaning weights and the post-weaning growth rates of rats may be indicative of the constancy of the ultimate weights between animals. In the ruminant data analysed herein the genetic

constancy seen in the laboratory animals should be absent, so that the high correlations between rates of gain and calculated birth weights could indicate that the compensatory mechanisms in ruminants may be superior to those of the rat.

SELECTION FOR BODY SIZE

The present results question the use of birth weight as an adequate criteria for selecting for adult size in cattle, as proposed by Kassab (1964). The relevant correlations between birth weights and subsequent weights, from the literature reviewed by Kassab, show a decline with age in heifers, especially in the data collected by Kassab, indicating compensatory growth. In bull calves the correlations persist with age, perhaps indicating a sex difference for compensatory growth in cattle. (In Chapters 2 and 3 no sex difference in the compensatory growth shown by sheep was noted. Widdowson (1976) did note a sex difference in the compensatory growth of rats; males showed a relative failure of 'catch-up' compared to females.) Kassab does not comment upon the clear decrease in the correlations with age, in his female animals, even though the estimates for females were based upon twice the number of animals that were included in his male groups. Despite this difference between the sexes he proceeded to pool the males and females to obtain the rather meaningless total correlation, irrespective of sex. Birth weights are relatively poor indicators of the inherent growth potential of a young animal as they mostly reflect the maternal influences. In other words, birth weights tell the agriculturalist what has happened in utero not what is about to happen at pasture. So selection for mature size would be more profitably made if more than two weighings were obtained, at least the birth and weaning weights, from individuals.

This study also indicates that selecting for a high rate of gain may not be identical to selecting for mature weight. Selection for a high rate of logarithmic gain would also, because of compensatory growth, lead to selection for low birth or weaning weights. The justification for selecting animals for high rates of gain or high

mature weights is not clearly established. What the breeders of meat producing animals would seem to desire is an animal that is early maturing, or has a particularly rapid early growth rate, without an increased mature size. The outcome of selecting for average daily gain has, as Taylor (1968, p.287) observes, "...gone towards changing mature body weight". But the evidence supporting the idea that one can 'bend' growth curves by selecting for animals of a similar mature weight but of different early growth rates seems tenuous. The author is unaware of published data describing, in laboratory animals, systematic attempts to 'bend' growth curves by selection. However Gray and Addis (1948, p.40) found "A straight line relation between the log body-weight and the reciprocal of age...for data derived from 11 different colonies,'; suggesting that genetically different animals do have growth curves of similar shapes. It seems odd that experiments to confirm the feasibility of the objectives of livestock breeders have not been given priority by mammalian geneticists. For example strains of rats of genetically different mature weights or growth rates could be used to test for differences between the strains in the residuals about the log-reciprocal equation (see Chapters 6 and 7). Differences between strains in the patterns of the residuals would confirm the feasibility of selection based on the shape of growth curves. Attempts have been made (i.e. in mice by Kidwell, Howard and Laird, 1969) to study the inheritance of the parameters of the Gompertz equation, without a great deal of success. Perhaps selection based upon the residuals about the lines rather than the values of the parameters would have been more successful.

Fitzhugh and Taylor (1971) present a method of selecting early maturing animals - those that reach a high percentage of their mature live weight at an early age. This method of selection seems to overcome the problem of selection for weight at a particular age, or selecting for live weight gain, leading to selection for mature weight. But the method, as described by Fitzhugh and Taylor, has its difficulties. In an earlier study by Taylor and Young (1967) virgin twin cattle were fed on 6 levels of constant food intake until 7 years of age yet surprisingly some treatment groups, especially those on the

higher feeding levels, continued to increase in weight despite their constant food intake. Breeding cows, under North American range conditions, were shown by Urick *et al*. (1971) to be still increasing in live weight at 11 years of age. These results illustrate that the concept of a measurable mature weight in cattle is difficult to substantiate. But mature weight, as was shown in the rat (Chapter 1), can be estimated. Perhaps Fitzhugh and Taylor should have recommended the use of a weight, at a fixed age or its estimate, rather than introducing the concept of a mature weight. This concept was traced by Fitzhugh and Taylor to Brody's work; again the profound influence of Brody on agricultural research is evident.

It was stated by Fitzhugh and Taylor that their method "....might also be applied with advantage to data involving frequent observations but where the mean growth curve is so irregular that the normal type of growth curve could not be meaningfully fitted" (p.717). This statement is not soundly based. For example in their own work Fitzhugh and Taylor combine adjacent live weights since "The genetic correlations between growth rate and weight or degree of maturity tended to change abruptly....To reduce these transient genetic associations... average weight or degree of maturity at the mid-point of the age interval was used...." (p.722). Thus their results demonstrate that combining weights leads to greater genetic correlations. That fitting growth curves, or using transformed weight-age scales, which combine all live weights, give superior estimates of mature weights or birth weights has also been shown in the present work.

Mature weight can not be measured precisely so that dividing all live weights by the mature live weight then analysing the resultant degrees of maturity, as suggested by Taylor and Fitzhugh, leads to the error associated with mature weight being incorporated into the errors in the estimates of the degrees of maturity at each age. For example if the mature weight is too high all the degrees of maturity will be too low and the vice versa. Despite their stating that logarithmic transformations of weight are desirable Fitzhugh and Taylor appear to use untransformed live weights to estimate the degree of maturity.

On an arithmetic scale the variation in live weight increases with increasing size so that the error attached to a mature weight would necessarily give large errors in the degrees of maturity at immature ages. Smith *et al.*, (1976, p.391) found that, "Correlations among degrees of maturity tended to be lower than corresponding correlations between body weights." showing that the large variation in mature weights does indeed inflate the variation in degrees of maturity compared to the variation in the actual immature weights. A superior approach would be for the data to be transformed to logarithms and the mature weight then estimated from all the data. This improved estimate could then be used to calculate the degrees of logarithmic maturity.

There is some evidence that the selection for degree of maturity in live weight may be associated with undesirable traits. McCarthy (1974) discusses his evidence that selection for high body weight at an early age gave fatter mice than selection for high body weight at a latter age. Selection for degree of maturity may not be desirable to the meat producer. Maturity is characterised by a high rate of fat deposition so that the finding that early maturing animals have an increased fat deposition at an early age is not wholly unexpected. Also as fat is the most variable component in the body it would be expected to be the body component most sensitive to selection pressure. For example in pigs,Webb and King (1976) showed that 11 years selection against backfat thickness reduced the thickness by about 40%. Clearly the selection of meat producing animals for degree of maturity should not proceed without adequate analysis of changes in body composition.

At the Fourteenth Easter School at Nottingham in 1968, McCarthy questioned whether it was possible to 'bend' growth curves, by selecting animals for rapid early growth rate, without increasing mature size. Since that time despite a large amount of research the evidence on this point, to the author, remains inconclusive. For example Dickerson *et al.*, (1972) give the growth curves, from birth to 26 weeks of age, for 7 breeds of sheep. These growth curves do not intersect but follow the same pattern, with the possible exception of the Corriedale whose birth weight seems large compared to its subsequent weights. Similarly

Smith *et al.*, (1976) show the growth curves for seven types of cattle; again the growth curves do not intersect. It could be argued that these breeds of sheep and cattle were not selected for earliness of maturity and so would not be expected to show growth curves of different shapes. Conversely if there is a reasonable amount of variation in earliness of maturity one would expect to see breed differences in this character.

There seems to be little evidence in the literature to support the idea that it is possible by selection to 'bend' or change the shape of mammalian growth curves. Samuel Brody's demonstration that the growth curves of different species have similar shapes suggests that the shape of the growth curve is an intrinsic property of growth. As there is apparently little variation between species in the shape of growth curves the within species variation may be very small. Bowman's (1968, p.293) invitation to undertake such "...unexciting but worthwhile research..." is accepted in Chapter 7 where, in rats, the effect of strain on the shape of the growth curve is studied.

TWIN EFFICIENCY

In estimating the twin efficiency values the correlation between slopes and constants was utilised in an analysis of covariance. The overall relationship between slopes and constants was negative, the growth lines being convergent, which would seem to be due to compensatory growth. Thus, as discussed earlier in this chapter, the majority of the variation in birth weight would seem to be due to environmental effects. The removal of the effect of birth weight on rate of weight gain by covariance should therefore give an estimate of the variation in rate of weight gain due to differences in mature weight. This should not be taken as an estimate of the genetic variation in mature weight due to both the birth weight being subject to genetic variation and the compensatory growth not being complete. Both these factors would tend to give an underestimate of the genetic variation in mature weight. so the within twin pair mean square (M_w) and the between twin pairs mean square (M_b) obtained from the analysis of covariance were used to

estimate the twin efficiency by the formula given by Dick and Whittle (1951), where

$$\text{var between/var within} = 0.5\ (M_b/M_w) - 1)$$

From Appendix Tables 4.1 and 4.2 it can be seen that estimates of twin efficiency for slopes and constants, using analysis of variance, range from 1 to 3. But following covariance the estimate of twin efficiency for live weight transformed to a 2 parameter logarithmic scale was 5.5 (Appendix Table 4.1) while for the three parameter case the estimate was 8 (Appendix Table 4.2). The increased value for the three parameter case is especially noteworthy and gives an estimate of twin efficiency equalling that obtained by Hancock for the absolute growth rate over the same period (4-88 weeks). However if the complete set of measurements, rather than the running averages, were available the efficiency estimated from the resultant slopes and constants would probably be greater. (Evidence to support this statement will be presented in Chapter 5). A refinement of the present techniques, shown in Chapter 10, involves the removal of the systematic variation about the line in an analysis of variance. In the present case this should further reduce the within-pair variation and increase the twin efficiency.

These results reaffirm that the number of animals required to demonstrate a statistically significant difference in live weight growth could be reduced by eight times or more by the use of monozygotic twins. Also by the use of linearised growth curves each animal could serve as its own control (Chapter 1), so linearisation could bestow further advantages to the efficiency of experimentation. Normal untreated controls should still be included in experiments; to account for non-random temporal changes common to treatments.

CHAPTER FIVE

THE REGRESSION OF LIVE WEIGHT AND AGE IN CATTLE

II Monozygous twin heifers at Massey

INTRODUCTION

This chapter embodies an analysis of a large amount of previously unpublished live weight data collected at Massey University from dairy heifers born from 1964 to 1968. Justification for the analysis of this data could be based purely on the grounds mentioned by Brumby and Hancock (1956), "....due to the extremely limited information available in this field, together with the high cost and appreciable time lag in obtaining such data, publication of these results is considered justified..." Both the large amount of information, from 65 pairs of twins, and the nutrition, pasture grazing from weaning (which differs from European methods of husbandry where the majority of published monozygotic twin growth data has been collected), further increases the value of the present data.

The present analysis shows some advantages which result from linearising heifer's live weight-age curves. The within and between twin variation is compared over the 5 years and the large variability in twin efficiencies between years is shown to be largely due to differences between twin pairs rather than due to differences within twin pairs. The effect of the frequency of measurement on the precision of these estimates is also illustrated.

Plots of arithmetic and logarithmic means and their standard deviations, for the live weights at the different ages, are used to quantify compensatory growth. The animals'growth lines will be shown to converge but they do not converge at a constant rate, compensatory growth appearing to occur at a constantly decreasing rate. Compensatory growth will be demonstrated in another way, and the author feels a more refined manner, by the regression of rate of weight gain on birth weight (both calculated from the regression equation relating log live weight to the log tangent of age). Differences within identical twin pairs should be due solely to environmental effects so the relatively large within pair differences in birth weight could be ascribed to differences in the pre-natal environment. Compensatory growth largely eliminates these environmental effects as the correlations between birth weights and rates of gain (within pairs, within years) were very close to unity.

These findings are followed by a general discussion of the physiology of compensatory growth in mammals.

THE DATA

The weighings of the Massey Identical Twin Dairy Herd, collected by Mr Geoff Raven of the Dairy Husbandry Department, were kindly made available by Professor D.S. Flux. The live weights were recorded in pounds, at the intervals shown in Table 5.1

TABLE 5.1

MASSEY MONOZYGOTIC TWINS, SCHEDULE OF WEIGHINGS

Year born	AGE (weeks)	
1964	1 - 8 weekly weighings	12 - 96 monthly weighings
1965	1 - 12 " "	16 - 96 " "
1966	1 - 24 " "	28 - 96 " "
1967	1 - 12 " "	16 - 96 " "
1968	1 - 12 " "	16 - 84 " "

The live weights from a total of 65 twin pairs were analysed. Fifteen pairs were classified as either Jersey crosses, Shorthorns, or Fresians. So 50 pairs were classified as Jerseys and 15 pairs as non-Jerseys.

Some data from animals born in 1969 was available but following weaning (at 12 to 16 weeks) the twins were grazed away from the university farms and were not weighed. When weighing was resumed the animals could be seen to have not gained weight during their absence from the university farms. This data could be of value for studying the effect of realimentation in monozygous twins but is unsuitable for the present purposes.

No experimental treatments were applied to any of the heifers during their first two years of life.

FIGURES 5.1A & 5.1B

RELATIONSHIPS BETWEEN MEANS AND STANDARD DEVIATIONS, ON ARITHMETIC AND TWO PARAMETER LOG METAMETERS, FOR THE MASSEY MONOZYGOTIC JERSEY TWIN HEIFERS WEIGHED FROM 1 TO 92 WEEKS OF AGE AND BORN IN 1966.

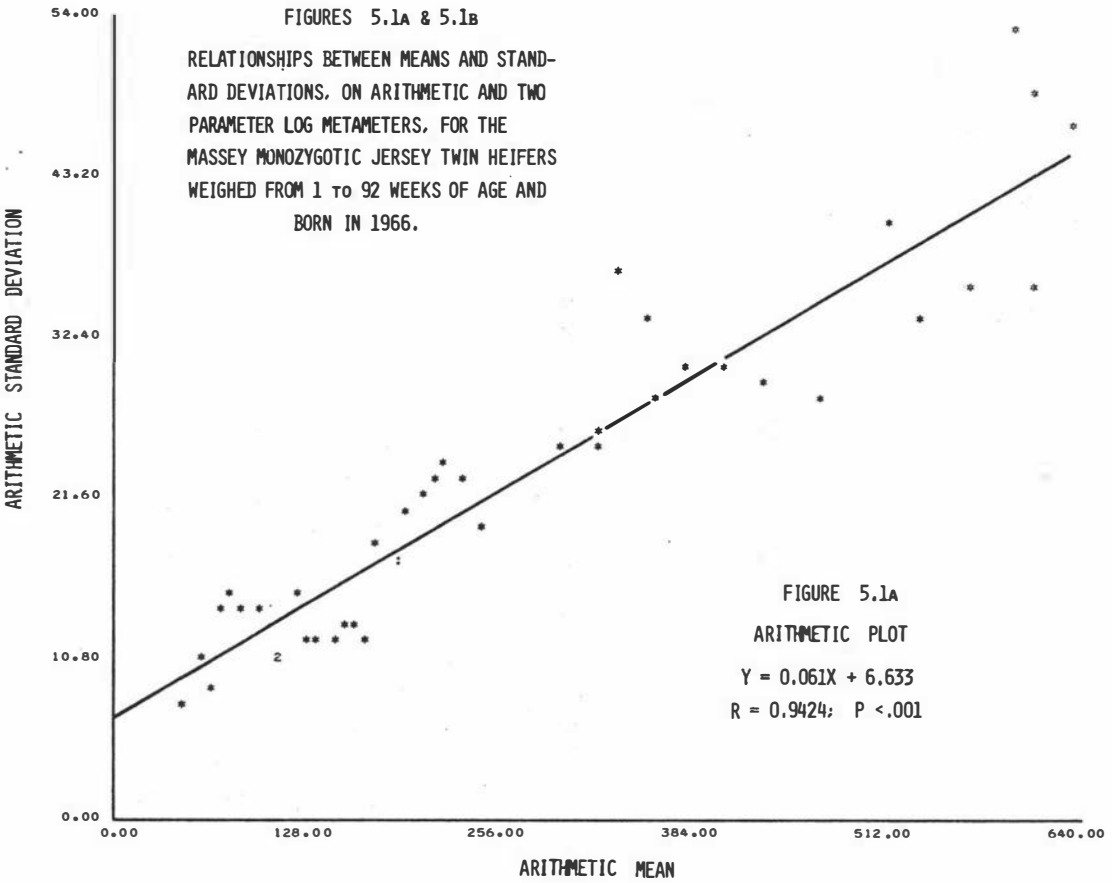


FIGURE 5.1A
ARITHMETIC PLOT
Y = 0.061X + 6.633
R = 0.9424; P <.001

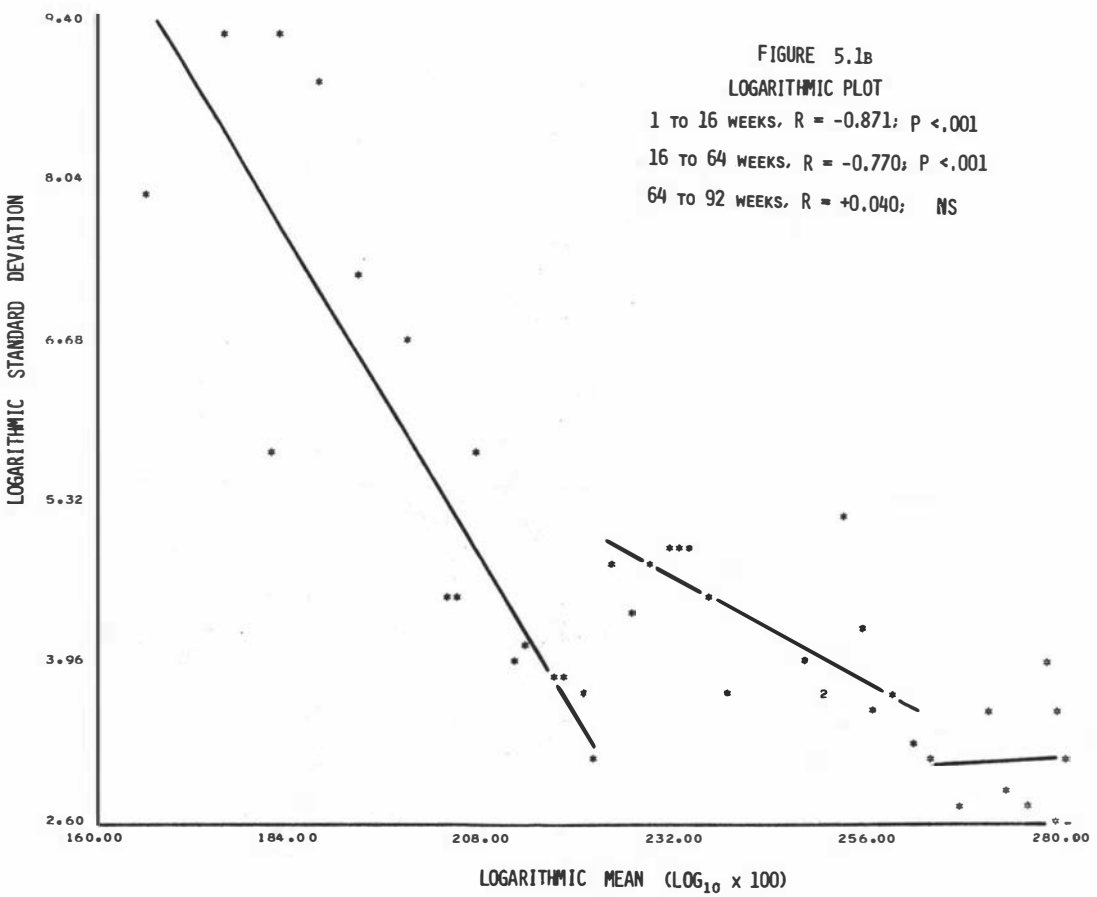


FIGURE 5.1B
LOGARITHMIC PLOT
1 TO 16 WEEKS, R = -0.871; P <.001
16 TO 64 WEEKS, R = -0.770; P <.001
64 TO 92 WEEKS, R = +0.040; NS

BIOMETRICAL CONSIDERATIONS

For animals classified as Jerseys, and within years, the mean live weights at each age were plotted against their standard deviations. The plots for different years were very different. For animals born in 1966 the regression equation describing the plot of the means and standard deviations, for the untransformed data, from birth to 64 weeks of age was,

$$Y = 0.06093\ X - 6.6327$$

while in the animals born in 1968 the relationship's equation was,

$$Y = 0.12640\ X - 0.5868$$

So to obtain a constant origin and to achieve normality, by a three parameter logarithmic transformation (see Chapter 2), 110 would have had to have been added to the live weights from animals born in 1966 while the addition of 4.5 would have given a constant origin to the plots for animals born in 1968. The large differences in these relationships between means and standard deviations, between years, did not allow the calculation of a single correction to a constant origin for all years. As comparisons between years were of interest the data was analysed using a two parameter log transformation of live weight even though within years the appropriate three parameter log transformation would have been superior (Chapter 4).

Hancock (1951) was able to show that the average percentage deviations from the mean set weights (for 10 sets of monozygotic twins) declined rapidly up to 12 weeks of age and then decreased slowly to 76 weeks of age when the stage of pregnancy caused a rise in the deviations. The plots of means and standard deviations (Fig. 5.1; Table 5.2) differ from the plots of the within set deviations presented by Hancock as both within and between set variations are included in the present plots; of which the between set variation provides the greater proportion (Table 5.3). However the present measure of variation (i.e. for the Jerseys born in 1966, Fig 5.1) again declined sharply to weaning (12-16 weeks of age), but then increased after weaning (presumably due to the between set variation since Hancock showed the within set variation did not increase at this age) followed by a statistically significant

slower decline in variation (compared to that before 16 weeks of age, Table 5.2) until the onset of pregnancy. The changes in standard deviation were similar in other years although not as clearly defined, partly because the measurements in other years were taken at monthly intervals from an earlier age (Table 5.1), and possibly due to differing environmental conditions in the different years.

TABLE 5.2

Massey Monozygotic Twins (Jerseys born in 1966)

Regression equations and the correlations between the mean live weights and their standard deviations, following live weight's transformation to logarithms, over the periods indicated.

AGES (weeks)	n	SLOPE ± S.E.M.	CONSTANT ± S.E.M.	CORRELATION r COEFFICIENT
1) 1-16	16	-0.1108 ± 0.0167	27.923 ± 3.344	-0.8714
2) 17-64	18	-0.0410 ± 0.0085	14.146 ± 2.086	-0.7696
3) 68-92	7	0.0058 ± 0.0649	1.604 ± 17.904	+0.0401

Hancock found that differences between animals in their stage of pregnancy caused the within pair variation to increase following conception. The correlations between means and standard deviations from the present study indicate that following 64 weeks of age the variation in live weight did not continue to decrease, on a logarithmic basis, confirming Hancock's findings. So for this reason, and because pregnancy also altered the shape of the growth curves, the regressions of live weight on age were calculated from the live weights up to 64 weeks of age.

For individual heifers in the Massey herd plots of arithmetic live weight against age showed an almost linear relationship from birth to 64 weeks of age. It was found that using logarithmic metameters the slope changed constantly, and increased, giving a curvilinear relationship. A suitable metameter for age should have

compared to the logarithmic, a contracted scale at the initial ages, but an expanded scale at later ages, to reduce the curvature (a positive quadratic) towards linearity. The log tangent transformation possesses these properties. A linear relationship between the log tangent of age (one week being equated to half a degree) and the log of live weight was indeed found. Whether the log tangent metameter would prove of value in other situations, for other data, is problematical. For each data set various relationships should be tested; applying transformations a priori should be avoided.

The slopes and constants for individual heifers, calculated following the transformation of live weight to logarithms and age (weeks/2) to its log tangent, are given in Appendix Tables 5.10 (Jerseys) and 5.11 (non-Jerseys). Slopes and constants were then subjected to analyses of variance. The slopes were also adjusted for differences in the constant by analyses of covariance. No comparisons of the within group regressions are given in the Appendices Tables as such comparisons, with only 2 animals per group, are not possible mathematically.

A COMPARISON OF TRANSFORMED AND UNTRANSFORMED REGRESSIONS

Average daily gain is sometimes calculated by a regression of arithmetic weight on arithmetic age (Chapter 10). This method of analysis was compared with the transformed regressions using the measurements from the Jerseys born in 1966. These animals were chosen as they were the most frequently weighed heifers and their transformed regressions had yielded the uncorrected 'F' values of the greatest size.

A comparison of these arithmetic (Appendix Table 5.9) and logarithmic (Appendix Table 5.6) analyses reveals the advantages of a logarithmic analysis. Since, for the arithmetic regressions, the correlations between slopes and constants, although negative, were non-significant, compensatory growth could not be demonstrated statistically. Therefore in the analysis of covariance the 'F' value for slope was only slightly altered by fitting the regression on birth weight.

FIGURES 5.2A & 5.2B
RELATIONSHIPS BETWEEN SLOPES AND CONSTANTS, FROM THE LINEAR EQUATIONS RELATING LOG LIVE WEIGHT TO LOG TAN AGE (WEEKS / 2), FOR MASSEY MONOZYGOTIC TWIN HEIFERS, BORN IN 1966 AND 1968.

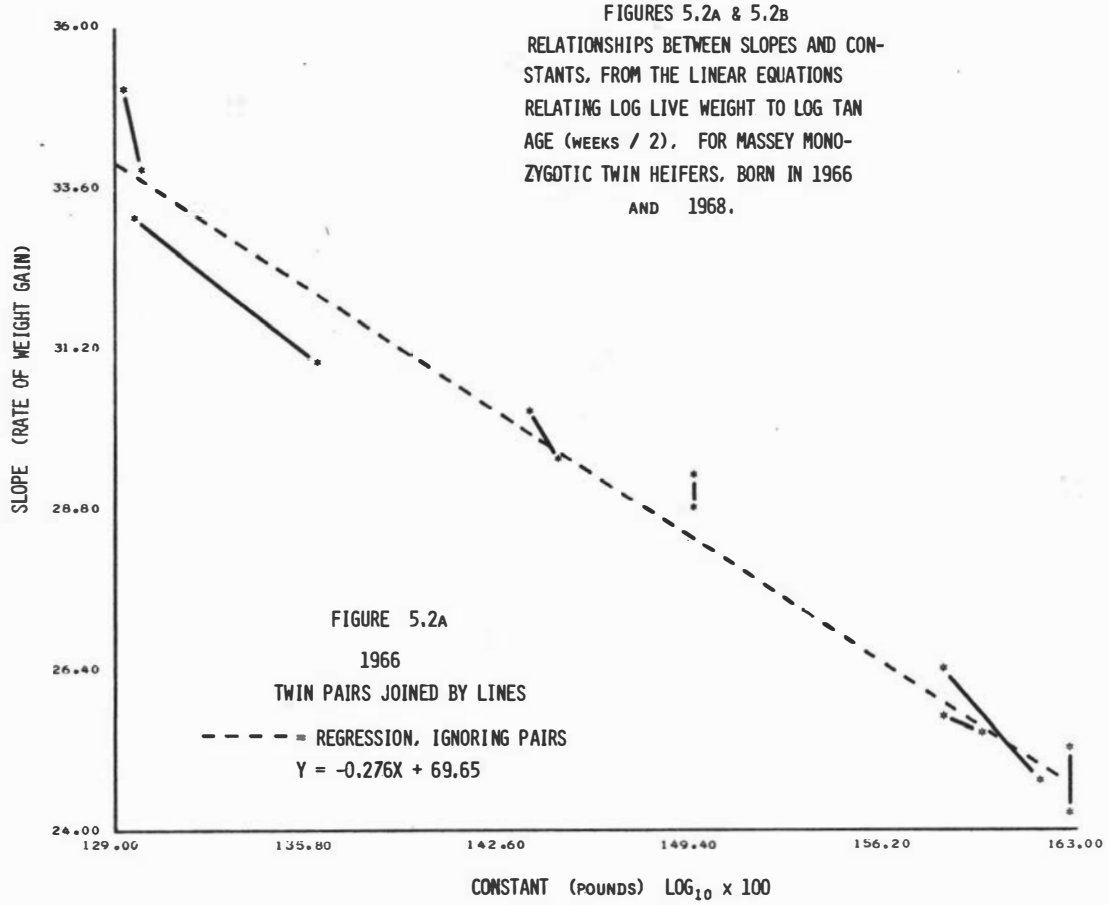


FIGURE 5.2A
1966
TWIN PAIRS JOINED BY LINES
- - - - = REGRESSION, IGNORING PAIRS
Y = -0.276X + 69.65

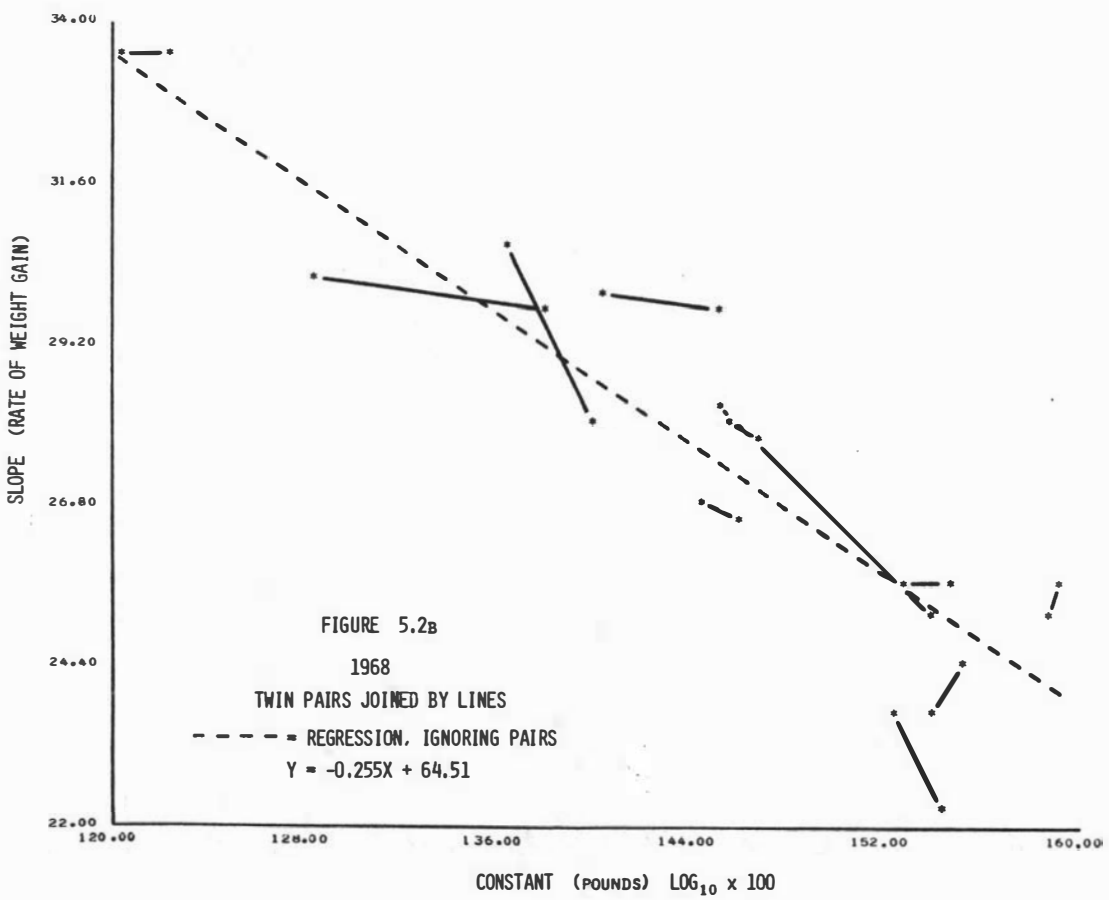


FIGURE 5.2B
1968
TWIN PAIRS JOINED BY LINES
- - - - = REGRESSION, IGNORING PAIRS
Y = -0.255X + 64.51

From the analyses of variance the 'F' ratios for slope and constant were, respectively, 15 and 8 times greater using the logarithmic scale. Although the 'F' value for the corrected slopes was only 30% greater for the transformed regressions the transformations used clearly give greater twin efficiencies than could be calculated on the original arithmetic scales.

RESULTS AND DISCUSSION

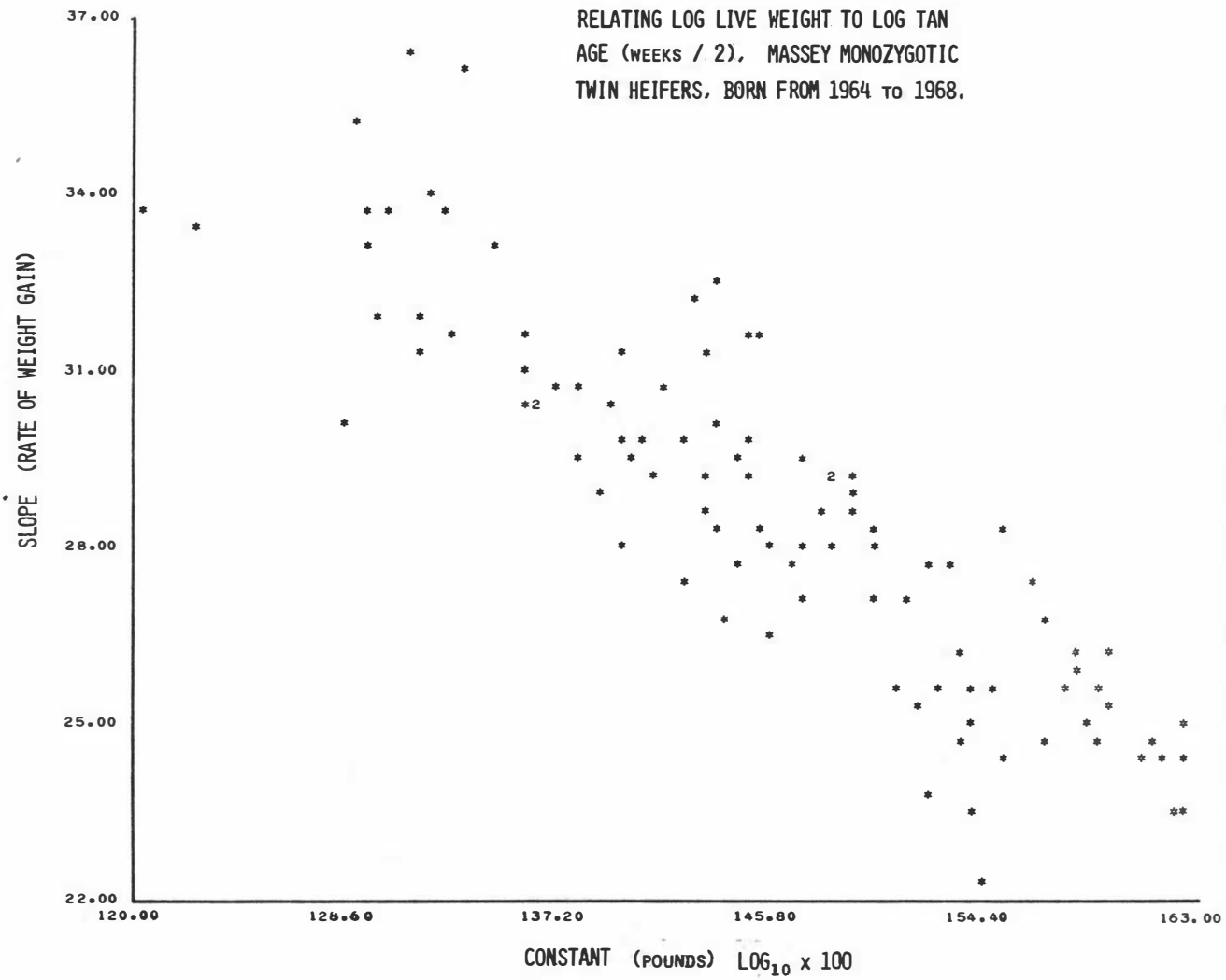
RELATIONSHIPS BETWEEN THE ESTIMATED SLOPES AND CONSTANTS

For the Ruakura identical twins there was a strong negative correlation between the calculated birth weights and the subsequent growth rates (i.e. Fig 4.3; R = -0.9667). Similar high negative correlations were present in the Massey identical twin data. The heifers born in 1966 showed the highest correlation (R = -0.990), possibly because of the greater number of measurements allowing a better estimate of the slopes and constants (Figure 5.2a). The reasons for the comparatively poor average within group regression for the 1968 group (Figure 5.2b) are discussed in a subsequent section. Figure 5.3 shows the slopes and constants for all the years combined in a single plot. The slopes and constants for the animals born in different years are, clearly, closely associated.

COMPARISON OF JERSEYS AND NON-JERSEYS

There was no obvious difference between Jerseys and non-Jerseys with respect to the log-log tangent equation's ability to describe growth. However this point was not closely examined, or considered, at the time of analysis. Plots of the residuals about the lines using the techniques described in Chapters 6 and 7, and a numerical test for their additivity (Chapter 10) would probably be useful to illustrate and detect possible differences in the shape of the growth curves.

FIGURE 5.3
THE RELATIONSHIP BETWEEN SLOPES AND CONSTANTS, FROM THE LINEAR EQUATIONS RELATING LOG LIVE WEIGHT TO LOG TAN AGE (WEEKS / 2), MASSEY MONOZYGOTIC TWIN HEIFERS, BORN FROM 1964 TO 1968.



Appendix Table 5.1 indicates similarities and differences between the live weights of Jersey and non-Jersey heifers. Although the Jerseys were slightly smaller than the non-Jerseys at birth (using the calculated birth weight) this difference was non-significant by analysis of variance. The rate of gain (the slope) was also less for Jerseys, although again the difference was statistically non-significant. The correlations between slopes and constants (ignorning twins) in Jerseys (-0.879) and non-Jerseys (-0.867) were very similar and there was no difference between these regressions (Appendix Table 5.1). Due to the high correlations between slopes and constants the error mean square was reduced by 76% following covariance so the non-Jerseys could be shown to have a higher rate of gain than the Jerseys, if the breeds were born at similar weights. Again the advantages of the analysis of covariance, when live weight data is analysed, are apparent.

Due to the difference in growth rate between Jerseys and non-Jerseys the non-Jerseys were excluded from the subsequent analyses. This was primarily because there were unequal numbers of non-Jerseys in the different years; their inclusion would have caused fluctuations of the between set variation depending on their number in any particular year.

COMPARISONS BETWEEN YEARS (IGNORING TWINS)

Appendix Table 5.2 shows that by analyses of variance no difference could be found between the slopes and constants for the Jersey heifers born in different years. The correlations between slopes and constants were high, the average within group correlation being -0.9205. Although there was no difference between the individual group regressions (of slopes on constants) the 10% level of significance was exceeded. When the effect of birth weight (the constant) was removed significant differences between the rates of gain in different years were revealed. The differences appear to be due to the heifers born in 1964, '65, and '66 having significantly greater rates of gain than those born, at a similar weight, in 1967 and 1968.

TABLE 5.3

MASSEY MONOZYGOTIC TWINS

Summary of Analyses of Variance (ANOVA) and Analyses of Covariance (ANCOVA) for heifers born in different years.

Between and within set mean squares for slopes and constants for the regressions of log live weight vs log tangent age (weeks/2).

		ANOVA				ANCOVA		AVERAGE WITHIN GROUP CORRELATION
		SLOPE (Y)		CONSTANT (X)		(Y) UPON (X)		
	(n)	MS BETWEEN	MS WITHIN	MS BETWEEN	MS WITHIN	MS BETWEEN	MS WITHIN	(r)
YEARS	pairs	'F'		'F'		'F'		
1964	13	23.06	1.119	147.7	10.06	4.113	0.148	
		20.6		14.68		27.76		-0.9369
1965	13	13.94	0.658	171.3	5.25	2.002	0.242	
		21.2		32.6		8.27		-0.8127
1966	7	24.81	0.579	345.9	3.97	0.401	0.102	
		42.8		87.1		3.92		-0.9210
1967	6	7.43	1.286	94.5	6.72	0.787	0.164	
		5.8		14.1		4.79		-0.9453
1968	11	18.49	0.932	238.5	9.85	2.704	0.538	
		19.8		24.21		5.03		-0.6893
ALL YEARS	50	18.38	0.934	193.2	7.58	4.113	0.276	
		19.7		25.49		14.89		-0.8428

BETWEEN SET AND WITHIN SET MEAN SQUARES (WITHIN YEARS)

Text Table 5.3 summarises the analyses of variance and covariance of Appendix Tables 5.3 to 5.8 where the within and between set variation is subdivided. The large differences between years for the 'F' values, in both the analyses of variance and covariance, are clearly apparent. A close inspection of the within and between set mean squares isolates the source of these differences to the between twin set mean squares. The approximate ratios of the minimum to the maximum mean squares were for the slopes (between 3: within 2), constant (between 8: within 5), and from the analyses of covariance (between 10: within 5). The between set mean squares were therefore almost twice as variable as the within set mean squares. In other words the value of identical twins for experimentation (using the present method of analysis) depends on the variability between twin pairs as the within twin pair mean squares, in different years, were similar.

The within twin pair mean square for the 1968 heifers (0.538) was unusually large. This was not due to the error term, before covariance, being large, but was due to the poor within group correlation ($r = -0.6893$) for the regression of slopes and constants. The plot of these values (Fig 5.2b) shows that four twin pairs were aberrant. Although, for these pairs, the slopes and the constants were similar they were positively correlated; all other pairs showed negative correlations. Within the four aberrant pairs the differences between the estimates were small compared to the errors attached to each estimate. Thus, for these pairs, although the errors attached to the estimates overlap, the analysis of covariance gives equal weight to each within pair regression. Thus the average within group correlation was, because of these four positive correlations, much smaller than for other years. The overall correlation (ignoring twin pairs) of $r = -0.913$, perhaps shows a 'true' refection of the relationship between the slopes and constants.

Text Table 5.3 also shows that the greatest twin efficiency is shown by the calculated birth weight. This again demonstrates the large environmental influence on the birth weight. The argument to justify this statement is as follows. In utero, differences of environment

between twin pairs would be expected to be greater than differences within twin pairs. Although on a logarithmic scale the differences within twin pairs at birth are large (compared to those at later ages) the differences at birth between pairs are much greater. So the environmental variation between pairs added to the genetic variation between pairs gives the large 'F' ratio of the constant. (The 'F' ratio of 87 for the constant in 1966 would appear to give the largest reported twin efficiency for live weight). The uncorrected slopes, being highly negatively correlated with the constants, also show large 'F' ratios. But the uncorrected slopes contain two opposite influences, the variation due to the birth weights differing, giving convergence of the lines, and that due to the mature weights differing, giving divergence. The corrected slopes only reflect the divergence of the lines. So from the 'F' values for uncorrected slopes and constants one can predict (assuming the correlations between slopes and constants to be similar) the size of the corrected slope 'F' value. For example if the uncorrected 'F' for slope is large compared to that for the constant then the corrected 'F' for slope should also be large (Table 5.3 1964), while if the uncorrected slope 'F' is small compared to the constant's 'F' the corrected 'F' for slope should also be small (Table 5.3, 1967).

It is worthy of note that the greatest twin efficiencies are in the 1966 group. It can be seen from Table 5.1 that these animals were the most frequently weighed of all the animals. Thus the within and the between animal mean squares are the smallest for both the corrected and uncorrected estimates for all the years. The uncorrected between animal mean squares are also the largest for both slopes and constants. But the corrected between animal mean square is small giving the small corrected 'F' for slope. But the advantage of more frequent weighing can be clearly seen. This tendency can also be seen from Tables 5.1 and 5.3 as the groups with the greater number of early weighings tend to have the greatest uncorrected 'F' values.

The corrected and uncorrected mean squares from the Ruakura and Massey animals, for the lines calculated using a two parameter log transformation of live weight, are not strictly comparable as age was, in the Massey study transformed to its log tangent, and in the Ruakura

study to its logarithm. Despite this a comparison of the uncorrected mean squares for slopes and constants (using the two parameter transformation of live weight) from the Ruakura and Massey animals is informative. If one compares these mean squares in Appendix Table 4.1 and Text Table 5.3 the between pair mean squares are of similar magnitude. But the within pair mean squares for the Ruakura twins were, compared to the maximum value in the Massey twins,

1) for the slopes 6.544 compared to 1.286 in 1967,
2) for the constants 20.371 compared to 10.06 in 1968.

This greater within pair variation would seem to be because the estimates of slopes and constants from the Ruakura animals were based upon only 8 measurements compared to a minimum of 20 for the Massey data.

DISCUSSION

LIVE WEIGHT-AGE GROWTH CURVES

Many of the conclusions reached in this work depend on the use of transformed weight-age scales. So a discussion of the methods of analysing live weight growth in cattle is particularly relevant. The use of the average daily gain is discussed and criticised in Chapter 10, as is the use of sigmoid equations.

The methods of analysing growth curves that were popularised by Brody (1928, 1945) have been widely used and advocated by agricultural scientists. Although some workers have noted that their data was poorly explained by these equations the assumptions and the data upon which Brody's equations were based do not seem to have been questioned.

Brody (1928, p.58) conceived of growth where '.....segments of constant growth rate are separated by relatively abrupt breaks, the abruptness of the breaks being of the order of metamorphosis in cold-blooded animals." This claim of abrupt changes in the course of whole body growth ignores homeorhesis, a basic property of higher animals, which counters such sudden changes. (Homeostasis may be an insufficient term to describe the control of body growth. Kennedy (1967) prefers

the term homeorhesis since, classically, homeostasis implies a steady state whereas growth involves a pathway of change. Kennedy (p.338) also preferred homeorhesis since "....it avoids confusion with physiological homeostasis, and because the requirements of growth may sometimes differ from, and even be sacrificed to, those of homeostasis.")

The live weight growth curve of the rat was divided into 2 phases by Zucker *et al* (1941a), but with a transition zone between the two phases rather than a sudden change in growth rate. The present emphasis on the importance of weaning to live weight growth is contrary to the emphasis on puberty, as an abrupt break, described by Brody. Brody divided live weight growth, irrespective of species, into self-accelerating and self-inhibiting phases (which were themselves divided into further cycles), the point of inflexion being puberty. However contrary to Brody's quite definite statements the application of his formulae to various domestic animal's growth has proved equivocal. In Shropshire sheep Mitchell (1962), using Brody's equations, found an inflexion at 1-2 months which was considerably before puberty which occurred on average at 5-7 months from birth. In Holstein cattle Mitchell showed an inflexion at 6 months of age, again earlier than normal puberty in dairy cows. Furthermore cattle and sheep display seasonal reproductive rhythms so puberty would obviously be affected by the season of birth despite the weight attained.

Hancock (1951) attempted to apply Brody's formulae, to the live weight growth of heifers, but found that the division of the self-accelerating phase into 3 cycles (from birth to 8, 8-16, and 16-24 weeks of age) was forced by breaks, ascribed by Hancock to changes in nutrition. For the live weight growth of Duroc-Jersey pigs Mitchell stated "....the data were not well described by the Brody growth curves" which sums up the evidence presented above. For a further discussion of the Brody growth curves see Chapter 6.

The rat live weight data collected by Donaldson, from the Wistar Institute colony, was used by Brody as evidence to support his "self-inhibiting and self-accelerating' phases and their intersection at puberty. However as early as 1926 Osborne and Mendel noted that the growth curves of rats raised upon improved diets did not contain post-

weaning points of inflexion. Zucker *et al.*, (1941a) showed in female rats an inflexion at weaning (3.3 weeks) rather than at puberty. They concluded that Brody's equations did not describe 'normal' rat growth due to the equations being based upon data '...obtained at a time when little was known concerning nutritional requirements'.

Thus for both domestic animals and for rats there is little justification, from live weight measurements themselves, for dividing post-natal body growth into pre- and post-pubertal periods and fitting different equations to each period.

But following Brody (1928, 1945) it is commonly stated that the post-natal live weight growth curve of cattle is sigmoid in shape (McDonald, Edwards and Greenhalgh, 1969; p.231: Roy, 1970; p.27: Butterfield and Berg, 1976; p.16: and Elsley, 1976). It is notable that these authors did not consider their statements warranted referencing; common knowledge, presumably, being their source. For individual heifers in the Massey herd, from birth to 64 weeks of age, the plots of arithmetic weight against age were essentially linear. Elsley (1976, p.325) admits that the growth curves of individual animals, in some experiments, may not be sigmoid but he states that the bulk of the available data shows a sigmoid pattern. In other words the mass curve is sigmoid whereas the growth curve of an individual does not necessarily follow the mass curve. But the mass curves of three breeds of dairy cattle (data of Morrison, 1956) do not show a sigmoid curve of the exaggerated form shown, for example, by McDonald *et al.*,(1969).

Support for the present author's doubts as to the sigmoid nature of the post-natal growth curves of cattle comes from a paper by Brown *et al.*, (1971) who fitted von Bertalanffys', Brodys', Gompertzs, the Logistic, and Richards',models to the post-natal weight-age curves of 151 Jersey and 147 beef females. They conclude that "A point of inflection (POI) was not indicated in 50% of the data suggesting that a sigmoid curve may not be necessary to describe the postnatal weight-age curves in cattle. A postnatal POI may only represent compensatory effects of various environmental restrictions, if so, simpler models may prove useful in growth analysis." These important conclusions deserve

confirmation and the deduction that simpler growth models may be applicable supports the use of the simple transformations of weight and age used in the present work.

Emphatic statements and assumptions that "...the growth curve of mammals and birds follows a sigmoid pattern." (Elsley, 1976; p324) should be avoided.

COMPENSATORY GROWTH

The relationships between means and standard deviations (Fig. 5.1 Table 5.2) indicate that post-natal compensatory growth in the heifer may proceed at a rapid rate to weaning, then at a reduced rate following weaning. Thus weaning seems to be a crucial stage in the live weight growth of the heifer. However in the rat compensatory growth seems to occur mainly following weaning. This is possibly because in litter bearing animals the number of pups per litter controls the amount of milk supplied to each pup and is therefore a major determinant of weaning size (Kennedy, 1957). This point is further discussed in Chapter 9. But for calves or lambs compensatory growth seems to occur during lactation (before weaning) as in these animals "......the capacity of the offspring largely governs the actual yield of milk produced." (Wallace, 1948; p152). The calf or lamb is born at a much greater physiological age than the rat. For example the calf's eyes are open at birth while a rat pup's eyes open from about 14-16 days of age. Thus weaning in the rat and birth in the ruminant may be roughly equivalent physiological ages.

The demonstration in both lambs and heifers that, on a logarithmic scale, the rate of weight gain following birth is negatively related to the birth weight, raises an interesting question. The differences in birth weight between individual animals imply that the pre-natal growth lines are divergent while, under similar conditions, the post-natal growth lines are convergent. It seem paradoxical that during foetal life, when the nutritional drain of the foetus on the dam is slight, the growth lines of young ruminants should diverge while during lactation, when the nutritional drain on the dam is greatest (Mitchell, 1962), the growth lines converge.

Hammond (1944) hypothesised that the foetal metabolic rate in early pregnancy was similar to that of the dam's nervous system so that during early pregnancy the growth of the foetus would not be adversly affected by restricting the caloric intake of the dam. He drew support for this idea from the work of Wallace (1948) who could show no effect of maternal nutrition on the weight of sheep foetuses at 91 days of gestation. But, as Everitt (1968, p144) points out, the degree of nutritional stress in Wallace's trials '.....was not particularly great.' The re-analysis of Wallace's results in Chapter 2, where no clear effect of plane of nutrition on the correlations between rates of gain and birth weights was demonstrable, supports Everitt's claim. Everitt reviews his own experimental evidence, and that of others, which indicates that the level of nutrition does affect ovine foetal weight in early gestation. But birth weight is more affected by a period of nutritional stress during late gestation than a similar stress in early gestation. Thus Hammond's theory of the inviolate nature of early foetal growth has been questioned. The greater effect in late gestation could be due to the amount of nutrient required by the foetus increasing exponentially as gestation progresses. At 75 days of gestation the ovine foetus weighs less than 200 grams but at term (about 150 days) the foetus can weigh 5000 grams. Clearly a greater nutrient requirement exists in late pregnancy so that a nutritional stress of constant magnitude could be expected to have a greater effect during the later phase of pregnancy. But if the same nutritional stress is imposed following birth (as in the study of Wallace, see Chapter 2) the growth lines of individuals then converge.

One is forced to conclude that it must in some way be advantageous to the species for the young animal to be retarded during foetal life rather than following birth. The effective post-natal mechanisms of compensatory growth obviously allow for retardations in growth rate during pregnancy to be made up, but they do not explain why retardation should occur during pregnancy. It seems that during pregnancy the survival of the mother, and thus of her foetus, is paramount; so the mother has priority in the partition of nutrients (Hammond). But following a successful parturition the chief function

of the dam is to give her young maximum nutrition, even at the expense of her own well-being. The effective post-natal compensatory mechanisms can then take advantage of this supply of nutrients. But if the dam has lost her foetus she has not 'wasted' her body reserves, and has these reserves ready for another pregnancy and lactation. Such mechanisms would be especially important if the ruminant, as suggested by Houpt (1970), evolved when the earth was subject to a period of dry climatic conditions.

A point that has not been investigated, to the author's knowledge, is whether or not, in cows and sheep, compensatory growth occurs in utero. Does compensatory growth function in utero to correct a retardation in growth incurred in early gestation by accelerating the rate of growth in late gestation? The experiments of Everitt (1968), although not aimed directly at this point, seem to show that a diminution in growth rate occurring during early gestation is not eliminated despite realimentation during late gestation. The absence of compensatory growth in suckling rats is described in Chapter 7 and discussed in Chapter 9. As birth in ruminants and weaning in rats appear to be equivalent physiological ages the absence of compensatory growth before birth in ruminants could be expected from these results in rats.

These consistently high correlations between birth weight and the subsequent rate of gain, for both the average within twin and the total (ignoring twins) regressions, are suggestive of very effective compensatory mechanisms in ruminants. This has been stated by others, for example Everitt (1968, p145) concludes that "...the phenomenon of compensatory growth...is especially important in the growth and production of domesticated species....'. Another argument suggesting that compared to rats ruminants have superior mechanisms of compensatory growth is presented in Chapter 4. The superior mechanisms for the preservation of mature weight in ruminants, by compensatory growth, can also be argued from an evolutionary standpoint.

Houpt (1970, p119) states that,

'Paleontologists suggest that much of the evolution of ruminants as a separate branch of mammals occurred during prolonged dry

periods of the earth's history. It is likely that the special digestive tract modifications present in modern ruminants were developed in response to such environmental pressures and that the ability to survive a long period of semi-starvation is the result of these adaptations.'

To take full benefit from favourable changes in nutrition due to climatic changes (i.e. due to rains following prolonged droughts), enhanced mechanisms for compensatory growth may have been especially advantageous to the survival of ruminants. These assumptions are supported by studies in Northern Australia (Vercoe and Frisch, 1974) where Zebu and Zebu-cross cattle, fed on a low quality ration, were less affected by starvation, and following realimentation ate more, grew faster and more efficiently, than Shorthorns. The tropical breeds seemingly possess compensatory mechanisms superior to those of European breeds, presumably due to selection pressure for these traits being maintained in the tropics.

An experiment by Brumby and Hancock (1956), using dizygous and monozygous dairy twins to estimate the genetic variation in body size by three methods, is pertinent to this discussion. The estimate of genetic variation derived from the within-set variations of dizygous and monozygous sets was assumed to be '....uncomplicated by the similarities of environment common to the twin sets but containing most of the non-additive genetic variations.'. The three estimates of genetic variation were similar for milk and milk-fat production but for measurements of body size the estimate derived from within-set variations of dizygous and monozygous twins was much less than the variation between twin sets. This difference between the estimates was ascribed to the pre-natal environment greatly affecting growth but not milk production. This finding of a large apparently non-genetic difference between twins in their pre-natal growth illustrates the importance of post-natal compensatory growth to cattle. Russell (1976) clearly demonstrated compensatory growth in cattle since the large differences in birth weight between single and twin calves (a twin being about 75% the weight of a single) were overcome by 18 months of age (a 1% difference remained).

Thus during pregnancy, nutritional stress can retard the weight gain of the foetal ruminant, but lactation supplies sufficient nutrient for post-natal compensatory growth to give a partly restored weaning weight even if the nutritional stress continues. Following weaning the young animal could remain at a low body weight until favourable conditions allow for a surge of growth, allowing reproductive and ponderal maturity.

So in the heifer and the lamb variations in birth weight are largely compensated for by the convergence of the pre-weaning growth curves, giving, by weaning, a reduced variation in the logarithm of live weight. This pre-weaning compensatory growth may be vital to the full expression of the genetic potential for growth. A restriction in growth before weaning may not be able to be completely overcome by the apparently slower post-weaning phase of compensatory growth. Most research has indicated that restrictions in growth following weaning can be overcome (Hansson and Claesson, 1960; Bondari and Wilham, 1977).

In a series of experiments, using monozygotic twin cattle fed on widely differing planes of nutrition from 1 to 25 months of age, Hansson and Claesson (1960) were able to demonstrate almost complete compensation in body weight following realimentation. They concluded that,

'Within a wide limit of variation in the level of nutrition the young animals continue to grow, but they do so at different rates and... reach practically the same final body development at maturity....heredity directs the development of the body to approximately the same body size and conformation at maturity, to a large extent independent of the feeding intensity.'

Bondari and Wil ham (1977) fed beef steers from one month after weaning (weaning at 200 days) with either a high or low plane of nutrition. After 100 days low plane animals were realimentated. At slaughter, after a further 100 days, both groups of animals (of the same age) had similar carcass weights, but the low-high group had significantly less fat. The authors concluded that 'Compensatory growth in this study is obviously of economic importance....'

Allden (1970, p1174), in his literature review of compensatory growth, states that 'However, there is no evidence to show that a calf subjected to nutritional deprivation from a very early age of suckling

will eventually become a cow of smaller stature.' Similarly Roy (1970, p34) advances an argument in support of the early weaning of calves when he states that '...much lower weight gains have been accepted during the first 3 months of life on the basis that such calves will be able to catch up as a result of subsequent compensatory growth.' The results from the present work do not support these conclusions.

But the importance of the post-natal period was shown by Everitt (1972) who fed steer calves, members of identical twin sets, on ad libitum or restricted milk intakes from birth to 16 weeks of age. The large differences in live weight between treatments, at weaning, were incompletely recovered at 400 days of age. In a series of similar studies, at Massey, Davey (1974) reached conclusions agreeing with those of Everitt '....the period from birth to 4 weeks of age is critical, so that low live weight gains over this period could have a permanent effect'. (Davey, 1974; p138). The present study supports these findings of Everitt and Davey and furthermore suggests that the post-natal period is 'critical' because at this time compensatory growth, to eliminate pre-natal differences in body size, is proceeding at a very rapid rate.

Surprisingly little is known about the physiological mechanisms controlling the increased efficiency of food utilisation attendant upon compensatory growth. Meyer and Clawson (1964), in a well controlled experiment, investigated some effects of 5 levels of nutrition in rats (for 21 days) and in sheep (for 42 days) followed by realimentation on an ad libitum diet. Wilson and Osbourn (1960) believed that a decrease in maintenance requirements occurred during starvation which influenced the efficiency of gain during realimentation. The findings of Meyer and Clawson (1964) however agreed with those of Cumming and Morrison (1960) who showed that a reduction in heat production of a starved animal could be due to a reduction in both activity and heat increment due to the low food intake. Cumming and Morrison concluded that food intake stimulation was not a major influence in compensatory growth. Allden (1970), in his review of the literature concerning compensatory growth, found no general agreement as to the contribution of increased food intake to compensatory growth. Saubidet and Verde (1976) restricted steers on 5 planes of nutrition then realimentated them ad libitum.

But the food intakes, upon realimentation, were similar leading these authors to write 'This led us to discard compensatory feed intake as a possible explanation for compensatory growth.' Thus there seems to be little basis for explaining the high growth rates of compensatory growth by a decreased energy requirement for maintenance or by an increased food intake.

Using young growing rats Sinha *et al.*,(1973) found that dietary restriction depressed plasma pituitary growth hormone (GH); but refeeding caused the hormone levels to rebound. However recently Mosier *et al.*, (1978) could find no marked hormonal changes associated with compensatory growth in young rats. This led these workers to conclude that 'Normal GH and SM (somatomedin) concentrations in plasma do not in themselves assure catch-up growth. The possibility that other, as yet unknown humoral or metabolic factors are involved is suggested.' In lambs the feeding of a low protein diet did not affect pituitary or serum GH, although serum insulin levels were decreased (Johns and Bergen, 1976). Clearly the endocrinology of compensatory growth is still to be defined.

This discussion indicates the major practical importance of compensatory growth. For example the mechanisms whereby the manipulation of nutrition induces the multiple release of eggs at ovulation, commonly known as 'flushing', could be elucidated when the mechanisms of compensatory growth are understood. The physiological basis to flushing and to compensatory growth, although possibly hormonal, remains to be determined. But the author believes that the untangling of the mechanisms involved in compensatory growth would prove to be of great practical value to both animal breeders and meat producers.

CHAPTER SIX

FURTHER ANALYSES OF RAT LIVE WEIGHTS

INTRODUCTION

This chapter contains further developments and extensions of the biometrical techniques described in earlier chapters. These more exhaustive methods will then be used, in the present and the following chapter, to further elucidate the changes in body size with age in the laboratory rat.

Bliss (1970) criticised the log-reciprocal equation preferring to use an asymptotic equation to mathematically describe the post-weaning live weight growth of the rat. Thus the present chapter, in Section One, contains a comparison of these two equations. Section Two deals with an unexpected finding, and to the author an extremely interesting discovery, encountered during the comparison of the equations in Section One. Section Two shows that there are long-term systematic variations in live weight about the general trend which cannot be explained by a simple linear equation.

CHAPTER SIX
SECTION ONE

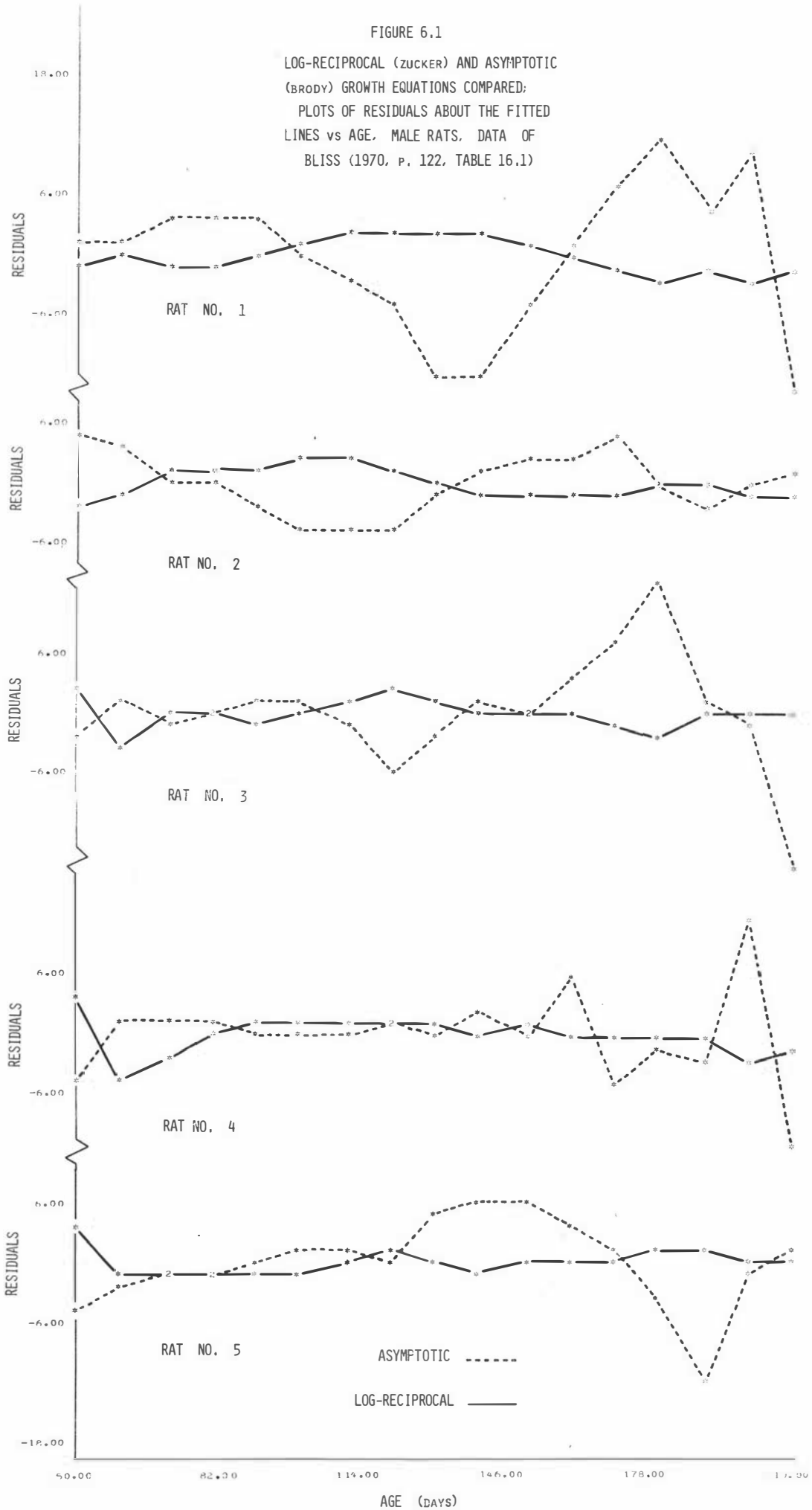
THE ZUCKER LOG-RECIPROCAL EQUATION COMPARED TO THE BRODY ASYMPTOTIC EQUATION.

The log-reciprocal equation was, in 1962, used by the National Academy of Sciences Committee on Animal Nutrition to represent the post-weaning growth in live weight of the laboratory rat. The report gives five independent references which all confirmed the Zuckers' equation. Dunn *et al*. (1947) were said by the Committee (1962, p.53) to have "....reported that in situations of excellent growth the curve arched above a straight line." However the article by Dunn *et al*. (1947) does not show that "....the curve arched above the straight line." What Dunn *et al* . (1947) hoped to convey was that using the Anderson-Smith diet (the optimal diet for rat growth according to Osborne and Mendel) the post-weaning growth of a rat could be resolved into two phases; before and after 14 weeks of age. However both phases could be fitted with the log-reciprocal equation. So even though doubt was cast upon the ability to explain post-weaning rat growth by one line the log-reciprocal equation was not actually questioned by their paper. Despite the majority of the published evidence confirming the log-reciprocal equation Bliss (1970, pp. 121-124) wrote that the weights of male rats agreed with the Zuckers' formulation up to 130 days, "...but then curved systematically about the regression."

Bliss preferred an asymptotic regression of the form used by Brody (1945). Briefly, this method involves the calculation of an asymptote from which the observed weights are then subtracted, transformed to logarithms, and plotted against the observed ages. Brody's methods have received little recent critical examination. This is surprising since Brody neglected the statistical approach to curve fitting in favour of an approach where the equations were fitted because their parameters possessed "...definite, rational, physical meaning".

FIGURE 6.1

LOG-RECIPROCAL (ZUCKER) AND ASYMPTOTIC (BRODY) GROWTH EQUATIONS COMPARED; PLOTS OF RESIDUALS ABOUT THE FITTED LINES vs AGE, MALE RATS, DATA OF BLISS (1970, P. 122, TABLE 16.1)



Bliss criticised the log-reciprocal equation on the grounds that the variation about the line increased with age. He then fitted the asymptotic formula to the mass curve and showed a similar effect. The variation about the line increased with age, "Because of differences in the (growth) rate, the variance about the mean weight increased progressively with age." Thus Bliss suggested that the variation about the line was systematically heterogeneous in both the log-reciprocal and the asymptotic regressions; yet he preferred the asymptotic method. Bliss's explanation of the systematically heterogeneous error associated with the asymptotic plot, that the growth lines diverged, is not convincing. The original data (Table 16.1, p.122) show that the percentage difference in the range of weaning weights (46 to 74 g) was larger than that at 210 days of age (480 to 620 g). This would cause the growth lines, on a logarithmic scale, to converge. To investigate these apparent inconsistencies the variation about the log-reciprocal and asymptotic lines was re-analysed using the animals numbered 1 to 5 in Bliss's Table 16.1.

Both log-reciprocal and asymptotic regressions were therefore fitted to the live weight-age curves of individual animals. The asymptotic regressions were calculated using the asymptotes supplied by Bliss. The percentage of the variation in live weight explained by the log-reciprocal equation in the 5 animals ranged from 99.3 to 98.4 percent while the percentages of the variation explained by the asymptotic regressions ranged from 98.6 to 96.6 percent. For each of the 5 animals the log-reciprocal equation explained a greater percentage of the variation in live weight.

The two equations were also compared by plotting the residuals (the observed minus the expected values) about the regressions for individual animals against age. Bliss contended that systematic deviations from the log-reciprocal line occurred only after 130 days of age; but Figure 6.1 shows that systematic departures from linearity are consistently present over the entire range of ages. The variation about the line for the asymptotic plot also showed serial correlation between the residuals, a situation not considered by Bliss. The variation about the line was much greater overall for the asymptotic

method and increased markedly with age. The smaller overall residual variation, and the absence of an increased residual variation with age, makes the log-reciprocal plot the better description of the data.

The greater residuals at the later ages in the asymptotic case must arise within animals and cannot be, as Bliss claimed, a between animal effect. The increased residuals at later ages are due to the variation in arithmetic weight (W) becoming greater as the limiting asymptotic weight (A) is approached. Therefore the variation in log (A-W) also increases rapidly as W approaches A. The example quoted by Zucker (1941a, p.459) to illustrate this point is a good one, "Assuming an A of 270 gm., a 1 gm. change in a rat weighing approximately 240 gm. should according to this function (the asymptotic) represent the same change in growth status of the animal as an 8 gm. change in a rat weighing approximately 30 gm. As a rat approaches the limit weight the normal fluctuations around the equilibrium weight determined for that time by its inherent growth curve should be limited to fractions of a gram and finally approach 0."

The asymptotic equation involves an additional parameter, the asymptote. The estimate of the asymptote is subject to error which, because of the estimation procedure, affects the other statistics of the regression potentially giving them a larger error than that of the more simple log-reciprocal equation. The log-reciprocal equation is also to be preferred as it requires less calculation, especially if, as Bliss suggests, an asymptote requires to be calculated iteratively.

In using the asymptotic formula Bliss assumes that the weight of the rat 'plateaus' by 300 days of age. But in Chapter 10 evidence will be presented to show that at no time can a 'true plateau' or asymptote be said to be reached in a rat's live weight growth.

The serial correlation between the residuals about the log-reciprocal plots was an unexpected finding. The extremely high correlations between live weight and age, commonly approaching 0.999, give a very small amount of unexplained variation which was assumed to be random. The Zuckers also made this assumption, but Bliss drew attention to serial correlation about the fitted lines. This stimulated the analyses of Section Two of this Chapter and those of Chapter 7.

SUMMARY

In this section several statistical analyses, and some practical reasons, were presented to show that the log-reciprocal equation, rather than an equation of the asymptotic type used by Brody, provides a superior description of post-weaning live weight growth in the laboratory rat.

CHAPTER SIX

SECTION TWO

THE "LINEAR" RELATIONSHIP BETWEEN THE LOGARITHM OF RAT LIVE WEIGHT AND THE RECIPROCAL OF AGE: THE PRESENCE OF SYSTEMATIC OSCILLATIONS ABOUT THE FITTED LINE.

INTRODUCTION

An analysis of live weights, in a time series, is usually considered completed when the general trend has been fitted. But, as Sholl (1954) forcibly argues, fitting the general trend should be looked upon as merely the first step. Sholl discussed a time series, recorded in the eighteenth century by Count Philibert Gueneau de Montbeillard of his son's height changes from birth to seventeen years of age, and detected periodicity in the deviations from the trend of the growth curve; a yearly (13 month) oscillation operated in the young French boy's growth.

Sholl (p.231) stresses that a time series may contain one or all of three components.

1) A general trend.
2) An oscillation about the trend.
3) A random component.

Sholl described the general trend as the line depicting the track of a railway on a continental map; the general direction of the railway is shown but the small variations do not appear. The task is to decide whether or not the small variations are random.

Sholl states emphatically (p.225) that for the fitted general trend "....no demonstration of closeness of fit can ever prove the curve to be that one which is in any sense the unique 'true' curve." This idea, due to its importance, has been repeatedly stated in the literature, and will be reiterated in the present work. (But although one cannot prove a curve to be the 'true' one, curves can be rejected for both biological and statistical reasons).

Zucker and Zucker (1942) justified their growth equation using two criteria for adequacy of fit. The first criterion being that the ratio of the standard deviation to the mean at each age should be constant. Although Zucker and Zucker state that in their data this criterion was fulfilled, the data from intact rats, presented in Chapter 2 (Figure 2.4), questions the generality of their statement. The second of their testing criteria, the agreement between observed and

calculated values, was rejected by Zucker and Zucker (1942, p.453) ".....because deviations are of constantly decreasing biological significance...." Although this may be true for arithmetic deviations, for logarithmic deviations this would not be so; the residuals should have been tested. The tests for adequacy of fit, for the mass curves, performed by Zucker and Zucker were therefore incomplete. For this reason, and also because in the present case lines were fitted to individual animals, a complete re-examination of the adequacy of fit of the log-reciprocal equation was necessary.

Zucker *et al*. did attempt to fit their equation to individual rat's live weight curves (1941b, p.130-2) and concluded that " ....rats of our colony adhere to a single slope with moderate error." Their main interest was the "....growth performance of the rat." with respect to nutrition. This may have caused a bias toward the position that individuals, under identical nutritional and environmental conditions, should grow at the same rate. They observed individual rats to grow "...in a series of parallel lines on log-reciprocal paper."

This conclusion, that individual lines were parallel, may be the reason why the Zuckers analysed only the standard deviations and means of the mass curves. That the scatter about an individual's curve would be reflected in the mass curve (or the mean and standard deviation at each age) has been used in this thesis as a basis for selecting a normalising metameter for live weight. But in rats, lambs, and heifers the transformation of live weight to logarithms can produce a negative relationship between means and standard deviations. This was due to the convergence of the lines for individual animals because of compensatory growth. As individual lines are clearly not parallel the errors about the mass curve are poor predictors of the scatter about an individual's line.

An example may more clearly illustrate this argument. It is possible that on a chosen metameter, although the coefficient of variation at each age is constant, an analysis of individual curves would show the variation to be increasing with age; the constant coefficient of variation in the mass curve being due to the counter-balancing effect of the individual lines converging. This illustrates the dangers of using

the means at each age to select the normalising and linearising metameter for individual lines. A better criterion may be to select the metameter on the basis of the residuals about the lines fitted to individual animals. Problems can be seen to arise if, in the analysis of growth curves, the between animal variation is not separated from the within animal variation. The confusion resulting if these sources of variation are not differentiated was seen in the previous section where Bliss seems to have misinterpreted the live weight data he analysed. These points will be emphasised in the present section by a comparison of some properties of the mass and individual curves fitted to the same data.

Several sets of live weight measurements, from rats subject to various treatments, will be re-analysed to investigate possible non-random fluctuations in the residuals about individual animals' log-reciprocal lines. The biological significance of the results is then discussed.

THE DATA

Serial correlations between the residuals about the log-reciprocal plots for individual animals were investigated using weekly weighings, from weaning, collected at Massey over two periods of time, by

1) Sommerville and Tarttelin (1977)* from female rats
   a) neo-natally ovariectomised and treated with testosterone propionate
   b) neo-natally ovariectomised and treated with oil,
   c) neo-natally sham ovariectomised and treated with testosterone propionate

and
   d) neo-natally sham ovariectomised and treated with oil, (90 µg of testosterone propionate and ovariectomy both on day 2) and weighed weekly to 18 weeks of age, groups 1a and 1b were also weighed 3 times a week to 21 weeks of age.

* unpublished observations

2) Tarttelin and Clark (1975) from female rats injected with
   a) 90 µg of testosterone propionate on days 3 and 5 following birth,
   and b) oil injected at the same ages,

and weighed weekly until 14 weeks of age.

The animals used in these two experiments at Massey were kept under very similar conditions to those described in Chapter 1. Other weighings obtained from rats at Massey by Twine and Tarttelin (1976) and by the author (those of Chapter 1) were also re-analysed for the present purposes.

Weekly weighings, to 18 weeks of age, of intact female rats (treated with oil, 10, 30, 90 and 270 µg of testosterone propionate at 2 days of age) from the colony at the University of California at Los Angeles (U.C.L.A.) were kindly supplied by Dr M.F. Tarttelin and were utilised to ascertain the generality of the results obtained from the Massey animals. The management of the U.C.L.A. animals, described by Tarttelin *et al.* (1975), was similar to that for the Massey animals.

BIOMETRICAL CONSIDERATIONS

Sholl (1954) fitted a polynomial equation to the cubic term, to data, then tested the residuals for departures from randomness. The present growth curves could have been similarly analysed but the parameters of a polynomial equation are difficult to interpret biologically (see Chapter 1). This difficulty of interpretation would extend to the residuals about such a curve. The justification of the log-reciprocal equation resides in the simplicity of the biological interpretation of its parameters. This emphasis on simplicity may not please the empirical mathematician, or the worker who prefers another growth equation. However to quote Kendall (1973, p.53)

"....trend-fitting and trend estimation are very far from being a purely mechanical process....there is great scope - even a necessity - for personal judgement. To a scientist it is always felt as a departure from correctness to incorporate subjective elements into his work.

The student of time series cannot be a purist in that sense. What he can do, of course, is to make available the primary data on which he worked and explain unambiguously how he treated them; anyone who disagrees with what has been done can then carry out his own analysis."

The present data is available, as punched cards, from the Department of Physiology and Anatomy, for such a purpose.

The statistical analyses of this chapter were executed on the Massey University IBM 1620 computer using a version of the FORTRAN regression program BAR3 which was written at the University of New England by E.J. Burr in 1969. The data was punched onto cards. BAR3 transformed the live weights to logarithms to the base 10 and ages (in weeks) to their reciprocals and then calculated and printed the regression equations. Using these equations BAR3 then calculated the residuals and produced the scattergrams on the line printer, some of which are reproduced in the text.

DURBIN-WATSON TEST FOR INDEPENDENCE OF RESIDUALS

Although it can be seen from the Text Figures that the residuals about the log-reciprocal lines show systematic patterns it was considered necessary to numerically confirm this subjective impression. In 1950 J. Durbin and G.S. Watson proposed a statistical test for the independence of residuals, subsequently termed the Durbin-Watson test. The Durbin-Watson statistic (d) is defined in terms of the observed residuals as

$$d = \sum_{i+1}^{n} (e_i - e_{i-1})^2 \; / \; \sum_{i=1}^{n} e_i^2$$

The test is related to the first autocorrelation coefficient (r_1) of the residuals as

$$d = 2\,(1-r_1)$$

Thus if the correlation between the residuals is high (i.e. when they are non-random) then (d) is near zero and indicative of positive serial correlation; if the residuals are uncorrelated (d) is near 2.

FIGURES 6.2A & 6.2B
THE RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO INDIVIDUAL INTACT OIL INJECTED FEMALES RATS vs AGE.

FIGURE 6.2A
DATA OF TARTTELIN & CLARK.
MASSEY COLONY.

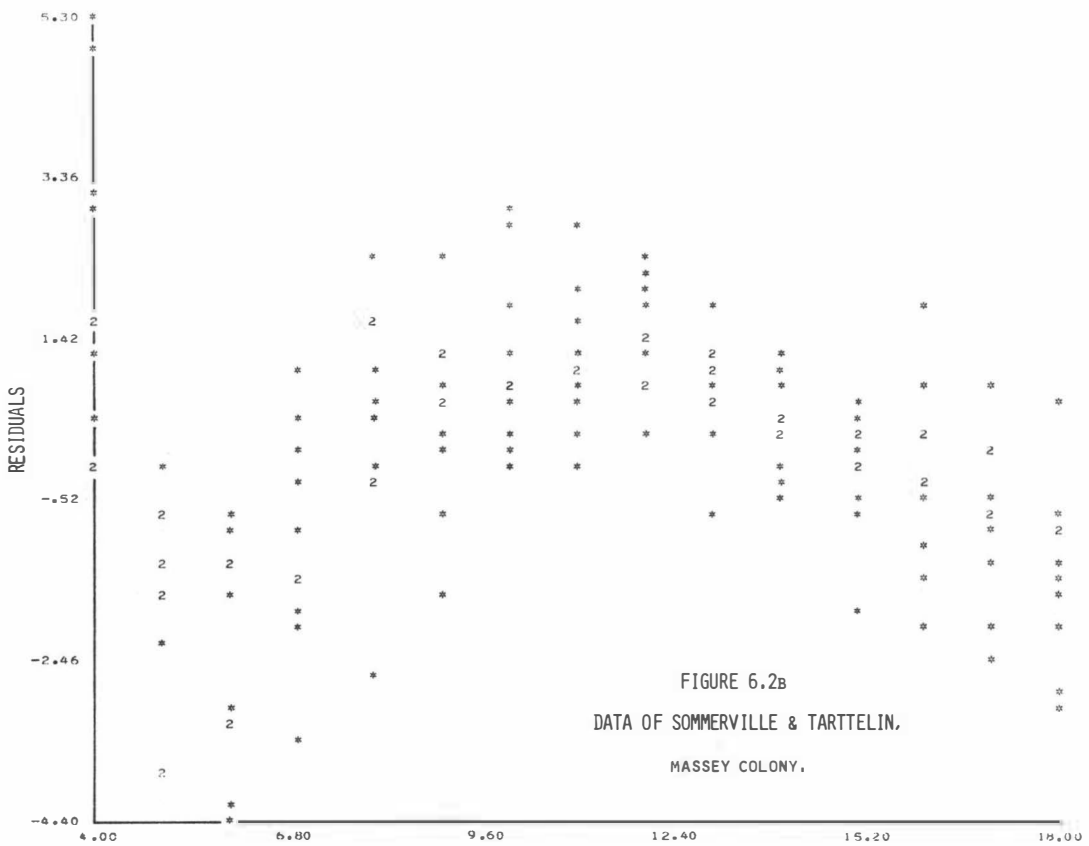


FIGURE 6.2B
DATA OF SOMMERVILLE & TARTTELIN.
MASSEY COLONY.

Plots of the residuals against age for the Massey animals injected with oil on days 3 and 5 (Tarttelin and Clark) and those sham ovariectomised and injected with oil (Sommerville and Tarttelin) are shown in Figures 6.2a and 6.2b. The visual impression of serial correlation between the residuals about the log-reciprocal lines of individual animals was confirmed by statistically highly significant Durbin-Watson Tests (indicating positive serial correlations between the residuals).

Calculations of Durbin-Watson statistics were also performed by the program BAR3 run on the Massey IBM 1620 computer.

RESULTS AND DISCUSSION

DATA FROM THE MASSEY COLONY

The live weight data collected by Sommerville and Tarttelin was the first data to be systematically tested for the presence of serial correlations between the residuals about the log-reciprocal plots for individual animals. Both Durbin-Watson statistics and plotted scattergrams confirmed in all 4 treatment groups that individual animals showed patterns in their residuals, when plotted against age, that were similar to those of Figure 6.2b and were statistically significant. As it was possible that the observed patterns in the residuals were due to systematic errors of measurement several other sets of data were also analysed. The results from the normal intact female rats weighed by Tarttelin and the author have been illustrated in Figure 6.2a. Again the individuals from the other treatment groups in their study showed comparable patterns in their residuals. Re-analysis of the data from the Massey rats described in Chapter 1, and the data obtained by Twine and Tarttelin (unpublished observations), also yielded patterns of residuals consistent with those of Figures 6.2a and 6.2b. The striking similarity between the plots from four different experiments

FIGURES 6.3A & 6.3B

THE RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO INDIVIDUAL NEO-NATALLY OVARIECTOMISED FEMALE RATS, DATA OF SOMMERVILLE & TARTTELIN, MASSEY COLONY

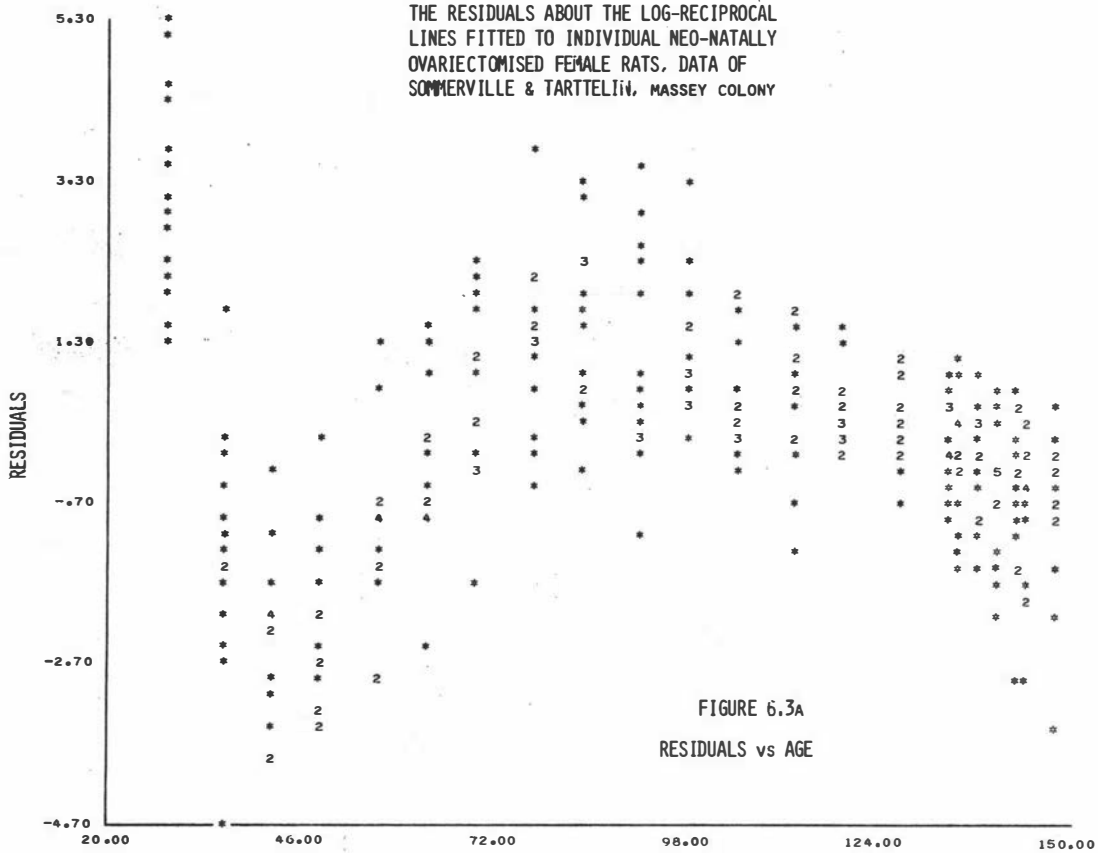


FIGURE 6.3A

RESIDUALS vs AGE

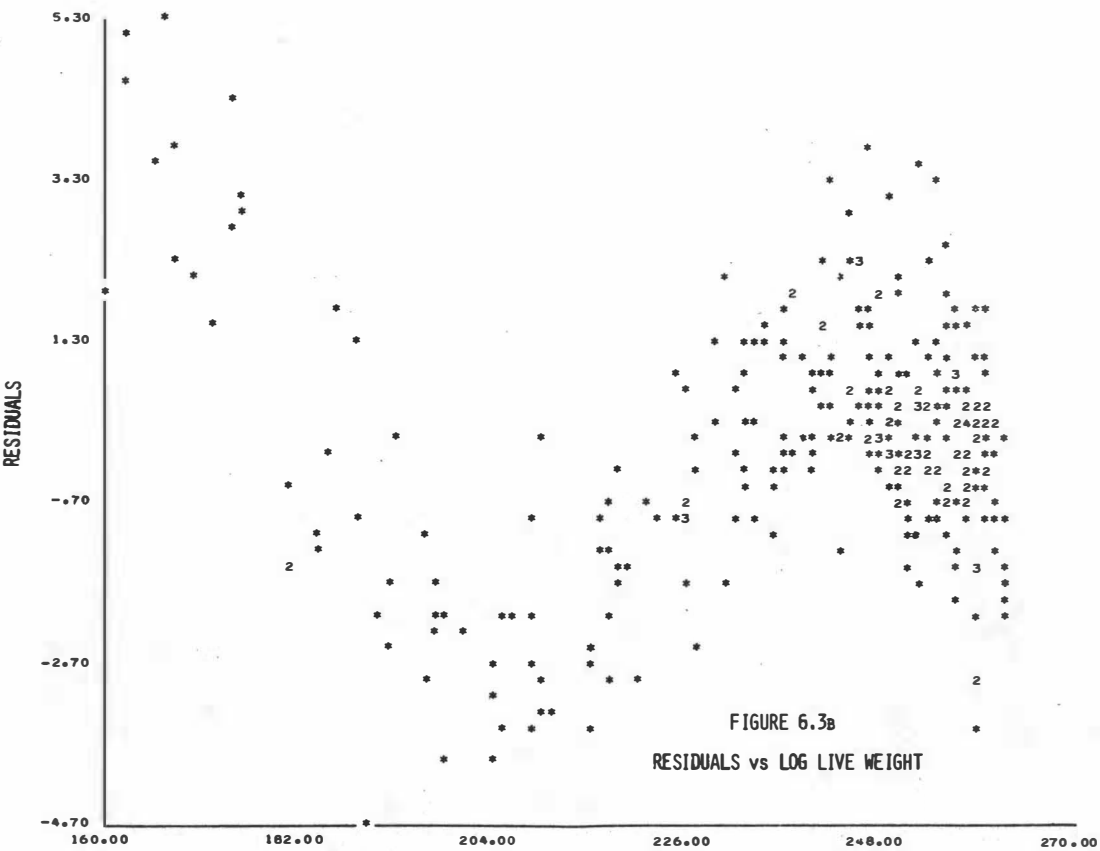


FIGURE 6.3B

RESIDUALS vs LOG LIVE WEIGHT

using the Massey animals, spanning three years, indicates that the serial correlations were not an artifact due to systematic errors of measurement; they were clearly a repeatable biological phenomenon. Further support for this interpretation of the results was obtained from plotting the residuals against the logarithm of body weight rather than against age. If the deviations were due to errors of measurement being different at successive ages then as the animals were of different weights at the various ages the residuals would not be expected to show serial correlations to the same extent when plotted against the observed body weights. But serial correlation was again present, perhaps to an even greater degree. The residuals for the neo-natally ovariectomised animals kept by Sommerville are plotted against age (Fig 6.3a) and against log body weight (Fig 6.3b) to illustrate this idea.

For a group of rats, plots of the residuals about the individual lines, as a function of age, show that at early ages the range of the residuals is greater than at later ages (Figures 6.2a, 6.2b, and 6.3a). Compensatory growth would again seem to be indicated since when the residuals are plotted against log body weight the range at a given body weight is less variable than that at a given age (Figures 6.3a and b): the suggestion being that at early ages there is a greater range in body weights (on a logarithmic scale) and thus a greater spread of residuals since the residuals are related to weight more closely than to age. To fit a mathematical function to the residuals, log body weight would serve as a better independent variable than would age, due to the superior homoscedasticity of the residuals with respect to log body weight. Log body weight is also to be preferred since the form of the relationship between the residuals and log body weight appears to be a simple wave-like function, compared to the more complex function evident when age is the independent variable.

The concept of an ultimate body weight (developed in Chapter 1) implies that at a given body weight the ideal rate of gain, to achieve the ultimate weight, is to a high degree predetermined, irrespective of the age of the animal. Thus if retarded animals are realimentated on an unlimited food supply their rate of gain should be more closely related to their body weight than to their age; this has been demonstrated

FIGURES 6.4A & 6.4B

THE RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO INDIVIDUAL FEMALE RATS vs LOG LIVE WEIGHT, DATA OF TARTTELIN ET AL. (1975), U.C.L.A. COLONY.

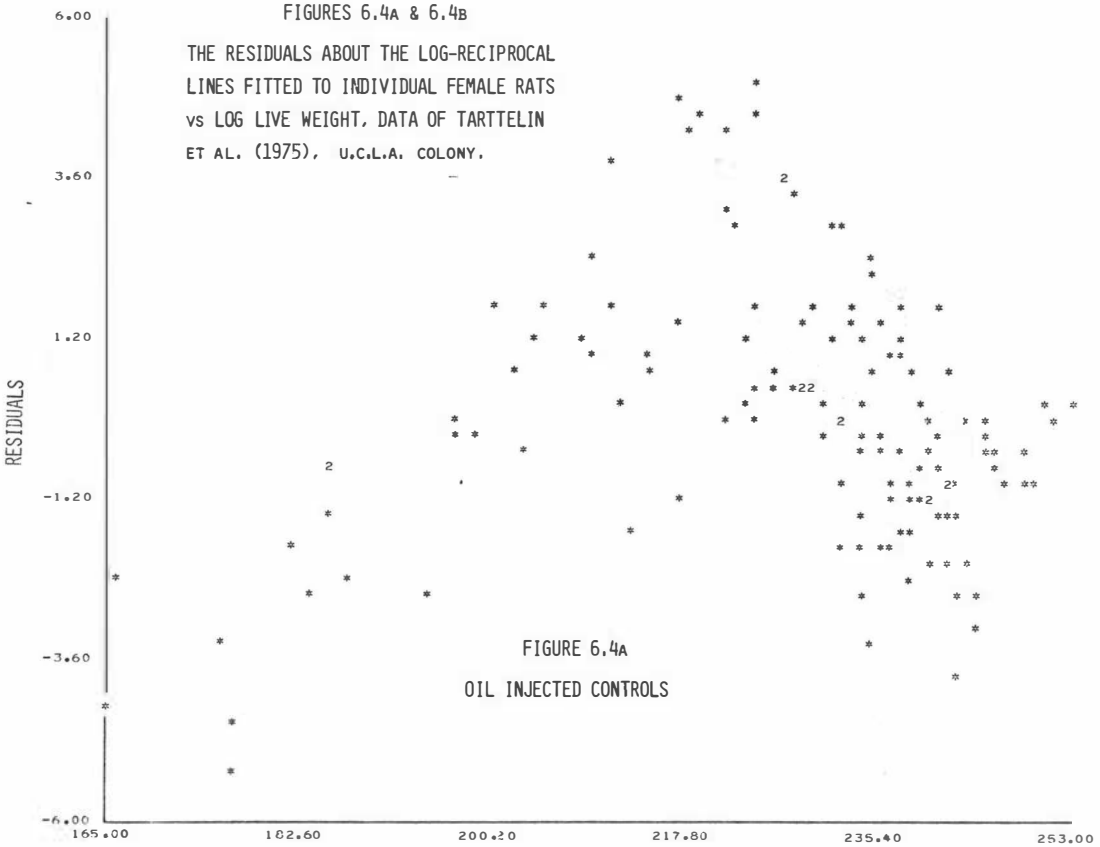


FIGURE 6.4A

OIL INJECTED CONTROLS

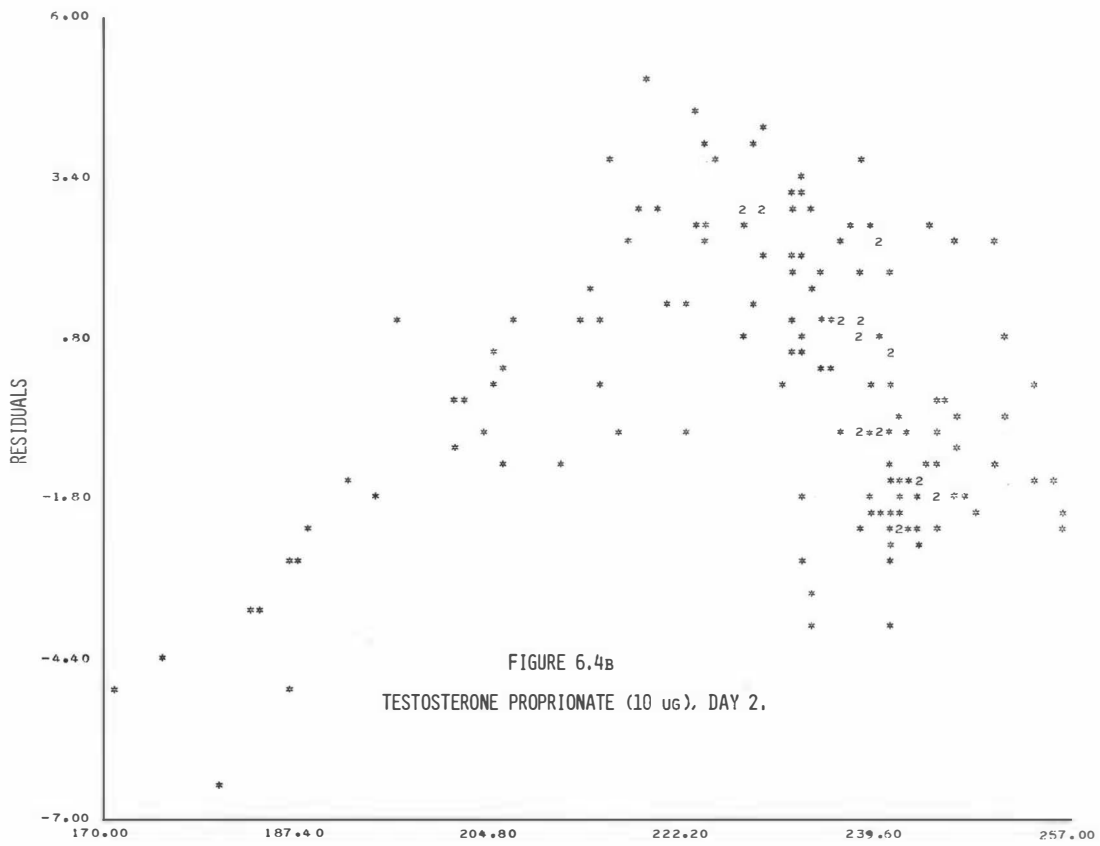


FIGURE 6.4B

TESTOSTERONE PROPRIONATE (10 UG), DAY 2.

FIGURES 6.5A & 6.5B

THE RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO INDIVIDUAL FEMALE RATS vs LOG LIVE WEIGHT, DATA OF TARTTELIN ET AL. (1975), U.C.L.A. COLONY.

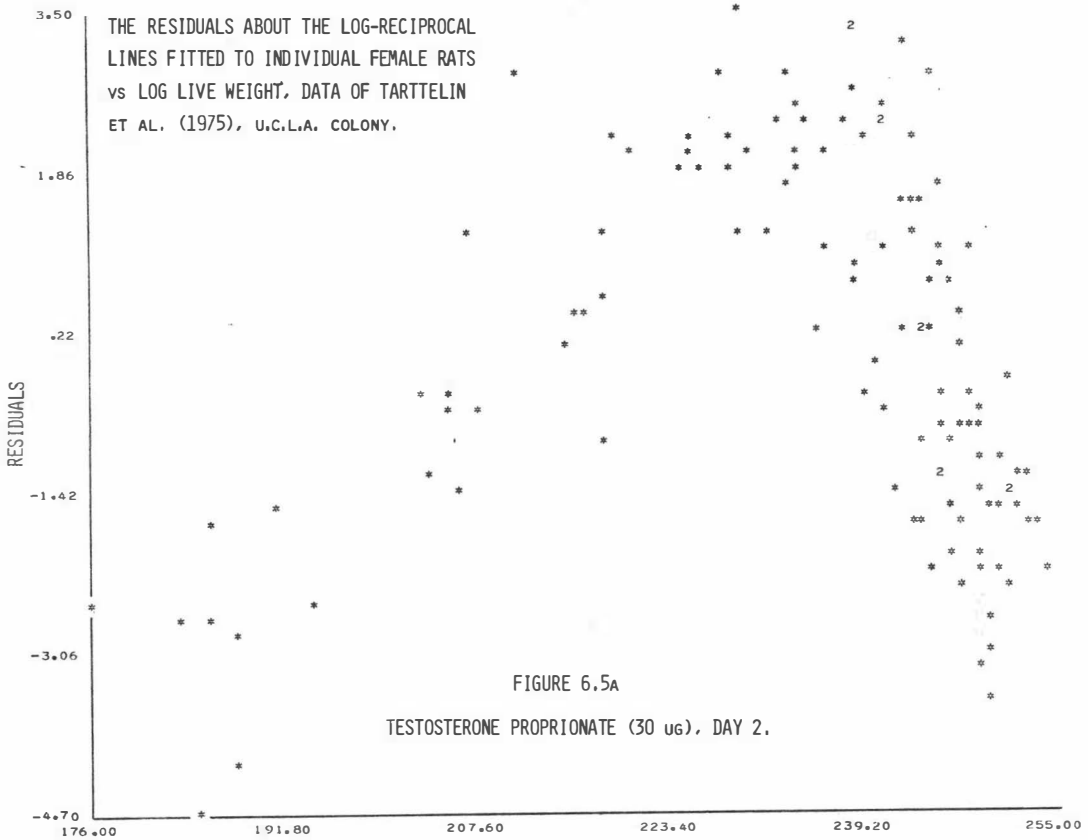


FIGURE 6.5A

TESTOSTERONE PROPRIONATE (30 UG), DAY 2.

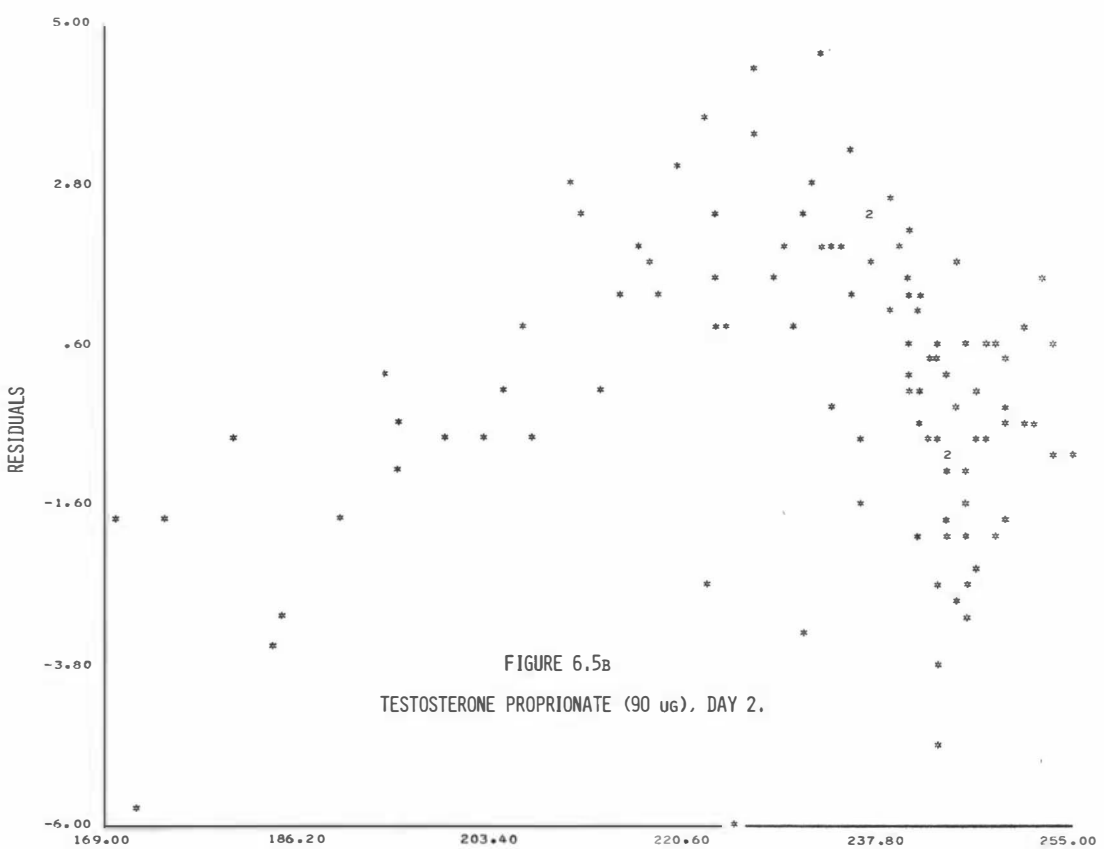


FIGURE 6.5B

TESTOSTERONE PROPRIONATE (90 UG), DAY 2.

FIGURES 6.6A & 6.6B

THE RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO INDIVIDUAL FEMALE RATS vs LOG LIVE WEIGHT, DATA OF TARTTELIN ET AL. (1975), U.C.L.A. COLONY.

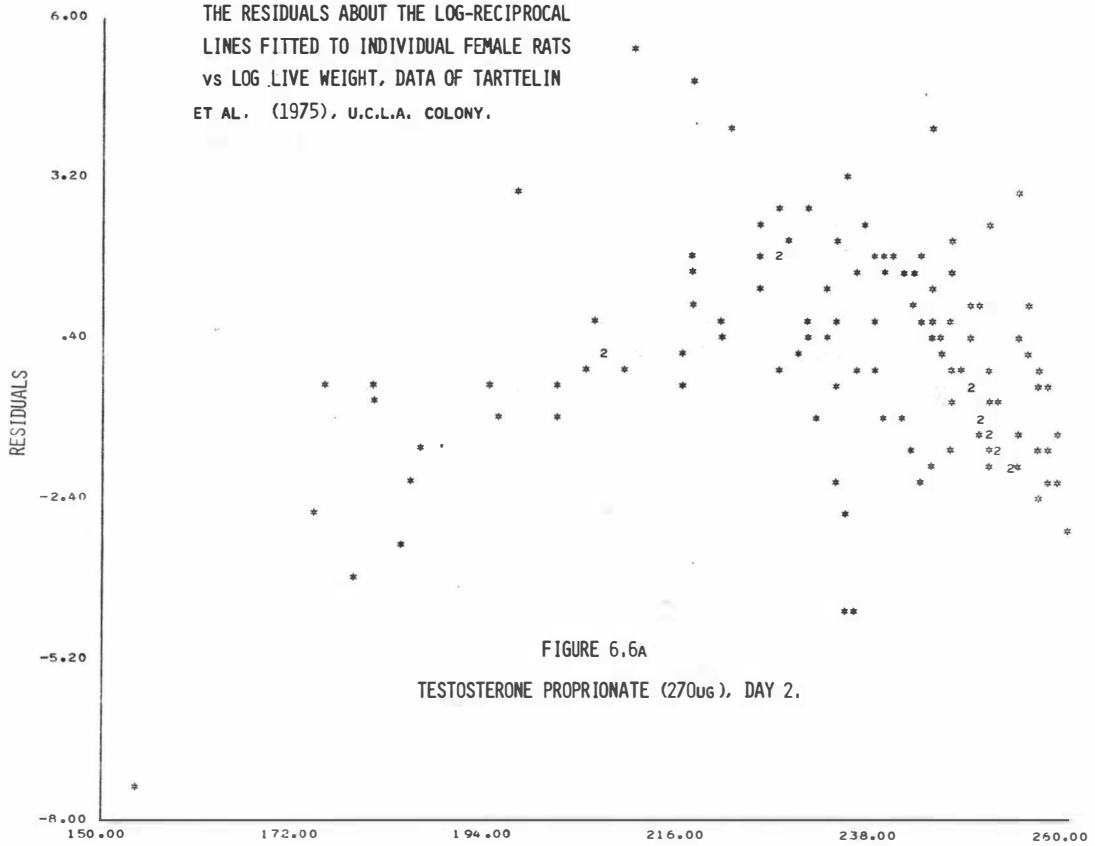


FIGURE 6.6A

TESTOSTERONE PROPRIONATE (270UG), DAY 2.

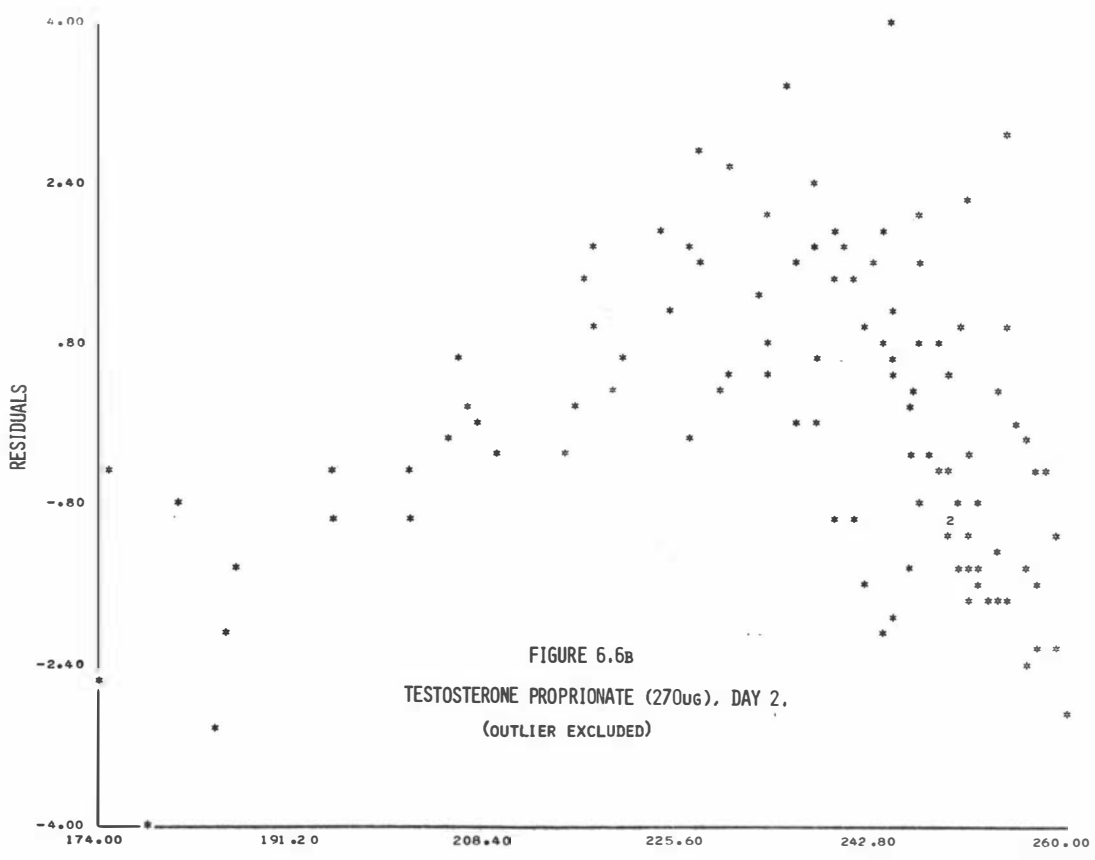


FIGURE 6.6B

TESTOSTERONE PROPRIONATE (270UG), DAY 2.
(OUTLIER EXCLUDED)

(Widdowson and McCance, 1963).

Therefore it is logical that the swings around the general trend of live weight increase should be more closely related to weight, rather than to age, if one accepts that the growth rate, or the pattern of growth, depends more on an animal's physiological age than its chronological age. Size here being a measure of the stage of development or physiological age of the rat.

A variety of reasons indicate that plotting the residuals about the log-reciprocal equation against the logarithm of body weight is superior to plotting the residuals against age.

STRAIN DIFFERENCES IN PATTERNS OF GROWTH?

The only other set of body weight measurements from individual rats, of another colony, that were available to the author were those made at U.C.L.A. by Tarttelin *et al*. (1975). This data proved valuable to the present work in several ways. The residuals (plotted against log body weight) about the log-reciprocal lines are illustrated for the rats injected with either oil (Fig 6.4a), 10 µg (Fig 6.4b), 30 µg (Fig 6.5a), 90 µg (Fig 6.5b), or with 270 µg (Figs 6.6. a and b) of testosterone propionate two days following birth.

The plotted residuals clearly show systematic deviations but the patterns of the deviations are fundamentally different from those for the Massey animals. In the U.C.L.A. rats there is not the early decline from 4 weeks of age to a nadir at about 6 weeks of age which was seen in the Massey animals, instead a steady rise from the smallest weights occurs. The U.C.L.A. sham rats reached a peak in their residuals, in terms of body weight expressed as $\log_{10} \times 100$, at about 220. The Massey rats peaked later, at around 230 (Fig 6.10a), thus for the U.C.L.A. sham rats a greater part of the latter downward trend in the residuals was available. In these animals some evidence of a turning point at around 250 was evident while the Massey animals, due to their later peak, are seen to be still on a declining phase. These differences between the colonies could be due to a number a factors.

For example Zucker *et al*. (1941a, p 433) write,

"As far as post-weaning growth is concerned, animals raised in large litters are too small at weaning for the log reciprocal equation fitted to the subsequent course of their growth, and, strangely enough, those raised in small litters are too heavy, and come down onto the log reciprocal equation from above."

Despite the plausibility of this argument it can not explain the difference between the colonies. The plots of residuals against age, for the Massey animals, invariably show the residuals to be initially above the fitted line irrespective of the weaning weights of the animals. Furthermore the plots of the residuals against log body weight indicate that if an animal were large at 4 weeks of age it would tend to be below the fitted line, and if it were small, to be above the fitted line; the complete opposite to the effect described by Zucker *et al*. (1941a).

The diets used at U.C.L.A. and at Massey differed. The Zuckers showed, by re-analysing data from the literature, that diet could have a major influence on the shape of growth curves. Although the diet seems a likely cause of the differing patterns of growth the diets used at Massey and U.C.L.A. were formulated to give adequate nutrition.

It is possible that the difference between Massey and U.C.L.A. rats represents a strain difference in the pattern of growth. Although the animals are reputedly both of the Simonsen strain of Sprague Dawley ancestory, they have probably been subjected to differing selection pressures and therefore now represent sub-strains of the Sprague Dawley. Again, although an attractive hypothesis, there seems little evidence for such major strain differences in patterns of growth (as discussed in Chapter 4).

In spite of the conclusion from the preceding paragraph, which was based upon a review of the literature, the possibility of strain differences in patterns of growth was investigated. Therefore live weight measurements from albino rats from the following sources were re-analysed,

1) the U.C.L.A. Sprague Dawley rats,
2) the Sprague Dawley rats of Acheson *et al*. (1959),

and 3) the Sherman rats of Zucker *et al*. (1941b).

FIGURES 6.7A & 6.7B

THE RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO INDIVIDUAL FEMALE RATS vs LOG LIVE WEIGHT, DATA OF TARTTELIN ET AL. (1975) U.C.L.A. COLONY. (WEANING WEIGHT INCLUDED)

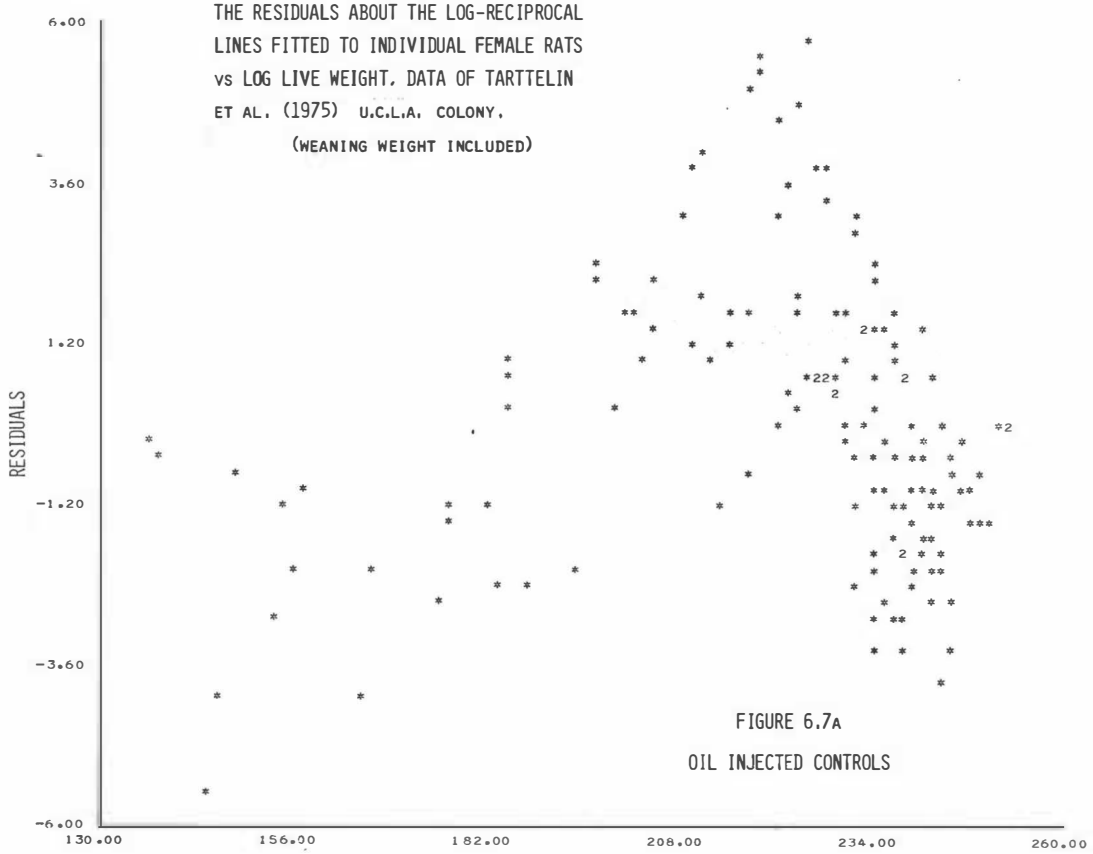


FIGURE 6.7A

OIL INJECTED CONTROLS

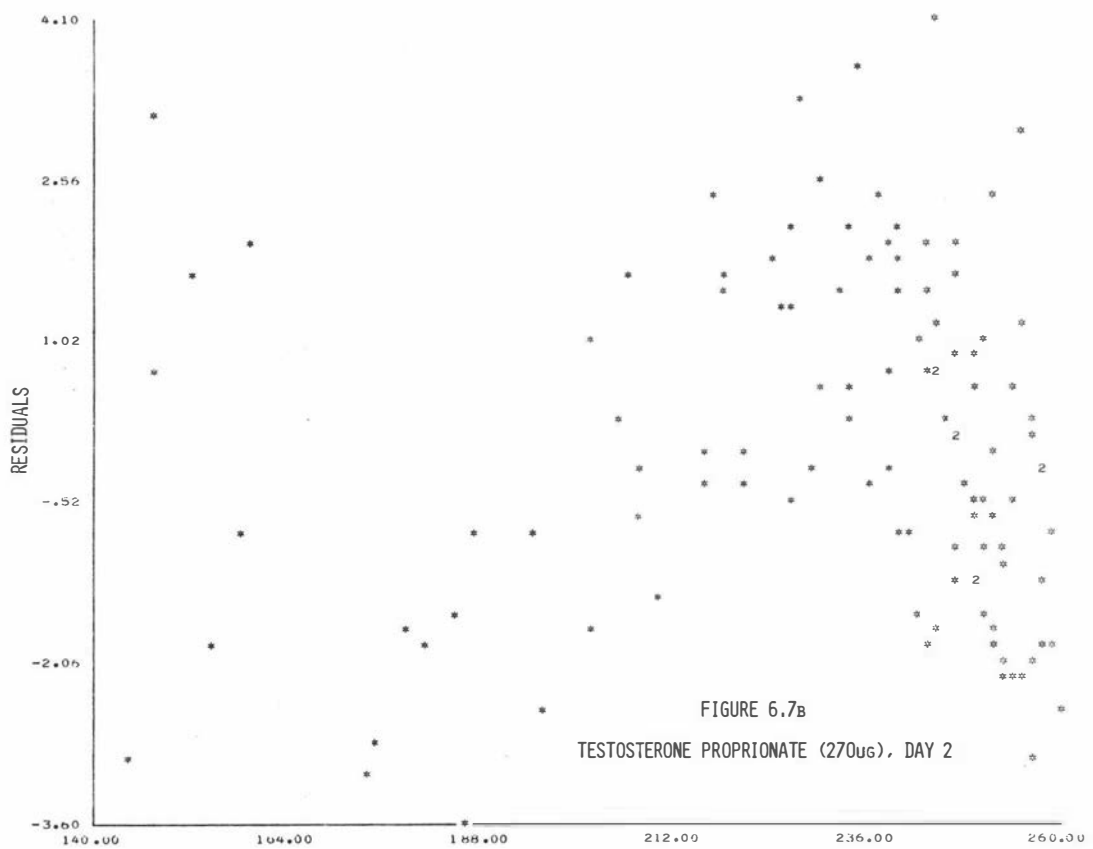


FIGURE 6.7B

TESTOSTERONE PROPRIONATE (270UG), DAY 2

The following proposal seemed a logical starting point for the analyses. It was hypothesised that the U.C.L.A. pattern of growth was an abbreviated version of the Massey pattern; the difference being that the whole pattern was displaced to the left in the U.C.L.A. animals. A logical consequence of this interpretation of the results is that at earlier ages, or smaller live weights, the residuals for the U.C.L.A. animals should climb back towards the fitted line. As the weaning weights were available from the U.C.L.A. rats the hypothesis could be directly tested.

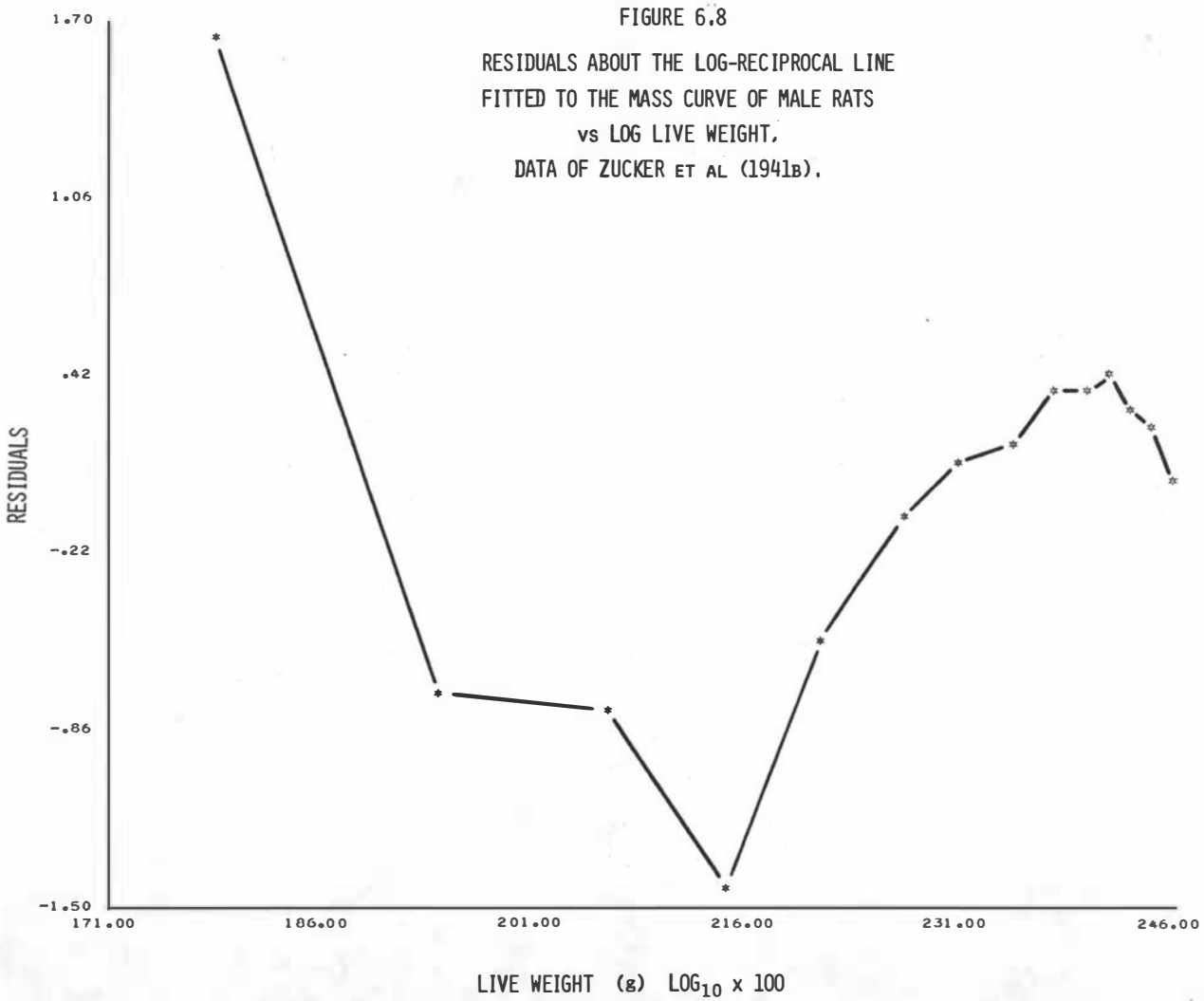
In Chapter 1 the live weights at weaning (week 3) were excluded from the regressions for statistical reasons, as outliers, and because they had been similarly excluded by Zucker and his colleagues. As the weaning weights definitely were outliers from the Massey regressions, and since differences between strains were not suspected, the weaning weights were originally also excluded from the regressions for the U.C.L.A. animals. At the time no statistical check on whether the week 3 weights were outliers was performed for the U.C.L.A. data.

Therefore the data from the U.C.L.A. animals injected with oil on day 2 was re-analysed with the weaning weights now included in the regressions. The resultant residuals about the log-reciprocal lines fitted to the live weights of individual animals are shown plotted against log live weight in Figure 6.7a. There is a suggestion from this figure that the residuals may be following an upward trend at the smallest observed weights. The evidence is however suggestive rather than conclusive. But clearly, for the U.C.L.A. data, the original exclusion of the week 3 weights from the regressions cannot be justified statistically.

This inconclusive result was however not without its benefits as it stimulated the following line of reasoning and analysis. The next section of this chapter will show that the pattern of growth is shifted to the right in androgenised rats. Therefore if an initial downward swing in the residuals was to be found in the U.C.L.A. animals it should occur in the animals treated with the largest dose of testosterone. Figures 6.7b confirms these deductions; the

FIGURE 6.8

RESIDUALS ABOUT THE LOG-RECIPROCAL LINE
FITTED TO THE MASS CURVE OF MALE RATS
vs LOG LIVE WEIGHT,
DATA OF ZUCKER ET AL (1941B).



residuals for the rats treated with 270 μg of the testosterone propionate on day 2 do show an initial downward trend when plotted against log body weight. Their oil injected controls do not show this effect to the same extent (Fig 6.7a). The different patterns of growth for the Massey and U.C.L.A. colonies therefore seems to be due to the same basic pattern being transposed in time. The patterns of growth therefore indicate that the Massey rats may be a late maturing strain compared to the U.C.L.A. animals. This finding accords with the average age at vaginal opening (a measure of maturity) being at 35 days in the U.C.L.A. colony (Tarttelin, pers. comm.) compared to 43 days in the Massey colony (see Chapter 1).

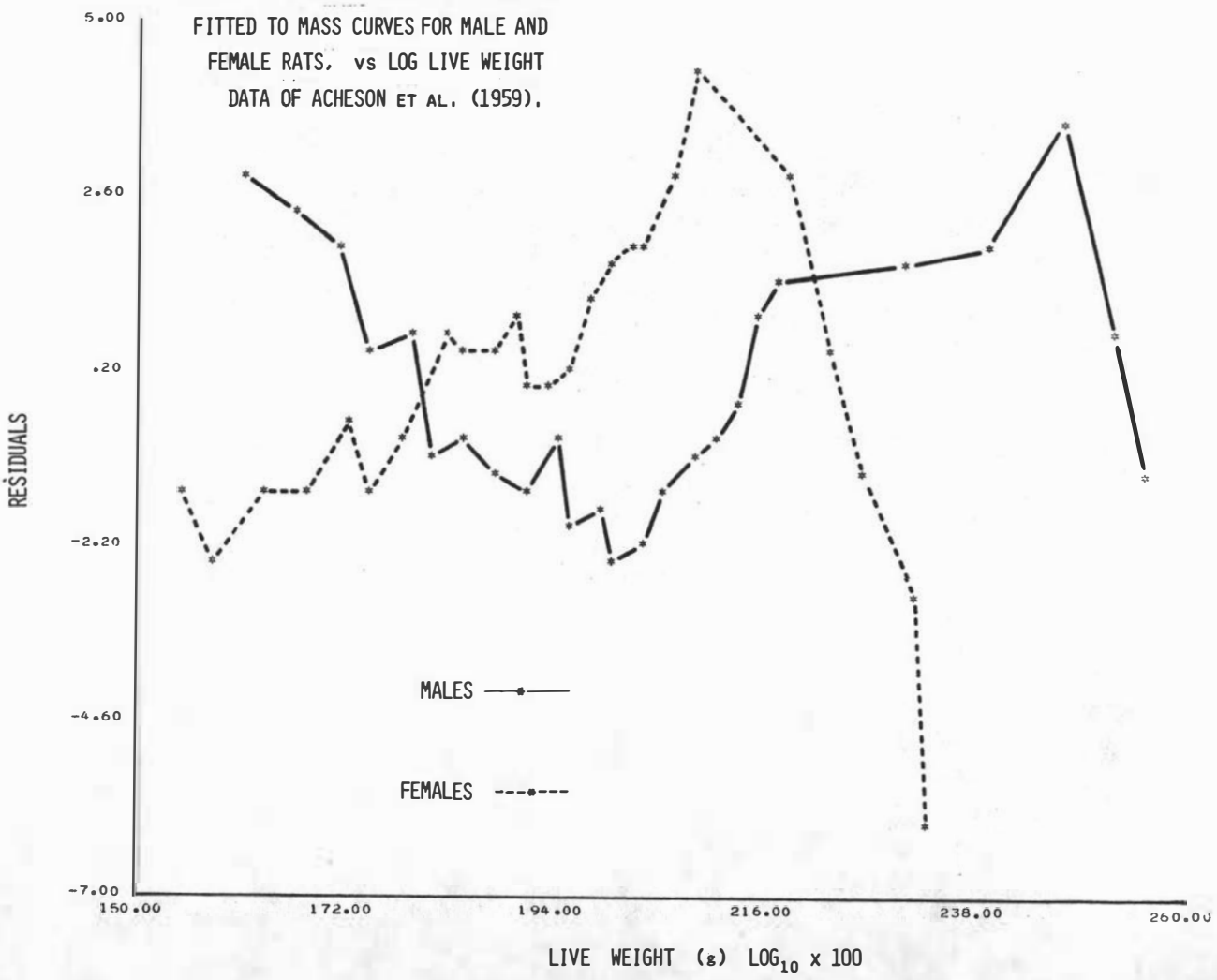
The pattern of growth around log-reciprocal lines was also studied using literature data. But since measurements from individual animals are rarely published it was necessary to use average weights obtained from groups of animals. An obvious source of data was the mean weekly live weights from male and female rats, published by Zucker *et al*. (1941b), to which the log-reciprocal equation was first applied. Other data was considered (that of Smith and Bing, 1928; Hughes and Tanner, 1970) but since it was based upon decreasing numbers of animals (mixed cross-sectional and longitudinal data) it proved to be highly variable. The data of Acheson *et al*. (1959) was also used since, like Zucker (1941b), these workers used the same animals for all weighings.

The author's persistence in this matter was rewarded when, for the male data of Zucker *et al*. (1941b) the residuals (Fig 6.8) about the fitted lines showed a pattern very similar to that obtained from the Massey rats. Acheson *et al*. (1959) weighed male and female rats frequently until 110 days of age (see Chapter 7 for further details). When the complete range of their measurements was fitted with the log-reciprocal equation the pre-weaning weights were well above the fitted line. Thus only post-weaning weights (21 to 110 days) were used in the following analyses.

From the data obtained by Acheson's group plots of the residuals for males against log live weight were very different from those for the females (Figure 6.9). The pattern for the males was similar to that for the animals raised at Massey and for the Zuckers' animals

FIGURE 6.9

RESIDUALS ABOUT LOG-RECIPROCAL LINES FITTED TO MASS CURVES FOR MALE AND FEMALE RATS, vs LOG LIVE WEIGHT DATA OF ACHESON ET AL. (1959).



FIGURES 6.10A & 6.10B

THE RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO INDIVIDUAL FEMALE RATS vs LOG LIVE WEIGHT, DATA OF TARTTELIN AND CLARK, MASSEY COLONY.

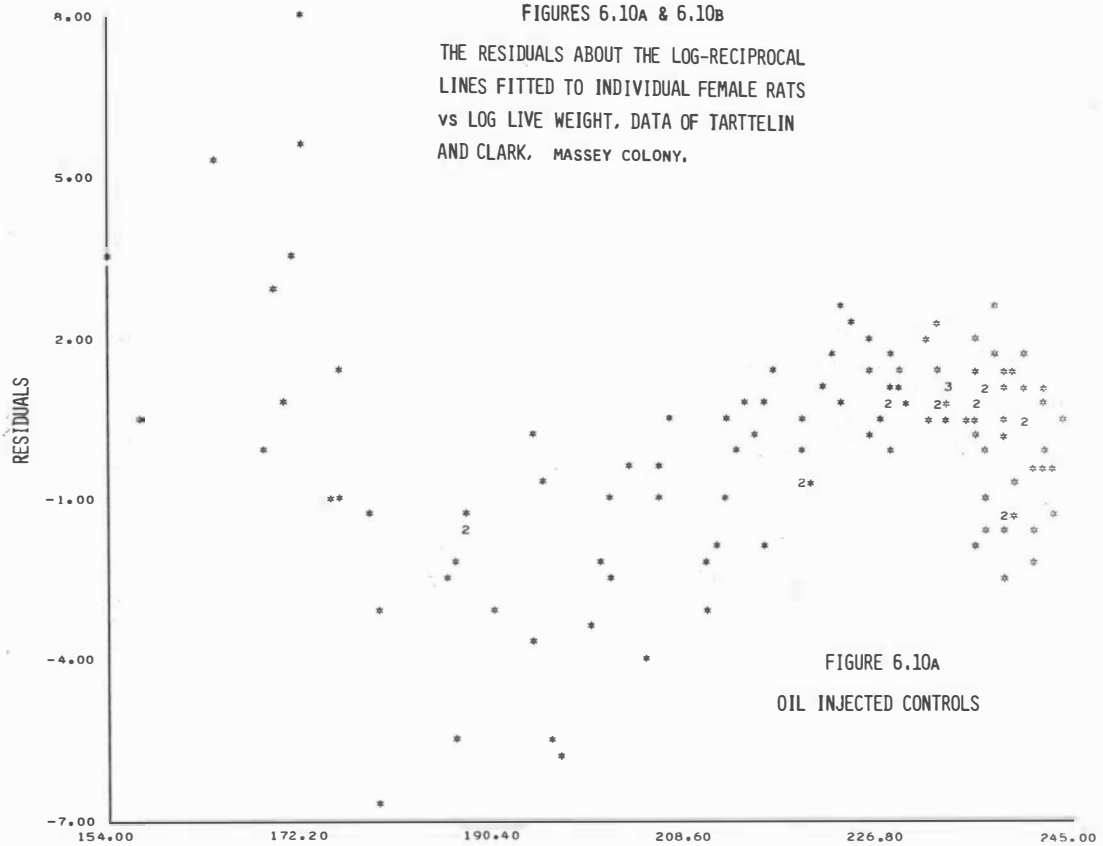


FIGURE 6.10A

OIL INJECTED CONTROLS

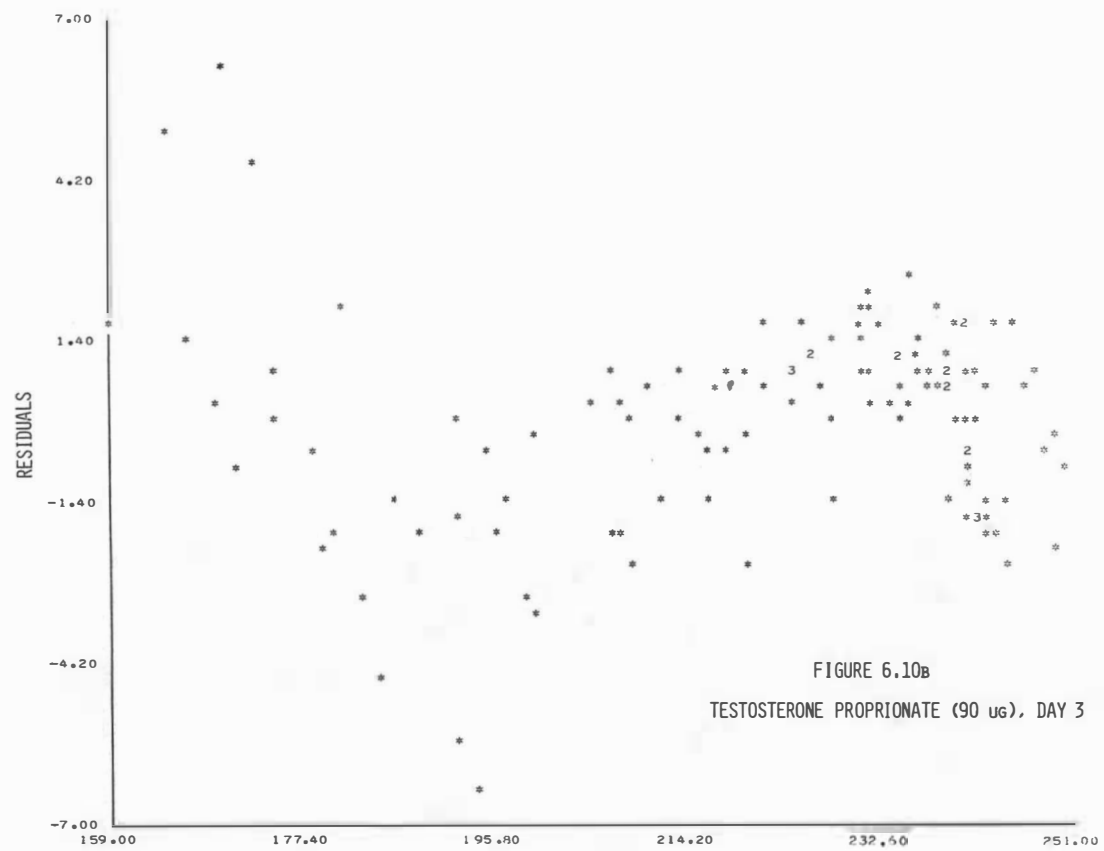


FIGURE 6.10B

TESTOSTERONE PROPRIONATE (90 UG), DAY 3

while the females possessed a pattern similar to that shown by the U.C.L.A. animals.

It must be emphasised that these results were obtained from mass curves, so direct comparisons with the earlier results, from individual curves, may be misleading.

THE EFFECT OF NEO-NATAL TESTOSTERONE ON THE PATTERN OF LIVE WEIGHT GROWTH IN FEMALE RATS.

An interesting feature of the data collected by Tarttelin and his colleagues is that testosterone propionate treatment affected the pattern of the residuals. The turning points, or maximum positive residuals, seem to occur at greater body weights with increasing doses of hormone. For example the oil injected controls' maximum (expressed as $\log_{10} \times 100$) is at 220, that for the 10 µg at 225, the 30 µg at 230, as is the 90 µg, while that for the group that received 270 µg of testosterone propionate seems to turn after 235 (Figures 6.4 to 6.6, a and b). It seems that the hormone prolongs the early growth phase and the effect seems to be dose dependent. A similar effect can be seen in the data collected at Massey by Tarttelin and the author, where testosterone propionate treatment delayed the maximum peak from 230 in controls to around 235 in the treated animals (Figures 6.10a and b).

Neo-natal androgen treatment could have another effect; reducing the range of the residuals at a given body weight. The residuals appear to be less variable as the dose of androgen is increased, especially if the outlying animal in the 270 µg group is excluded (see the next paragraph). This result is not apparent from the findings of Tarttelin and Clark (Figures 6.10a and b). But a smaller dose of androgen was used in their study and the hormone was injected not on day 2 but on day 3 when androgen may have a lesser effect on subsequent growth (Tarttelin *et al*.1975).

FIGURES 6.11A & 6.11B

GROWTH CURVES OF NEO-NATALLY OVARIECTOMISED FEMALE RATS, DATA OF SOMMERVILLE AND TARTTELIN, MASSEY COLONY.

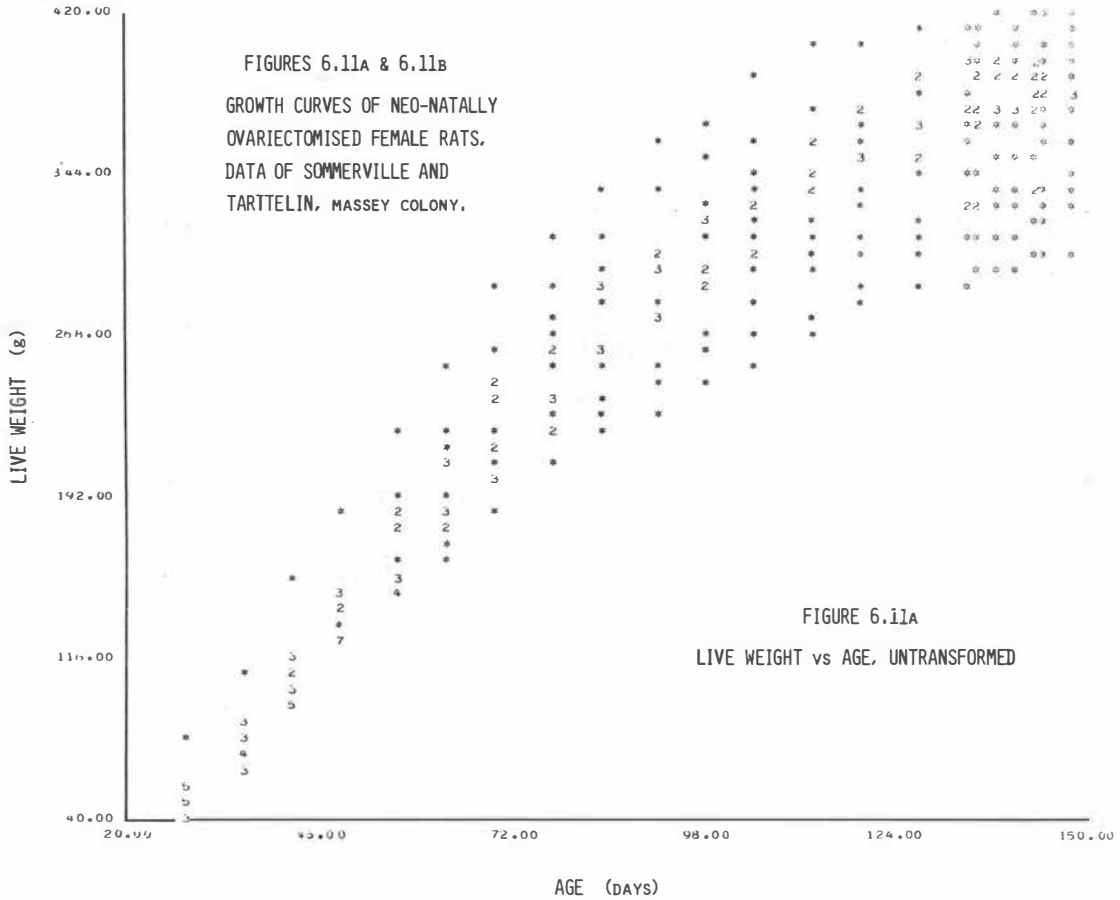


FIGURE 6.11A

LIVE WEIGHT vs AGE, UNTRANSFORMED

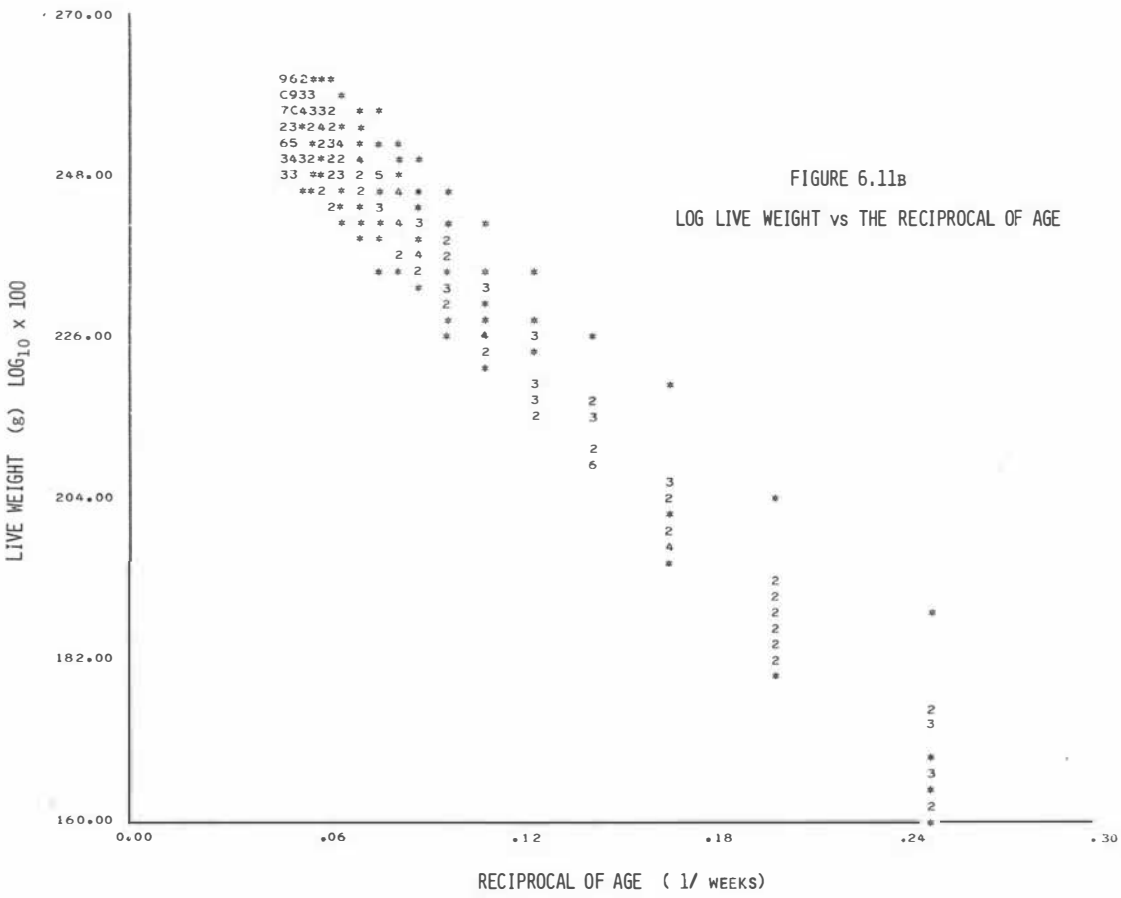


FIGURE 6.11B

LOG LIVE WEIGHT vs THE RECIPROCAL OF AGE

FIGURES 6.12A & 6.12B

THE RESIDUALS ABOUT INDIVIDUAL AND MASS CURVES, vs LOG LIVE WEIGHT, NEO-NATALLY OVARIECTOMISED FEMALE RATS, DATA OF SOMMERVILLE AND TARTTELIN, MASSEY COLONY

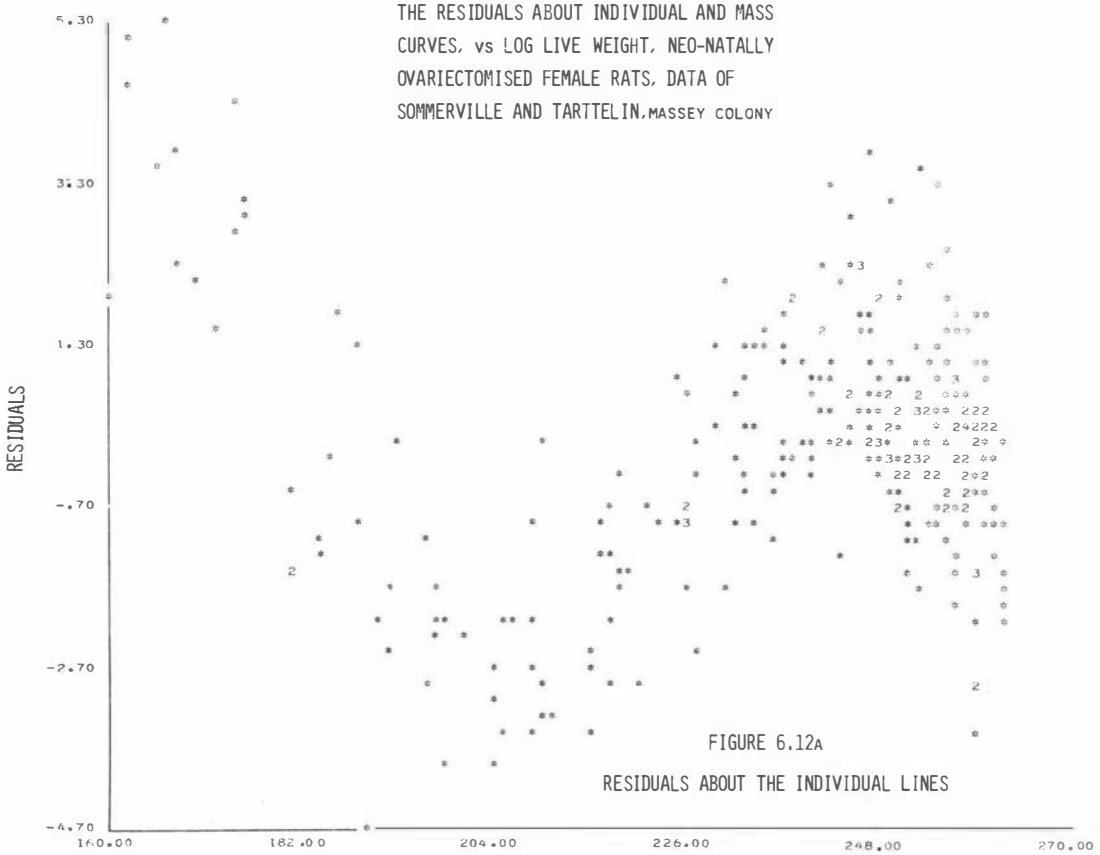


FIGURE 6.12A

RESIDUALS ABOUT THE INDIVIDUAL LINES

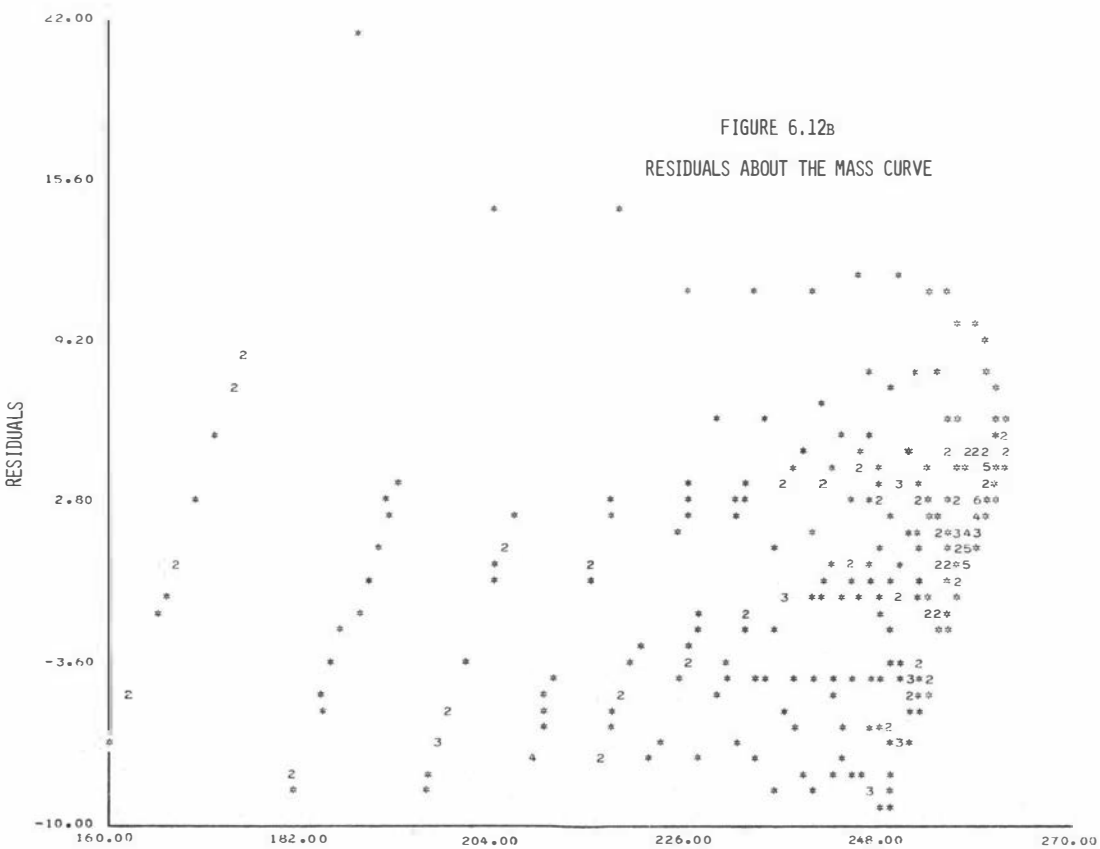


FIGURE 6.12B

RESIDUALS ABOUT THE MASS CURVE

In Figure 6.6a one animal is an outlier, compared to the rest of the group, as its residuals show an early peak at 215 and a descent to 235. This suggests that the animal was either not successfully injected with androgen or that it was a runt. Therefore this animal was excluded and the residuals were replotted (Figure 6.6b). In the 90 µg group (Figure 6.5b) a group of residuals also appear to be outliers and could also be excluded and the data replotted. So the pattern of residuals can also be used to detect animals or groups of points that differ from the norm. Although this method allows possible outliers to be detected the actual rejection of aberrant observations from regressions should be based on numerical tests (see Bliss, 1967; pp.442-4).

INDIVIDUAL AND MASS CURVES

Systematic departures from linearity around the log live weight vs. reciprocal of age plots for individual animals have been amply illustrated. Also of considerable interest was the variability about the combined or mass curve; was it also systematic?

It was apparent from Section 1 of this Chapter that the failure to separate the between-animal variation from the within-animal variation led to confusion. Therefore, using the data from neo-natally ovariectomised female rats collected by Sommerville and Tarttelin, log-reciprocal lines were computed both for each individual animal and for the mass curve for all animals. For completeness Figure 6.11a shows the untransformed growth curves for these animals while Figure 6.11b gives the transformed values for the same animals. When plotted against log body weight the residuals about individually fitted transformed lines (Fig 6.12a), when compared to those for the mass curve (Fig 6.12b), clearly show that the between-animal variation is large compared to the within-animal variation. Of greatest importance is the obvious impression that the residuals about the mass curve do not give the same clear pattern of systematic variation shown for the residuals about the individual lines. It could be concluded from

Fig 6.12b that no serial correlation existed and that since the errors were random the log-reciprocal equation could therefore be said to provide a complete explanation for post-weaning live weight growth in the rat.

These results illustrate both the value of fitting lines to individual animals' growth curves and the way that mass curves can conceal rather than reveal. Sholl (1954, p. 226) reiterates the importance of this effect; a view that is commonly not understood, appreciated, or heeded by many authors and for these reasons is repeatedly restated in this work,

"Workers have often made a kind of average curve from measurements on different individuals at different ages. Such a combined set of data is quite adequate for the study of the distribution of sizes of individuals at different ages but is quite useless, and in fact misleading, for studying the growth of individuals or the process of growth itself. Although sharp changes in the growth rate of individuals may occur at ages which are approximately the same for different people, the study of the curve of combined sizes obtained from various individuals will lead to the damping out of such changes."

Brody (1945, p.549) also stressed the importance of the study of the individual "The average differed in fundamental characteristics from the separate curves. Significant undulations observed in the average were absent in the individual growth curve. Undulations and changes in skewness are often the result of the averaging process, unrelated to the biology of growth. In brief, the growth process in an individual is not the smooth sigmoid curve represented by average curves. The average curve represents properties of the mathematical averaging process often absent in individuals."

Medawar (1945) describes a situation where dramatic differences between the mean and individual curves occurs, "At puberty, for example, the growth of individual boys shows a marked spurt, whose time of onset is normally distributed through the male population about a mean value of approximately 14½ years. The 'adolescent growth component' of the mass-curve of male growth, to which Robertson fitted the logistic equation, does not occur in the curve of individual growth:

it is essentially a transformed curve of normal distribution, indicating the time of onset in development of the adolescent spurt."

What is probably the clearest example in the literature of differences between individual and mass curves, and the most startling, is a hypothetical case conceived of by Medawar (1950). He hypothesises that if a change in a variable from one level to another were step-like (ie. involved two 90^{o} changes), and the steps occurred at different times from the average, then the combined change would actually resemble a sigmoid curve!

The original general statement of these ideas, and probably the most extreme view, can be traced to Claude Bernard and his classic "Introduction to the Study of Experimental Medicine,"

"By destroying the biological character of phenomena, the use of averages in physiology and medicine usually gives only apparent accuracy to the results.....So in physiology, we must never make average descriptions of experiments, because the true relations of phenomena disappear in the average..... In the cases just considered, averages must therefore be rejected, because they confuse, while aiming to unify, and distort while aiming to simplify. Averages are applicable only to reducing very slightly varying numerical data about clearly defined and absolutely simple cases....."

A BIOLOGICAL EXPLANATION FOR THE SWINGS IN THE RESIDUALS

Attempts to mathematically describe normal changes in body size during active growth have occupied the time of many men. But their analyses have usually assumed that under uniform environmental conditions body size will increase as a simple function of age; the theoretical growth curve is assumed to be a smooth curve. Therefore any deviation from this simple relationship would be ascribed to either environmental effects or random errors. This was the view held, perhaps naively, by the author before the results of this chapter were obtained. These results clearly show that in individual animals although the relationship between log live weight and the reciprocal

of age is essentially linear there are systematic departures from this relationship; indicating that the log-reciprocal plot is far from a complete explanation of the post-weaning growth in live weight of the laboratory rat.

If, as current research suggests, body size is closely monitored and controlled, in both the long and the short-term, one would expect body size to oscillate or weave as it increases during normal active growth. Although this idea seems logical no-one seems to have recently considered this possiblity. The literature from the first part of this century abounds with discussions of the cyclical nature of growth (reviewed by Zucker *et al*. 1941a). Two points of view were current at that time, one that growth cycles were consecutive in time (the view of Brody) the other that cycles were simultaneous in time (the view of Robertson). But 'processes' or 'mechanisms' would more precisely describe what these authors meant when they used the term 'cycles'. But from the present work post-weaning live weight changes in the rat can be shown to be explained by a linear process and a long-term cyclical oscillation around this trend. This should not necessarily be taken to imply that several different mechanisms (either simultaneous or consecutive) are controlling the live weight changes. A more simple explanation would be that the linear trend indicates the ideal state that homeorhesis intends while the oscillations indicate the actual errors from this ideal. But this simple explanation seems contrary to the evidence of the illustrations which clearly show that the residuals for all the individuals, within a treatment group, follow a similar pattern of change when plotted against either log body weight or age. If the residuals represented the mechanism controlling size 'hunting' for the ideal then one would not expect the patterns of the residuals for individuals to follow such similar patterns. The residuals for different animals would probably follow different patterns. The proposal that post-weaning growth occurs in 'cycles', in the sense used by Brody or Robertson, may be the best interpretation of the present results.

Ideas advanced by Zucker *et al*. (1941a) can be adapted to help explain the observed pattern of growth. Zucker *et al*. used two equations

to describe normal post-natal live weight changes in the rat. A log-log equation explaining the changes up to 6 weeks of age and the log-reciprocal equation describing those following 4 weeks of age. The transition from one equation to the other was equated with the change from what Zucker *et al* term histo-differentiation (cell hyperplasia) to auxano-differentiation (cell hypertrophy) and with the nutritional changes associated with weaning. These ideas may help explain the pattern of change in the residuals, in the Massey animals, where the initial downward trend could be due to the decline in cell hyperplasia occurring before the mechanisms controlling cell hypertrophy are fully functional. The rising phase could represent cell hypertrophy being maximal with the declining phase showing a relative slowing in cell hypertrophy. Whether the damped sinusoidal pattern of the residuals (plotted against log body weight) continues (ie. that further cycles occur) remains to be established. A later rise above the line, perhaps due to a relative rise in the rate of fat deposition, is possible.

There is other evidence to support the division, at the times indicated by the present work, of live weight growth into two post-natal phases. Evans, in a series of classic experiments (reviewed by Simpson, Asling, and Evans, 1950), hyophysectomised suckling and weanling rats at several ages to find that "The capacity to increase in size was lost at a constant age, approximately 30 days chronological age." (Simpson *et al*. 1950; p.23). This age is a week or so before the declining phase of the residuals, in the Massey animals, is reversed. It may be that the pituitary takes some time to organise growth following assuming control of changes in size. This evidence adds further support to the swings in the residuals being of physiological significance. As the residuals appear more closely related to weight than to age the assumption of Simpson *et al*., that the ability to increase in size is lost at 'a constant age', seems questionable. The residuals suggest that the pituitary's control may begin at a constant weight or physiological age. This point, which seems to have not been directly tested experimentally, could be elucidated by hypophysectomising rats of widely different weaning weights to see if weight increase did cease at a fixed weight or at a fixed age.

CONCLUSIONS

The analyses of this chapter clearly illustrate that the post-weaning pattern of live weight growth in the rat can be measured as the residuals around log-reciprocal lines. There are clear differences in these patterns between strains of rats and, within strains, between the sexes. These differences seem to be caused by the same basic pattern of growth being transposed in time (physiological or chronological).

The sex difference in the pattern of growth could be related to early maturity in female animals since the swings in the residuals, or perhaps the changes in the rates of cell hyperplasia and hypertrophy, occur before those in the male. The early diminution of the rates of these processes giving the resultant smaller size of the female. But there seems to be a sex by strain interaction in the magnitude of this effect. For example, in the Massey colony the sex difference appears to be small while the data obtained by Acheson's group suggests a large difference. The effect of neo-natal ovariectomy or testosterone treatment supports this view of the nature of the differences as in these treatments the pattern of residuals was advanced in time; perhaps towards the male pattern?

The effect of neo-natal ovariectomy was also of interest since at first the author held the opinion that the swings in the residuals could be due to the effects of sex steroids. The early relative decline in growth in the Massey female animals being an effect of oestrogen, the subsequent upward swing therefore being the 'escape' from this inhibition. However the occurrence of the same basic pattern in the residuals for ovariectomised, male, and intact female animals contradicts this theory. The sex steroids may alter the position of the basic pattern but they do not seem to have as great an effect on the pattern's shape.

Frisch (1974) has proposed that puberty is triggered by a change in the rate of metabolism when an animal reaches a 'critical weight'. This assertion was challenged by Tanner (1974) since he found that in young girls the menarche was more closely related to skeletal

age than chronological age but that this relationship did not pertain for other events marking puberty (breast development, pubic hair appearance). As Donovan (1974) points out the relationship between Frisch's 'critical weight' and the menarche need not be causal. Since the residuals were closely related to weight, as is vaginal opening in the rat (Kennedy, 1969), it was natural to attempt to relate the patterns of growth of individuals to their age at puberty (measured by vaginal opening). Fortunately Sommerville kept records of the vaginal opening dates from her animals, but the time of vaginal opening did not seem to be related to any particular phase of the pattern of growth as it occurred during both the relative declines and rises in the rate of growth. This evidence supports a theme of this thesis, that puberty is a minor event in terms of the live weight growth of the rat.

The difference between strains, in their pattern of growth, was an unexpected finding which the author finds difficult to explain. The Massey rats reached weights comparable to those of the U.C.L.A. strain yet the patterns of growth to attain these weights were very different. Although Acheson's female rats reached 210 grams at 110 days of age and the weights of the U.C.L.A. females reached 300 grams at this age, the patterns of the residuals in these two strains were very similar. There seems, therefore, no clear indication that the pattern of growth is related to the final size of a strain. The results show differences between individuals in their patterns of growth. The strain difference in these patterns indicates that the pattern of growth has a genetic basis. Contrary to the negative findings discussed in Chapter 4 it seems that the selection of domestic animals for pattern of growth (degree of maturity) may prove to be possible.

Finally, to return to the initial section of this Chapter, it is clear from the present analyses that individual growth curves yield more information than do mass curves. The analyses of this Chapter were hindered by the lack of published data from individual animals, a situation that has been deplored in the past, yet shows no signs of

being remedied. As this Chapter began with the work of Sholl it is apt to end with his advice to the publishers of data,

"Adequate growth curves for a score of individuals would be of more value for investigating changes in shape and size than hundreds of curves founded on the mean values of different groups at different ages."

Sholl, (1950, p. 473)

CHAPTER SEVEN

'LINEAR' RELATIONSHIPS BETWEEN AGES, BODY WEIGHTS, AND BODY LENGTHS, AND SYSTEMATIC DEPARTURES FROM THESE RELATIONSHIPS, IN LABORATORY RATS

INTRODUCTION

The rat live weights analysed in previous chapters were obtained at weekly intervals. As suggested in Chapter 10 more frequent live weight measurements, followed by the application of the techniques of statistical analysis developed in this thesis, should give more precise estimates of growth rates and live weights.

Acheson *et al.* (1959) seem to be the only authors who have published comprehensive longitudinal data where body lengths and weights were frequently measured on the same animals. Hughes and Tanner (1970) initially measured 71 male and 48 female rats but by the end of their study only 38 male and 24 female rats remained. Such mass curves, based on different individuals at different ages, present difficulties of analysis and interpretation that are not present in pure longitudinal data. For the present purposes, regrettably, the measurements from individuals were not given in Acheson's work; only the means at each age being published. Similar measurements given in the older literature have not been re-analysed since the diets used in many early studies have been criticised as being nutritionally inadequate (Zucker *et al.*, 1941b). Zucker and his associates showed that the body weight-age curves from much early data did not conform to the log-reciprocal equation; it is probable that the body length-age curves, and perhaps even the allometric relationships would also be affected. So the data collected by Acheson and his associates was used to investigate several topics, germane to the present work, which have either been poorly researched or have completely escaped investigation.

Although live weight is the most frequently used index of body size concurrently taken measurements of linear dimensions not only give additional information but in some cases may provide a superior index of body size (Tanner, 1976). To quote Acheson *et al.* (1959, p292),

'It is urged that free use of total length....would add much to the value of nutritional, endocrinological and other experiments in which growth is studied. To date, weight increment has too frequently been the only measure of growth.'

Eleven years later, despite the huge volume of recent literature concerning live weight changes in the rat (see Wade, 1976), Hughes and Tanner (1970, p349) were forced to say that 'Previous studies on body dimensions of rats are not numerous'.

Many attempts have been made to fit equations to live weight-age growth curves, but the relationships of linear dimensions to time have not been as closely scrutinised. Because of the constant relationship between an individual rat's weights and lengths, said to pertain from weaning (Freudenberger, 1932), one would expect body weights and lengths to show relationships of the same form, with respect to age. This was recognised and theoretically analysed by Laird *et al* (1968). In the rat the reciprocal of age and the logarithm of live weight follow a linear relationship after 4 weeks of age (Chapters 1 and 6). The logarithm of live weight and the logarithm of conception age were shown by Zucker and his associates to be linearly related from birth until 6 weeks of age; this finding has not, apparently, been subsequently confirmed. Thus plotting the logarithm of length against the reciprocal of birth age, from 4 weeks onwards; and the logarithm of length against the logarithm of conception age, from birth to 6 weeks of age, should give the linear relationships found for body weights. These relationships are of great importance if, as suggested in 1959 by Acheson and his colleagues, linear measurements are to be used in experimentation for the routine measurement of body growth. Such linear relationships bestow many advantages during the analysis and interpretation of data, as was shown in Chapter 1 for body weight.

Allometry was the name given by Julian Huxley (1932) to a method of studying the relative growth of body parts by plotting the logarithms of body dimensions against one another. The technique, and especially its statistical validity, will be examined in Chapter 8. Despite their frequent measurement of body weights and lengths Acheson *et al*.(1959) and, for example, Hughes and Tanner(1970), did not make use of allometry to study changes in body shape with age. Of great interest in the present re-analysis was whether sex differences in body weights, body lengths, and allometric relationships were present, and if so, at what age they became established.

As Acheson and his associates made daily measurements from 16 to 40 days following birth their measurements were especially valuable for studying the serial correlations between the residuals about the fitted lines, using the methods developed in Chapter 6. The daily measurements allowed short-term fluctuations or rhythms to be detected; obviously this was not possible using the weekly measurements of previous chapters. Such rhythms were not considered by Acheson and his colleagues and no similar studies in laboratory animals, where daily measurements have been made, are currently known to the author. Therefore the investigation of live weight data for short-term fluctuations in growth rate seems to be a new avenue of study.

To supplement published data a simple experiment was devised where male and female rats were frequently weighed, from birth to 50 days of age.

To summarise: the aims of the study were to investigate the relationships between,

1) the logarithm of live weight and the reciprocal of age,
2) the logarithms of age, weight and the linear dimensions,
3) the logarithms of lengths and weights (allometry),
4) the pattern of the residuals about these linear regressions,

and 5) study the sex differences in these relationships.

EXPERIMENTAL PROCEDURES

The following is a brief summary of the materials and methods used by Acheson *et al.*, (1959) in their original paper. Ten male and thirteen female Sprague Dawley rats were housed in groups of not more than three per cage at 69^{o}F (20^{o}C) and fed a commercial rat diet. The linear measurements (in centimetres) and body weights (in grams) were obtained daily from the conscious animals from 16 to 40 and at 50, 60, 70, 90, and 110 days from birth; giving 30 measurements for each variable from each animal. Lengths were measured using a measuring board developed by the authors. Means and standard deviations for tail lengths and total lengths (body plus tail)

were obtained directly and appear in the tables published by Acheson *et al.*, (1959). Body lengths were not directly measured by Acheson *et al.* (1959) but were obtained for the present analyses by subtracting the mean values for tail length from those for total length.

The male and female rats for the experiment at Massey were raised, housed, and fed in a way comparable to the animals described in Chapter 1. Males and females were housed in separate cages. The experiment (initiated by Dr M.F. Tarttelin) used animals subjected to three treatments; the offspring of female rats that had either been fed a ration containing testosterone propionate during late pregnancy (days 15 -21) or had been injected with the androgen at the same time, and the offspring of untreated control females. For the daily weighings (from 30 to 50 days of age) weighings were planned to be at the same time (12 noon) on each day but the weighing on one day, at about day 36 in most rats, was made early in the morning (9 am). This weighing gave weights that were consistently several grams greater than expected so these weights were excluded from the following analyses. The animals were weighed to the nearest tenth of a gram, it is usual practice in most laboratories to weigh rats to the nearest gram.

BIOMETRICAL CONSIDERATIONS

The transformation of live weight to logarithms has been shown in this work to be desirable for both statistical and theoretical reasons. A case can be made for linear dimensions of the body being analysed without transformation as it can be argued that they are proportional to the cube root (approximated by the logarithm) of volume (weight). This argument can be extended by suggesting that an increase in weight is due to a multiplicative effect (to be analysed logarithmically) but a change in length is due to an additive effect (to be analysed arithmetically). The author held these views until they were made untenable by the experimental evidence of Hughes and Tanner (1970) and Acheson *et al.* (1959) shown in Table 7.1.

TABLE 7.1

Means and standard deviations for various linear dimensions in rats

SOURCE	VARIABLE	MEAN ± STANDARD DEVIATION
Hughes & Tanner	Tail length (birth)	20 mm ± 2
" "	" " (190 days)	200 mm ± 20
" "	Nose-rump length (birth)	50 mm ± 2.5
" "	" " " (90 days)	220 mm ± 9
Acheson *et al*	Total length (16 days)	170 mm ± 6.5
" "	" " (110 days)	440 mm ± 16

Table 7.1. indicates that the standard deviation increases progressively with the mean; a logarithmic transformation is therefore indicated.

These results therefore forced a reconsideration of the biologically based arguments presented which would theoretically discount such a transformation. It was suggested that weight increases as a simple cubic function of length. This will be questioned later in this chapter as plots of log body length against log body weight changed slope continuously. Therefore length is not necessarily normally distributed on an arithmetic metameter. The second argument, that length increase is an additive process, can also be questioned as the rate of increase in length at an age is obviously not independent of the length at that age.

Justification for the transformation of lengths to logarithms could be based solely on the empirical grounds that a linear relationship with age or weight results. This justification is affirmed in this chapter as such linear relationships will be demonstrated.

Means and standard deviations for body lengths may not always be correlated simply because the spread of the data is insufficient. For example, over a similar range of ages a significant relationship may be obtained between the arithmetic means and standard deviations for live

TABLE 7.2

Percentages of the variation (R^2) explained by log conception age, the reciprocal of birth age, and by allometry, for the periods shown, for body weight (body wt), total length, and for tail length.

			X = AGE			ALLOMETRY	
AGE	EQUATION	SEX	BODY WT	TOTAL LENGTH	TAIL LENGTH	BODY WT vs TOTAL LENGTH	BODY WT vs TAIL LENGTH
16 to 110 days	LOG vs LOG	F	87.2	94.1	91.2	99.6	98.2
		M	92.6	95.2	94.5	99.0	98.9
	LOG vs RECIP.	F	96.6	93.7	98.6	-	-
		M	96.1	93.6	96.4	-	-
16 to 38 days	LOG vs LOG	F	98.4	99.7	99.0	98.3	96.4
		M	99.4	99.8	99.3	99.4	98.0

F = FEMALE RATS

M = MALE RATS

DERIVED FROM THE DATA OF ACHESON ET AL. (1959)

weights, but not for the lengths of the same animals, because the live weights have a greater range. This effect may be present in the results in Chapter 8 for weights and lengths.

RESULTS AND DISCUSSION

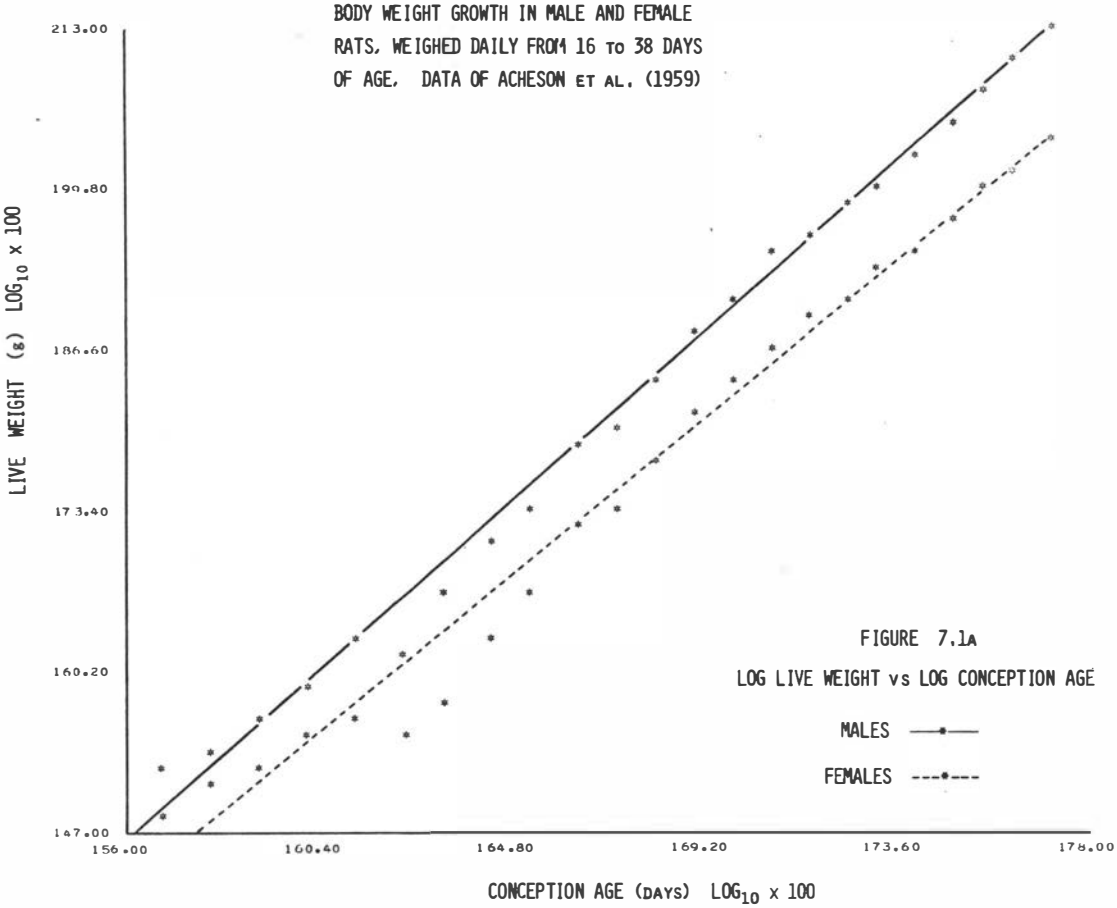
A fundamental difference between the two data sets of this chapter is that measurements from individuals were analysed in the author's study while Acheson and his colleagues published only mean measurements at each age. Thus the curves fitted for the linear measurements will be the mass curves.

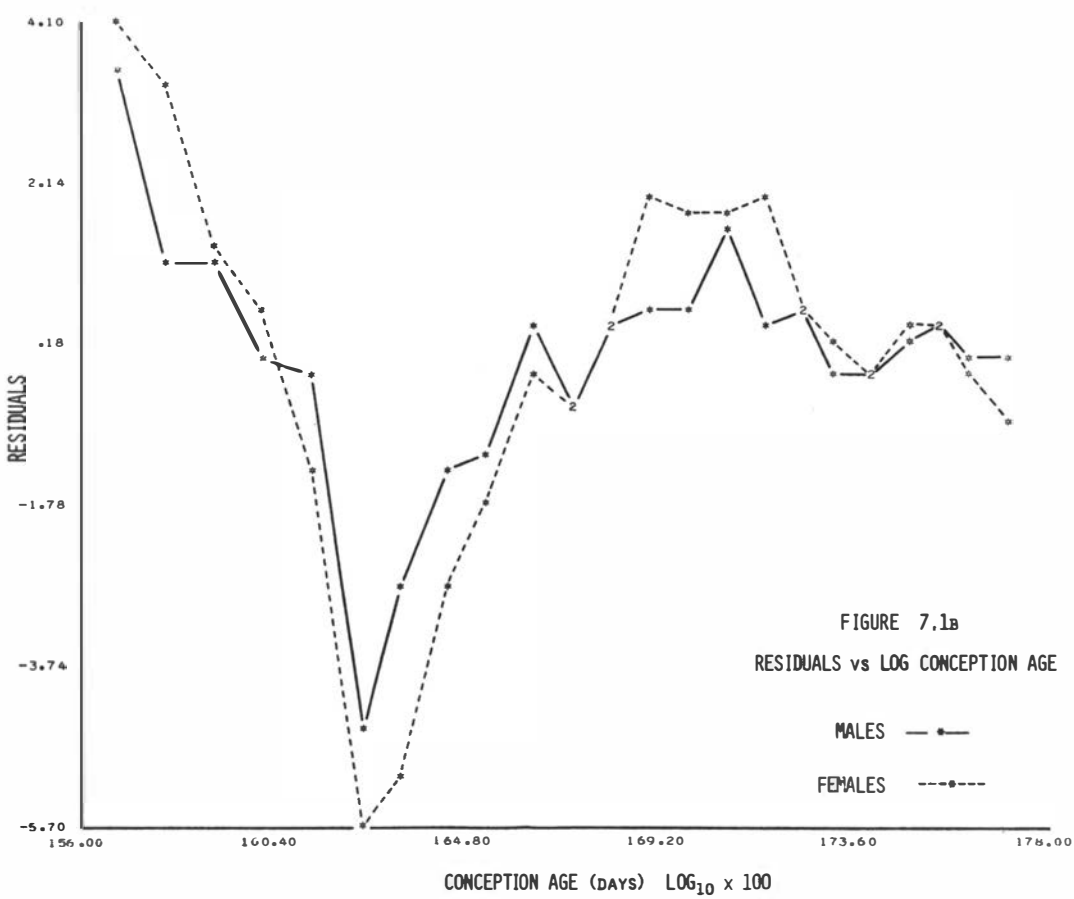
RELATIONSHIPS TO AGE

Each variable was initially transformed to logarithms and regressed against log conception age, (birth age + 21 days) then against the reciprocal of birth age, for the entire range of measurements (16 to 110 days). The result for live weight confirmed the finding of Zucker *et al.*, (1941a); more than one equation was needed to adequately describe the changes in live weight over this range of ages as the fits for both equations were generally poor (Table 7.2). Linear dimensions were also found to show poor relationships to time over this range of ages. The log-reciprocal equation provided the superior explanation of the complete range of data, especially for females where the reciprocal of age explained 98.6% of the variation in the logarithm of tail length. In contrast to the regressions against time the explanation of body weight by total length and by tail length was excellent, especially in males (Table 7.2). Thus over the complete range of ages lengths and weights were more closely related to each other than to age. This result seems relevant to a finding of Chapter Six where the pattern of growth (the residuals around log-reciprocal lines) was shown to be more closely related to the physiological age (represented by the log of live weight) than to the chronological age of the animal. In the present case length (a measure of physiological age) explains a greater proportion of the variation in

FIGURES 7.1A & 7.1B

BODY WEIGHT GROWTH IN MALE AND FEMALE RATS, WEIGHED DAILY FROM 16 TO 38 DAYS OF AGE. DATA OF ACHESON ET AL. (1959)





live weight than does chronological age.

As the Zuckers recommended that the log-log equation should be fitted up to 42 days of age, and Acheson and his co-workers give daily weighings to 40 days, it was convenient to test the log-log equation over this range of ages. But the measurements at 39 and 40 days of age were found to be well above the calculated line, especially for male and female total lengths. For example; in the regression of male total length against the logarithm of age the expected total length at 40 days was 145.804 while the observed value of 147.567 gives a residual of 1.763, which, when compared to the RMS residual (0.512), shows the point to be a possible outlier. As it was proposed to regress all pairs of variables it was simpler to have equal numbers in all regressions, thus the day 39 and day 40 measurements were dropped from all the regressions, whether or not they were outliers, with respect to age, in their particular case.

So for the measurements made from 16 to 38 days of age variables were regressed against the logarithms of conception age, and for allometry against one another. The regression statistics are given in pairs for females (a) and for males (b) in Appendix Tables 7.1 and 7.7.

LOG LIVE WEIGHT vs LOG CONCEPTION AGE

Appendix Table 7.1 and Text Figure 7.1a confirm, apparently for the first time since the original publication of Zucker *et al.*, (1941a), that the log of live weight is linearly related to the log of conception age, up to about 6 weeks of age, in both male and female rats. But although the multiple correlation coefficients (R^2) show that in females 98.4%, and in males 99.4%, of the variation in live weight is explained by log conception age the Durbin Watson statistics indicate that the residuals are strongly correlated (Appendix Tables 7.1a and 7.1b). The plotted residuals clearly indicate the presence of systematic departures from linearity (Fig 7.1b).

In the Massey experiment 35 live weights were obtained from each individual affording a definitive test of the log-log equation. The untransformed growth curves for control males (Figure 7.2a), control females (Figure 7.3a) and for the female offspring of dams that were

FIGURES 7.2A & 7.2B

GROWTH CURVES OF MALE RATS WEIGHED
FROM BIRTH TO 49 DAYS OF AGE,
DATA OF TARTTELIN & CLARK,
MASSEY COLONY.

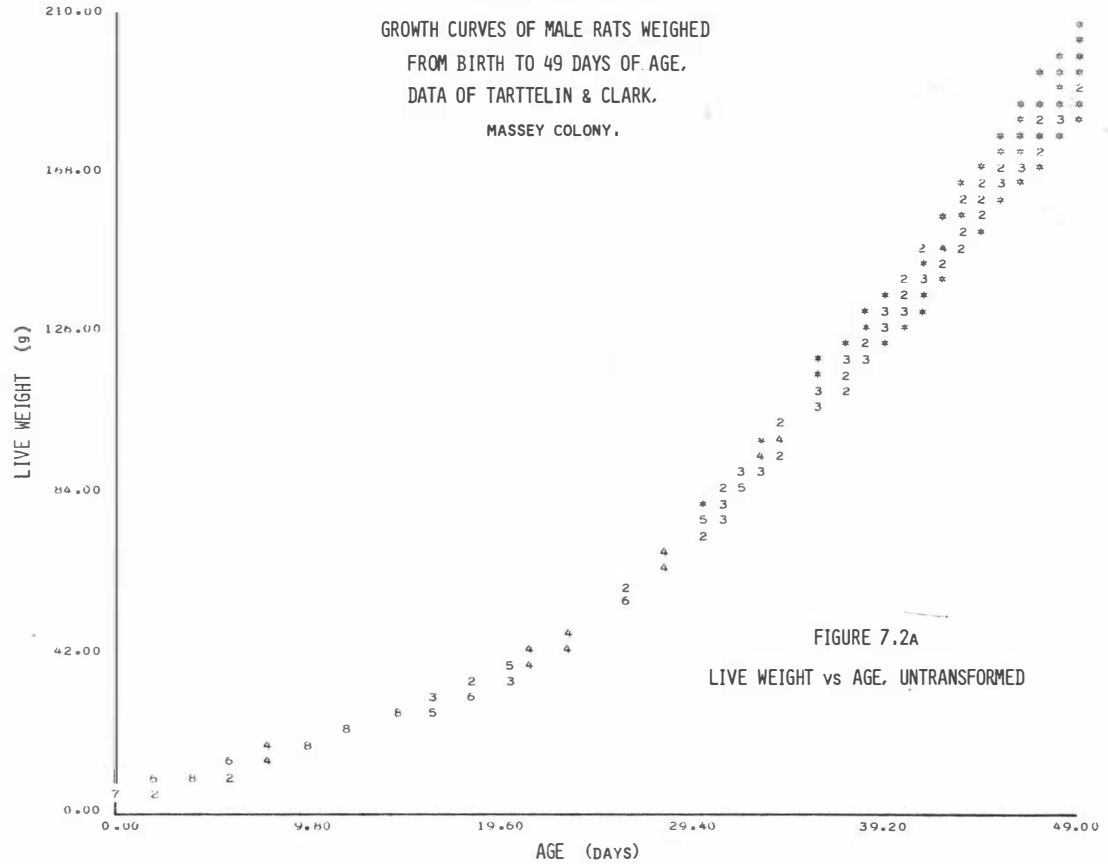


FIGURE 7.2A

LIVE WEIGHT vs AGE, UNTRANSFORMED

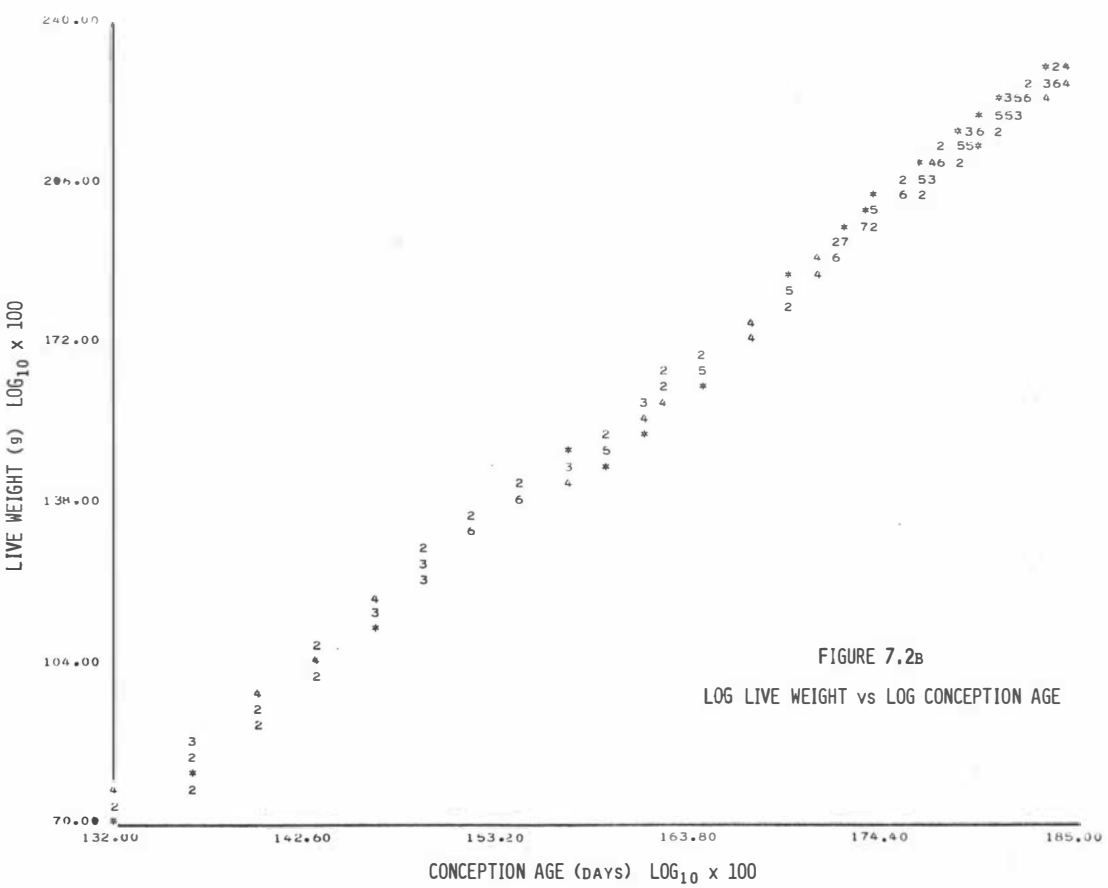


FIGURE 7.2B

LOG LIVE WEIGHT vs LOG CONCEPTION AGE

FIGURES 7.3A & 7.3B

GROWTH CURVES OF FEMALE RATS WEIGHED FROM BIRTH TO 49 DAYS OF AGE, DATA OF TARTTELIN & CLARK, MASSEY COLONY.

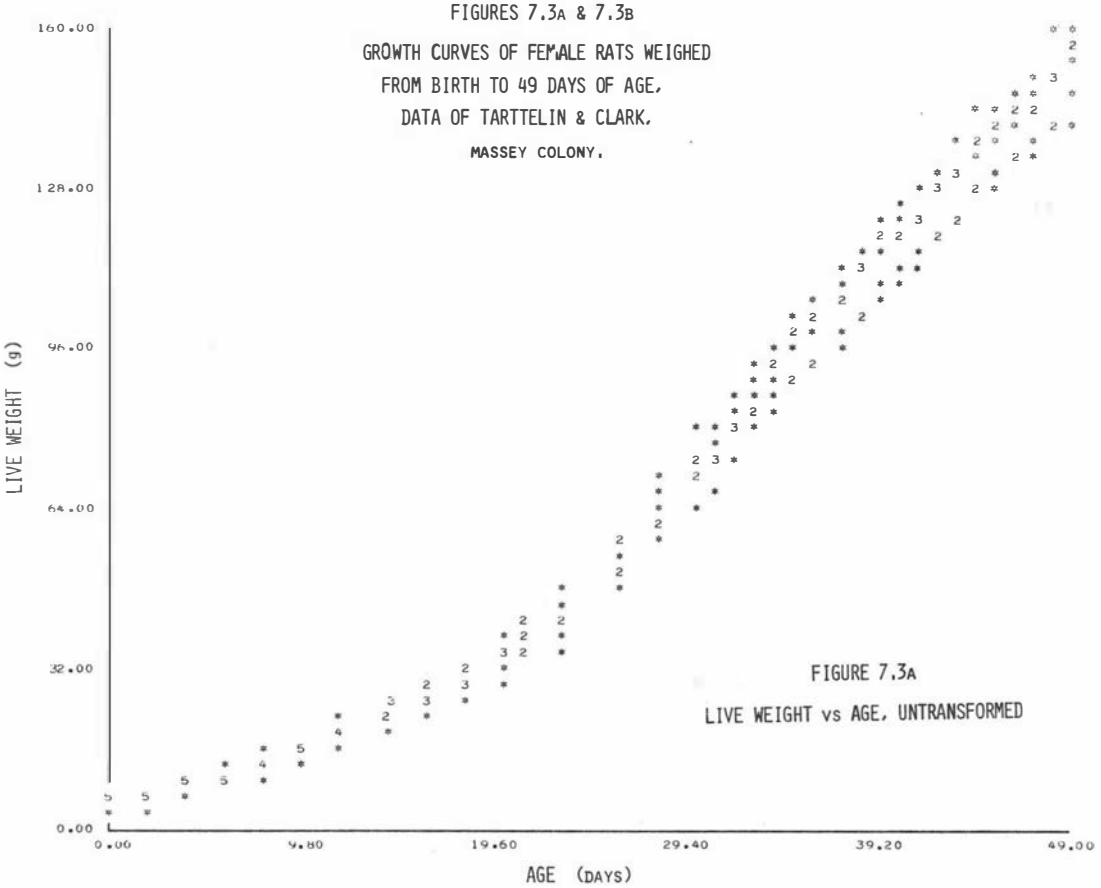


FIGURE 7.3A

LIVE WEIGHT vs AGE, UNTRANSFORMED

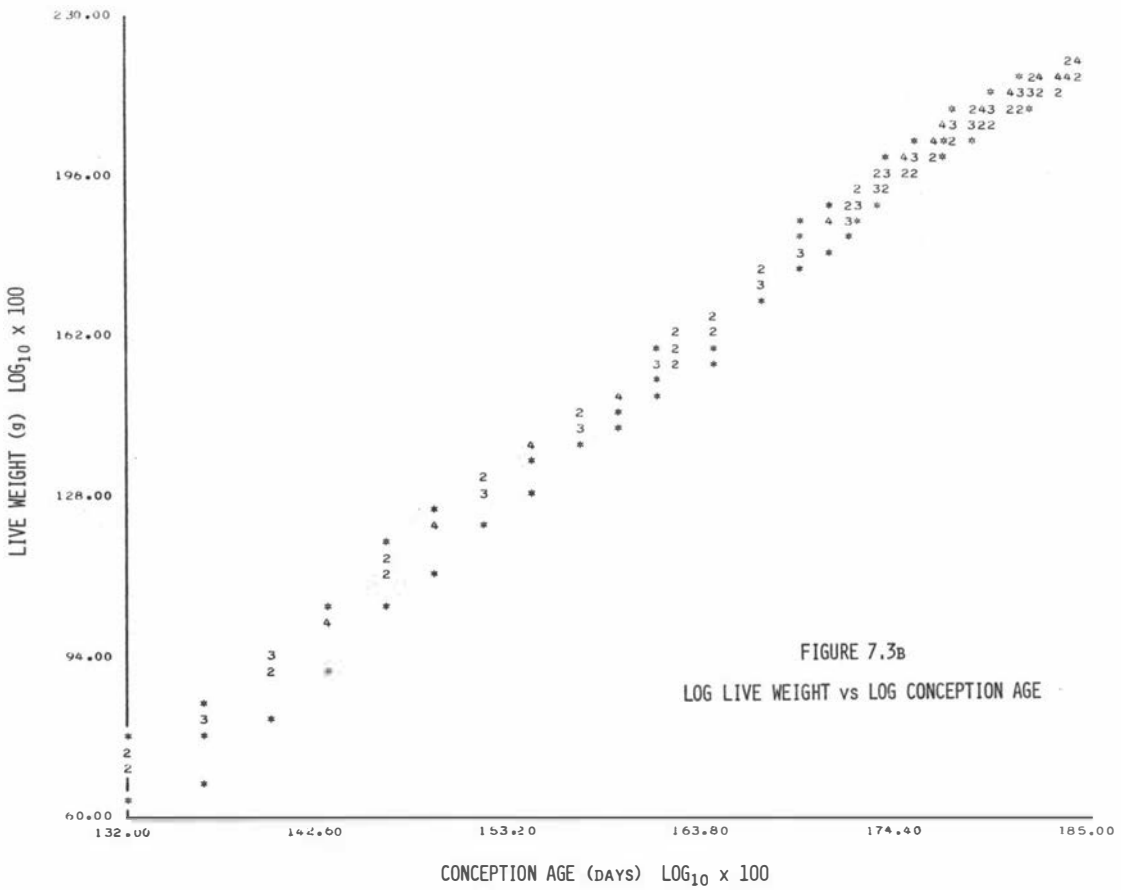


FIGURE 7.3B

LOG LIVE WEIGHT vs LOG CONCEPTION AGE

FIGURES 7.4A & 7.4B

GROWTH CURVES OF FEMALE RATS TREATED PRE-NATALLY WITH ANDROGEN AND WEIGHED FROM BIRTH TO 51 DAYS OF AGE, DATA OF TARTTELIN & CLARK, MASSEY COLONY.

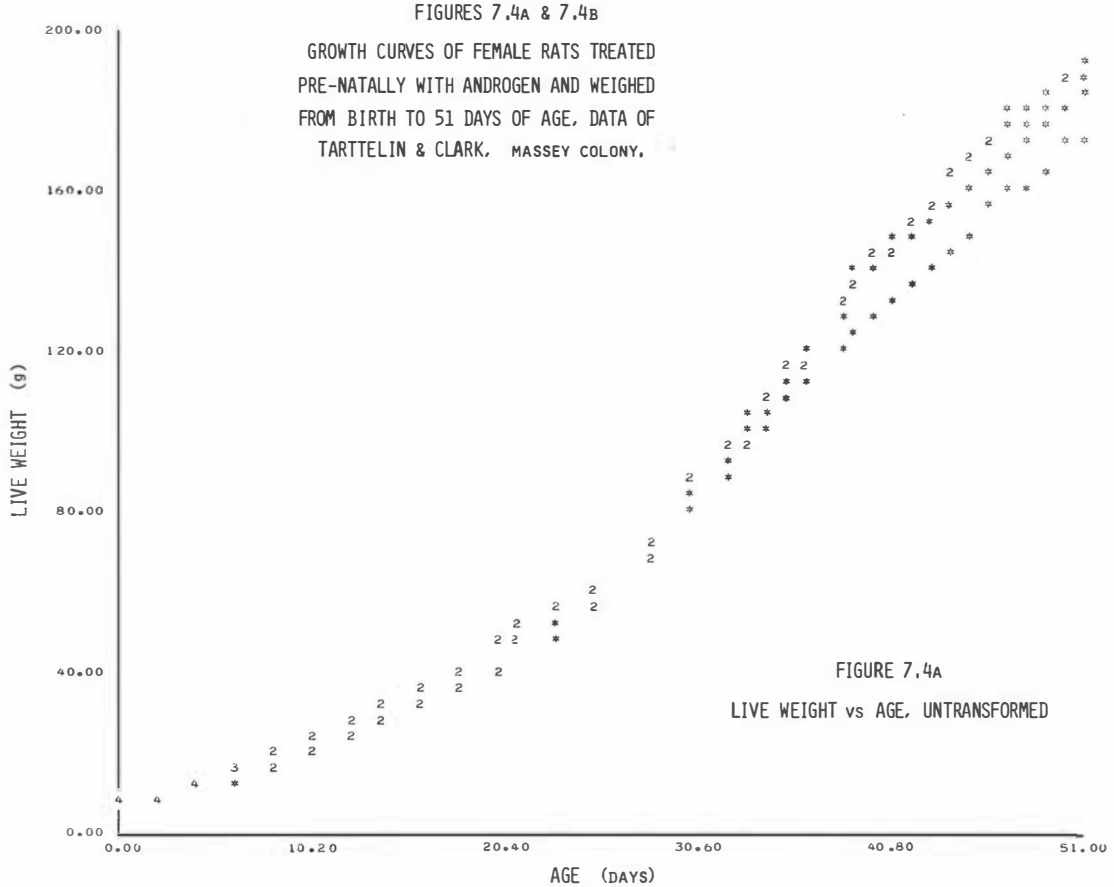


FIGURE 7.4A

LIVE WEIGHT vs AGE, UNTRANSFORMED

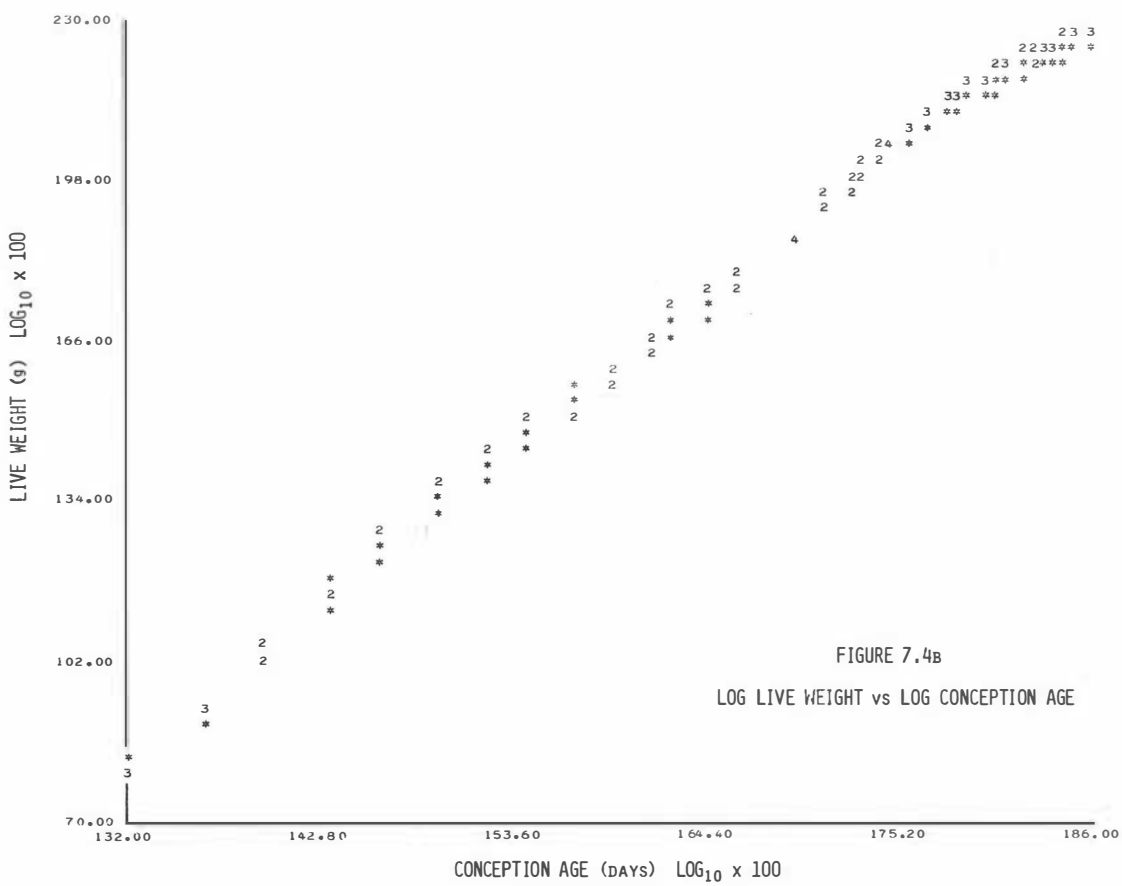


FIGURE 7.4B

LOG LIVE WEIGHT vs LOG CONCEPTION AGE

fed testosterone propionate (Figure 7.4a) appear to be of very similar shape. The animals shown in each illustration are all from the same litter. Beneath the untransformed curves are the curves obtained by transforming both conception age and live weight to logarithms (Figs. 7.2b, 7.3b, 7.4b); the curves have clearly been linearised. Table 7.3 shows the multiple correlation coefficients (R^2) for the regressions of log weight on log conception age for the animals shown in the Figures. The greatest amount of unexplained variation in the 18 animals' regressions was half a percent and the smallest 8 hundredths of a percent (in animal 46). For animal 46 the mean square ascribed to the slope of the line, by analysis of variance, yielded an 'F' value of 41212.5 when compared to the scatter about the line; despite such clear statistical grounds for assuming the relationship to be strictly linear even in animal 46 the Durbin-Watson 'd' value was 1.030 ($p < \cdot 01$; for 32 d.f.) indicating serial correlation in the residuals. The cause of the significant Durbin-Watson statistics can be seen when the residuals about the log-log lines fitted to individuals are plotted,

1) vs Age
   a) for males (Figure 7.5a),
   b) for females (Figure 7.5b),
2) vs Log live weight
   a) for males (Figure 7.6a),
   b) for females (Figure 7.6b),

and 3) for pre-natally androgenised females
   a) vs log live weight (Figure 7.7a),
and b) vs age (Figure 7.7b).

The pattern revealed in these figures clearly shows the complexity of the early growth phase in the rat. What appears from the untransformed and the transformed growth curves to be a simple logarithmic or exponential increase in live weight is shown by examining the residuals about the general trend to involve a systematic wave-like oscillation in the growth rate.

In both sexes the birth weights are above the fitted line, growth then falters for a few days, presumably due to the neonate adapting to its new environment. A surge of growth then follows but, curiously,

TABLE 7.3

MULTIPLE CORRELATION COEFFICIENTS:

from the linear regressions of log live weight on log conception age for individual rats kept at Massey and weighed from birth to about 50 days of age

MALES		FEMALES		FEMALES FED TP	
RAT NO.	R^2	RAT NO.	R^2	RAT NO.	R^2
40	.9992	48	.9967	29	.9974
41	.9973	49	.9967	30	.9967
42	.9976	50	.9970	31	.9950
43	.9985	51	.9970	32	.9959
44	.9986	52	.9966		
45	.9975	53	.9965		
46	.9992				
47	.9980				

FIGURES 7.5A & 7.5B

RESIDUALS ABOUT THE LOG-LOG LINES
FITTED TO INDIVIDUAL RATS vs AGE,
DATA OF TARTTELIN & CLARK,
MASSEY COLONY.

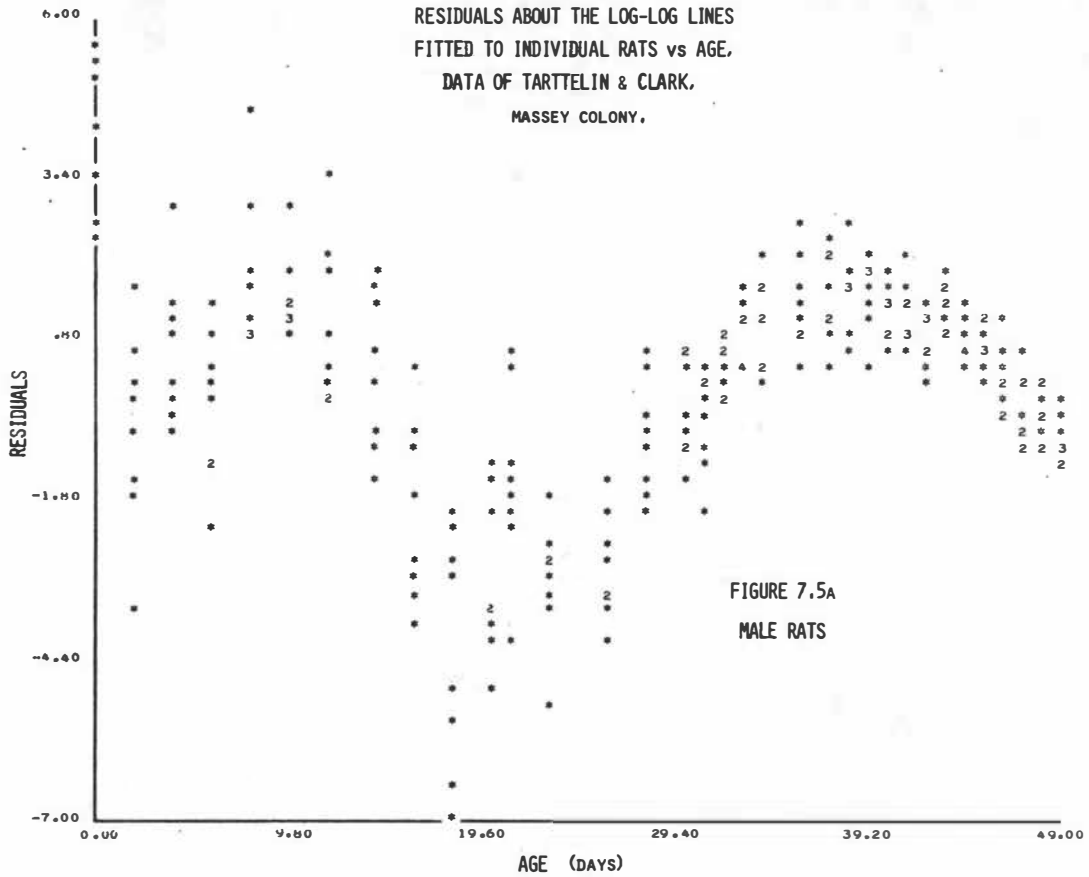


FIGURE 7.5A
MALE RATS

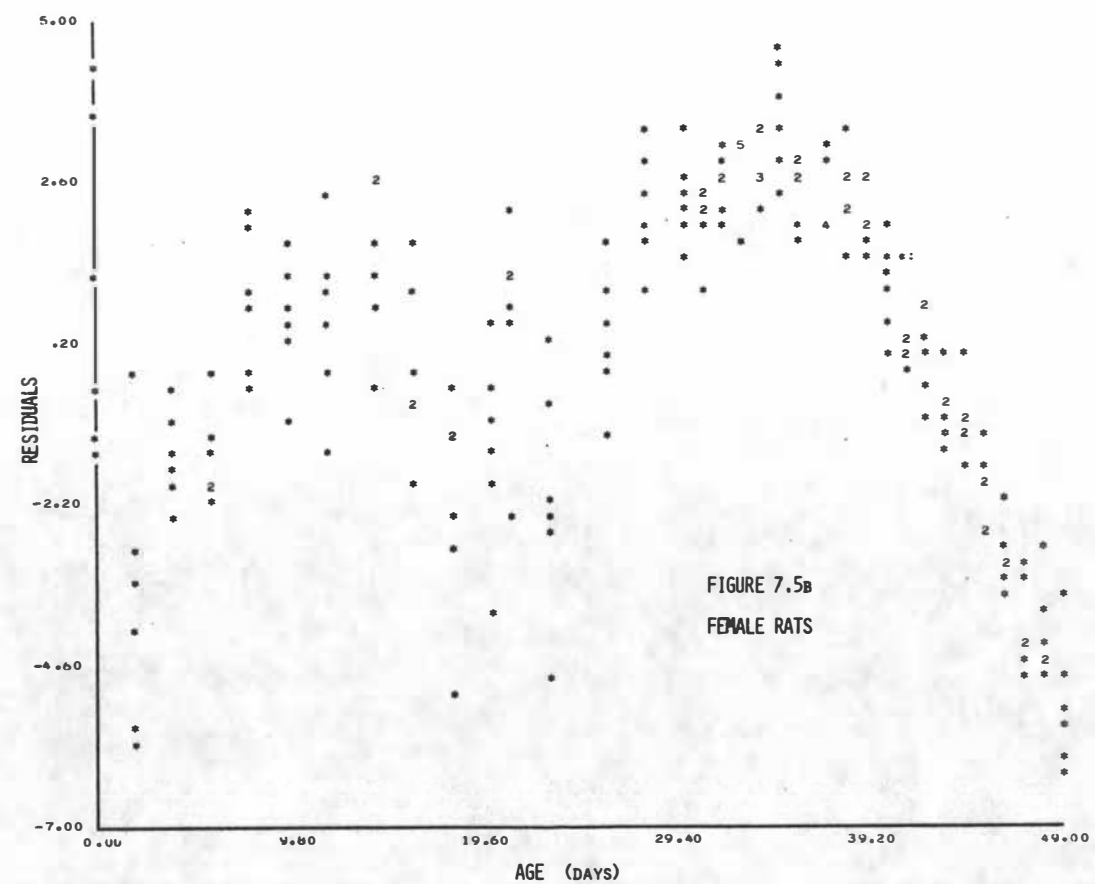


FIGURE 7.5B
FEMALE RATS

FIGURES 7.6A & 7.6B

RESIDUALS ABOUT THE LOG-LOG LINES FITTED TO INDIVIDUAL RATS, vs LOG LIVE WEIGHT, DATA OF TARTTELIN & CLARK, MASSEY COLONY.

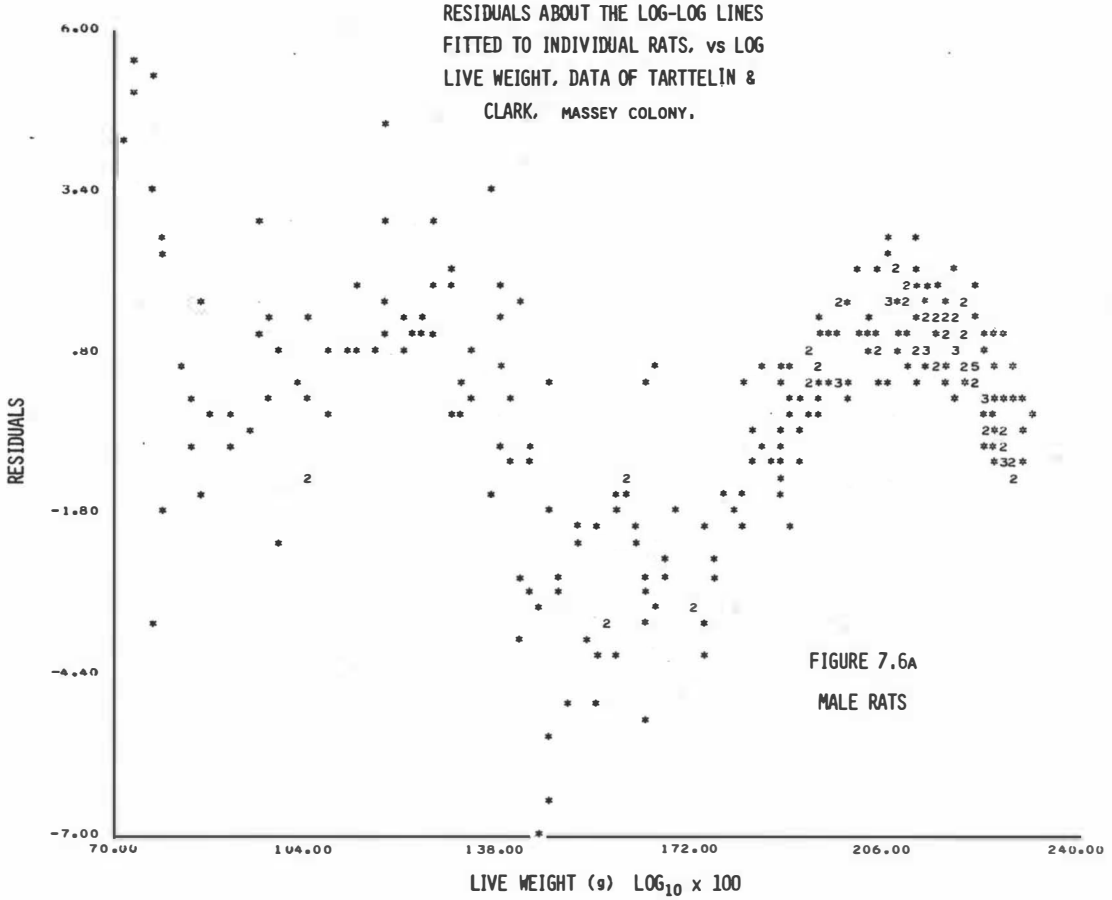


FIGURE 7.6A

MALE RATS

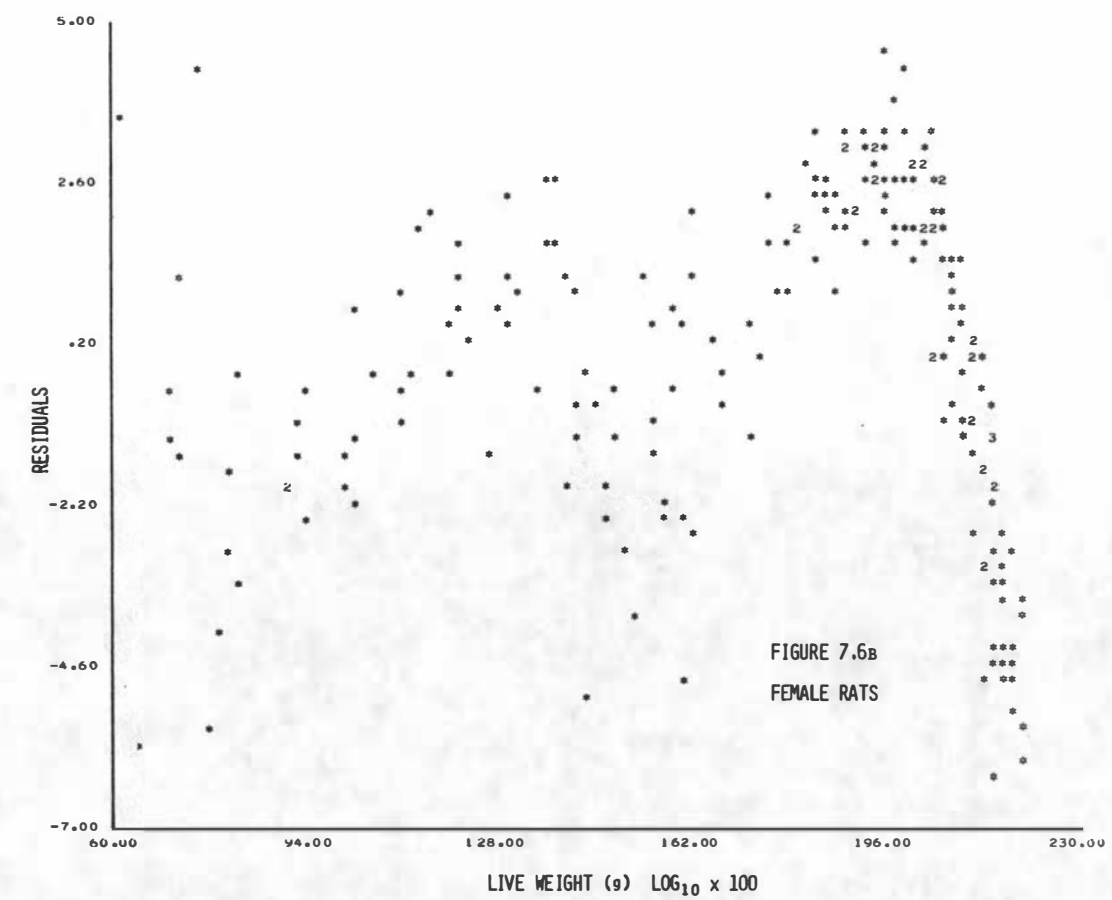


FIGURE 7.6B

FEMALE RATS

FIGURES 7.7A & 7.7B

RESIDUALS ABOUT THE LOG-LOG LINES FITTED TO INDIVIDUAL FEMALE RATS TREATED PRE-NATALLY WITH ANDROGEN. DATA OF TARTTELIN & CLARK. MASSEY COLONY.

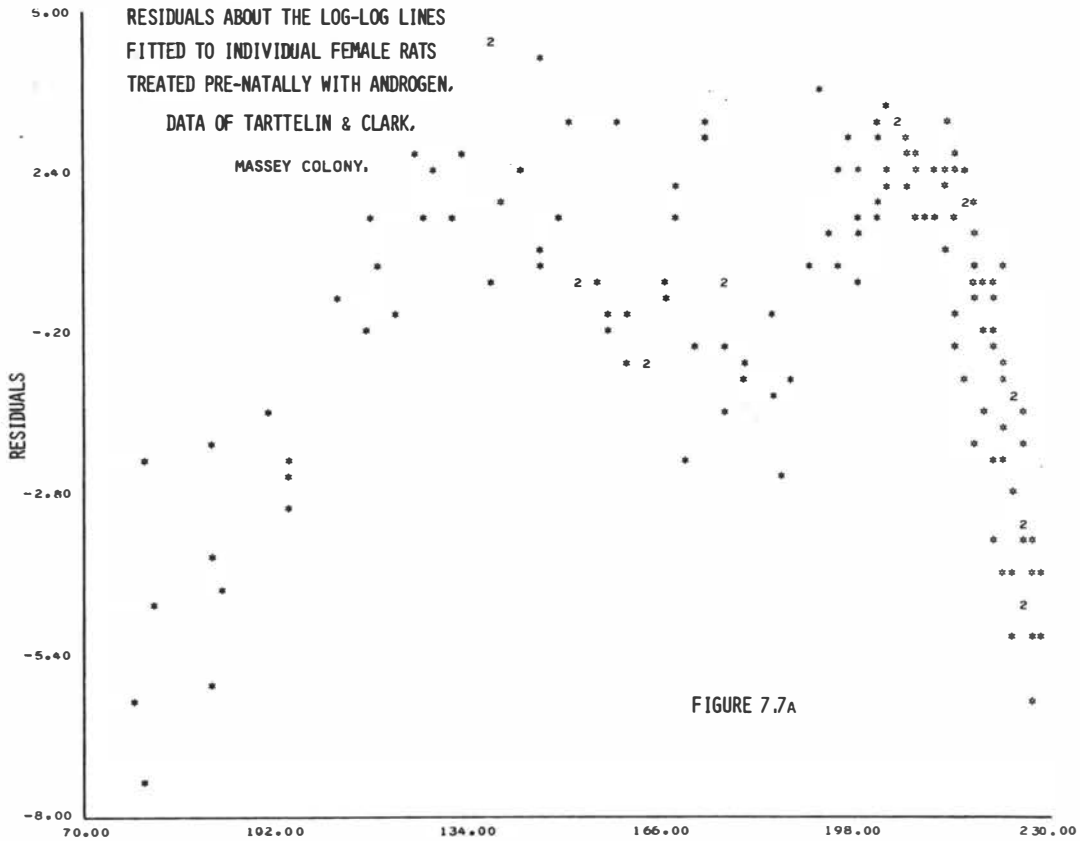


FIGURE 7.7A

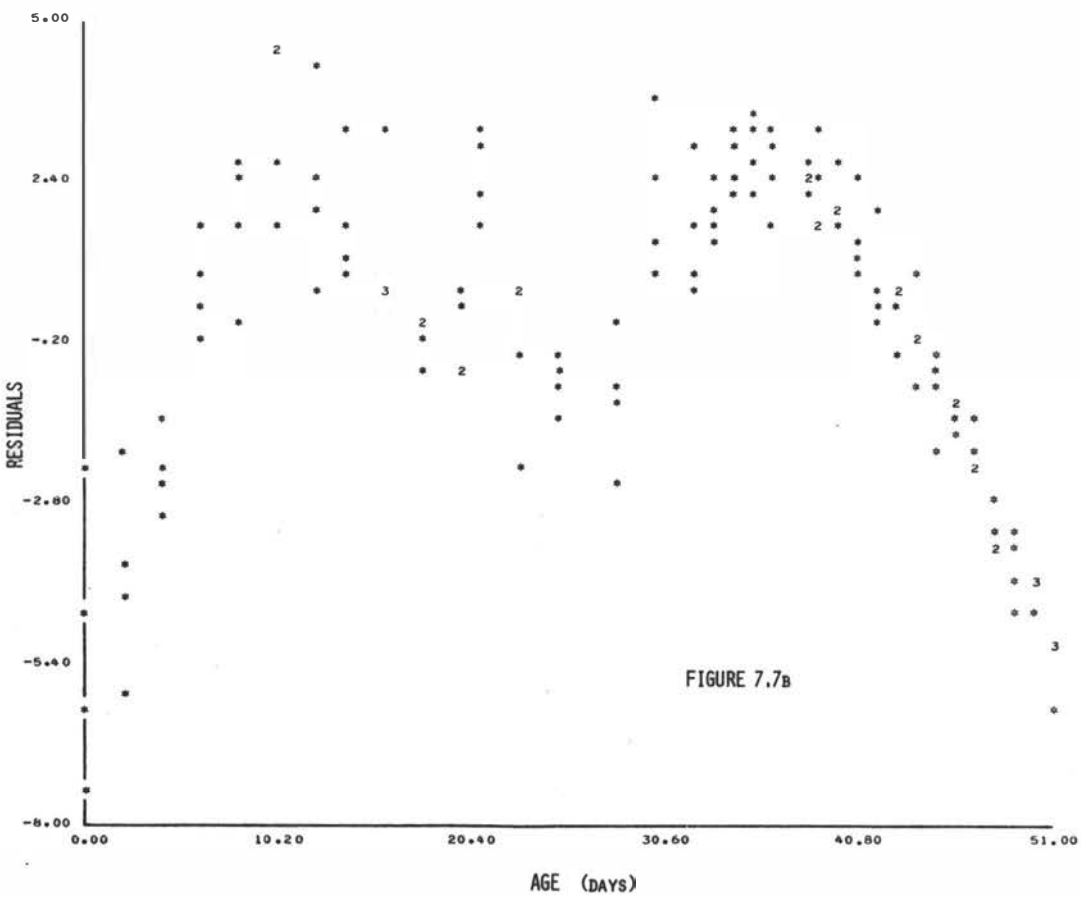


FIGURE 7.7B

this surge is halted at about 8 to 12 days of age when the rate of gain again slows. This downward trend continues until about weaning when a new surge of growth occurs which proceeds until about 35 to 40 days when another diminution in the rate of gain is evident. It is obviously tempting to relate these swings in the residuals to the onset and decline of lactation, the development of homeothermic mechanisms, the change of diet at weaning, and the onset of hypophyseal control of hypertrophic growth following the hyperplastic phase of growth. But since the present results appear to be novel, and thus unconfirmed, and based upon few animals, these relationships have not been investigated in detail. The strain difference in the pattern of growth shown in Chapter 6, albeit for a later phase of growth, also warns against over-generalisations based upon a small sample from only one strain of the laboratory rat. These effects are considered in more depth in Chapter 9.

Untreated females (of Figures 7.5b and 7.6b) and pre-natally androgenised females (Figures 7.7a and 7.7b) clearly show different patterns of growth. The most obvious difference being that the treated females show no early decline in growth rate. This effect could be an 'aging' effect so that the early initial downward trend in the growth rate is absent in the treated animals. Another possibility is that androgen interferred with the normal hormonal balance of pregnancy giving a retarded birth weight. A comparison of the male and female offspring following androgen treatment may clarify this point. The initial upward phase extends to a greater weight than in controls and the following downward trend also continues for longer in the treated animals. The lack of a deleterious effect of weaning should be noted. The upward trend of the 'second wave' may be shorter in the controls while the final downward trend may start at a greater weight in the androgenised animals. The general impression is that the pattern of growth has been advanced in time (shifted to the right) in the androgenised animals. The pattern is clearly different from that of males and still closely resembles the female pattern especially with respect to the final downward trend.

FIGURES 7.8A & 7.8B

TAIL GROWTH IN MALE AND FEMALE RATS MEASURED DAILY FROM 16 TO 38 DAYS OF AGE, DATA OF ACHESON ET AL. (1959)

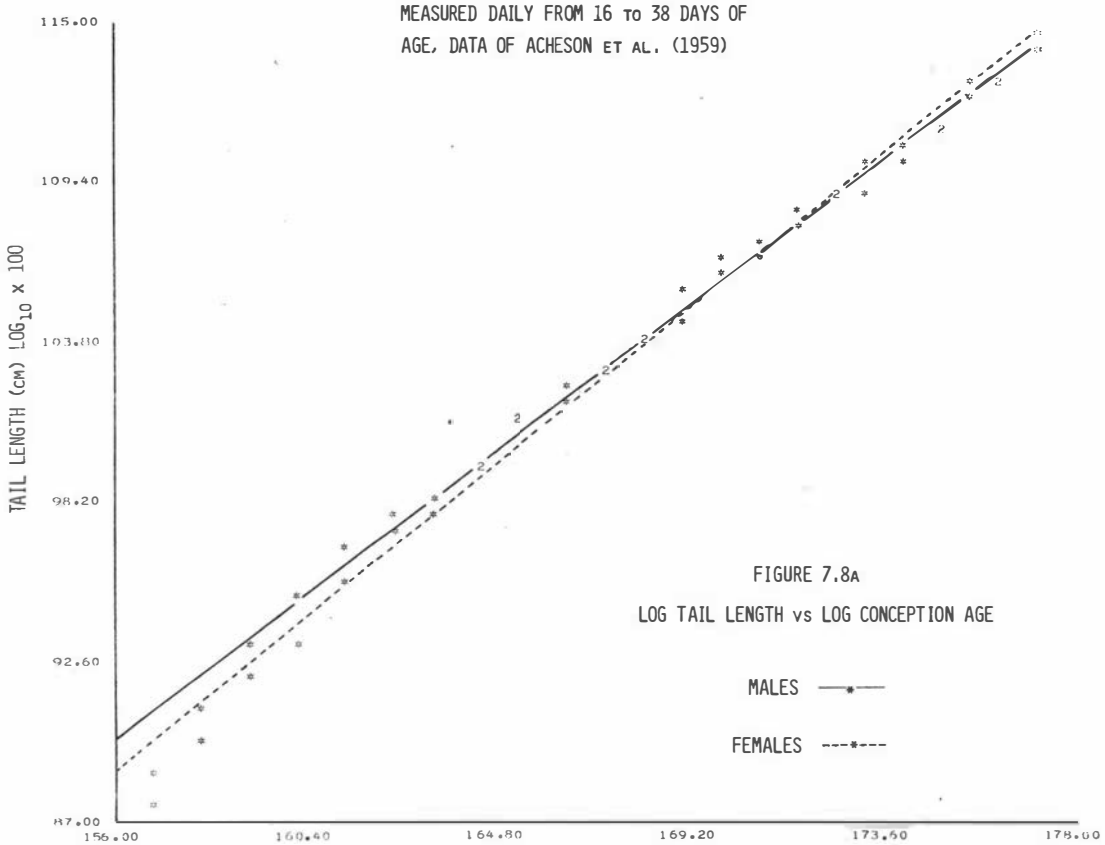


FIGURE 7.8A

LOG TAIL LENGTH vs LOG CONCEPTION AGE

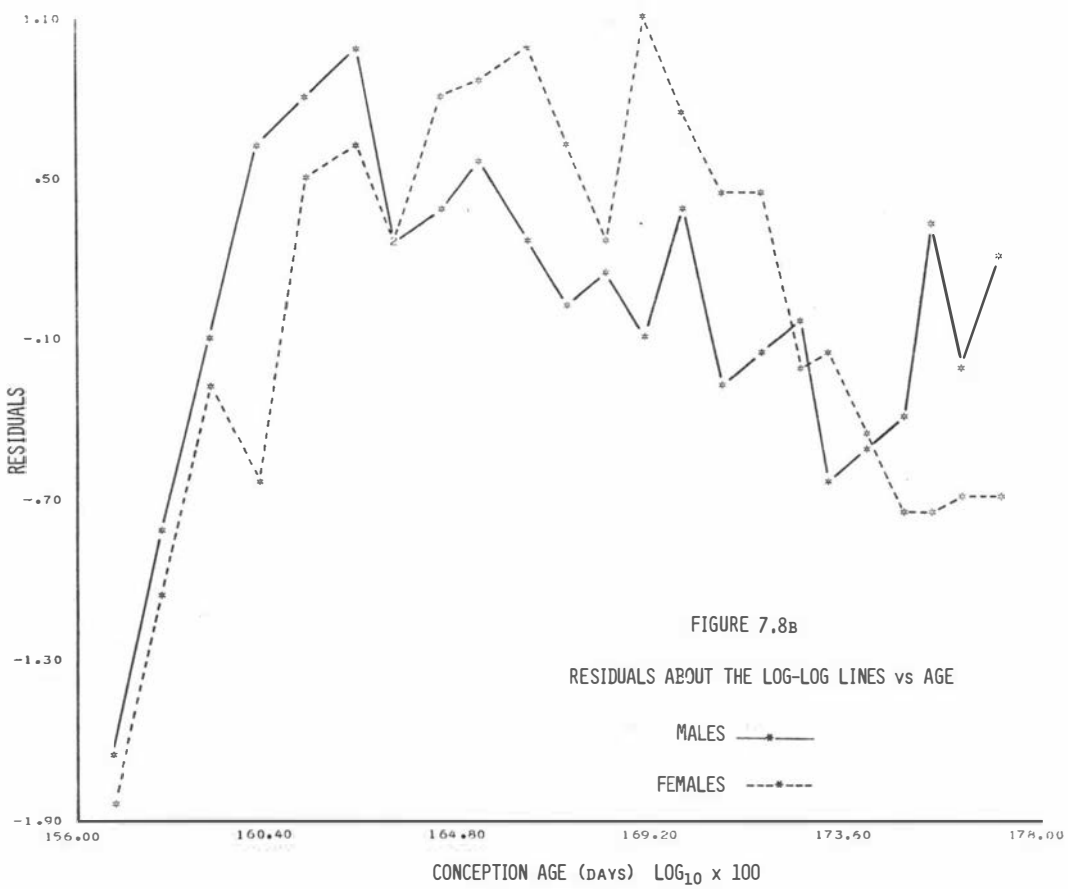


FIGURE 7.8B

RESIDUALS ABOUT THE LOG-LOG LINES vs AGE

Plotting the residuals against age (Figures 7.5a and 7.5b) gives patterns for males and females that differ. Males show an earlier peak, at about 8 days of age, compared to about 12 days in the females. But when plotted against log live weight this sex difference disappears (Figures 7.6a and 7.6b). A very similar pattern of growth, using log live weight as the X variable, can be seen for both sexes up to the last downward swing in the residuals. The final upward stroke of the residuals does, however, persist to a greater weight in the males. This implies that the logarithmic phase of growth, which was described in Chapter 6 as the period of cell hyperplasia, continues for longer in the male rat. Also the cessation of the logarithmic phase appears more abrupt in the female compared to the slow turning of the residuals displayed by the males. As discussed in Chapter 1 it may be quite wrong to ascribe these changes in growth in the female to the onset of the oestrous cycles. In the Massey colony vaginal opening occurs on average at 43 days of age while the change in the pattern of growth is initiated well before this time. And again a similar but modified pattern occurs in treated females and in males so the role of the sex hormones would seem to be to modify, rather than to create, the basic pattern of growth.

THE RELATIONSHIPS OF LINEAR DIMENSIONS TO CONCEPTION AGE

The analyses of the logarithms of total length (Table 7.2), tail length (Table 7.3), and body length (Table 7.4) regressed against log conception age for the data of Acheson *et al*. (1959) are tabulated in the Appendices. The logarithms of total length and tail length show an excellent agreement with the linear relationship demonstrated for body weight; log conception age accounting for 99.7% and 99.0% of the variation in females and 99.8% and 99.4% in males (for total and tail lengths respectively). The excellent fits for tail length are shown in Fig 7.8a. But again the remaining variation, usually less than half a per cent, shows significant serial correlation (Fig 7.8b) as demonstrated for the log- reciprocal equation in Chapter 6. Thus the log-log equation is

FIGURES 7.9A & 7.9B

THE GROWTH IN BODY LENGTH OF MALE AND FEMALE RATS MEASURED DAILY FROM 16 TO 38 DAYS OF AGE, DATA OF ACHESON ET AL (1959).

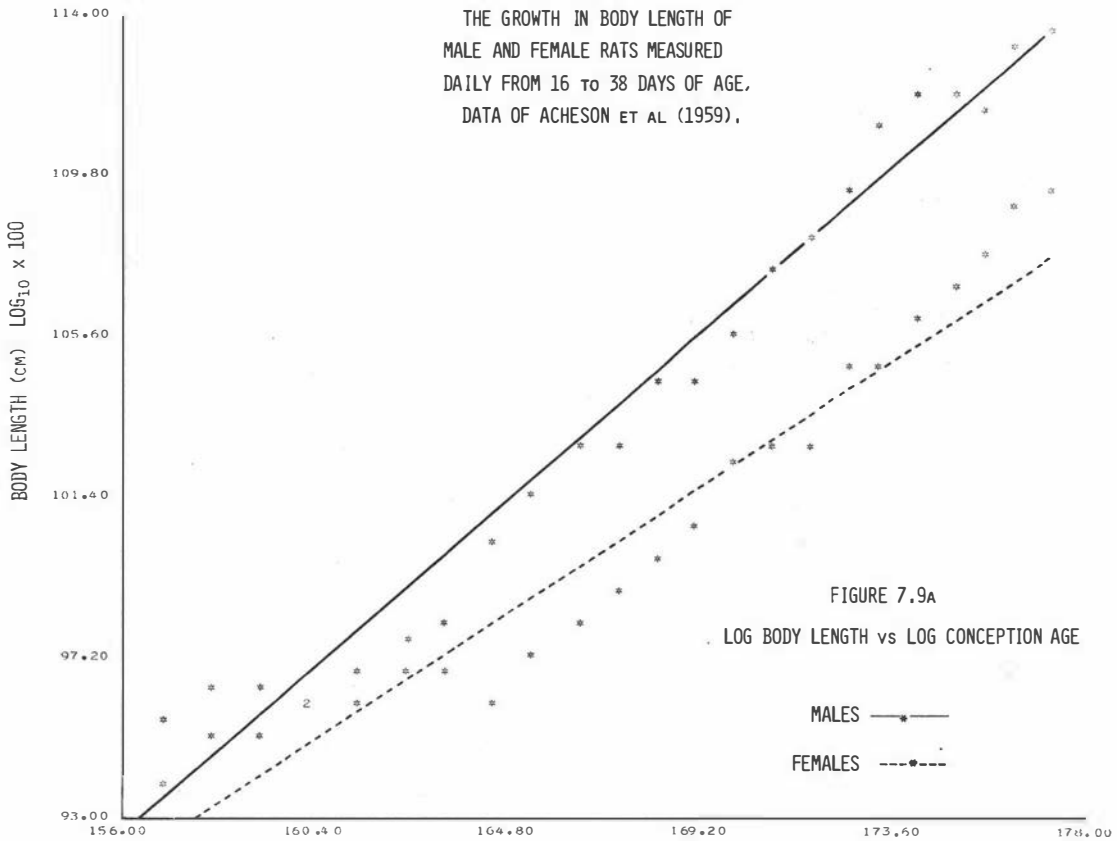


FIGURE 7.9A

LOG BODY LENGTH vs LOG CONCEPTION AGE

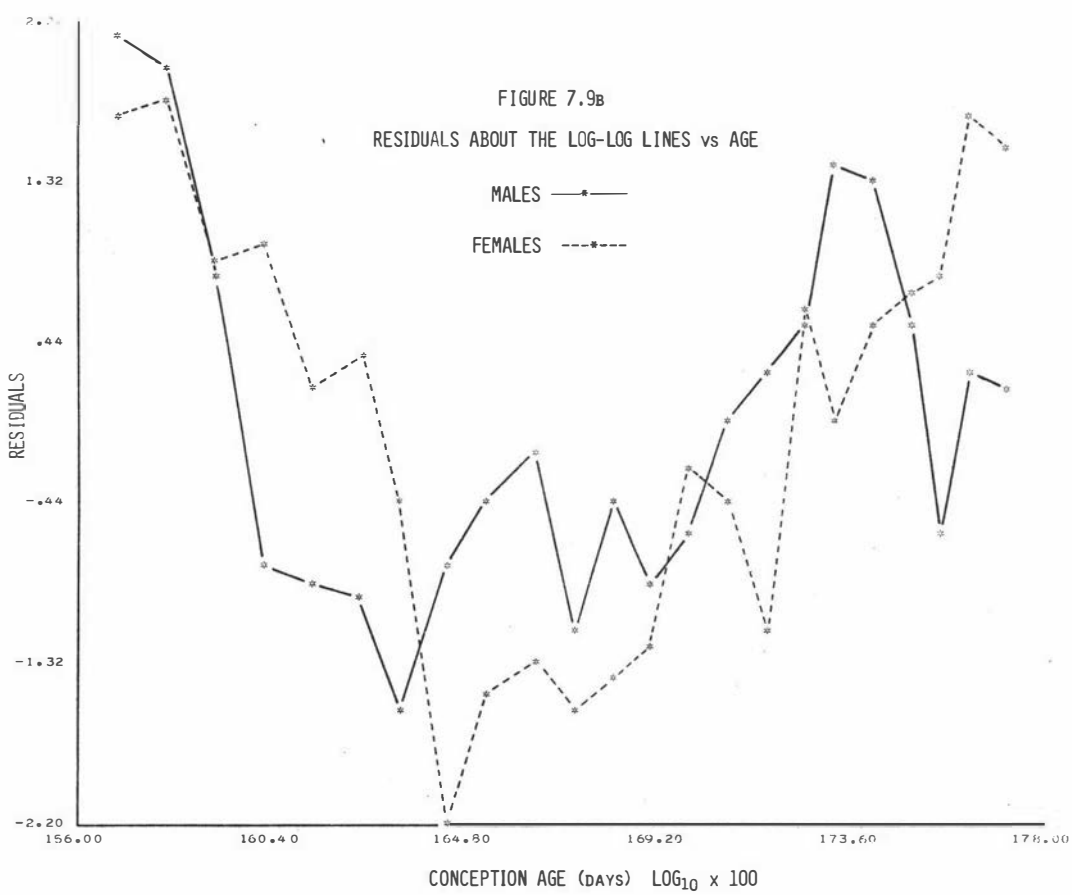


FIGURE 7.9B

RESIDUALS ABOUT THE LOG-LOG LINES vs AGE

not the definitive model of rat growth, explaining all the systematic variation in live weights, total lengths or tail lengths (c.f. Zucker *et al.*, 1941).

The derived variable, body length (nose-rump length) shows a comparatively poor relationship to log age (Appendix Table 7.4). In females, 5.9% of the variation is unexplained and in males 2.6% is unexplained by the log-log equation. That this poor fit is not entirely due to the increased random scatter about the line, resulting from combining the errors from the two parent measurements, is apparent if the residuals are inspected (Appendix Table 7.4 or Fig. 7.9b). Moreover the Durbin Watson statistics indicate that serial correlation is present, with the residuals indicating that addition of a quadratic term in log age would give a better description (Fig. 7.9a)

Figure 7.9b illustrates that the poor fit for body length is due to the pattern of body growth differing from that shown by the tail. An inspection and comparison of Figs. 7.1b, 7.8b and 7.9b reveals that the residuals for body length and body weight follow a pattern that differs from that shown by tail length. For example, the residuals clearly show the tail undergoes an enhanced growth before weaning while the growth of the body is, in comparison, temporarily slowing. Body weights reached a nadir immediately following weaning (at 21 days) but from the residuals it can be seen that body length reached a nadir at 22 days in males and at 23 days in females (Fig. 7.9b). In Fig. 7.8b a small effect of weaning on tail growth seems possible at 22 days. So the adverse effect of weaning on bone growth is 'cushioned' by other tissues of the body being depleted or starved of nutrient, while bone growth is continued for as long as possible in the face of the nutritional crisis. This is reminiscent of Hammond's (1944) theory of 'Priority of partition of nutrients according to metabolic rate'.

That the tail is little affected by weaning, while the growth of the more rostral vertebrae is inhibited, suggests that the organism attaches an importance to the growth of the tail at this early age. This observation could be due to the major role of the tail in thermoregulation in the rat. Abrams (1966) reviews the evidence that the rat's ability to control body temperature develops between 10 and

20 days of age. A rapid growth of the tail would therefore be desirable toward the end of this process. It would not be desirable for the tail to grow quickly at an earlier age since excess heat would then be lost while the neonate was poikilothermic.

The earlier maximum growth rate of the body length compared to the tail length is also in general accordance with the well known cephalo-caudal maturity gradient (Huxley, 1932). But the timing of the peak in tail length growth suggests that the thermoregulatory explanation of this phenomenon may also be of importance.

ALLOMETRY

Using Acheson's data the following plots were made for both male and female animals, over the entire range of ages,

1) log body weight vs log total length (Figure 7.10a),
2) log body weight vs log body length (Figure 7.10b),
3) log body weight vs log tail length (Figure 7.11a),

and 4) log body length vs log tail length (Figure 7.11b).

The changes in tail growth at weaning, with respect to age, have already been demonstrated. The allometric plots involving tail length (total length includes the tail) also show a change in slope at weaning (Figures 7.10a and 7.11a and b). In contrast the plots of log body weight against log body length show no major change at weaning (Figure 7.10b). This is due to body length and body weight showing the same pattern of growth in time as illustrated in Figures 7.1b and 7.9b where their residuals show a similar pattern of change with time. Following weaning the three plots of log body weight against the linear dimensions all show negative curvature to various degrees; the greatest being for body length, the least for tail length (especially in males). The allometric regressions between 16 and 38 days of age show a similar negative curvature (Appendices Tables 7.5, 7.6, and 7.7).

Further discussion of the allometric curves, especially emphasising the sex differences shown, follows later in this chapter.

FIGURES 7.10A & 7.10B

ALLOMETRIC RELATIONSHIPS
BETWEEN BODY LENGTHS AND WEIGHTS
FOR MALE AND FEMALE RATS,
DATA OF ACHESON ET AL. (1959).

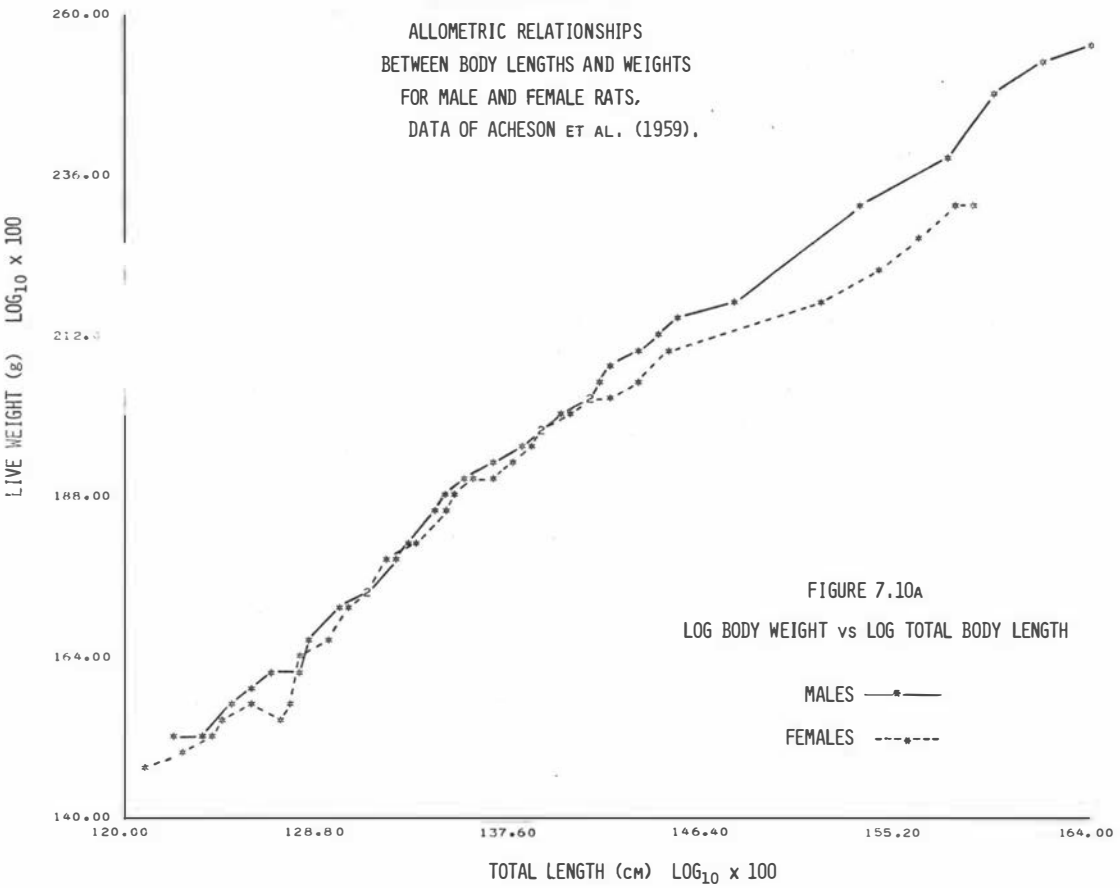


FIGURE 7.10A

LOG BODY WEIGHT vs LOG TOTAL BODY LENGTH

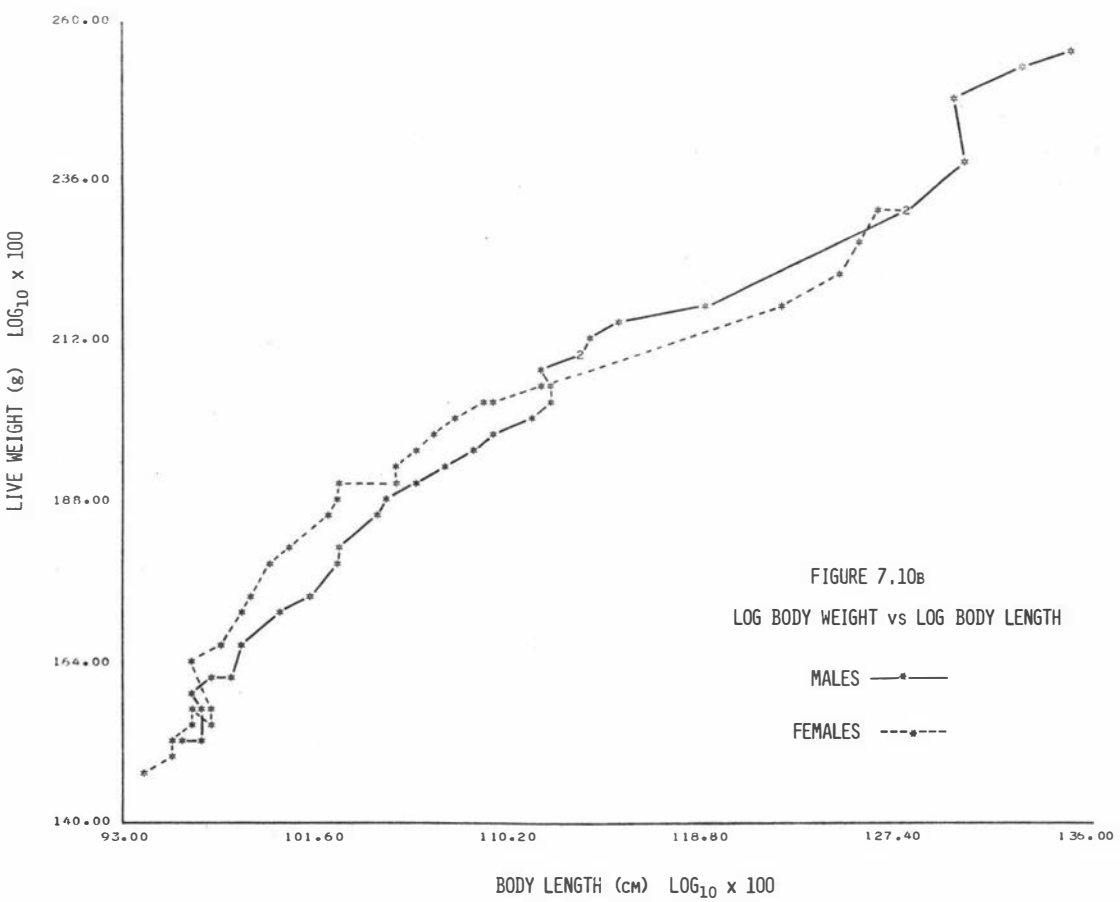


FIGURE 7.10B

LOG BODY WEIGHT vs LOG BODY LENGTH

FIGURES 7.11A & 7.11B

ALLOMETRIC RELATIONSHIPS
BETWEEN BODY LENGTHS AND WEIGHTS
FOR MALE AND FEMALE RATS.
DATA OF ACHESON ET AL. (1959).

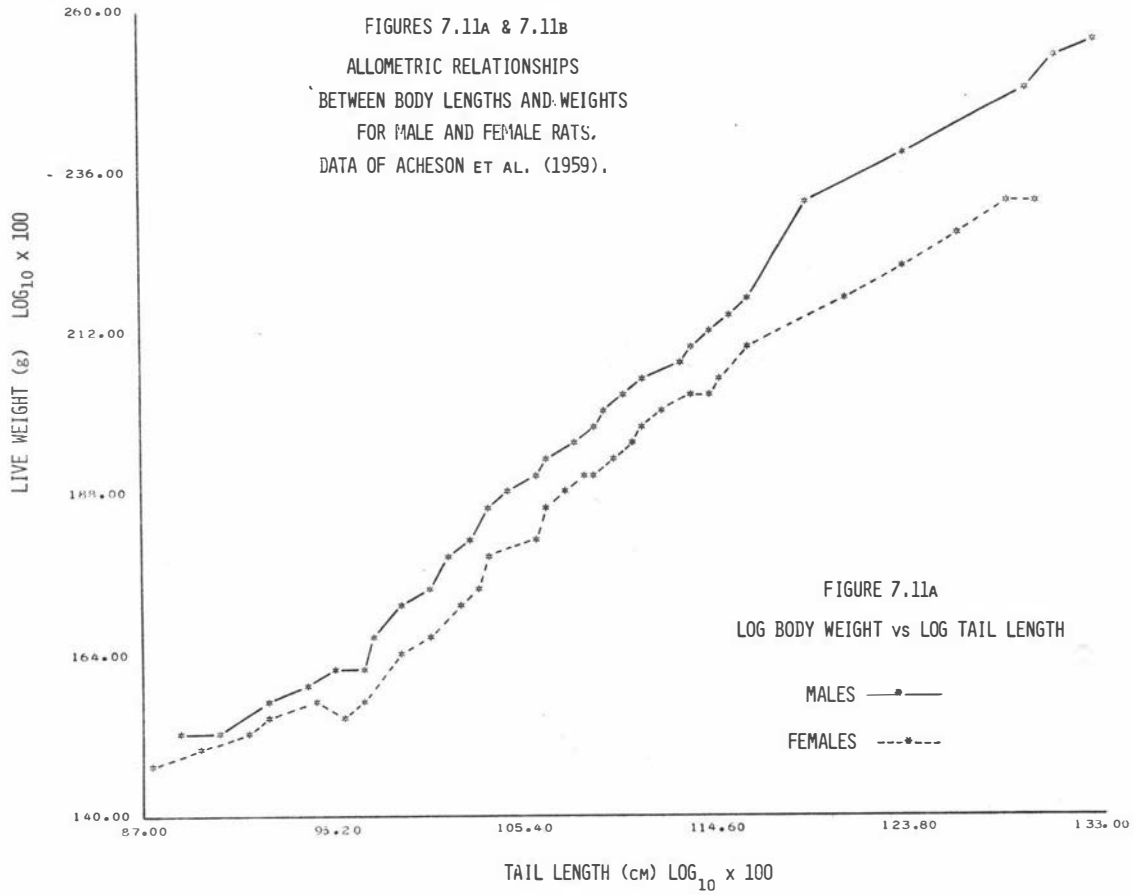


FIGURE 7.11A
LOG BODY WEIGHT vs LOG TAIL LENGTH

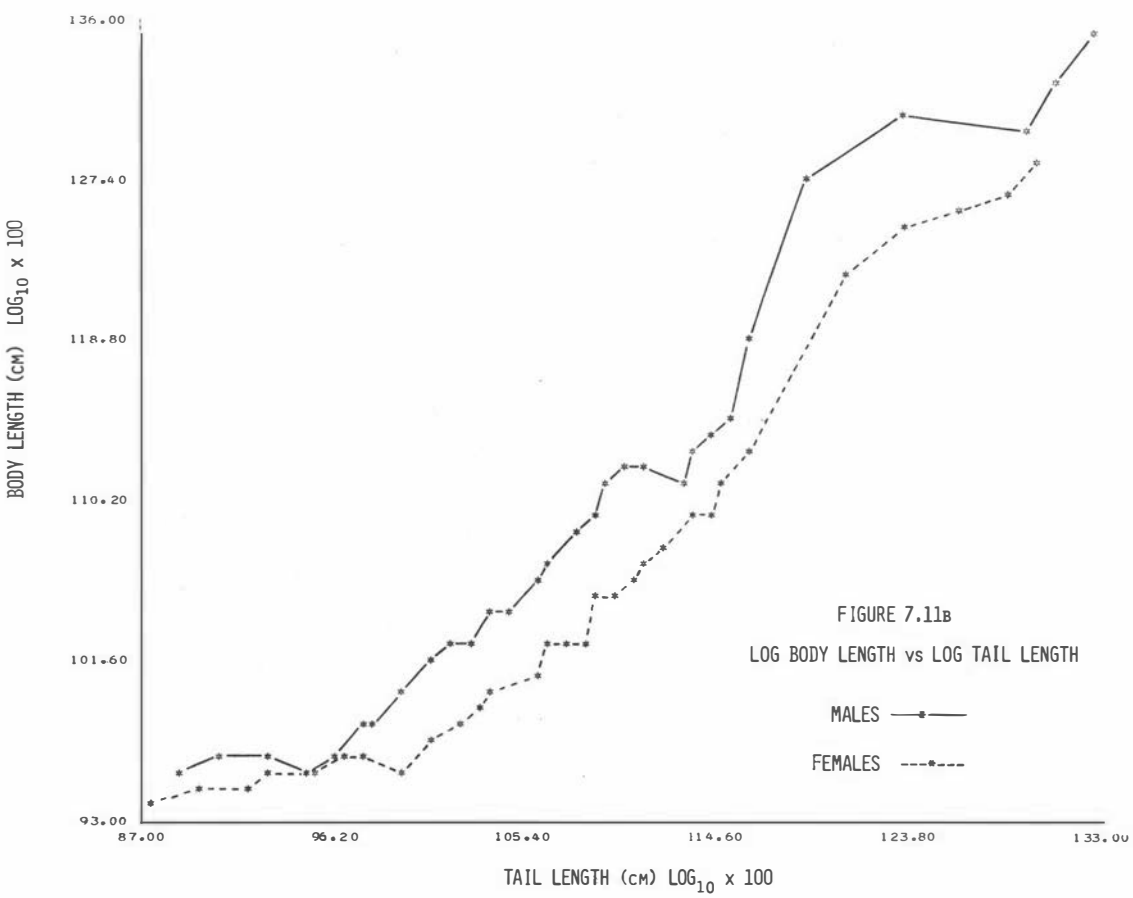


FIGURE 7.11B
LOG BODY LENGTH vs LOG TAIL LENGTH

SEX DIFFERENCES IN GROWTH

A comparison of Figures 7.1a, 7.8a and 7.9a clearly shows that from 16 to 38 days of age there were the following differences between the sexes;

1) males had greater body weights, the difference increased slowly with age,
2) males had greater body lengths, the difference increased markedly with age,

but 3) the sexes did not differ in tail length.

These differences between the sexes were reflected in the allometric plots. Females had, between 16 and 38 days of age,

a) at a given total length a similar body weight (Figure 7.10a),
b) at a given body weight a greater tail length (Figure 7.11a),
c) at a given body length a greater body weight (Figure 7.10b),

and d) at a given body length a greater tail length (Figure 7.11b) than male rats.

With age the males developed a greater body weight at a given total length and a given body length. The greater tail length of females at a given body weight or body length remained with age.

Drawing together the evidence from the temporal and allometric plots provides some new and interesting conclusions. Tail lengths were not different in males and females between 16 and 38 days of age and sex differences in body weight that were present before weaning persisted largely unaltered at least until 38 days of age. But the linear growth of the body was retarded in the female, thus allometry showed that the female had a greater weight at a given length up to 38 days of age. If only total lengths had been used no effect of sex would have been apparent, the opposite nature of the growth of the tail and body lengths, in the combined measurement, giving little overall change in body weights' relationship to length.

In Chapter 8 it will be shown that ovariectomy at 28 days of age results in a longer and heavier animal than does ovariectomy at 49 days of age in the rat. This finding supports the present finding that linear growth is inhibited in the female rat before puberty. The

ovary would seem to secrete biologically active oestrogens before puberty.

The surprising lack of an effect of sex on tail growth from 16 to 38 days of age, yet a clear sex difference in the growth of the more rostral vertebrae has not, to the author's knowledge, been reported previously. Acheson *et al*., (1959) did not calculate the body lengths so they did not report this effect. Re-plotting the data of Hughes and Tanner also shows that females have, at a given body weight or body length, a greater tail length.

These results suggest that the responses of tail length and body length to oestrogen differ. But Paesi and De Jongh (1954) have shown in young hypophysectomised female rats (24 days old and weighing 29 to 35 g) that following 12 days oestrogen treatment (using 7 doses of oestrogen) tail length growth was greatly reduced. This clear demonstration of a response of the tail to oestrogen, in a pre-pubertal animal, is contrary to Kennedy's statement (1970, p320), 'After weaning and before puberty.....Oestrogen is again without effect either on intake or on growth.' Whether Kennedy is referring to linear growth or live weight growth is not clear. The different responses of tail and body lengths to oestrogen may be a dose related phenomenon, the tail being less sensitive to oestrogen.

Data will be presented in Chapter 8 to support the idea that part of the post-ovariectomy increased efficiency of food utilisation, increased body weight, and increased body length could be due to an increased skin size and therefore a decrease in heat loss. The allometric relationship between body weight and body length also changed following ovariectomy to give an increased weight at a given body length, again decreasing heat loss. Part of the greater efficiency of food utilisation of the male rat (Morris, Palmer and Kennedy, 1933) could also be due to the differences in allometry demonstrated herein. For example, the tail of the female is of greater length relative to body weight and body length than in the male which would further increase the surface area to volume ratio of the female, perhaps causing increased heat loss, and therefore contributing to the decreased efficiency of food ultilisation in the female rat .

FIGURES 7.12A & 7.12B

POST-WEANING RATE OF WEIGHT GAIN vs DAY 28 LIVE WEIGHT (BOTH ESTIMATED FROM THE FITTED LOG-RECIPROCAL LINES), DATA OF TARTTELIN & CLARK, MASSEY COLONY.

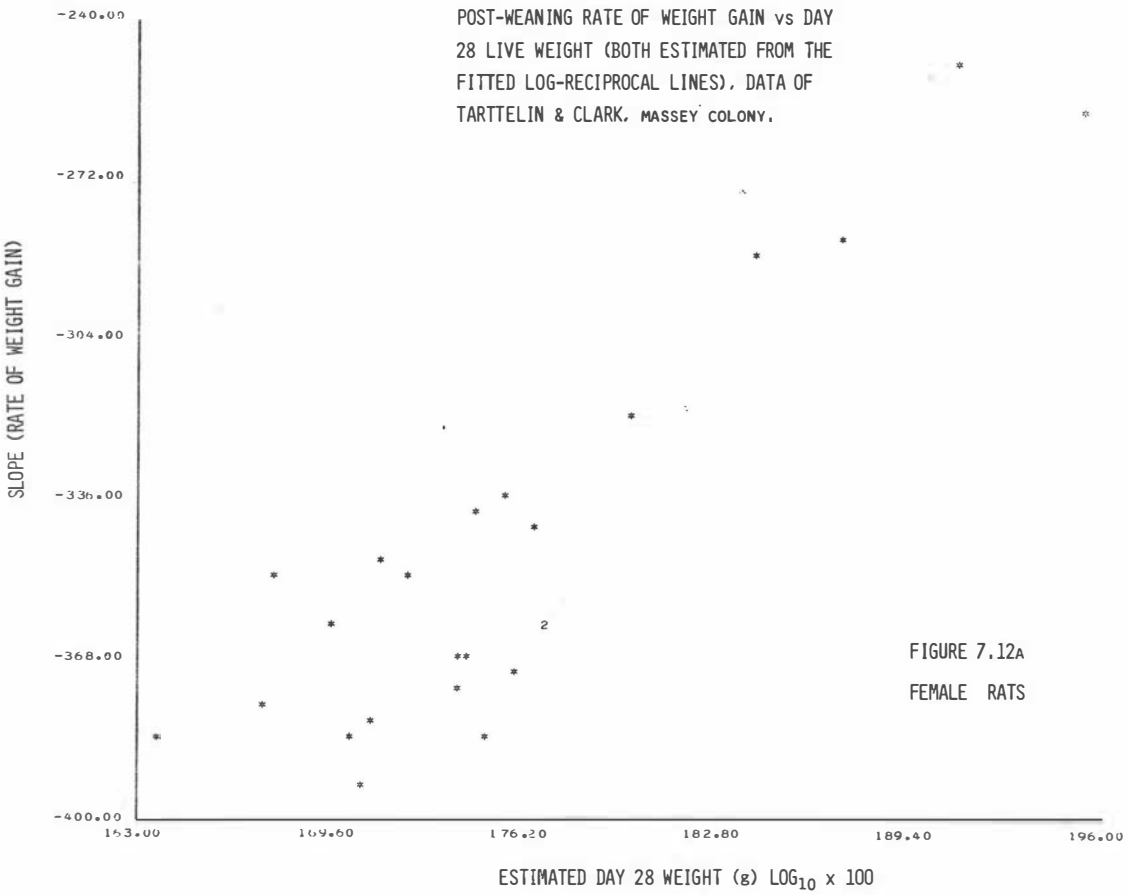


FIGURE 7.12A

FEMALE RATS

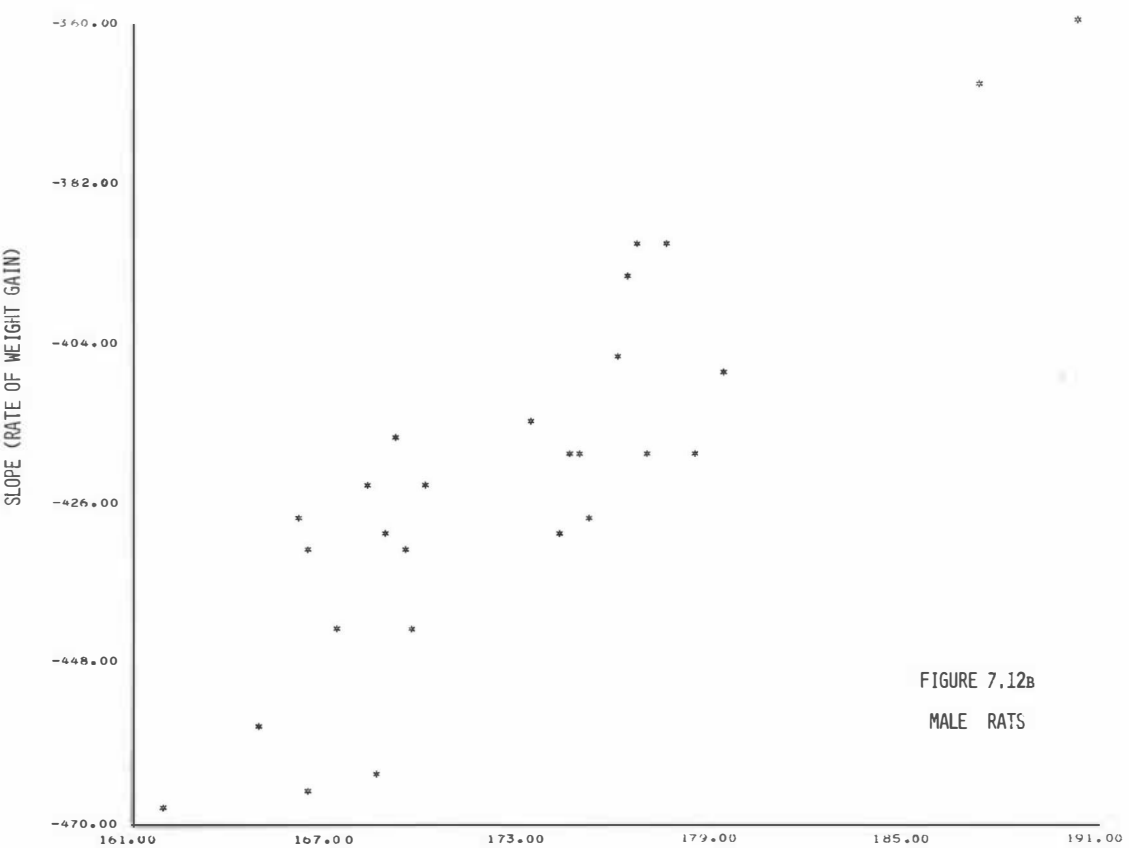


FIGURE 7.12B

MALE RATS

FIGURES 7.13A & 7.13B

ULTIMATE WEIGHT vs DAY 28 WEIGHT (ESTIMATED FROM THE LOG-RECIPROCAL LINES FITTED TO INDIVIDUAL RATS), DATA OF TARTTELIN & CLARK, MASSEY COLONY.

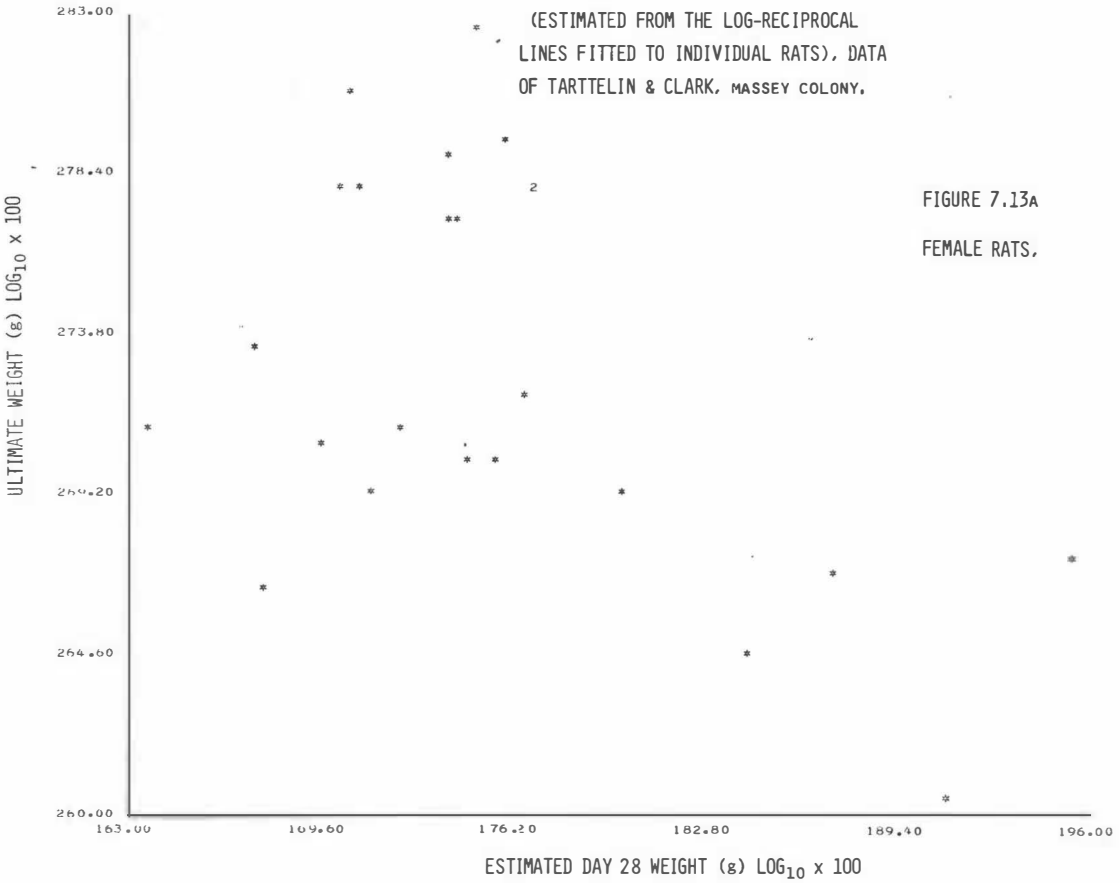


FIGURE 7.13A

FEMALE RATS.

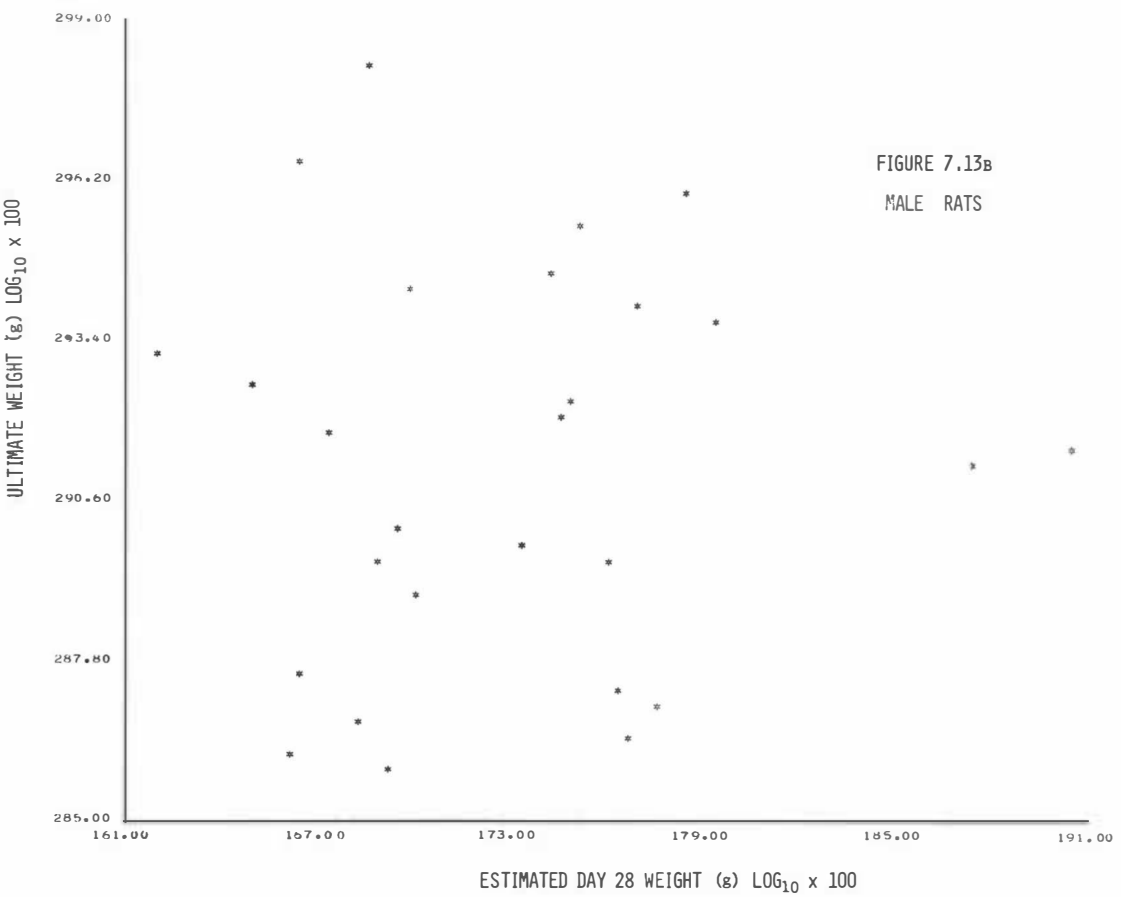


FIGURE 7.13B

MALE RATS

BIRTH, WEANING, AND ULTIMATE WEIGHTS: COMPENSATORY GROWTH

In dairy heifers (Chapter 5) the percentage of the variation in the rate of weight gain (slope) explained by the birth weight (constant) depended on the number of measurements made. Similarly a birth weight calculated from a number of measurements was shown in Chapter 2 to explain more of the variation in rate of gain than the measured birth weight. As 35 measurements were obtained from each animal in the present study it was logical that the findings of Chapter 1, concerning compensatory growth in the rat, should be re-examined and extended.

Log live weights were regressed against the reciprocal of age (in weeks) to obtain slopes, constants and the estimated weights at 28 days of age. Weights from 26 to 50 days were used, the earlier weights being greatly above the fitted line. Figures 7.12a (females) and 9.12b (males) show the extremely close relationship between the weight at 28 days of age and rate of weight gain (slope). In Chapter 1 about 40% of the variation in the rate of weight gain could be explained by the measured weaning weight. But both the male and female rats of the present study showed extremely high correlations between the slopes and the calculated day 28 weights with about 80% of the variation in growth rate being explained by the weaning weights. The relationships between the day 28 weights and the ultimate weights are shown in Figures 7.13a (females) and 7.13b (males). The lack of dependence between these two estimates clearly shows that weaning weight in the rat is largely controlled by external environmental influences.

Widdowson (1976a) presents a re-analysis of her classic experiment on compensatory growth in the rat, where suckling rats were cross-fostered to give large and small litters, which indicates that compensatory growth in the male is incomplete compared to that of the female. The present results do not support Widdowson's conclusion, they show that the day 28 weight explains a similar proportion of the variation in the subsequent growth rate of both male and females. Furthermore day 28 weight and ultimate weight are largely unrelated in both sexes.

Jinks and Broadhurst (1963) studied the inheritance of body weight in the rat using the refined techniques of the diallel cross. Weights were obtained from the offspring at birth, 21, 50, and 100 days of age. Heritability at birth and at 100 days was high while it was lower at 50 days and completely absent at 21 days of age. Monteiro and Falconer (1966) measured a small amount of genetic variation at weaning (3 weeks of age) but a large amount of genetic variation at 1 week of age in mice. Following several re-analyses of their data Jinks and Broadhurst could show '....no entirely satisfactory explanation of the absence of heritable differences in body weight at weaning'. However litter size and maternal ability were mentioned as possible factors involved. As weaning weight is largely environmentally determined, while ultimate weight is genetically controlled, post-weaning compensatory growth naturally follows. This evidence clearly supports the present findings of very high correlations between weaning weight (or currently day 28 weight) and the subsequent rate of weight gain and the lack of correlation between weaning weight and ultimate weight.

Few studies have attempted to analyse the source of the variation in weaning weight. In the earlier study, described in Chapter 1, no systematic measurements were taken before weaning. However in the present study tri-weekly weights were available from birth to weaning. These weighings were therefore transformed to logarithms and regressed against the logarithm of conception age to obtain, for each animal, estimated birth weights and slopes (the pre-weaning growth rates). The explanations of the variation in live weight (R^2) were frequently over 99.5%.

Birth weight was significantly influenced by genotype in the study of Jinks and Broadhurst. Therefore if the genes controlling body size equally influence both birth weight and ultimate weight one would expect a positive correlation between these two weights. (Estimates of the ultimate and day 28 weights were those previously calculated using the log-reciprocal equation applied to the weights obtained from 26 to 50 days of age. Therefore for individual animals estimates of birth weight and pre-weaning growth rate were obtained from different measurements to those used to estimate post-weaning growth rate and the day 28 and ultimate weights). But for the female rats (over all three treatments)

FIGURES 7.14A & 7.14B

THE EFFECT OF BIRTH WEIGHT ON THE SUBSEQUENT GROWTH IN LIVE WEIGHT OF FEMALE RATS. DATA OF TARTTELIN & CLARK MASSEY COLONY.

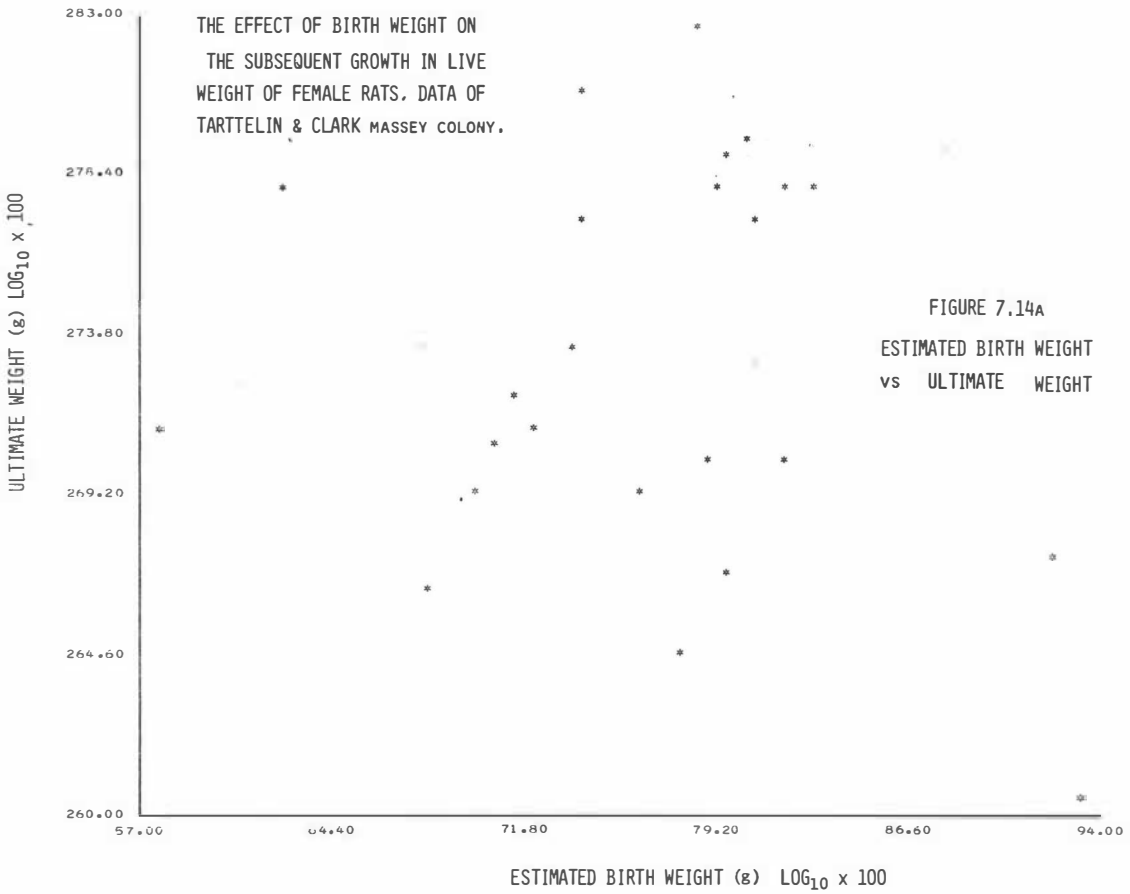


FIGURE 7.14A

ESTIMATED BIRTH WEIGHT vs ULTIMATE WEIGHT

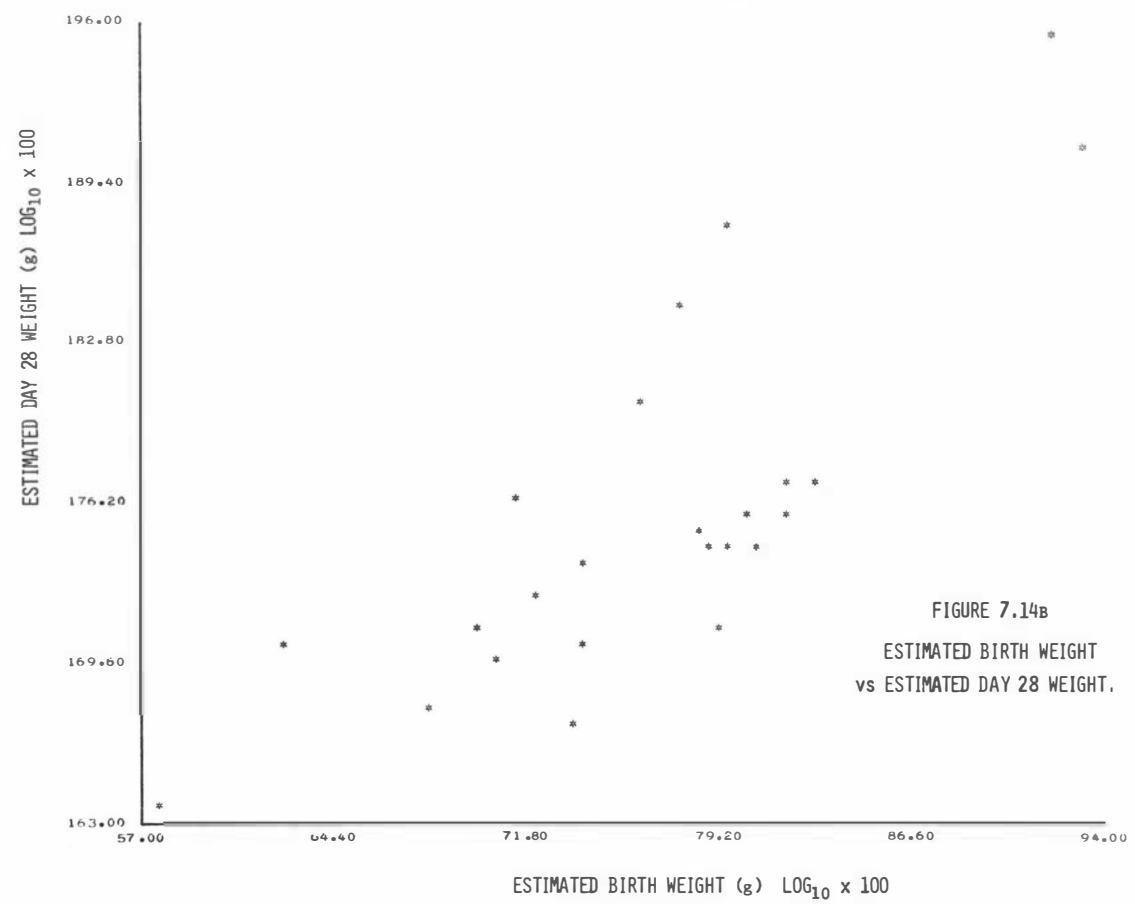


FIGURE 7.14B

ESTIMATED BIRTH WEIGHT vs ESTIMATED DAY 28 WEIGHT.

less than 2% of the variation in ultimate weight was explained by the calculated birth weight (Figure 7.14a). Similarly, for female rats, the pre-weaning growth rate was negatively correlated with the post-weaning growth rate (i.e. animals with a rapid pre-weaning growth rate tended to have low post-weaning growth rates) but the pre-weaning growth rate explained only 19% of the variation in the post-weaning growth rate ($P < .05$). In comparison the calculated weight at day 28 explained almost 80% of the variation in the post-weaning growth rate. McCance and Widdowson (1974) hypothesised that the rate of growth following weaning is determined by either the actual weight at weaning or the pre-weaning growth rate. The present analyses suggest that the actual weight at the initiation of compensatory growth, rather than the path by which the weight was attained, controls the subsequent growth rate.

Throughout this thesis it has been repeatedly shown that on a logarithmic metameter growth rate is negatively related to initial weight. But in the present study the calculated birth weight was exceptional as it explained only 4% of the variation in the pre-weaning growth rate. This means that differences in live weight between animals at birth were largely maintained until weaning and that there should be a strong positive correlation between birth weight and weaning weight (or in the present case the day 28 weight). Figure 7.14b confirms this idea as the birth weight explained over 62% of the variation in the day 28 weight ($P < .001$) and the pre-weaning growth rate explained only 16% of the variation in the day 28 weight ($P \simeq .05$). The later correlation was also positive, indicating, on a logarithmic scale, the divergence of the pre-weaning growth lines.

Therefore the variation in weight at day 28 was mainly due to pre-natal influences but the day 28 weight was also positively related to the pre-weaning growth rate, presumably a post-natal effect of the litter size or the lactational or mothering ability of the dam. The range of litter sizes was 3 to 13 offspring, a greater range may inflate the amount of variation in day 28 weight due to post-natal influences. These results clearly show that the mechanisms controlling compensatory growth are not developed until about weaning in the rat

FIGURES 7.15A & 7.15B

RESIDUALS ABOUT THE LINES RELATING
LOG LENGTHS TO LOG CONCEPTION AGE
vs LOG CONCEPTION AGE
DATA OF ACHESON ET AL (1959)

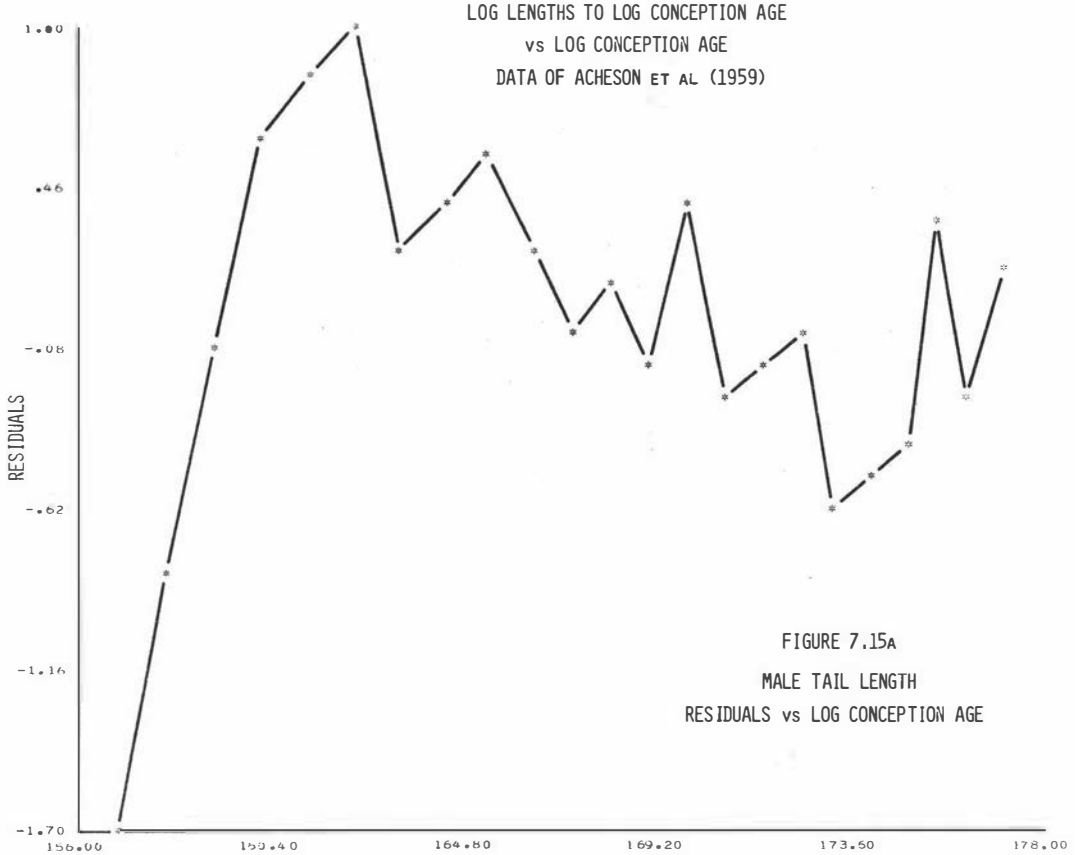


FIGURE 7.15A
MALE TAIL LENGTH
RESIDUALS vs LOG CONCEPTION AGE

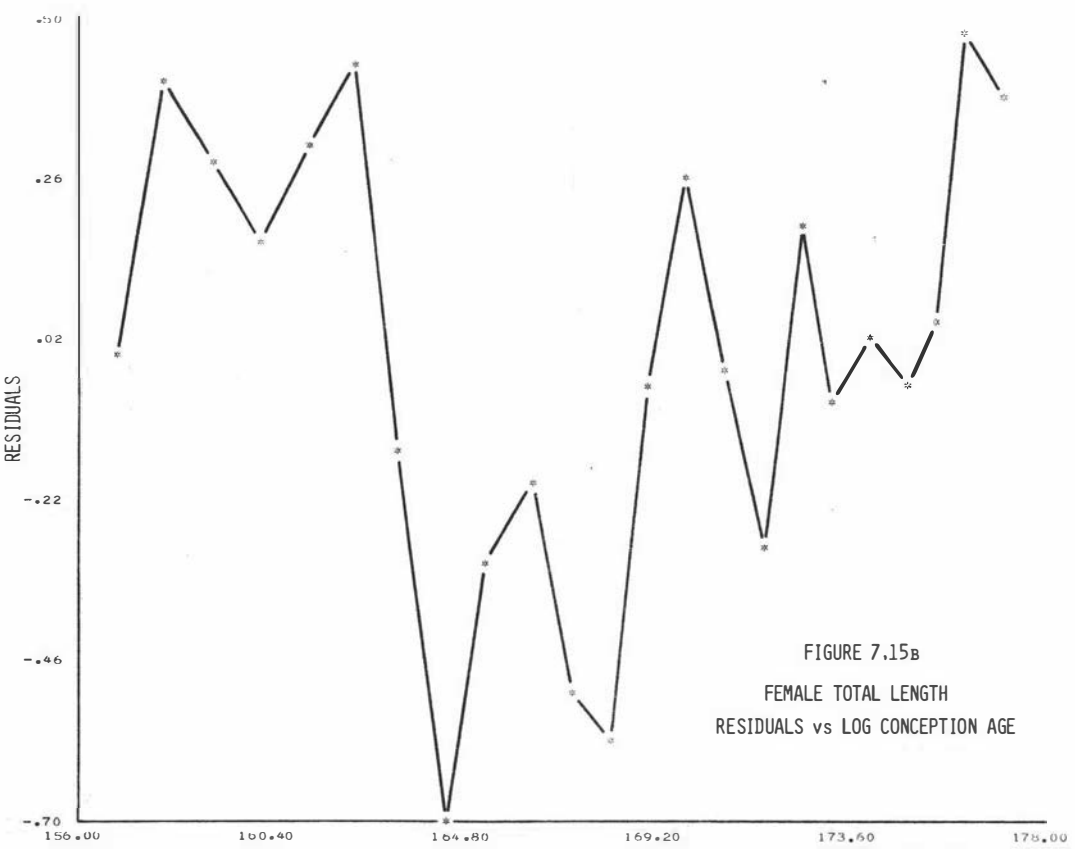


FIGURE 7.15B
FEMALE TOTAL LENGTH
RESIDUALS vs LOG CONCEPTION AGE

as differences between animals at birth appear to be maintained until weaning.

EVIDENCE FOR FOUR DAY CYCLES OF BODY GROWTH IN THE RAT

It was noted earlier that when log tail length was plotted against log conception age that the residuals deviated quadratically about the fitted line. It may have been prudent to drop the first few (pre-weaning) measurements as they seem to represent a pre-weaning growth phase, and test the post-weaning data for linearity; initially this was not done. Despite the remaining curvature a close inspection of the male data reveals the possibility of short-term growth cycles (Figures 7.8b, 7.15a). If, in Figure 7.15a, one starts at 18 days of age and inspects the values to 35 days, the male tail lengths that are most below the general downward trend are at 22, 26, 30 and 33 days, while those most above the general trend are at 21, 24, 29, 32 and 35 days. Both the maxima and minima indicate that the measurements fluctuate cyclically with a phase of about 4 days. In addition the measurements tend to occur in step-like sequences at 19, 20 and 21; 22, 23 and 24; 30, 31 and 32; and at 33,34 and 35 days. The point at 28 days is the only one to disrupt this pattern. For the female tail length residuals (Fig 7.8b) a short-term cycle could be present, the variation appears to be wave-like. After 35 days, approximately the time of puberty in the rat, the patterns are disrupted in both males and females.

The residuals for body length show evidence of cyclicity, the initial 'stepping', in the females (Fig. 7.9b) being notable. Residuals for total length, in females, showed a clearly cyclical pattern. Thus they have been plotted, for comparison, with the residuals for male tail length (Fig. 7.15a). For the females (Fig.7.15b) nadirs can be identified at 19, 23, 27, 31 and 35 days with peaks at 17, 21, 25, 29, and 32 days of age. These cycles seem to be one day behind, or out of phase from, the male cycles in tail length (Fig 7.15a) This is an important finding (providing that all the animals were born and weighed on the same days), with respect to the aetiology of the

FIGURES 7.16A & 7.16B

RESIDUALS ABOUT THE LOG-RECIPROCAL LINES vs AGE
FEMALE RATS MEASURED FROM 21 TO 38 DAYS OF AGE, DATA OF ACHESON ET AL. (1959).

FIGURE 7.16A
BODY WEIGHT
RESIDUALS vs AGE

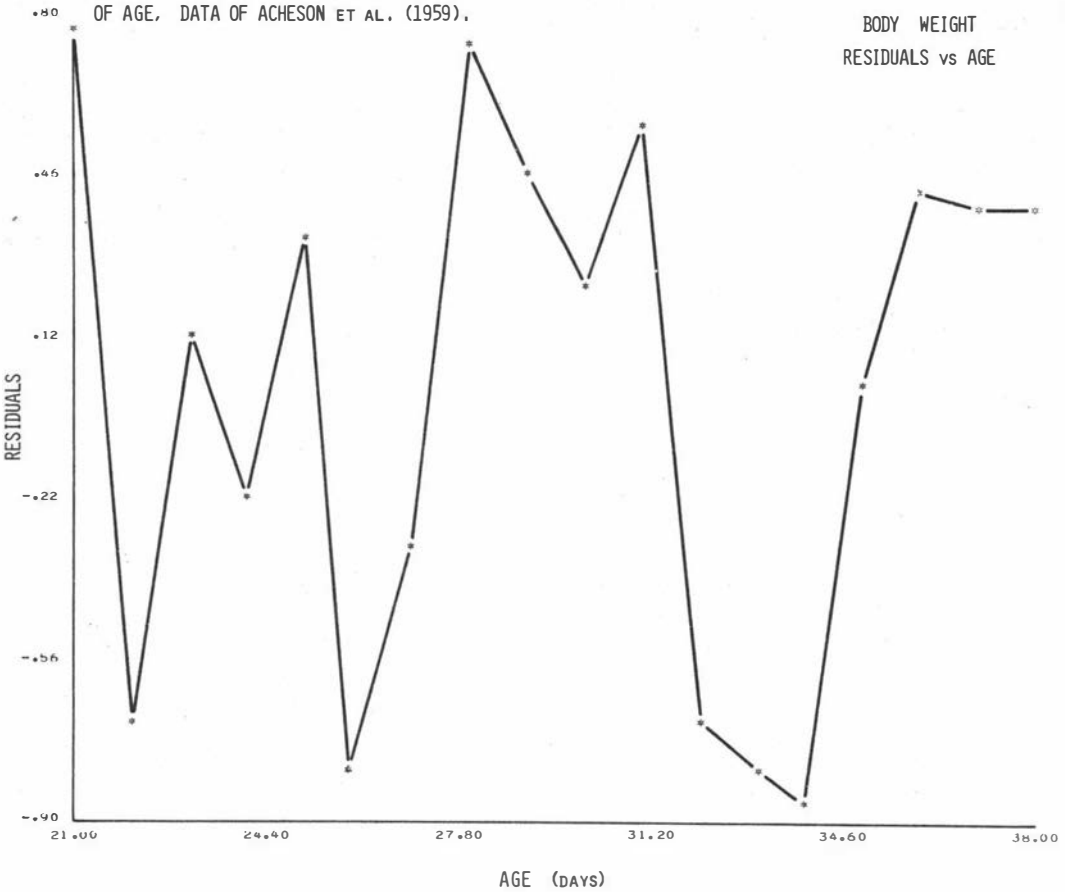
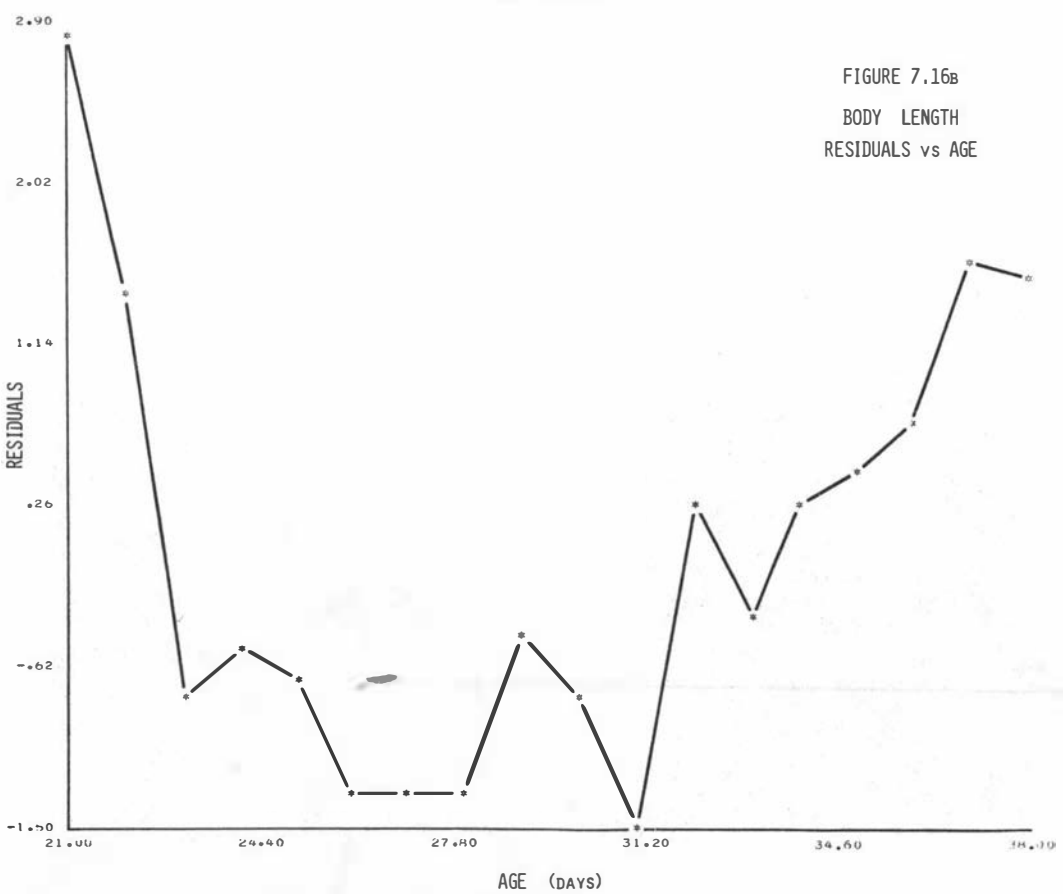


FIGURE 7.16B
BODY LENGTH
RESIDUALS vs AGE



the cycles, as it counts against the cycles being due to systematic errors of measurement or to a regularly fluctuating environment.

Appendix Tables 7.1 and 7.7 show that the residuals for body weight were approximately three times greater, relative to the means, than those for the linear dimensions. In addition the large long-term fluctuations in the residuals for body weight, especially that occurring before weaning, made it difficult to detect possible short-term fluctuations in growth rate. The major long-term fluctuation in the residuals was therefore eliminated by analysing only post-weaning weighings. Instead of the log-log equation the log-reciprocal equation was used since, for Acheson's female rat live weight data from post-weaning ages, this relationship appeared to be linear (Figure 6.9). The residuals about the log-reciprocal line for body weight show systematic short-term swings (Figure 7.16a) as do those for body length (Figure 7.16b). The maxima and minima of the cycles appear to be of about 4 days duration.

Unlike the published data of Acheson the individual rat weights were available from the Massey experiment and could be tested for short-term rhythms. The residuals about the log-reciprocal lines fitted to the daily weights (from about 30 to 50 days of age) of individual animals, are plotted against age in,

1) Figures 7.17a, b, c, and d for four male rats,
2) Figures 7.18a, b, c, and d for four female rats

and 3) Figures 7.19a, b, c, and d for four pre-natally androgenised female rats.

The positive quadratic for androgenised females, the negative quadratic for males, and the largely stationary trend for the females again shows the basic differences in the patterns of growth between the sexes and treatments. The females can be seen to show a greater amount of variation, than the males, about the general trend. This pictorially illustrates the greater amount of unexplained variation in the females compared to the males (Table 7.3). The androgenised females appear to be intermediate between the sexes in this regard.

The pairs of animals shown in Figures 7.17 to 7.19 were littermates and the members of a pair were housed in the same cage.

FIGURES 7.17A & 7.17B

RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO THE DAILY LIVE WEIGHTS OF MALE RATS vs BIRTH AGE, DATA OF TARTTELIN AND CLARK, MASSEY COLONY.

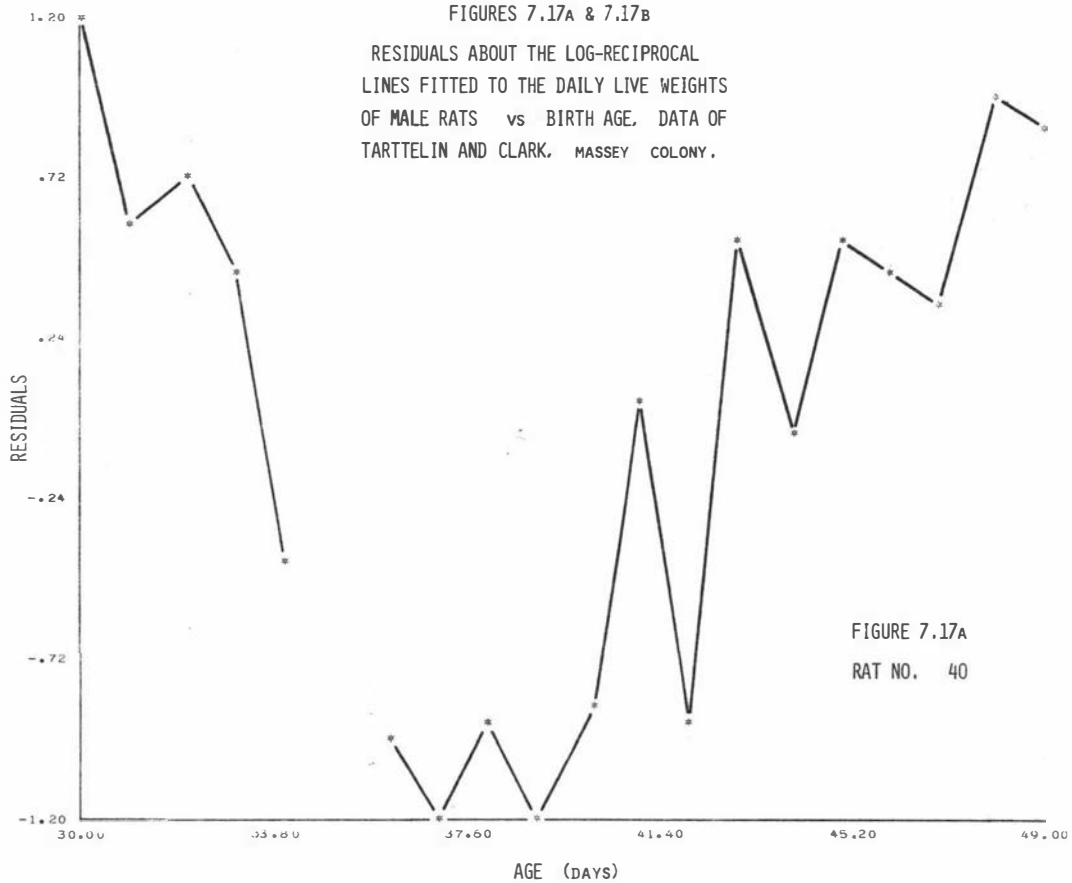


FIGURE 7.17A
RAT NO. 40

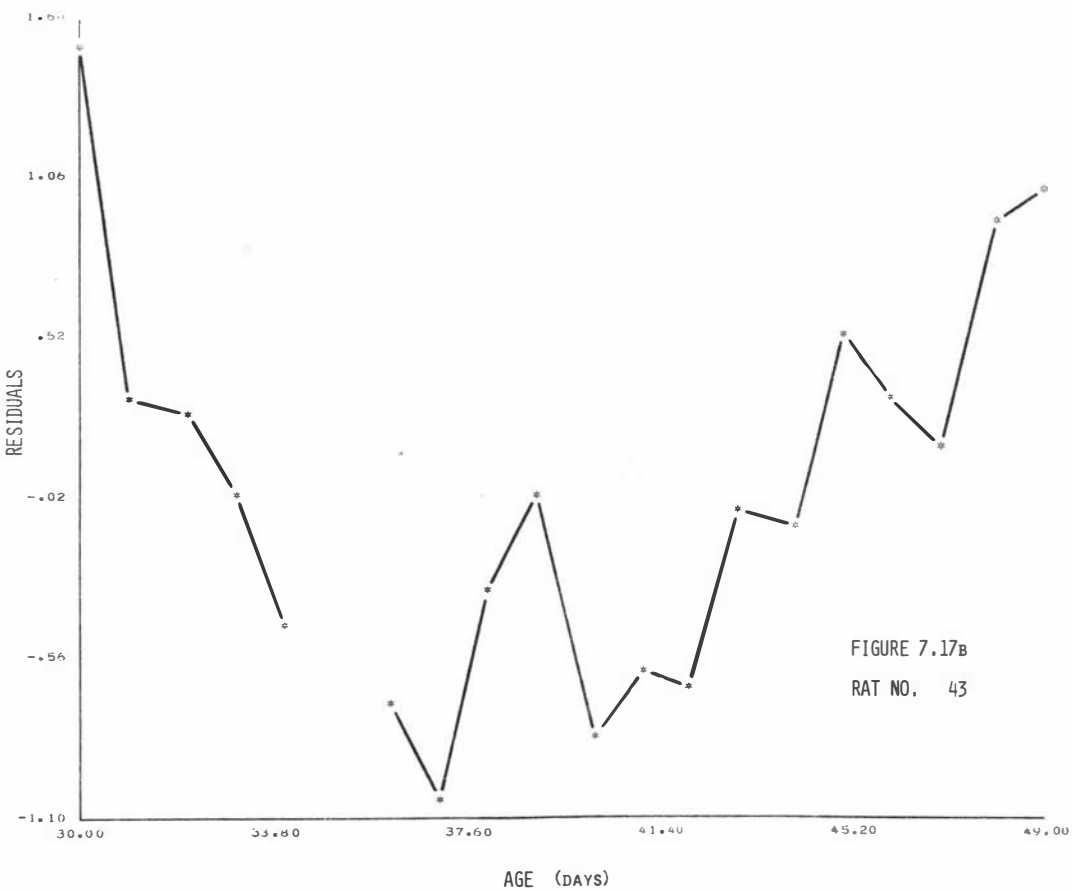


FIGURE 7.17B
RAT NO. 43

FIGURES 7.17c & 7.17D
RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO THE DAILY LIVE WEIGHTS OF MALE RATS vs BIRTH AGE, DATA OF TARTTELIN AND CLARK, MASSEY COLONY.

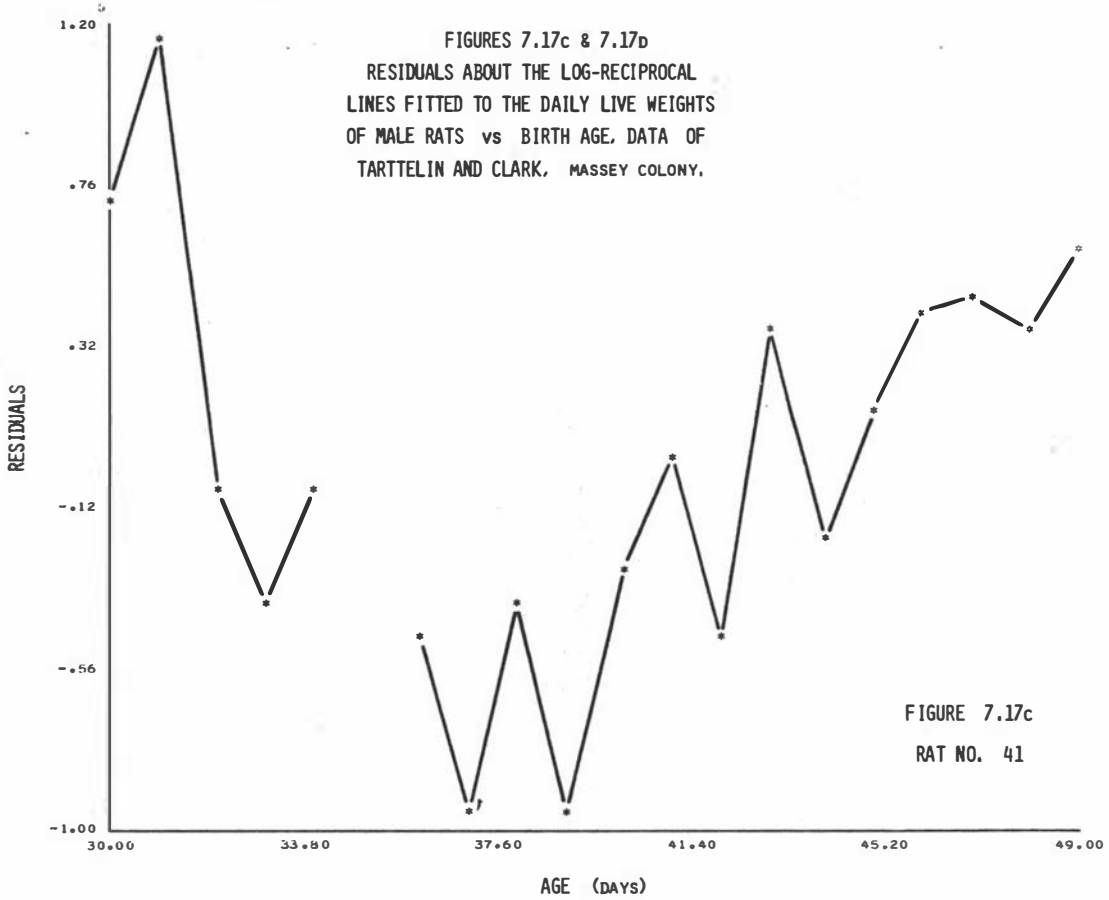


FIGURE 7.17c
RAT NO. 41

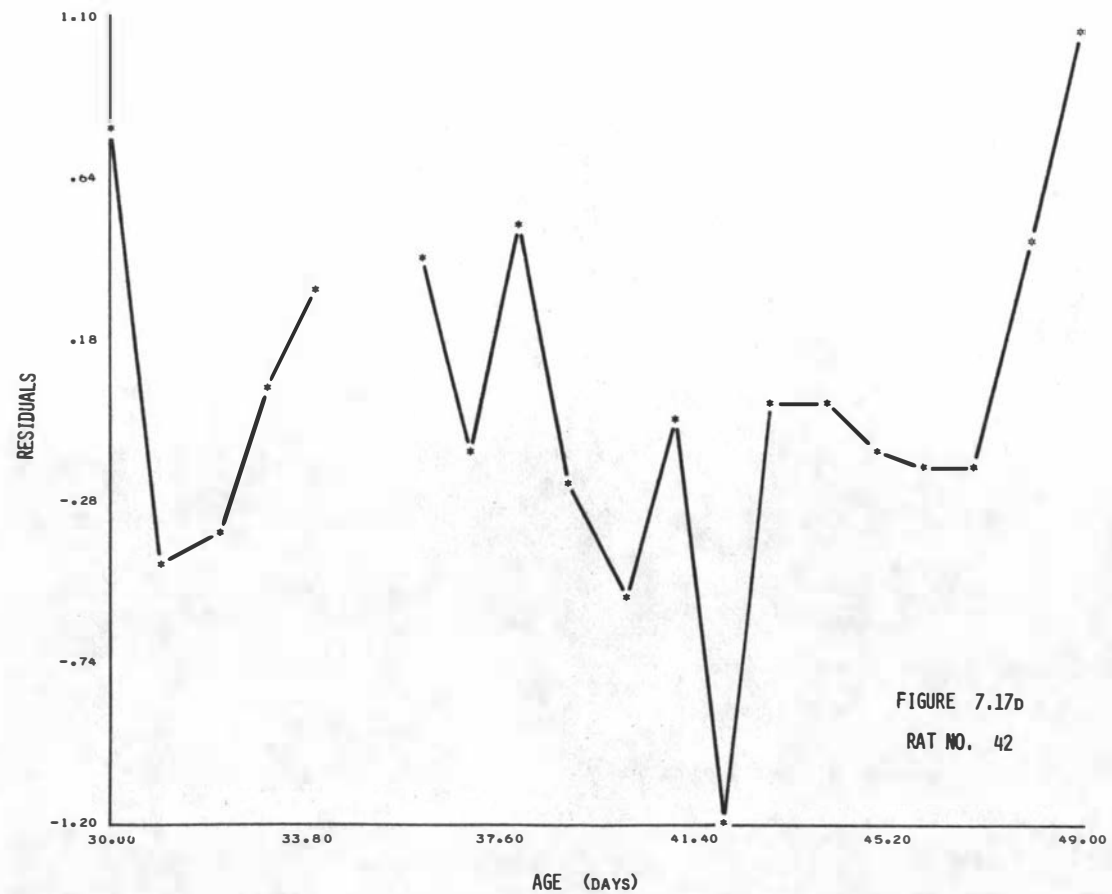


FIGURE 7.17D
RAT NO. 42

FIGURES 7.18A & 7.18B

RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO THE DAILY LIVE WEIGHTS OF FEMALE RATS vs BIRTH AGE. DATA OF TARTTELIN AND CLARK, MASSEY COLONY.

FIGURE 7.18A

RAT NO. 33

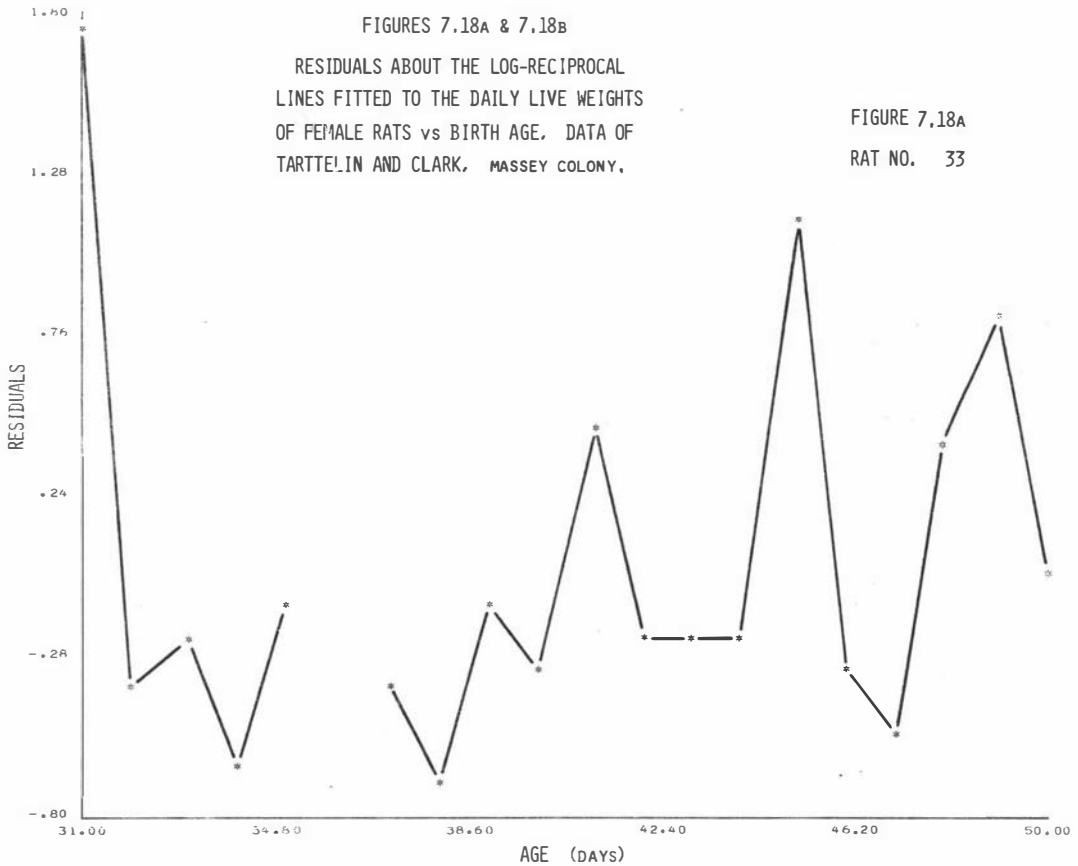
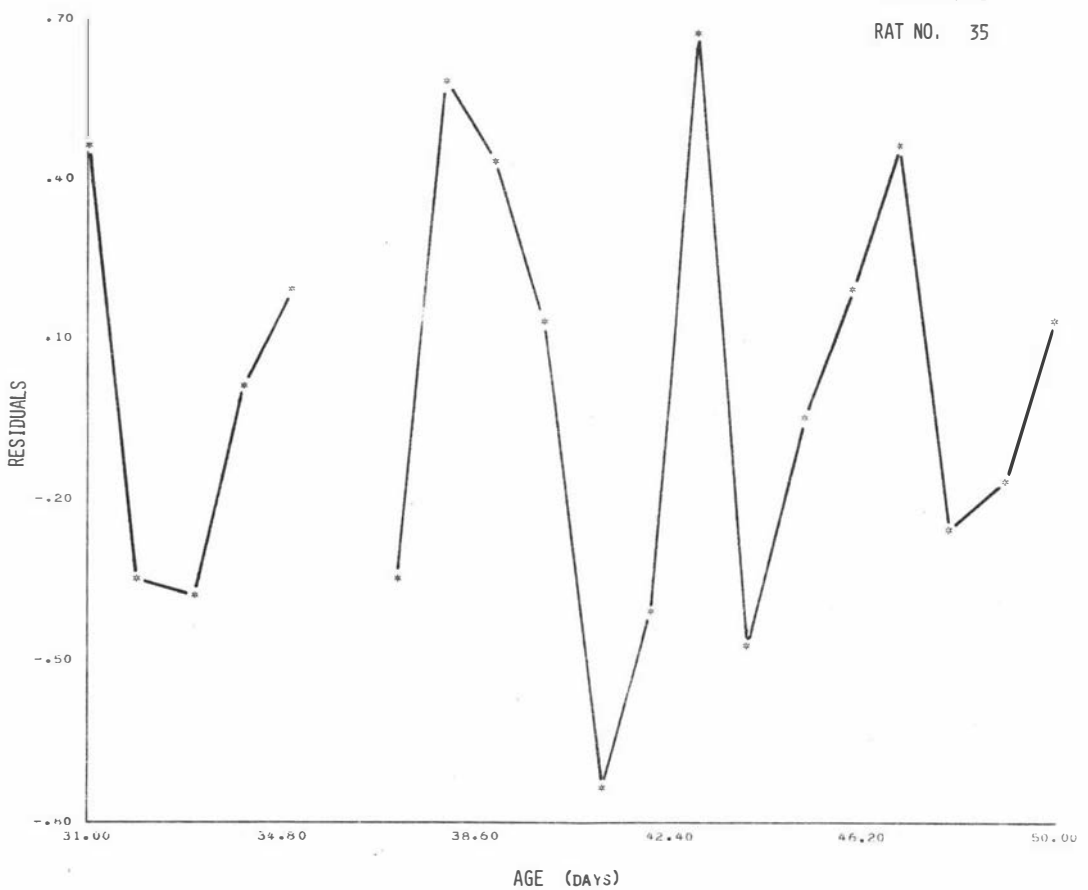


FIGURE 7.18B

RAT NO. 35



FIGURES 7.18c & 7.18d

RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO THE DAILY LIVE WEIGHTS OF FEMALE RATS vs BIRTH AGE, DATA OF TARTTELIN AND CLARK, MASSEY COLONY.

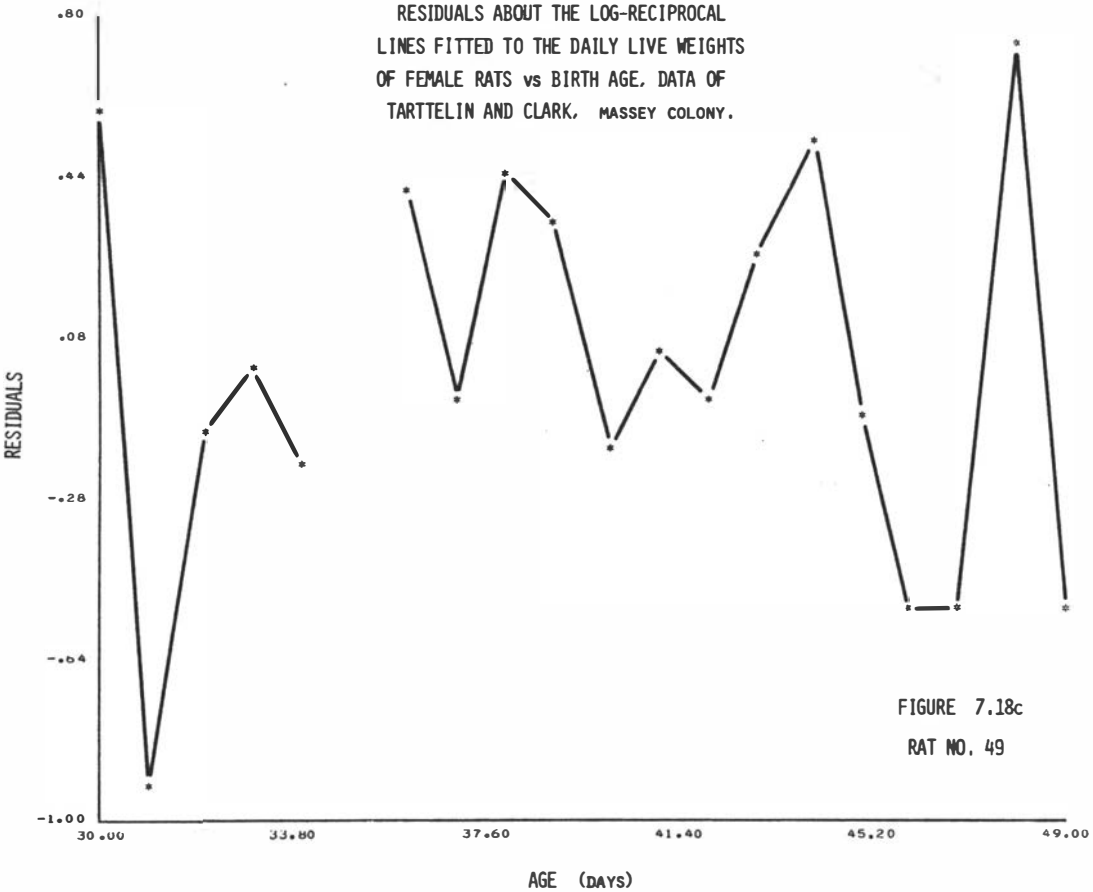


FIGURE 7.18c
RAT NO. 49

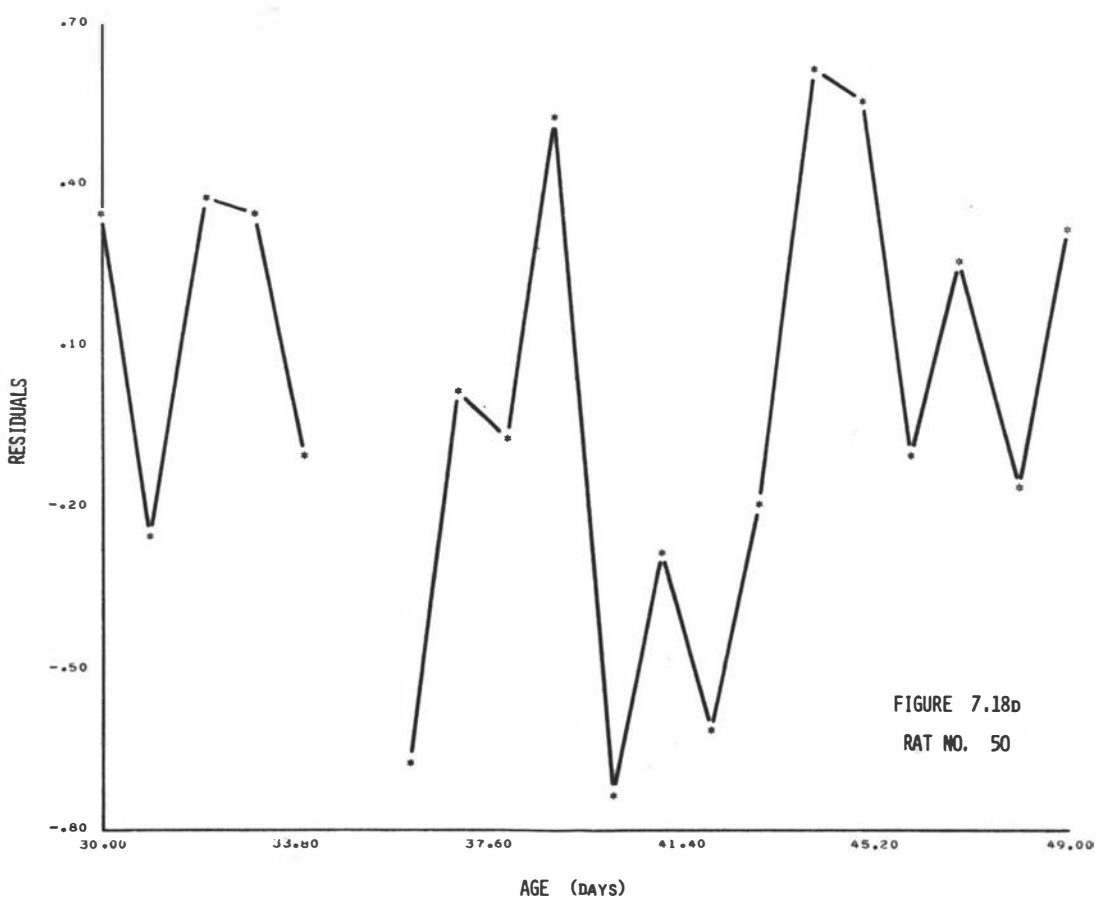


FIGURE 7.18d
RAT NO. 50

FIGURES 7.19A & 7.19B

THE RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO PRE-NATALLY ANDROGEN TREATED FEMALE RATS vs BIRTH AGE, DATA OF TARTTELIN AND CLARK, MASSEY COLONY.

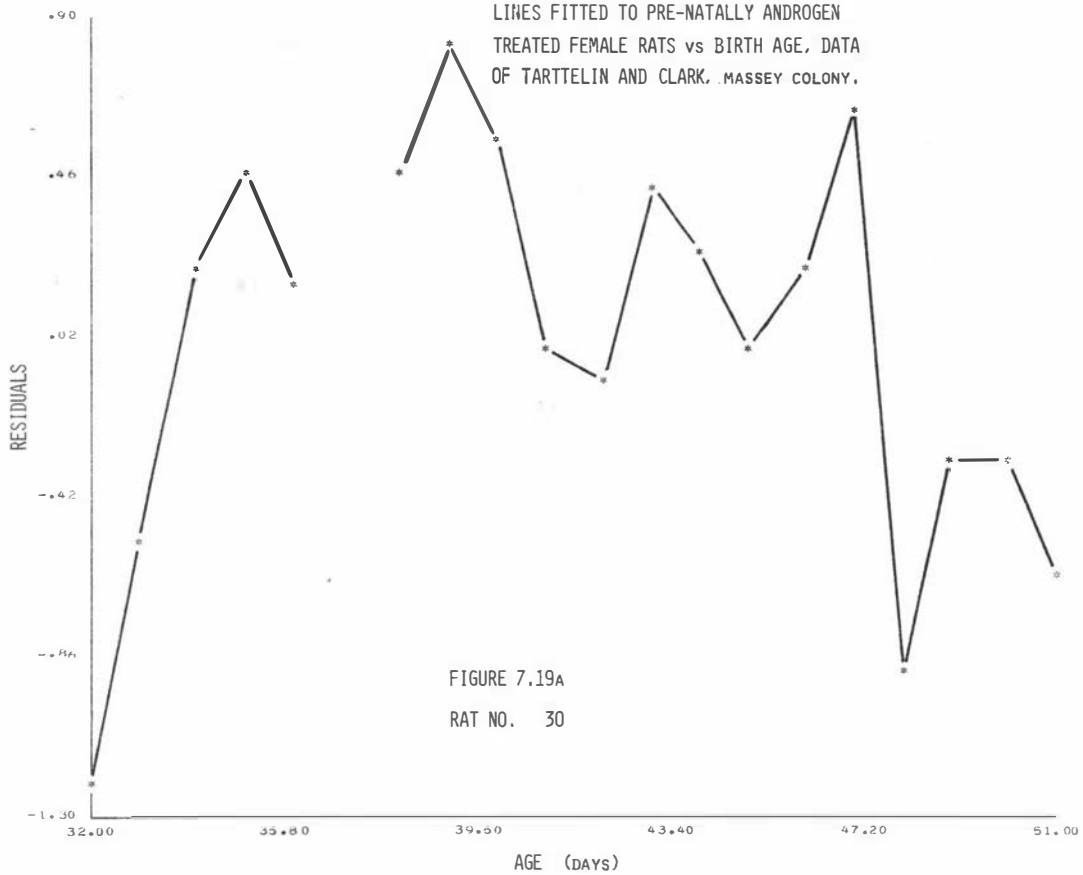


FIGURE 7.19A

RAT NO. 30

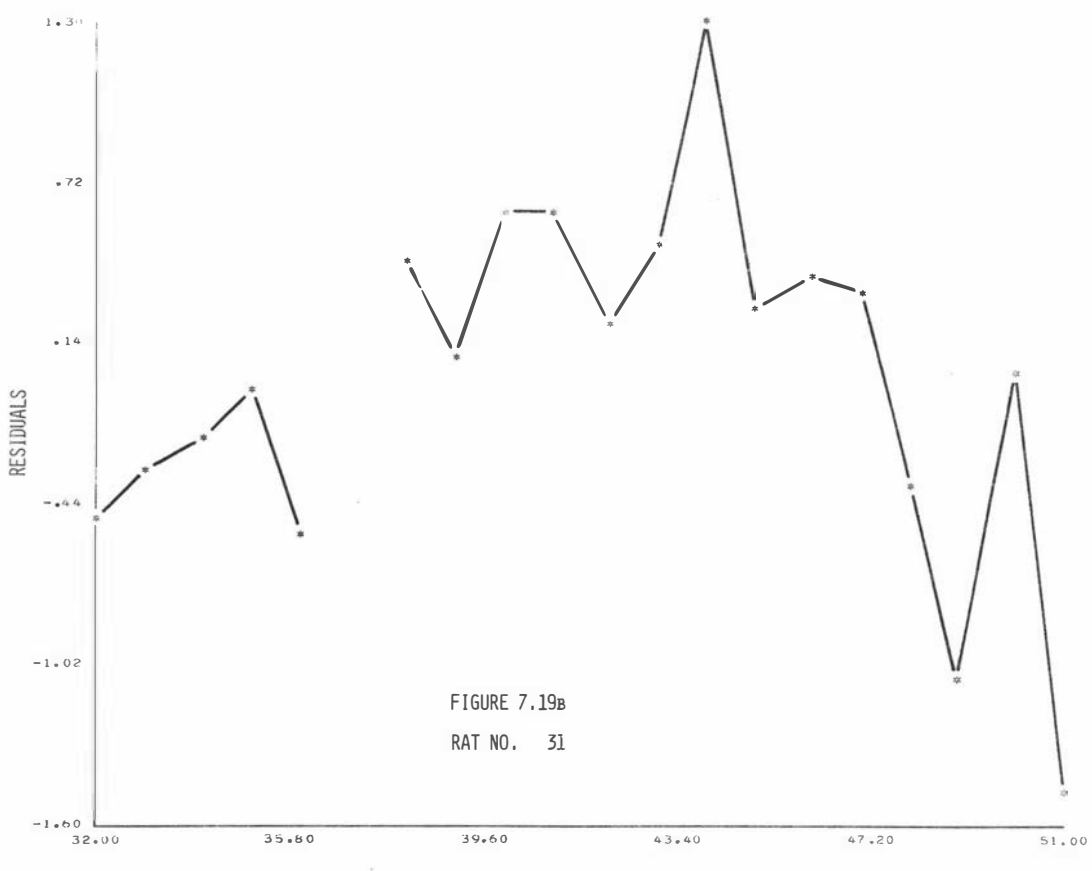


FIGURE 7.19B

RAT NO. 31

FIGURES 7.19c & 7.19D
THE RESIDUALS ABOUT THE LOG-RECIPROCAL LINES FITTED TO PRE-NATALLY ANDROGEN TREATED FEMALE RATS vs BIRTH AGE, DATA OF TARTTELIN AND CLARK, MASSEY COLONY.

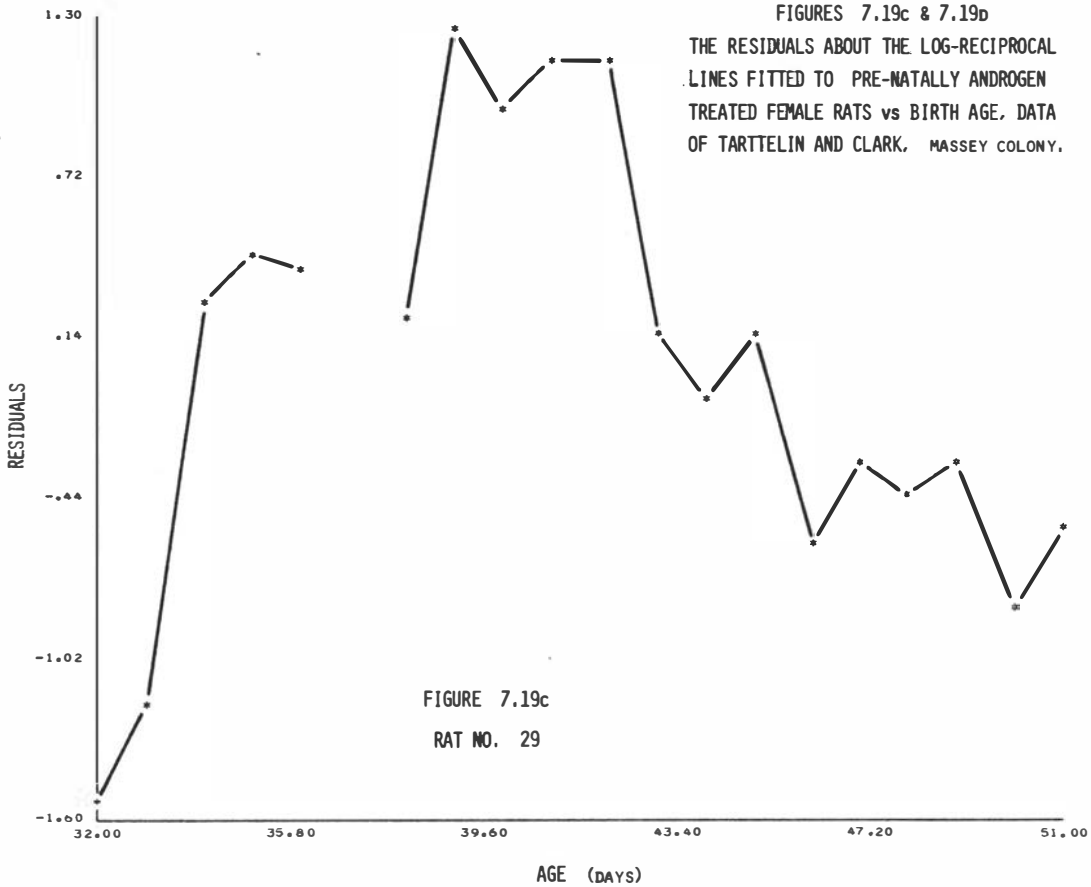


FIGURE 7.19c
RAT NO. 29

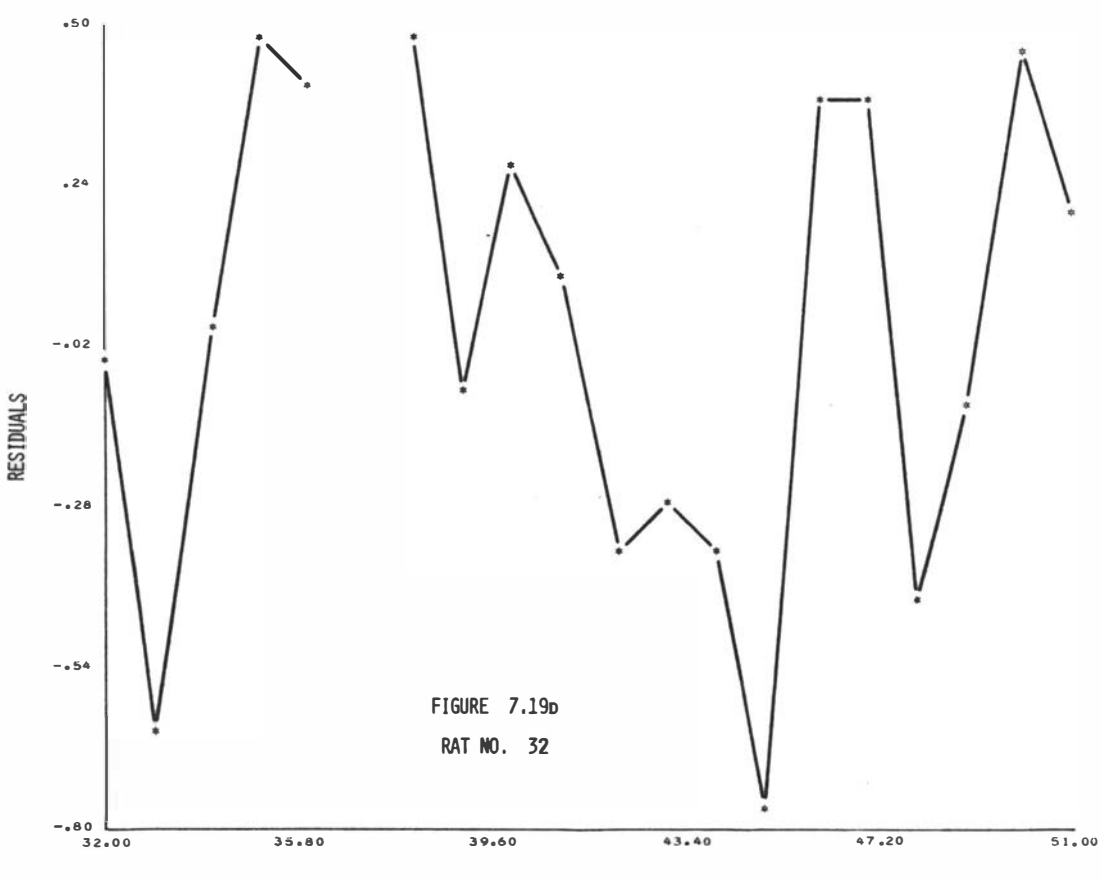


FIGURE 7.19D
RAT NO. 32

Therefore if the short-term swings in the residuals were due to environmental influences the pattern of residuals should be similar in these pairs of animals.

A careful study of the peaks and troughs, especially for the females, leaves the impression that the swings in the residuals are out of phase. For the males there is some evidence that the peaks and the troughs occur on the same days in the animals illustrated. Although the patterns of residuals from the males do not show convincing evidence of short-term cycles the normal and the treated females both show 4 day cycles of live weight growth. Unfortunately vaginal smears were not taken so that it was not possible to correlate the changes in weight with the events of the oestrous cycle. But it seems unlikely that the animals had started cycling at the earliest ages shown and the first few oestrous cycles in the rat are usually irregular. Thus the presence of short-term growth cycles, despite the lack of evidence for them in the Massey males appears to be confirmed. Their aetiology remains to be determined.

There was a large negative quadratic element remaining in the residuals for the male rats. For Acheson's body weight and body length data the removal of curvature in the general trend allowed a clearer picture of the short-term changes in the residuals to emerge. Therefore the removal of the quadratic trend in the Massey male animals may have helped the detection of short-term rhythms. This avenue of study was not followed. A definitive experiment to ascertain the aetiology of these short-term cycles in the females would be to ovariectomise suckling or weanling animals, weigh them daily, and then test for cycles in the residuals about the general trend of growth.

CONCLUSIONS

The analyses of this chapter demonstrate, apparently for the first time, that in the rat body dimensions and body weight adhere to the same general relationship to age from 16 to 38 days of age:

the logarithms of lengths and weights are linearly related to the logarithms of conception age. The daily measurements obtained by Acheson were well suited to test both the log-log and the log-reciprocal equations. However despite these relationships for the mass curves the individual curves could conceivably follow a different pattern of change. But the live weight changes of individual animals from birth to 50 days of age, obtained from the Massey colony, were also well described by the log-log and log-reciprocal equations.

In Chapter Six it was shown that there were systematic fluctuations in growth rate about the fitted log-reciprocal line. The residuals about the log-log plots of individual animals' growth curves presently display similar fluctuations. Following birth, growth slowed for a few days, then accelerated, slowed again to weaning, then accelerated again after weaning and slowed once more after about 40 days of age. These changes in live weight do not seem to have been described previously. But similar changes in the growth rates of linear dimensions have been noted by Hughes and Tanner (1970) who plotted the absolute growth rates (mm/day) for tail length and nose-rump length against post-conception age. Hughes and Tanner (p367) noted

'Nose-rump and tail length show a curious earlier feature. Both increase in velocity from 23 d to a small peak at 30 d, fall to 40 d and then increase to their major peak. This occurs in both sexes. It is unlikely to be due to sampling error.'

Their graphs of the absolute velocities of live weight gain did not give a similar early peak in growth rate but their live weight graphs (p359) do show an early neo-natal change. The apparent difference between the growth of the body's weight and length could be due to the arithmetic scale not revealing changes which a logarithmic scale would reveal. This again emphasises a recurrent theme of this work, this time restated by Meredith and Meredith (1958).

'As emphasised by Minot over 60 years ago, biologic growth is depicted more adequately through 'percentage increments' than through 'absolute increments'. This perspective is illustrated by noting that while two absolute increments of 1.0 kg are identical mathematically,

they are not identical biologically when one is added to an organism weighing 4.0 kg and the other augments an organism weighing 40 kg.'

Thus equivalent absolute (arithmetic) growth rates at birth and at 100 days of age are clearly not equivalent biologically. In the present work the use of logarithmic scales emphasises the importance of the early growth phase and shows the changes in live weight on this scale to be of a regular cyclical nature. These effects are not clearly apparent when arithmetic scales are employed with live weight (Tanner and Hughes) but they are seen with tail and nose-rump lengths.

The adverse effects of weaning on growth rate are commonly alluded to. But for the author's data, plots of the residuals about the log-log equations show the opposite effect. Following weaning a surge of growth occurred while before weaning a relative decline in growth rate was taking place. Some authors (Hughes and Tanner, 1970) advocate late weaning in rats (at 30 days of age) but the decline in growth rate which starts before weaning suggests that early weaning may halt this decline in growth rate and be beneficial in terms of ultimate weight. Further work in this area is necessary but the pre-weaning decline in growth rate could be associated with competition between litter mates and the dam for food. An investigation of the residuals about the growth curves of individual rats weaned at various ages, in different sized litters, and with the availability of solid food also controlled, could shed some light on these processes.

The development of the mechanisms controlling compensatory growth was investigated, using the data from the Massey colony, by studying the correlations between the calculated variables; birth weights, pre-weaning growth rates, day 28 weights, post-weaning growth rates, and ultimate weights. Compensatory growth following day 28 was shown, with no sex difference in the effect, when about 80% of the variation in post-weaning growth rate was explained by the day 28 weight. As birth weight and pre-weaning growth rate did not show a statistically significant relationship birth weight and day 28 weight were shown to be strongly positively correlated. There was a weak correlation between day 28 weight and pre-weaning growth rate. It seems that

compensatory growth is initiated at about the time of weaning and is controlled by the actual weight at initiation rather than by the growth rate before its initiation.

The allometric relations of tail length to body weight show negative curvature. In marked contrast the same relationships for the data of Hughes and Tanner (1970) and Mosier (1969) show positive curvature following weaning. At weaning in these studies, as in the present work, a change in growth rate occurred. The difference in curvature could be a strain difference as Mosier used Long-Evans rats, Hughes and Tanner hooded rats, and Acheson used Sprague-Dawley rats. It is possible to select and breed strains of mice which possess differing body weight to tail length ratios *(Munford and Cockrem, see footnotes), while similar work in rats is reviewed by Robinson (1965). Another difference between the experiments were the environmental temperatures, 72-78°F (Mosier), 69°F (Acheson *et al*), while Tanner and Hughes do not give this information. Rodent tail length varies greatly with environmental temperature (Harrison, 1963; Chevillard *et al*., 1963; Munford and Cockrem, unpublished studies) which could also account for part of the differing allometric relationships in the various studies.

But the important result is that curvature is definitely present in the allometric plots; the relationships between the variables cannot be considered as truly linear, just as the relationships between the variables and time show long-term systematic departures from linearity. This emphasises what Reeve and Huxley stated in 1945 (p138), 'One of the most serious difficulties, and often a neglected one, in studies of relative growth, is how to decide whether the growth trend of the data is adequately represented by a straight line on a double logarithmic grid'. They go on to say that workers seem so convinced of '...the universal significance of the simple allometry formula....' that straight lines are fitted when a non-linear equation would clearly provide a superior explanation. Breaks in allometry can sometimes be explained by a curvilinear relationship. Moss and Baer (1956) reported that the relative growth of certain skull bones was best explained by two straight lines with a distinct transition point. Ford and Horn (1959) disagreed; they concluded that the specific growth rates changed

* Cockrem, F.R.M. (1959) Nature 183: 342-343.
Baker, R.L. & F.R.M. Cockrem (1970) Genetics 65: 505-523.

continuously, with no part of the relative growth plot being represented by a straight line, a conclusion reached by Barton and Laird (1969) from a different approach. Most allometric relationships are derived from experiments where the data is collected following cross-sectional slaughter rather than by longitudinal studies on the same individuals. In cross-sectional studies the scatter about the lines is large. Such large, apparently random, variation may obscure the possible systematic deviations occurring. The advent of electronic computers allows the easy calculation and display of all the residuals about the fitted lines, allowing an easy check for departures from linearity or systematic trends. The techniques described by Richards and Kavanagh(1945) for testing for linearity in allometry could also be useful.

The use of total body length as an indicator of the growth of the body as recommended by Acheson, is of doubtful value as it combines tail and body lengths which show fundamental differences in their relationships to age. It has been said that 'It seems unlikely that the growth of the tail in an appreciable way should differ from the growth of the rest of the skeleton' (Reuter, 1976; p380). The above evidence questions this assumption and thus the conclusions reached by Reuter concerning body growth as they were based upon tail lengths. Similarly Mosier (1969, 1971) used tail growth as an index of whole body growth.

The sex differences in growth found before puberty supplement and extend the findings of Chapter 1. The clear sex difference in body length up to 38 days of age, with no difference between the sexes in tail length over this range of ages, seems to indicate that the tail's response to sex hormones differs from that of the other vertebrae. In Chapter 8 a clear inhibition of body length by oestrogen treatment, and a marked growth in length following ovariectomy, will be demonstrated Thus oestrogen would appear to be the hormone inhibiting bone growth in the female rat. The above evidence suggests either that oestrogen does not inhibit bone growth directly, since bone in two sites differs markedly in its response to oestrogen, or that the response of bone to oestrogen is site dependent.

The greatest difference between the sexes was between the body length-age curves; females having a markedly reduced body length, a trend which developed from a small difference at weaning to a large difference before puberty. This inhibition could be due to the pre-pubertal ovary, pre-pubertal ovariectomy followed by daily measurements of body length may settle this point. The small difference in body weight at weaning was maintained to puberty and appeared to increase slightly (the male slope although higher, was non-significantly so). An allometric plot showed that the female did have an inhibition of linear growth relative to growth in body weight: before puberty at a given length the females were heavier than the males.

These results do not support the conclusions reached by Hughes and Tanner (1970, p368)

'Our measurements show little sign of sex dimorphism in shape.'

They reached this conclusion despite a plot of arithmetic tail length against arithmetic nose-rump length revealing clear sex dimorphism. Re-plotting their data, logarithmically, shows sexual dimorphism in the relationships of live weight to nose-rump length, nose-rump length to tail length, and live weight to tail length.

Systematic departures from the fitted lines, describing the general trends, have been shown for linear dimensions in the present chapter, as they were for body weights in the previous chapter. But in the present chapter, due to the more frequent measurements, deviations could be seen to follow 2 patterns,

1) there is what is termed the long-term variation about the lines; i.e. a quadratic curvature for tail length, or the more complex apparently sinusoidal pattern for body weight,
2) there appears to be a regular short-term cyclical pattern of variation with a phase length of about 4 days, for both pre-pubertal males and females, and for both body weights and the linear measurements.

The demonstration of short-term cycles in mean data is surprising, one would expect the cycles of individuals to be out of phase, thus obscuring the cycles. That short-term cycles were found in the mean measurements suggest either that the cycles were related to age or were

due to environmental influences affecting all animals equally. But the live weights from individual Massey animals also showed cycles and the cycles were out of phase in different animals. This questions the environmental explanation of the cycles but does not help explain the synchronous nature of the cycles in the data of Acheson *et al.*, (1959).

Short-term cycles in bone growth have recently been detected by Tam *et al.*, (1974). They showed that fluctuations in the growth rate of bone could be measured in both rats and rabbits following regular multiple injections of radio-labelled tetracycline. The period of the oscillations seemed to be constant, but the period (24 to 36 hours in rats) was much shorter than the period reported herein. Carlson (1977) fitted polynomials to a high order then differentiated, to give the specific growth rate, and he found that the specific growth rate in chickens did not decay uniformly with time as it contained 'bumps'. This evidence questions the traditional view of unimpeded body weight growth which Mitchell (1962, p541) envisages '...the smooth course that growth must pursue, rather than the zig-zag pattern secured by a point-to-point diagram.'

The demonstration that cyclical variations, both long-term and short-term, in body weights and lengths occur, whatever their aetiology, has far-reaching implications for research where weights or dimensions are measured. For example the variability of live-weight measurements in domestic animals is legion (Hughes, 1976). That some of this variation could be cyclical seems to have been considered by few workers.

But sequential daily weighings of cattle have shown that live weight changes follow a cyclical pattern (Maymore and Sircana, 1930, quoted by Baker and Guilbert,1942). Baker and Guilbert weighed seven heifers and one steer on 69 consecutive days using both 12 hour fasting (shrunk weights) and ad libitum (full weights) feeding regimes. They fitted lines to each individual's weights to show that significant runs in the signs of the residuals occurred. For both periods (shrunk and full) significant 'F' ratios were obtained when the ratios of the variances common to individuals and that due to individual differences were compared. Thus in both shrunk and full periods significant systematic oscillations in the residuals occurred.

This work seems to have been misinterpreted by Koch *et al.*, (1958) who state that the cyclical variations in live weight were due to 'An animal that had an above average fill on the first weight day would also tend to have an above average fill on the following weight day.' D. Clark (1974) echoed this interpretation of Baker and Guilbert's results. But Baker and Guilbert found using shrunk weights that although they eliminated some of the variation between days, common to all animals, the pattern of change in individuals remained. Baker and Guilbert did not show that the oscillations were due to rumen fill.

Despite his quoting of the work of Baker and Guilbert, D. Clark (1974) states (p10) '....most of the experimental error is due to random fluctuations in live weight'. However regular fluctuations in the average daily gains of young bulls, weighed every two weeks, are clearly discernable from a figure in Clark's own Appendices (p151).

It may be important that Baker and Guilbert did not control the environmental temperature which in their first experimental period varied from 55^{o}F to 81^{o}F while in the second period the range was 45^{o}F to 69^{o}F. The correlations between the deviations in live weight and the daily range in temperature was significant for the first period but not in the second. The confounding of this effect with the imposition of the treatments (full and shrunk weights) does cloud the interpretation of their results regarding the efficacy of using shrunk weights to reduce the error variance in live weights.

In the present analyses the systematic short-term changes in live weight could be due to variations in food or water intake but the short-term systematic changes in the linear dimensions require a different explanation. The generality of regular fluctuations in growth rate was hypothesised by Huxley (1932, p203) who called the finding a '....curious but possibly important fact'. The author also believes in the generality of such fluctuations. Huxley concluded that the fluctuations in growth rate's ' ....physiological basis is at present quite unexplained.' However if growth is closely monitored and controlled then the classical picture of the controlled variable weaving about the ideal state describes the fluctuations observed.

As Needham (1964, p19) states, with reference to body growth, 'A tendency for physiological processes to oscillate is probably the rule rather than the exception'.

An obvious further analysis would involve fitting a periodic or cyclical regression to the residuals about the general trend. The use of log body weight as the independent variable seems to give the simplest function, a sine curve. But the analysis is complicated by the damped nature of both the amplitude and perhaps the period, which must be estimated. For these reasons, and because of time, this important part of the work was not incorporated into this thesis. However in Chapter 10 the separation of this systematic variation, from the overall scatter about the lines, has been achieved by an analysis of variance. The systematic variation about the lines can also be compared, testing if the swings about the lines are in phase with one another.

But if interest centres on the deviations themselves a mathematical description of them should be found. The maxima and minima of the long-term swings (about the log-reciprocal lines) for individuals could then serve as data for comparison or for regression against independent variables. An obvious choice being a test for the relationship between maxima, about the log-log lines for the Massey animals, and the age of puberty.

Another analysis could involve the fitting of the general trend then a cyclical function to the generated residuals and then by quasi-iterative procedures obtaining the best fit for both equations (see Kendall, 1973). An increased precision in estimating the growth rates (the general trends) should be possible by this method.

But to the author the main thrust of this chapter is the demonstration that valuable insights into the nature of the mammalian growth process can be gained by this simple approach to the analysis of growth curves. The fitting of mathematical functions to the trends in the residuals would allow objective statistical tests of what are presently subjective impressions concerning the pattern of growth. Therefore further refinements of the present techniques and analyses using larger samples of animals (and different strains of animals) should give clearer indications of the factors affecting, and the mechanisms controlling, mammalian live weight growth.

CHAPTER EIGHT

SOME EFFECTS OF OVARIECTOMY AND OESTROGEN TREATMENT ON THE BODY COMPOSITION AND THE ALLOMETRIC RELATIONSHIPS BETWEEN BODY CONSTITUENTS OF THE RAT

INTRODUCTION

The increased body weight (BWt) of the ovariectomised (OvX) laboratory rat has been attributed to the development of obesity (Kennedy, 1969; Wade, 1972; Redick *et al*.1973). This conclusion stems from Stotsenberg (1913) and Slonaker (1930) who both stated, without chemically analysing body composition, that ovariectomised rats are obese. But chemical analysis has produced conflicting results. Compared to intact animals, the weight of body fat in ovariectomised rats, as a percentage of body weight, has been reported as being,

1) increased (De Smet, 1953; Leshner & Collier, 1973)
2) decreased (Galletti & Klopper, 1964)

and 3) unchanged (Reed *et al*.1932; Holt *et al*. 1936; Bogart *et al*. 1944; Nyda *et al*.1948).

Oestrogen is the principal ovarian hormone affecting rat body weight (Tartellin & Gorski, 1971 & 1973). It is a logical progression from the conclusion that ovariectomy increases both body fat and body weight to Kennedy's (1969) statement that in young rats oestrogen treatment will reduce body fat. However the available evidence indicates that doses of oestrogen near the physiological range increase the percentage body fat (Loeb, 1942; Nyda *et al*. 1948; Ebling & Hale, 1966). There is also some *in vitro* biochemical evidence that oestrogen can have a lipogenic effect (Gilmour & McKerns, 1966; Watkins *et al*. 1972). These results obviously require verification as the assertion that ovariectomy is lipogenic and oestrogen treatment lipolytic has been used to support current ideas on the mechanisms controlling energy balance (Wade, 1976).

Holt *et al*. (1936) could show no change in body composition following ovariectomy so they attributed the increased body weight to 'increased growth'. This conclusion has been supported by Tang (1941), Grunt (1964), and Nyda *et al*.(1948) who all noted either increased bone or body lengths in ovariectomised rats. The possibility that an increased body length could explain the increased body weight was not considered by Wade (1972, 1976). However Kennedy (1970) states that the nature of the body weight increase following ovariectomy seems to be governed by the stage in development reached at the time of ovariectomy. In the studies

quoted animals were ovariectomised at widely differing ages, yet no consistent change in body composition with age at ovariectomy is apparent. This inconsistency could also result from differences in the age at slaughter, and the interval between ovariectomy and slaughter, in these studies. So the present experiment was designed to study the effects of both age at ovariectomy and age at slaughter by ovariectomising rats at 3 ages and slaughtering them at 4 ages.

The large between-animal variability apparent in body composition data seems to be due to real differences between animals rather than to errors from the analytical techniques employed. Thus in the present study approximately 30 animals were allocated to each ovariectomised group. However traditional techniques for the chemical analysis of body constituents, although accurate, are slow, as well as possessing other disadvantages. The large number of chemical analyses this experiment entailed necessitated the development of rapid procedures for both processing and analysing the tissues (see the Addendum to this Chapter).

The relative growth of body parts has been successfully analysed by allometry. In the rat Kennedy (1950) obtained a linear relationship between log body weight and log body length. Changes in this relationship are said to be gradual following weaning (Freudenberger, 1932). The use of length as the independent variable for the analysis of body composition avoids several statistical and practical difficulties implicit in the use of body weight as the independent variable, or the denominator in ratios or percentages. The statistical validity of allometry has been questioned, which could account for the lack of information on the shape or form of the body following ovariectomy or oestrogen treatment. Allometry allows, for example, changes in the body length to body weight relationship and the increased skin size following ovariectomy, found by Reed *et.al.* (1932), to be investigated. The consequences of these changes, particularly regarding their effect on heat loss, will be discussed.

TABLE 8.1

EXPERIMENTAL DESIGN

AGE AT SLAUGHTER (weeks)	7	9	12	15
TREATMENT	RATS / GROUP			
Control	6+	8	8	12
OvX W4	8 C	7	8	9
OvX D2	6*	8	8	8
OvX W7		7 B	7	6
OvX + EB¢			7 A	7

A = Groups included in Analysis A

B = Groups included in Analysis B

C = Groups included in Analysis C

\+ = processed tissues lost for two rats

* = processed tissues lost for group

¢ = Oestradiol Benzoate 2½µg/day

MATERIALS AND METHODS

The husbandry, treatment, and other details relevant to the rats used in this experiment are given in Chapter 1. The experimental design, including the number of animals in each treatment and slaughter group, is shown in Table 8.1. Many of the carcasses and skins from animals slaughtered at week 7 were accidentally destroyed during preparation. Other discrepancies between the numbers of animals in Tables 8.1 and 8.3 are due to the measurements not being recorded from some animals.

Before slaughter, whilst the rats were deeply anaesthetised, nose-anal lengths were measured by the author, in triplicate, using the following technique. The rat was laid upon its back, on a ruler, with its nose at the zero mark. A small amount of backward pressure was exerted on the tail and a pin was placed alongside the anus and the measurement obtained. Rats were then jugulated, the skin (including the paws) was removed (from the tail, body, and the head) and immediately weighed. The inguinal, retroperitoneal, and gonadal fat depots were dissected free, blotted, weighed, and stored separately at -30°C in Tyrodes Ringer. For animals slaughtered at week 9 omental and mesenteric depots were also separately analysed; at the other slaughter times these depots remained with the gut and were analysed as part of the carcass. The gut was emptied of its contents and weighed. The carcass, including the head and tail, was weighed and then combined with the gut and all the organs except the pituitary and the liver.

The skin and carcass were separately processed and analysed by a method developed by the author. The details of this method are given in the Addendum to this Chapter. Briefly, this method involves homogenising, freeze-drying, oven-drying, weighing (to obtain the dry matter), and then grinding before storage in air tight bottles. Lipids were extracted by a methanol: water: chloroform procedure. Ash was determined by firing triplicate aliquots in a muffle furnace. 'Protein' was estimated as the difference between the dry matter and the fat plus the ash. The validity of this estimation was verified by prior experiment (see the Addendum).

Individual fat depots were analysed separately by techniques similar to those used in the skin and carcass analyses. In the depots protein was assumed to be the difference between dry matter and lipid weights. For the present analyses wet weights and component weights for each depot were added to either the carcass (gonadal, retroperitoneal, omental, and mesenteric) or the skin weights (the inguinal depot).

BIOMETRICAL CONSIDERATIONS

In Chapter One a log transformation of rat body weight removed heteroscedascity. Similarly the tissue component weights for the skin, carcass, and whole body were transformed to logarithms (log) because the means and standard deviations were strongly correlated on the original scale, but were unrelated or had a greatly reduced correlation coefficient on a transformed two parameter log scale (Table 8.2). Skin ash weights were below 1.0 g so 1.1 was added to all these weights before taking logarithms to avoid negative logarithms. On the original scale mean nose-anal lengths and standard deviations were unrelated.

The convenient methods used in Chapter 1 for the analysis of body weight could not be used for body lengths or tissue weights as they were measured only once in each individual, at slaughter. The analysis of this data was complicated by imbalance in both the number of classes and the number of observations within the classes (Table 8.1). As the number of classes was unbalanced variables were analysed in 3 separate 2 way analyses of variance, where, due to unequal subclass numbers, the method of fitting constants by least squares was employed. Significant mean squares were then subdivided to give comparisons based on one degree of freedom. It is evident from Table 8.1 that some subclasses are included in more than one analysis, which would increase the chances of non-significant effects being described as significant. However if comparable analyses were made effects were only described as being statistically significant if the same significant effects were demonstrated in each analysis. The analyses of variance and followup comparisons are more fully described in Chapter Ten.

TABLE 8.2

Carcass, skin, and whole body constituent weights: correlations between means and standard deviations on arithmetic and logarithmic metameters

	Correlation Coefficients (r)					
Constituent	Total	Water	Dry Matter	Fat	Ash	Protein
Carcass						
Arith.	0.578*	0.771***	0.429^{+}	0.822***	0.697**	0.575*
Log.	-0.047	0.113	-0.162	0.408	0.543*	-0.076
Skin						
Arith.	0.677**	0.718**	0.651**	0.762***	0.664**	0.646**
Log.	0.2	0.216	0.149	0.111	0.129	0.175
Whole Body						
Arith.	0.649**	0.735**	0.607*	0.778***	0.659**	0.630**
Log.	0.13	0.172	0.076	0.226	0.131	0.085

$^{+}$ $0.05 > P < 0.10$

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

PERCENTAGE COMPOSITION

Body composition is frequently expressed as a percentage, or as weights per 100 g of body weight. However these expressions involve the use of the ratio of two variables which may themselves be normally distributed, but their ratio has a distribution which may be difficult to transform to normality. So unless a normalising metameter is found neither a mean nor a variance can justifiably be calculated for a number of ratios. This makes one value of the ratio as good an estimate as several values and makes comparisons between groups impossible. As body component weights were lognormally distributed an attempt to express the data as percentages would actually give a difference, rather than a ratio.

Skin and carcass composition was initially analysed using the untransformed data expressed as percentages. However this method of analysis, besides being statistically unsound, had practical disadvantages. For example, if the skin and the carcass components were expresssed as a percentage of whole body weight it was difficult to decide if the skin, or the carcass component, was proportionately normal in size. As the skin and carcass made up the whole a change in one would be reflected, on a percentage basis, in the other. Similar reasoning applies to all compositional analyses where a change in one component will, on a percentage basis, affect all other components.

The analysis of body composition using percentages can be challenged both statistically and practically. However percentages are the commonest mode of expressing such data so percentage results are given to allow comparison with other similar studies.

When percentage carcass composition results are presented it is usual to simply state that differences between groups were due to components being increased, decreased, or not significantly altered. That these differences could be real changes (due to a real physiological effect) or passive changes (artifacts due to another part changing) seems to have been ignored by many investigators.

Consider the case where the absolute weight of a component (W) increases while the absolute weights of all other components (X,Y, and

Z) are unchanged. On a percentage basis the % of W would increase while for X,Y, and Z a decline in % would occur. If the percentage change in W is small then X,Y, and Z may all decline, but perhaps by a statistically non-significant amount. However if less error is associated with X than Y or Z then a statistically significant difference may be shown only for X, despite X,Y, and Z all being decreased by an equal amount. Thus careful study of the relative sizes of the errors, as well as the directions of the changes in composition, should precede any attempts at definitive statements on the nature of changes in percentage composition.

When real percentage changes occur in two or more components the difficulties of interpretation increase. But if a real change in composition is due to only one component (W) then a percentage analysis calculated following the exclusion of that component (W) would show no differences between the remaining components (X,Y, and Z). A practical example of this technique is where Elsley *et al*. (1964), in their re-analysis and re-interpretation of the data collected by McMeekan (1940) and Palsson and Verges (1952), compared compositions on a fat-free basis to show that changes in fat were the major cause of changes in composition due to differing planes of nutrition. So in the present study components were expressed and analysed as % wet weight, % dry weight, and as a % of the wet fat-free weight.

An example from Table 8.5 where the carcasses of ovariectomised and control rats are compared in Analysis B, illustrates the advantages in interpretation gained by these modes of expressing the data. The % carcass wet weights show % water to be increased and % fat and % ash to be decreased by ovariectomy. From the means (Appendix Table 8.14) the % protein was also decreased but the difference failed to reach statistical significance. One could conclude that the only real change was in the % water, while all the other components were passively decreased. This conclusion can be tested from the % dry weight analysis where water is excluded; the conclusion is rejected since the % fat and % ash still show a decrease in ovariectomised rats. But on a dry basis the % protein was increased, while on a wet basis the % protein was reduced because on a dry basis, fat, ash, and protein make up the whole, a decrease in both the % fat and % ash must increase the

% protein. Thus it seems established that the fat and the ash show real % decreases following ovariectomy, while the % protein is passively changing. If the fat is now excluded from the analysis then the relatively small real decrease in ash should result in a small increase in both protein and water; if both the protein and the water are passively changing. However on a fat-free basis the % protein was decreased while the % water was increased. This indicates that following ovariectomy the % water shows a real change which is large enough to cause the % protein to show a passive decrease.

ALLOMETRY

Allometry is a useful method for the expression of compositional data as it avoids both the practical and the statistical objections inherent to the use of percentages or ratios. Although allometry has been subject to criticism (Richards & Kavanagh, 1945; Sholl, 1954), due to both the independent and dependent variables of the regression being subject to error, it can be mathematically justified (Berkson, 1950; Lindley, 1953). Using the mathematical model underlying regression analysis, that errors are independent of true values, there is no satisfactory estimate of the relationship between two variables both subject to error, unless the errors are known or similar knowledge is available. Berkson (1950) found that the simplicity of the problem was contradicted by the complexity of the solutions so he proposed a new model, providing a simple solution, which was justified mathematically by Lindley (1953). Berkson's model assumes that random errors are independent of the observed values, rather than the true values. This assumption, which allows the usual least squares solution, greatly extends the applicability of regression techniques. In the present case it allows the solution by least squares of the allometric equation,

$$\log y = \log b + k \log x$$

to be justified.

Bliss (1967) argues that '...the size of both the part and the whole, or of two different parts of an organism, are subject to

similar errors'. However nose-anal length measurements are subject to a small error (Hughes & Tanner, 1970), which would be expected to be less than, and independent of, the errors for the component weights. So pairs of measurements from individuals were regressed with log nose-anal length being the independent variable and log weight the dependent variable. For each variable the heterogeneity of the variance was tested using the ratios of the scatter about the regression lines ($S^2_{max}/\Sigma S_i^2$). A common error variance about the lines could then be assumed and lines could be combined for comparison. An analysis of variance for replicated regressions in a one way classification (Bliss, 1967) separated the variation due to position, non-parallelism, and error.

Differences in slope between treatment groups were analysed using as the error term a function of the error mean square (EMS) of the analysis of variance,

$$S_b = \sqrt{EMS} \;/\; \sqrt{(x^2)}$$

Testing for differences due to position is more difficult than testing for differences in slope. One would expect that differences in slope would be accompanied by differences in position, but this is not always the case (see carcass protein, Appendix Table 8.32). Also a difference in position may not be detected if only the constant (when x equals unity - in the logarithmic case) is tested. This is because the error (S_a) at a position (x) is,

$$S_a = \sqrt{EMS} \times \sqrt{1/n + ((x-\bar{x})^2 \;/\; (x^2))}$$

so that the error varies with the distance from the mean value, and will be minimal when $x = \bar{x}$ and increases to the constant. So one would expect differences between lines to be more easily detected at the mean value than at the constant. But this is not always the case. If two lines intersect near their mean values of x then they may still differ in position at their constants, but this would seem to be of little biological interest (Sholl, 1954) as in the measured or sensible range the lines may not differ in position. Testing for differences in position solely at the mean, rather than at the constant, is argued against by both the above situation and by the present case where the lines differed in their mean values of x. Lines were tested for differences in their constants, using the above error term. In

addition 95% confidence limits were computed for values of x from 16 to 25 cm (the observed range of body lengths) and plotted graphically to provide a pictorial analysis and to overcome the difficulties mentioned above.

With nose-anal length as the independent variable allometry asks, would there be a difference in composition at a given length?; while percentage analysis enquires, would there be a difference in composition at a given body weight? Each method of analysis shows effects the other cannot or does not show. One could regress log component weights and log BWt, but these pairs of variables are subject to similar errors which are not independent. In addition the component parts are included in the body weights. Thus the advice of Reeve & Huxley (1945, p.138) was heeded '.....to plot a part against another dimension which includes that part will always tend to obscure a change of proportions, and should generally be avoided'.

TABLE 8.3

Nose-anal length analyses

Treatment	Main Class Means No.	Length (cm)	Source of variation	d.f.	F value
Analysis A					
			Treatment	4	12.115***
OvX D2	16	22.028	1. All OvX vs C + EB	1	32.47 ***
OvX W4	17	22.879	2. C vs EB	1	0.00
OvX W7	13	22.588	3. OvX D2 vs OvX W7	1	6.327*
Control (C)	20	21.799	4. OvX W4 vs OvX D2 + OvX W7	1	8.049**
Oestrogen (EB)	14	21.689	Residual Mean Square	70	0.373
Analysis B			Treatment	3	20.893***
OvX D2	24	21.181	1. All OvX vs C	1	33.051***
OvX W4	24	22.156	2. OvX D2 vs OvX W7	1	16.275***
OvX W7	20	21.818	3. OvX W4 vs OvX D2 + OvX W7	1	12.758***
Control (C)	28	21.14	Residual Mean Square	84	0.312
Analysis C			Treatment	2	19.231***
OvX D2	29	20.495	1. All OvX vs C	1	15.48 ***
OvX W4	31	21.224	2. OvX D2 vs OvX W4	1	22.218***
Control (C)	32	20.523	Residual Mean Square	80	0.374

* P <0.05

** P <0.01

*** P <0.001

RESULTS

BODY WEIGHT

As each rat was weighed at weekly intervals the log of body weight and the reciprocal of age could be regressed to obtain a linear equation. Slopes (rates of gain) and constants (ultimate body weights) were then analysed as data. The results for body weight are described and discussed in Chapter 1.

NOSE-ANAL LENGTH

The means and the analyses of variance are given in the Appendices (Tables 8.28, 8.29, & 8.30). A summary, of the main class means, analyses of variance, and the comparisons, is given in Text Table 8.3. Only the results for the treatment main effects and follow-up comparisons are presented in the text as no significant interactions were found. The effect of age was, as expected, statistically highly significant.

Ovariectomised animals showed an increased length compared to intact animals (Analyses B & C) and compared to intact plus oestrogen treated animals (Analysis A). D2 OvX rats were shorter than OvX W7 animals (Analyses A & B). OvX W4 rats were longer than OvX D2 rats (Analysis C) and OvX D2 plus OvX W7 animals (Analyses A & B). Oestrogen treatment reduced the nose-anal length of OvX W7 rats to a value no different from that of intacts (Analysis A).

CONSTITUENT WEIGHTS

The sub-class and main-class means and the analyses of variance with follow-up comparisons are given in the Appendices for Analysis A (Tables 8.1, 8.2, 8.3), Analysis B (Tables 8.4, 8.5, 8.6), and Analysis C (Tables 8.7, 8.8, 8.9) for the skin, carcass, and the whole body respectively.

TABLE 8.4

Statistically significant (P at least <0.05) effects of treatment and age on log constituent weights.

	SKIN						CARCASS						WHOLE BODY					
	WET	W	DM	F	A	P	WET	W	DM	F	A	P	WET	W	DM	F	A	P
ANALYSIS A																		
OvX vs C + EB	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
EB vs C										+								
OvX (D2 vs W7)							-		-	-	-	-			-		-	-
OvX (W4 vs (D2+W7))			+			+												
ANALYSIS B																		
OvX vs C	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
							I	I				I						I
Ovx (D2 vs W7)	-	-				-	-	-	-	-	-	-	-	-	-		-	-
		D																
OvX (W4 vs (D2+W7))						+	+	+	+			+	+	+	+			+
LINEAR AGE	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
QUADRATIC AGE	↑	↑			↑	↑	↑	↑	↑		↑	↑	↑	↑	↑		↑	↑
ANALYSIS C																		
OvX W4 vs C	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
	I	I	I	I	I	I	I	I	I			I	I	I	I	l		I
LINEAR AGE	↑	↑	↑	↑	↑	↑	↑	↑	↑		↑	↑	↑	↑	↑	↑	↑	↑
QUADRATIC AGE	↑	↑	↑		↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑

KEY

+ = first mentioned treatment is the greater

- = first mentioned treatment is the smaller

I = significant interaction, difference increasing with age

D = significant interaction, difference decreasing with age

↑ = linear increase with age or positive quadratic effect of age

WET = WET WEIGHT, W = WATER, DM = DRY MATTER, F = FAT, A = ASH, and P = PROTEIN

EFFECT OF TREATMENT

The analyses of variance partitioned the variation into that due to treatment, age, and their interaction. However the effect of age is considered to be of lesser importance than the interactions. The interactions however, were mainly due to treatment differences becoming greater with age. Table 8.4 summarises the effects of treatment and age on body constituent weights.

Following ovariectomy the weights of all skin, carcass, and whole body components were significantly increased, compared to intact and intact plus oestrogen treated animals, with one notable exception; in all three analyses the weight of carcass fat remained unaffected by ovariectomy. Oestrogen treatment of OvX W7 rats resulted in all the component weights of skin, carcass, and whole body being similar to those of intacts, with one exception, the weight of carcass fat being significantly increased ($P < .01$) by oestrogen treatment. Conversely one could state that oestrogen treatment of ovariectomised animals reduced the weight of all body components except carcass fat. The weight of skin fat in oestrogen treated animals was indistinguishable from that of intacts. Whole body fat appeared to be increased by oestrogen, but the high variance associated with the fat weights probably masked the difference, as it only attained significance at the 10% level.

The differences in live weight due to age at ovariectomy (Chapter 1) were accompanied by differences between body components. D2 OvX rats had a lower live weight than OvX W7 rats. This was due to all the whole body component weights, except that of fat, being smaller in D2 OvX animals. In the carcass all components were significantly smaller in D2 OvX rats. The skin wet weight of OvX D2 rats was smaller than that of OvX W7 rats due to a reduction in the water and protein. This effect, of only the water and protein being altered, was repeated in the OvX W4 vs OvX D2 + OvX W7 comparison. Here the live weight difference in favour of OvX W4 animals was reflected in a significantly greater weight of whole body water and protein in the OvX W4 rats. The carcass of OvX W4 animals was heavier than that of D2 OvX + W7 OvX animals, the difference being due to water and protein. The skin of W4 OvX rats contained a greater weight of protein than did OvX D2 &

OvX W7 rats, yet the overall skin weights did not significantly differ.

EFFECT OF AGE AT SLAUGHTER

Table 8.4 indicates that for the skin the wet, water, dry matter, ash, and protein weights, expressed as logarithms, increased with age due to significant linear and quadratic effects. However log skin fat increased linearly with age, the quadratic effect being non-significant. A similar effect occurred in the carcass, but carcass fat increased with a small, but significant (Analysis C, $P < .01$), quadratic effect. In Analysis B carcass, skin, and whole body fat all increased linearly, while in the other components the quadratic effect was also highly significant. Nose-anal length increased with age with both linear and quadratic effects being significant. However arithmetic rather than log length was analysed.

INTERACTIONS

Oestrogen treatment for 5 weeks may have caused a greater accumulation of carcass fat than treatment for 2 weeks, as the interaction for the control *vs* oestrogen comparison reached significance at the 10% level.

From Analysis B a significant interaction ($P < .05$) for skin water indicated that at week 9 the OvX W7 animals contained a larger amount of skin water than at weeks 12 and 15, compared to OvX D2 rats.

There were many other significant interactions which, without exception, showed that the differences between treatments increased linearly with age at slaughter (or as body weight increased).

FIGURE 8.1

ALLOMETRY FOR WET WEIGHTS

ALLOMETRIC REGRESSIONS OF LOG WET WEIGHTS UPON LOG NOSE-ANAL LENGTHS,
WITH 95% CONFIDENCE LIMITS FOR EACH REGRESSION.

(SHORT CROSS BARS, INTACTS; LONG CROSS BARS; OVARIECTOMISED).

——— = INTACT + EB TREATED RATS (N = 46)

– – – – – = OVARIECTOMISED RATS (N = 76)

(NOTE: SKIN - BOTTOM AND RIGHT HAND AXES; CARCASS AND WHOLE BODY - TOP AND LEFT HAND AXES)

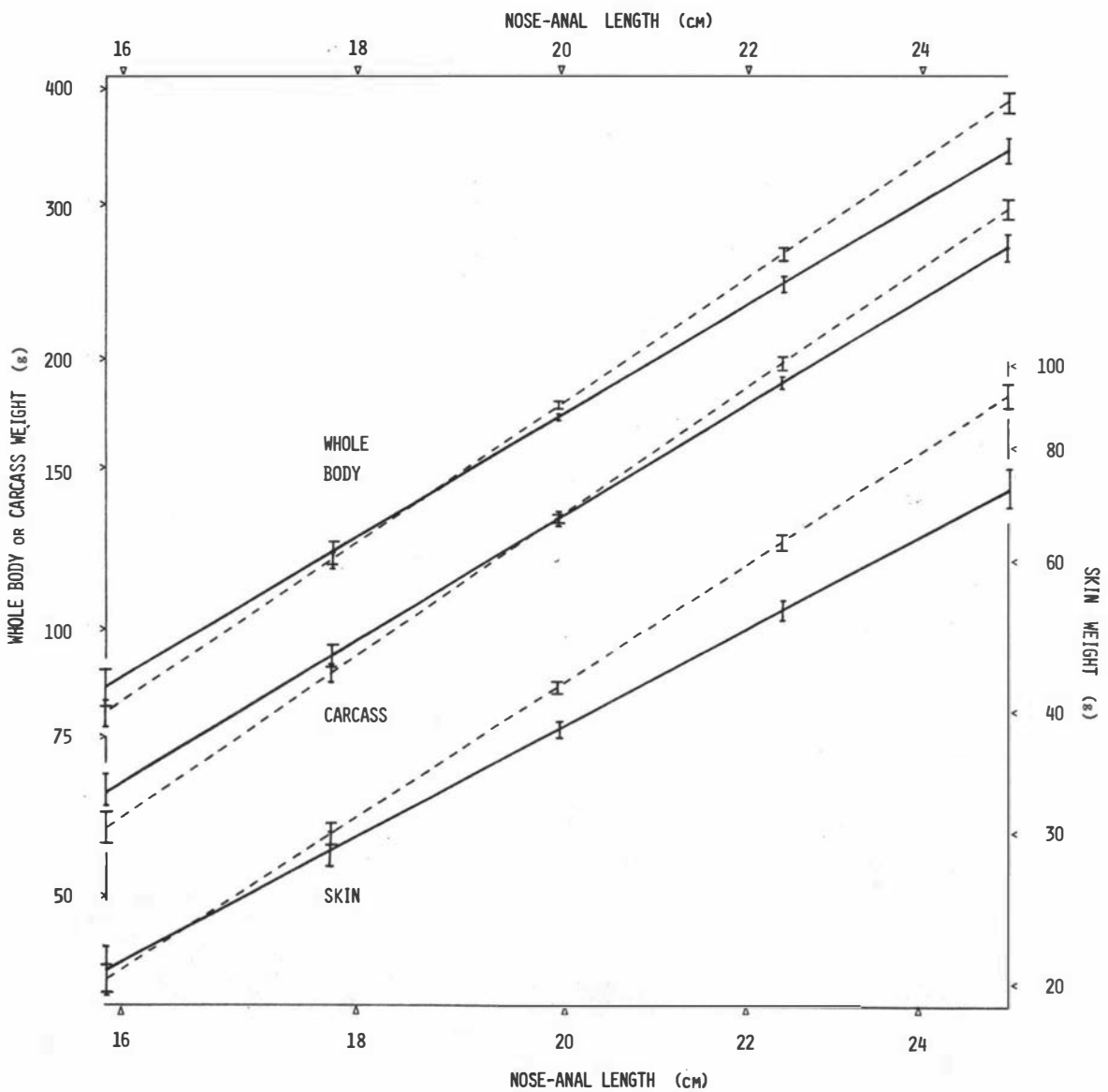


FIGURE 8.2

ALLOMETRY FOR THE SKIN

ALLOMETRIC REGRESSIONS OF LOG SKIN CONSTITUENT WEIGHTS UPON
LOG NOSE-ANAL LENGTHS, WITH 95% CONFIDENCE LIMITS FOR EACH REGRESSION
(SHORT CROSS BARS, INTACTS; LONG CROSS BARS OVARIECTOMISED).

——— = INTACT + EB TREATED RATS (N = 46)

– – – – – = OVARIECTOMISED RATS (N = 76)

(NOTE: FAT - BOTTOM AND RIGHT AXES; WATER, DRY MATTER, PROTEIN - TOP AND LEFT HAND AXES).

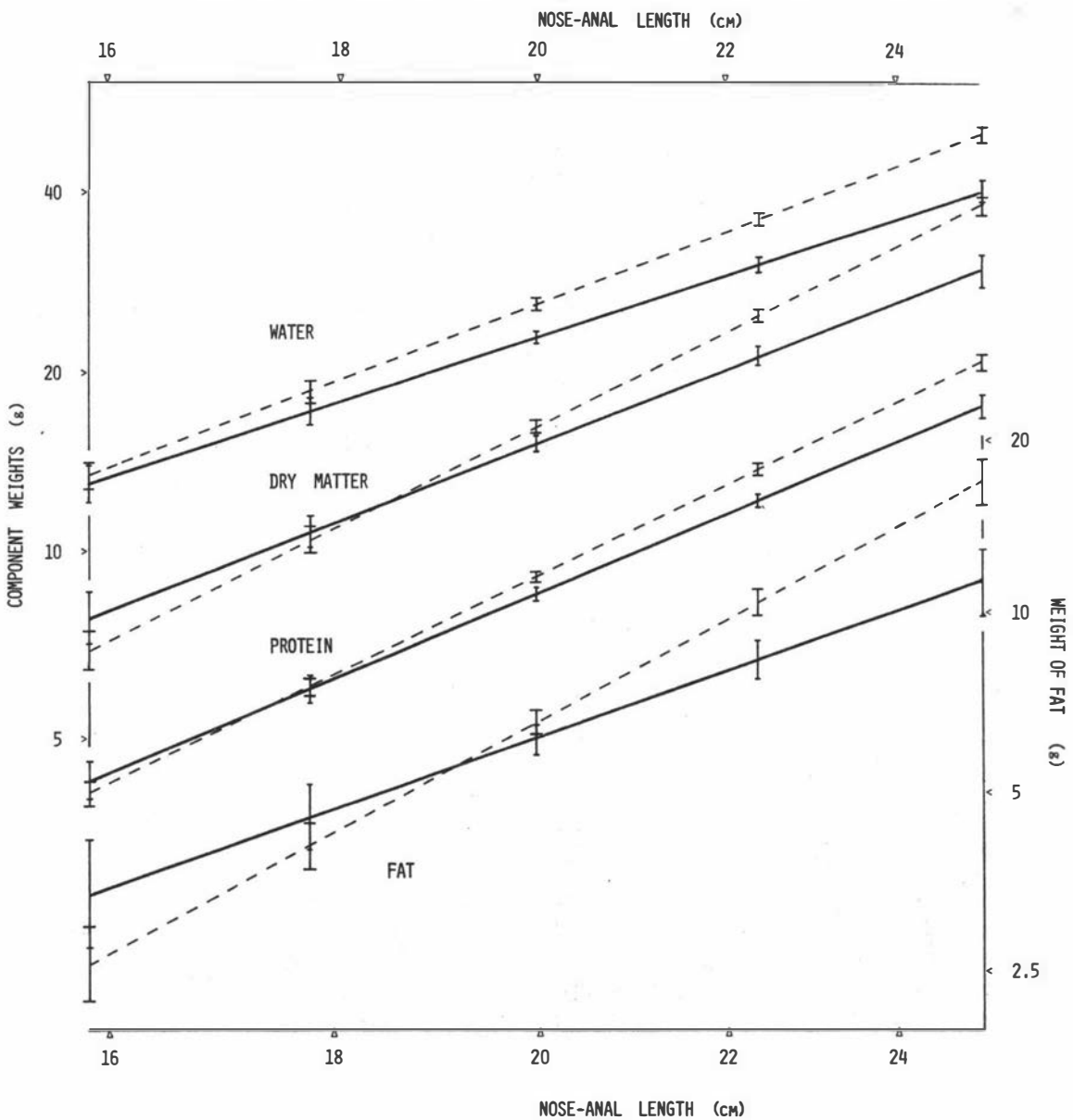


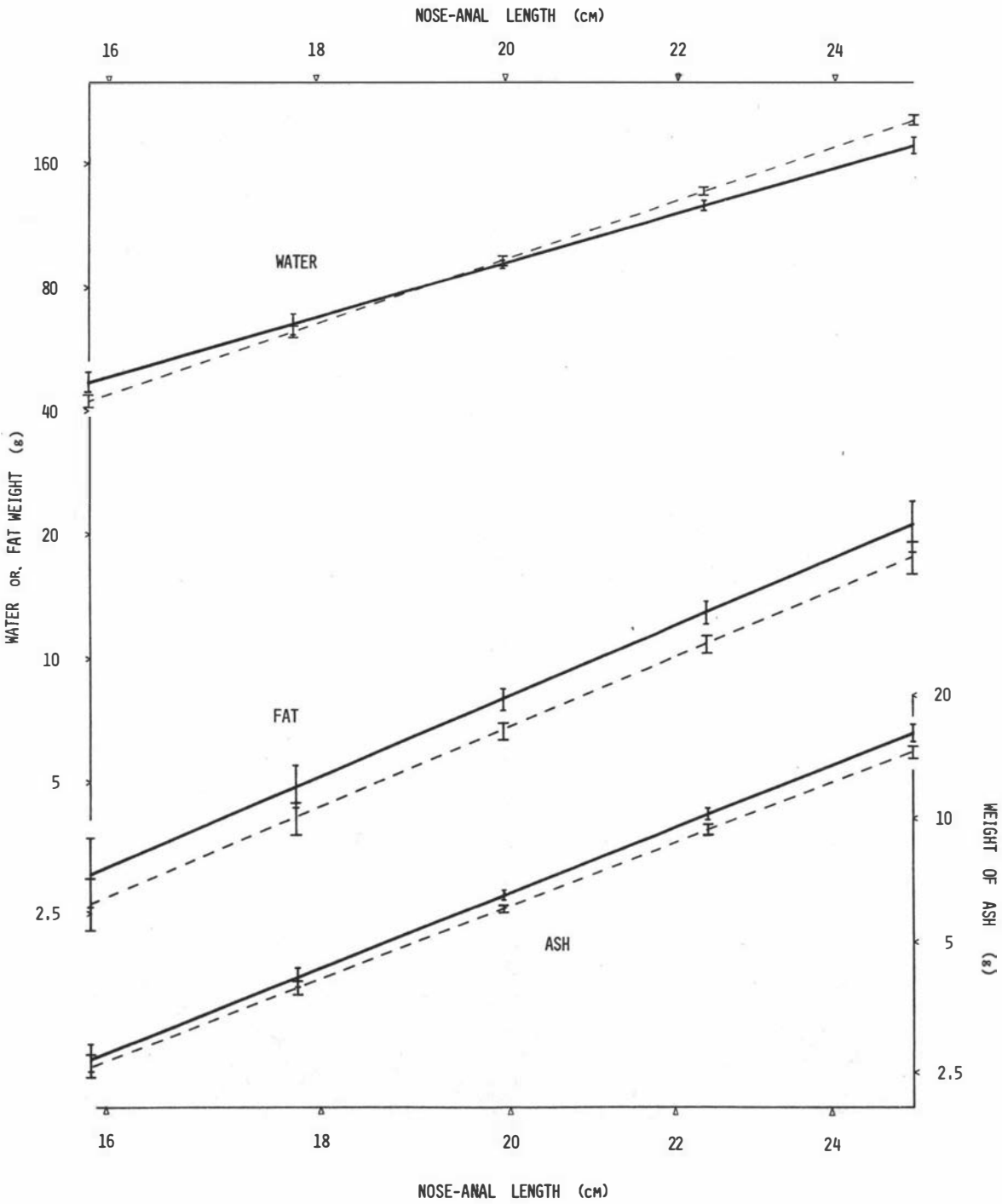
FIGURE 8.3

ALLOMETRY FOR THE CARCASS

ALLOMETRIC REGRESSIONS OF LOG CARCASS CONSTITUENT WEIGHTS UPON LOG NOSE-ANAL LENGTHS, WITH 95% CONFIDENCE LIMITS FOR EACH REGRESSION (SHORT CROSS BARS, INTACTS; LONG CROSS BARS, OVARIECTOMISED).

——— = INTACT + EB TREATED RATS (N = 46)

– – – – – = OVARIECTOMISED RATS (N = 76)

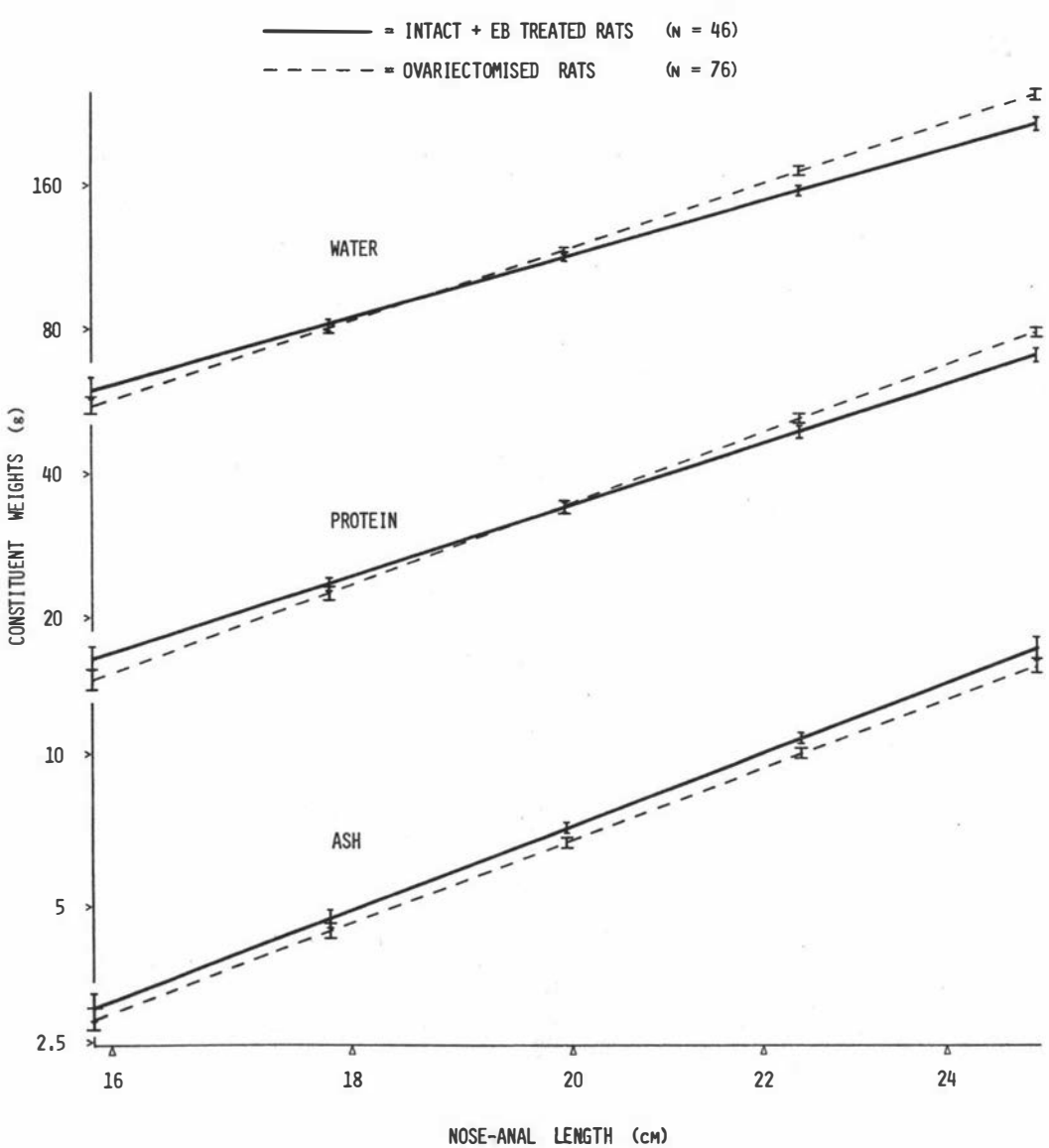


(NOTE: ASH - BOTTOM AND RIGHT AXES; WATER AND FAT - TOP AND LEFT HAND AXES).

FIGURE 8.4

ALLOMETRY FOR THE WHOLE BODY

ALLOMETRIC REGRESSIONS OF LOG WHOLE BODY CONSTITUENT WEIGHTS UPON LOG NOSE-ANAL LENGTHS, WITH 95% CONFIDENCE LIMITS FOR EACH REGRESSION (SHORT CROSS BARS, INTACTS; LONG CROSS BARS, OVARIECTOMISED).



ALLOMETRY

The equations, for the log body component weights regressed against log nose-anal lengths, and the analyses of variance comparing the slopes and constants, are shown in Appendix Tables 8.31 (skin), 8.32 (carcass), and 8.33 (whole body). The statistically significant and biologically important effects are illustrated for the ovariectomised vs intact + EB comparison in Figures 8.1 (wet weights), 8.2 (skin), 8.3 (carcass), and 8.4 (whole body).

It is clear from Fig. 8.1 that over the observed range of body lengths the wet weights of skin, carcass, and whole body of ovariectomised rats are relatively larger than those from intact and EB treated animals of comparable length. This was numerically confirmed when both the slopes and the constants were significantly different for each of these tissues. The skin wet weights of ovariectomised rats appear to increase, relative to those of intact and EB treated rats, before the wet weights of the carcass (Figure 8.1).

Skin water, dry matter, fat, and protein were also greater for ovariectomised animals, compared to intact and EB treated animals, due to both slopes and constants differing (Figure 8.2). The change in skin water appears to occur at a shorter body length, and thus at an earlier age, than does the change in dry matter (Figure 8.2). Skin protein may also change before skin fat. Skin ash was unaffected by treatment.

Age at ovariectomy also affected body form. Allometry showed skin wet weights of OvX D2 animals to have a higher slope than W7 OvX rats, giving a significant difference in position. But for the carcass wet weights the effect of age at ovariectomy was reversed, as the slope for W7 OvX animals was higher than that for D2 OvX animals (although the difference only approached significance, $P < .10$) giving a significant difference in position over the measured range of lengths. The difference in wet skin weight was due to skin water but the change

in carcass wet weight was probably due to changes in dry matter and protein, although these changes only approached statistical significance. The opposite nature of the effects on carcass and skin is illustrated by age at ovariectomy not affecting any whole body parameter.

Only one significant difference between the relative growth rates of EB and intact groups' components was shown; carcass fat being relatively increased by EB. The results suggest differences between other components which may have reached significance if either a greater range of length measurements, or numbers of rats, had been used.

The increased relative wet carcass size of ovariectomised rats was accompanied by an increased slope and constant for water, and an increased slope, but no change in position (by analysis of variance) for protein (Fig 8.3). This situation of a difference in slope with no associated significant change in position, although difficult to interpret, could indicate no real difference, in the measured range, between the regression lines. In contrast to water and protein the fat and ash were relatively greater in intact and EB groups due to the lines differing in position but being parallel (Fig 8.3).

Whole body allometry (Fig 8.4) reflected the changes in skin and carcass. Whole body water and protein were relatively increased by ovariectomy, slopes and constants both being increased. Whole body fat was unaltered by treatment but intact and EB rats again contained more ash at a given length due to their positions, rather than their slopes, differing.

PERCENTAGE COMPOSITION

The sub-class means, main-class means, the analyses of variance, and the follow-up comparisons (for individual degrees of freedom) are presented for the % wet weights and % dry weights in Appendix Tables 8.10 to 8.18 and for the % fat-free weights in Appendix Tables 8.19 to 8.27 for Analyses A, B, & C and for the skin, carcass, and the whole body. A summary of this data is presented in Text Table 8.5 for skin,

TABLE 8.5

STATISTICALLY SIGNIFICANT (P at least <0.05) EFFECTS OF TREATMENT AND AGE ON PERCENTAGE COMPOSITION

	SKIN									CARCASS									WHOLE BODY									% SKIN WHOLE BODY					
	% WET				% DRY			% F-F			% WET				% DRY			% F-F			% WET				% DRY			% F-F					
	W	F	A	P	F	A	P	W	A	P	W	F	A	P	F	A	P	W	A	P	W	F	A	P	F	A	P	W	A	P	WET	W	D	F	A	P
ANALYSIS A																																				
OvX vs C + EB				-				+		-	+	-	-		-		+	+	-	-	+	-	-			-	+	+	-	-	+	+	+	+	+	+
																			I										I							
EB vs C	-	+	+	+	+		-	-	+	+	-	+			+		-		+		-	+			+		-		+			-		-		
	I	I	I		I		I		I		I	I			I		I				I	I			I		I		I			I				
OvX (D2 vs W7)											+	-			-		+														+		+		-		
																																			D		
ANALYSIS B																																				
OvX vs C	+		-	-	+		-	+	-	-	+	-	-		-	-	+	+	-	-	+		-			-		+	-	-	+	+	+	+		+
	D							D		D			I																								
OvX (D2 vs W7)		+			+		-	+		-		-			-		+			-								+		-	+	-	+	+		-
								D		D										D									D		D	D		D		D
LINEAR AGE	↓	↑		↑		↓		↓		↑	↓	↑	↑	↑				↓	↑		↓	↑	↑	↑				↓	↑	↑	↓	↓	↓	↓	↓	↓
QUADRATIC AGE	↑	↓		↑	↓		↑														↑	↓		↑	↓		↑									
ANALYSIS C																																				
OvX W4 vs C	+	-	-		+	-	-		-		+	-	-			-	+	+	-	-	+		-			-		+	-	-	+	+	+	+		+
	D	D			I		I						I						I	I			I			I			I				I	I			
LINEAR AGE	↓	↑		↑		↓		↓		↑	↓	↑	↑	↑			↓	↓	↑	↑	↓	↑	↑	↑				↓	↑	↑	↓	↓	↓		↓	↓	
QUADRATIC AGE				↑	↓																													↓			

KEY (symbols in addition to those of 8.4)

↓ = linear decrease with age, negative quadratic effect with age

% WET = % of WET WEIGHT, % DRY = % of DRY WEIGHT, % F-F = % of FAT-FREE WEIGHT, and D = DRY MATTER

carcass, and whole body weights expressed as % wet weight, % dry weight, and as a % of the fat-free weight. All differences shown in Table 8.5 were statistically significant at the 5% level of probability or better (see the Appendices for the actual 'F' values). The results will be interpreted using the technique of sequential omission of components that was described in Biometrical Considerations.

SKIN

Analyses B & C show that following ovariectomy, on a wet basis, water was increased but fat, ash, and protein decreased; the exclusion of water showed ash and protein to again decrease while fat was increased. The exclusion of fat gave results comparable to those calculated on a wet basis. The significant interactions for water and fat as % wet weight in Analyses B & C, were reflected in Analysis A where only the decrease in protein reached significance. Thus following ovariectomy % skin fat passively changed while % ash and protein were decreased compared to intacts and the % water was increased.

On a wet basis EB treatment decreased % water and increased fat, ash, and protein percentages, but the exclusion of water caused the % protein to decrease while the % fat increased with ash being unchanged. Excluding the fat gave results comparable to those obtained on a wet basis. The % dry results indicate that the increase in fat, and possibly the increase in ash, on a wet basis, were real effects; the protein seemed to passively change while the % water showed a real decrease. The interactions between age and treatment for the EB *vs* intact comparison indicate that the changes in skin composition are obvious only after prolonged EB treatment.

Ovariectomy at D2 increased skin fat, compared to OvX W7 rats on a wet and a dry basis. Although it is difficult to interpret the changes in protein and water it seems that if the change in protein was a real effect then it would be exaggerated in the % wet comparison due to the increase in % fat. Thus it is probable that the increase

in water is a real effect, as is the increase in fat, while protein changed passively.

CARCASS

The changes in carcass composition following ovariectomy were discussed in Biometrical Considerations and were shown to be due to % water increasing, % fat and % ash decreasing, and to % protein changing in a passive manner.

On a wet basis, compared to intacts, EB treated rats showed a reduced % water and an increased % fat; after excluding water the % fat was again increased while the % protein was now decreased; excluding fat showed only the % ash to be increased. Taken together these findings suggest that EB gave a real increase in the % fat and % ash of the carcass while the % protein and % water changed passively. The interactions exhibited suggest these effects are greatest after 5 weeks EB treatment.

Ovariectomy at D2 caused a real decrease in the % fat, and possibly a real decrease in the % protein, compared to OvX W7 rats.

WHOLE BODY

Table 8.5 shows that whole body composition is closely allied to carcass composition. The carcass comprises about 80% of the whole body so changes in the carcass composition would be expected to influence the whole body composition more than changes in the skin. So ovariectomised rats, compared to intacts, showed an increased % water and a decreased % ash. Fat was only decreased in Analysis A where the inclusion of the EB treated rats was probably influential. It may be recalled that in ovariectomised rats carcass fat showed a real decrease while skin fat passively increased. Thus whole body fat was not affected by ovariectomy. Again protein seemed to change passively.

The EB *vs* intact comparison gave identical results to those for the carcass (% fat and % ash increasing, % water and % protein changing passively).

D2 OvX rats showed an increased % skin fat but a decreased % carcass fat, compared to OvX W7 rats. Thus for the whole body Analysis A showed no change in any components, while Analysis B revealed, on a fat-free basis, that for the D2 OvX rats the % water was increased and the % protein decreased, compared to OvX W7 rats. However the interactions show that even these differences disappeared with age. So the differences between D2 and W7 OvX animals on a whole body basis were slight. The OvX W4 *vs* OvX D2 + OvX W7 comparison is not included in Table 8.5, because this comparison yielded no significant results in the carcass, skin, or the whole body. In other words the responses to age at OvX, when present, were linear.

EFFECT OF AGE ON PERCENTAGE COMPOSITION

The results from Analysis A, where animals of only 2 ages were compared, are not included in Table 8.5 as Analyses B & C provide similar results that are more informative.

On a % wet basis, in skin, carcass, and whole body, the % water decreased with age while the % fat, % ash, and % protein increased with age. In the skin and the whole body quadratic effects were significant. The quadratic effects for the % wet skin fat and % dry skin fat, in Analysis B, being due to the % fat being lower at week 12 than at week 9, then rising rapidly to be higher at week 15 than at week 9. Whereas the quadratic effects for skin fat revealed a tendency for skin fat to rise with age, the quadratic effects for % protein (Analyses B & C) plateaued at weeks 12 and 15. The quadratic effect for % skin water was negatively related to that for % skin fat; skin water continuing to fall with age.

On a fat-free basis water decreased linearly with age while ash and protein increased linearly with age.

SKIN COMPONENTS AS A PERCENTAGE OF THEIR RESPECTIVE WHOLE BODY COMPONENTS

Sub-class means, main-class means, analyses of variance, and follow-up comparisons are given in Appendix Tables 8.34, 8.35, and 8.36 for Analyses A, B and C respectively. The summarised results (Text Table 8.5) show that the ovariectomised rat developed a larger skin than the intact animal due to all components, with the possible exception of the ash, being redistributed towards the skin. The interactions in Analysis C indicate that compared to intacts the % fat that is in the skin of ovariectomised rats increases with age. EB treated rats had less of their water and fat in the skin than intacts. Animals ovariectomised at D2 generally had larger skins than W7 OvX animals, although these differences decreased with age. The OvX W7 animals at week 9 (immediately following ovariectomy) had exceptionally large skins due to a large amount of water and protein being laid down in the skin. This effect gave a series of interactions.

With age the contribution of the skin components to their respective whole body components linearly decreased.

HEAT LOSS

The skin of an animal is the isolating surface layer which controls the rate of heat loss according to Fourier's Law where,

$$\text{rate of heat flow (loss)} = \lambda \frac{S}{l} (T_i - T_s)$$

S = flux area (Surface Area)

l = thickness of surface layer

λ = heat conductivity

T_i & T_s = internal and skin temperatures, respectively.

Since skin size increased following ovariectomy thickness (l) may have increased. So mean body and skin weights, for OvX D2 and control animals, were obtained from the respective allometric equations (using 22.5 cm as the length for substitution). Body surface area and skin

thickness were calculated following Kleiber (1969).

Skin thickness was increased following ovariectomy by 15%. (Table 8.6).

TABLE 8.6

Treatment	A Body Weight log (gm)	B Surface Area $10 \times W^{\frac{2}{3}}$ (dm^2)	C Skin Weight (Kg)	Skin Thickness $= C/B \times 10$ (cm)
OvX D2	2.43059	4.17	.0658	.158
Control	2.38337	3.88	.0530	.136

Calculation of skin thickness, for OvX and intact animals of equal length.

CONCLUSIONS

The results for nose-anal length confirm that ovariectomy stimulates and EB inhibits the growth of the rat skeleton. The stage of development reached at the time of ovariectomy did affect body length, as Kennedy (1970) surmised. Neo-natal ovariectomy gave a smaller increase in length than did post-weaning or post-pubertal ovariectomy, parallelling the results for body weight and suggesting that perhaps the stress of surgery was greater neo-natally. OvX W4 animals reached a greater length than did OvX W7 animals, this follows Kennedy's (1970) reasoning that a true growth response to ovariectomy progressively disappears as age at ovariectomy increases.

Ovariectomy increased the absolute weights of all carcass, skin, and whole body components except carcass fat. Allometry showed the skin of ovariectomised rats to be relatively greater in size, compared to intact and EB treated rats, due to all components except ash being significantly increased. The carcass and whole body of ovariectomised rats were, by allometry also larger than those of intact and EB treated rats, but although water and protein increased, fat and ash were generally decreased, leaving dry matter unaltered. When the present study was initiated it was hypothesised that in young actively growing rats all the body weight increase following ovariectomy could be accounted for by an increased nose-anal length, but this hypothesis was negated since ovariectomy clearly changed the shape of the body.

Ovariectomy altered both the composition with respect to body length, and the composition with respect to body weight. As a percentage of their whole body weight ovariectomised animals contained more water but less ash. Whole body fat was not increased by ovariectomy but was redistributed with carcass fat being decreased and skin fat, which changed passively, accounting for a larger proportion of total body fat. The overall increase in skin size following ovariectomy was due to all components being preferentially deposited in the skin. All these effects were not permanent changes as they were reversed by, and were possibly due to, oestrogen. The dose of EB used appears to be supra-physiological for the rat as although there is a reversal of composition compared to ovariectomised rats there was also an over-correction compared to intact

animals. The body weight changes in Chapter 1 also indicated that the dose of EB was supra-physiological.

The body weight and body length of animals ovariectomised at D2 showed the smallest response following ovariectomy, but compared to intact controls, the compositional changes in some cases were the greatest. The response in skin composition following ovariectomy was greatest at D2 as was the redistribution of fat to the skin. In comparison ovariectomy at W7, by allometry, did not affect the size of the skin, but increased the carcass size. The opposite nature of these changes tended to cancel one another so that on a whole body basis age at ovariectomy had little effect on composition or allometry.

According to the reviews of Wade (1972) and Kennedy (1969) the weight increase of ovariectomy is due to an increased food intake and a decreased energy expenditure (as physical activity). It has been shown that high levels of energy intake will increase body weight in rats (Schemmel *et al*. 1969), pigs (Elsley *et al*. 1964), and in man, and that the increased body weight is almost entirely due to increased fat deposition. Restricting movement will also produce obesity in rats (Ingle, 1949). But as Kennedy (1970) states 'Evidently hyperphagia does not of itself stimulate growth'. Therefore, although ovariectomised rats may eat more food than intact controls (exceptions being from the work of Slonaker (1930) and Tang (1941) where ovariectomised rats ate less than intacts) the increase in body length of ovariectomised rats is probably not due directly to changes in food intake or exercise.

In rats compensatory or 'catch-up' growth affects neither tail length-body weight allometry (Mosier, 1969) nor body length-body weight relationships (Widdowson & McCance, 1960). But in the present study the allometric growth coefficients were dramatically altered by ovariectomy showing that post-ovariectomy growth involves effects and mechanisms not seen in compensatory growth. The above evidence indicates that ovariectomy initiates complex changes in body form which could not be accounted for by changes in behaviour (eating and exercise). Yet Kennedy (1969) quotes work by Sullivan & Smith (1957) to support the view that, 'The weight lost under the influence of oestrogen is not entirely fat, but is caused by a reduction in food intake and can be reproduced by

pair feeding'. Sullivan & Smith pair-fed treated and untreated intact male rats. However, the untreated pair-fed ovariectomised female rats of Meites (1950) and Glasser (1954), and the untreated pair-fed intact female rats of Husain & Pincus (1968), were significantly heavier than their respective oestrogen treated controls. Similarly Leathem (1952) found that force-fed oestrogen treated rats were lighter than controls. The contrary result of Sullivan & Smith may have been due to their use of male rather than female rats. Husain & Pincus concluded that the inhibition of body weight increase by oestrogens, despite pair-feeding, '.....suggests that some other mechanisms were involved besides decreased food consumption alone'. When ovariectomised and intact rats were pair-fed (Grunt, 1964) an increased body weight and nose-anal length was recorded for ovariectomised rats. This crucial experiment deserves repetition and extension. Thus contrary to recent reviewers' statements, the experimental evidence indicates that non-behavioural factors may be more important, to the body weight responses following oestrogen treatment or ovariectomy, than are behavioural factors.

Some recent reviews (Kennedy, 1969; Wade, 1972, 1976) do not mention that the efficiency of food utilisation is altered in many states leading to increased body size, but Valenstein *et al*. (1969) review the large amount of evidence that part of the body weight increase following hypothalamic lesions in the rat is due to 'metabolic factors'. Similarly genetically obese laboratory animals will maintain a higher body fat level if pair-fed to non-obese partners (Zucker & Zucker, 1963). The two rat strains bred by Palmer *et al*. (1946) differed in body weight when pair-fed due to differing efficiency of food utilisation. More specifically Tang (1941) showed that compared to intact controls, ovariectomy just before or just after puberty caused an average increase of 31% in the utilisation of dietary energy for body weight increase. Some of this increased efficiency may be due to the decreased activity shown by ovariectomised rats. But Kennedy (1967) notes that under normal laboratory housing conditions the contribution of activity to energy balance is quite small and that extra energy production, when an activity wheel is provided, is accompanied by a compensatory increase in food intake of about 50%. As changes in activity may be largely compensated for by increased food intake it is doubtful that changes in activity can account

for all the change in efficiency of food utilisation following ovariectomy.

In the present study rats ovariectomised at 4 weeks of age (pre-pubertally) diverged from the growth curve of intact controls from the point of ovariectomy as did rats ovariectomised immediately post-pubertally (Chapter 1). It is clear from the work of Kennedy (1967) and others that the level of voluntary activity in female rats increases at the 2nd or 3rd post-pubertal oestrous cycle. Thus the increase in the rate of gain immediately following pre-pubertal ovariectomy could not be initiated by a decrease in voluntary activity.

The research of Oscai *et al.*(1973) is also pertinent. Young female rats trained to swim for 30 hours a week showed a 46% increase in food intake but no change in body weight, compared to sedentary controls. This supports the view that energy input, as eating, will match energy output, as exercise, in female rats (this is not the case in male rats, see Oscai *et al.* for refs.). It is of immediate interest that exercising animals also had a smaller skin weight than sedentary controls, yet a larger eviscerated carcass.

An increase in skin size following ovariectomy was recorded by Freudenberger & Billeter (1935) and Tang (1941). Ebling and Hale (1966) confirmed earlier reports that oestrogen inhibits skin and hair growth. In the present study ovariectomised rats had a larger and thicker skin than intact and oestrogen treated rats. Although animals were shaved no estimates of hair length, thickness, or weight were made. An increased skin thickness would reduce heat loss since the environmental temperature used (24°C) was below the critical temperature for rats (27-29°C). In addition the changes in body shape, a larger weight at a given length, would cause the surface area to volume ratio to be smaller in ovariectomised rats, which would further reduce heat loss. Percentage skin composition was also altered following ovariectomy to favour the deposition of fat and to give a larger skin at a given body weight. However it was not possible to estimate the post-ovariectomy change in the thermal conductivity (λ) as the effect of skin composition on λ appears to not have been investigated. But since λ for fat is 1/3 that for water the increased skin fat of ovariectomised rats should further

decrease heat loss. It is possible that the reduction in heat loss in ovariectomised rats is of the order of 20%. Theoretically this could account for a very large part of the increased efficiency of the ovariectomised rat but these estimates should be tested experimentally before definite conclusions are reached.

The decreased skin size of the exercising rats of Oscai *et al.* could be an adaptation to lose heat more effectively. Pigs seem to lay down subcutaneous fat as an adap tation to low environmental temperature despite the high maintenance costs which seem to preclude such a deposition (Sorensen, 1962). In the present study allometry showed that following ovariectomy the skin appears to increase in relative size before the carcass. This result suggests that the increased relative skin size could be causative of part of the increased relative carcass size. Wade (1976) discusses results where ovariectomised rats, kept at an environmental temperature above their critical temperature, showed a blunted body weight increase. This supports the idea that heat loss plays a part in post-ovariectomy body weight changes.

The model for the control of body weight described by Wade (1976) ascribed the primary action of oestrogen to an inhibition of food intake via the 'hypothalamic body weight regulating mechanism'. Body weight is defined by Wade (1976) and by others as '....the mass of adipose tissue' (Woods, 1974). Under this model oestrogen is thought to reduce the mass of adipose tissue and thus reduce body weight. Although the equivalence of body weight regulation and adipose tissue regulation seems plausible for the so-called 'adult plateaued rat' Zucker *et al.*(1941) showed sufficient bone growth in rats up to 70 weeks of age to maintain the allometric relationship between bone growth and body weight. This result was confirmed by Berg & Harmison (1957). Thus normal growth gives changes in body weight in adult rats which are not entirely due to changes in fat. In the general case, of the growing animal, the equivalence of body fat and body weight is difficult to defend.

For example, in the present study ovariectomised and intact rats of widely different body weights had similar compositions, and Schemmel *et al.* showed, by altering diets, that animals of the same body weight could have different compositions. Carcass analysis of sheep and pigs,

over a wide range of ages and body weights, caused Elsley *et al.* (1964) to conclude that '.... fat deposition is not closely related to the growth of the fat-free body mass'. This is illustrated by comparing the within-group variation, and the coefficient of variation for body fat with those for other chemical consitituents, in the present study and, for example those of Elsley *et al.*, Morris *et al.*,(1933), and Reid *et al.* (1967). Body fat was shown to be the most variable component in the body, not the most stable as is suggested by the model proposed by Wade.

Kennedy (1969) states that oestrogen inhibits growth in young gonadectomised rats by, '.....reducing food intake and is accompanied by a low body fat'. Wade (1976) envisages that oestrogen indirectly reduces food intake by resetting a 'neural lipostat; or 'Bwt (fat) set-point' to a lower level. These views are not supported by the present study or by that of Nyda *et al.* (1948) as in both studies body fat was not reduced by oestrogen treatment yet linear growth and the rate of body weight gain were inhibited. Clearly oestrogen can inhibit growth and body weight independent of changes in body fat; supporting the non-equivalence of body fat regulation and body weight regulation in the growing animal.

An individual maintains a close relationship between its length and weight, under a variety of environmental and experimental conditions, during growth. (A discontinuity in this relationship at weaning (Mosier, 1969) confirms a previous finding (Zucker *et al.* 1941; Chapter 1) that the form of the body weight-age curve changes following weaning.) Mosier proposed that a mechanism '.... controls relative growth, and that linear growth is subservient to this mechanism', i.e. the relationship between length and body weight is controlled rather than length or weight. But the present study provides an exception to this proposed mechanism as the relationship between length and weight was altered by ovariectomy. Mosier's proposal may be unnecessary. It has been hypothesised that body length is controlled and that functional demands of the skeletal size determine the size of many other body organs (Goss, 1964; Bryden, 1969). This mechanism may be the main one regulating body weight. The 'hypothalamic lipostat' may regulate the amount of body fat but to call this a 'body weight set-point' mechanism would seem to be a misnomer, especially for

the growing animal. Another objection to the idea of a 'set-point' is that body weight does not, as is commonly believed 'plateau' in the rat. Body weight under favourable conditions, increases at a constant rate until at least 70 weeks of age (Zucker *et al.*,1941; Chapter Ten). So if there is a 'set-point' mechanism in older rats it must be a constantly changing 'set-point'. A mechanism encompassing an ultimate weight would, for the rat, seem more appropriate (Chapter 1). For similar reasons the neural 'set-point' mechanism has recently been criticised by Wirtshafter & Davis (1977) who describe '....a simple feedback control model which contains no set-point....'.

For the skin, carcass, and whole body it was clearly shown that the percentage water decreases but the percentages of fat, ash, and protein increase with age; a process termed the development of 'chemical maturity' by Moulton (1923). In addition the percentage contribution of the skin components to their respective whole body components declined with age. Table 8.5 shows that the effects of age and of ovariectomy on body composition are completely opposite; ovariectomy in the growing rat seems to slow, or delay, the development of chemical maturity and to increase the size of the skin. Conversely oestrogen treatment hastens the development of chemical maturity and decreases the size of the skin. A causal relationship between the demonstrated effect of oestrogen, of inhibiting the growth of the body's weight and length, and hastening chemical maturity, is possible. It is possible that the same mechanisms promote chemical maturity and inhibit growth; giving a unity of action to oestrogen.

ADDENDUM TO CHAPTER EIGHT

A METHOD FOR CHEMICALLY ANALYSING THE CHEMICAL COMPOSITION OF ANIMAL TISSUE.

INTRODUCTION

The first section describes a simple method of reducing tissues to a fine homogeneous dry powder using equipment found in most laboratories or obtainable cheaply from hardware stores. The second section describes an accurate and rapid method for analysing the powdered tissues for fat, ash and protein content.

SECTION ONE

THE PRIMARY PROCESSING OF TISSUES

Many methods of primary tissue reduction have been described, varying from mincing the whole fresh animal and sampling directly from the homogenate (Mickelsen and Anderson, 1959), freezing in liquid nitrogen and crushing, to drying the whole animal in an oven for several days before mincing (Leshner *et al*, 1972). These methods are usually time consuming, require heavy duty equipment, and contribute to an unpleasant working environment. They may even fail in their primary aim of reducing the tissues to a completely homogeneous state because mincing is not an adequate method of mixing (unless the sophisticated equipment described by Mickelsen and Anderson is used) and the wet homogenates tend to be unstable which would increase the sampling errors between replicates.

The method to be described was designed for rat tissues but has also been applied to oppossum, chicken, and pig tissues.

Hair is removed from the rat with electric clippers before slaughter. If required the skin can be removed from the carcass at this stage and can be processed separately. The tissues are immediately weighed to avoid loss of moisture, identified with a code pencilled into a small piece of card, and placed into a glass beaker containing a small amount of water (to facilitate the removal of the tissues) and autoclaved at 120^{o}C for 30 minutes. Tissues are then homogenized in a blender for 2-3 minutes with sufficient water to produce a smooth consistency and then poured into tared reusable aluminium foil baking

trays (20 x 15 x 3 cm). A wash bottle is used to rinse the remaining homogenate from the blender into the tray. Trays are frozen then freeze-dried for 3 - 4 days or until the frozen homogenate has shrunken and loosened from the tray. Trays are then dried in an oven (maximum temperature of 70°C to avoid the loss of volatile components) and weighed for dry matter calculation. The dried homogenate is removed from the tray, crushed with a mortar and pestle, ground in an electric rotary grinder for 20 - 30 seconds and stored in an air tight bottle. The grinder is easily wiped clean of powder between samples with a paper tissue.

DISCUSSION

Autoclaving is a critical part of the procedure as the high temperature and the moist heat break down tissues such as skin and bone which contain large quantities of elastic and collagenous tissue. The processing times indicated appear to be optimal for rat tissue but could be modified for other types of tissue. In the absence of an autoclave a large domestic pressure cooker could be used. Freeze-drying is the best way of drying the homogenate as the tissue remains in an expanded biscuit-like state which simplifies the final crushing and grinding and retains the fat even from very fatty tissues such as rat skin. Slow oven drying of the homogenate could be used if a freeze-drier is not available. The mortar and pestle breaks down the block of dried tissue, crushes any remaining pieces of bone and enables the use of a rotary grinder such as is used for coffee grinding. The pencilled cardboard labels remain legible and float on top of the homogenate so that the trays can be identified at any stage of the process. The fully processed powder can be stored in a freezer indefinitely or stored at room temperature for several months without obvious deterioration. Tissues can also be stored in the freezer at any stage of the process which allows the organisation of large batches of tissues.

SECTION TWO

ANALYSIS OF CHEMICAL COMPOSITION

Lipids

Lipids are extracted by the following modifications of the chloroform:menthanol:water procedure of Atkinson *et al*. (1972). A large volume of methanol (1 litre) and distilled water (900 ml) is pre-mixed to eliminate one pipetting stage and is stored in a refrigerator to facilitate pipetting. The constant delivery system for chloroform designed by Atkinson *et al*. (1972) was used but the disintergration system was re-designed using 25 ml McCartney bottles (commonly used for microbiological liquid cultures) into which is weighed a tissue sample sufficient to yield a maximum of 0.4 g of lipid (for rat carcasses about 2-3 g of powder). About 10 glass beads (5 mm diameter) are added to the bottles followed by 14.25 ml of methanol:water mixture and then 7.5 ml of chloroform. The bottles are immediately capped with 2 layers of thick polythene, a plastic sealing disc, and a plastic screw cap drilled off centre with an 18 gauge hole. The bottles are left overnight to digest the sample and are then shaken horizontally for 20 minutes on an electric flask shaker. The bottles are then centrifuged for 5 minutes at 3000 rpm in an upright position and are then inverted and spun for 1 minute at 1000 rpm which allows a 5 ml aliquot of the lower chloroform layer to be withdrawn through the 18 gauge hole by hypodermic needle into an accurate glass syringe without any losses through evaporation. The aliquot is expelled into a tared aluminium container (an autoclave charge capsule) and dried in an oven at 70°C for 2 hours. Sample duplicates differing by more than 2% are re-analysed. Calculation of the lipid content of a sample is as follows:

$$\text{Amount of fat in the original sample} = \frac{V_c \times W_z}{(V_z - (S_f \times W_z))\, W_s} \times 100$$

Where,

W_s = weight of sample of tissue,

V_c = volume of chloroform added (excluding ethanol normally present),

V_z = volume of the aliquot of the chloroform layer after extraction,

S_f = volume of 1 gram of fat,

W_z = weight of fat in the aliquot.

The recoveries of pure lipid from 9 assays are given in Addendum Table 8.1. Four arachis oil 'standards were routinely included in each batch of 72 bottles.

ADDENDUM TABLE 8.1

Statistical validifaction of lipid assay*

Assay	% recovery	Coefficient of variation (CV)
1	100.21	0.96
2	99.95	1.15
3	100.26	0.32
4	99.89	0.40
5	100.95	0.27
6	101.03	0.67
7	100.10	0.47
8	100.02	0.41
9	99.99	0.39
Mean	100.27%	0.56%

coefficient of variation between assays 0.42%

*Recoveries of arachis oil from 9 separate assays, 4 samples per assay.

Ash

Ash was determined by the standard method of firing triplicate samples of the dried tissues in a muffle furnace.

Protein

The difference between the dry matter percentage and the sum of the fat plus the ash gave the percentage protein (protein = DM - (fat + ash)) or more properly the residue. This method has been shown by others (Barnicoat and Shorland, 1952; Everitt and Jury, 1966) to give a reasonable estimate of the percentage protein and also glycogen only constitutes a small proportion of a rat's body weight (0.2%, Mickelsen and Anderson, 1959).

Despite the literature support for this method of calculating protein the accuracy of the calculated estimate of protein was tested by analysing 14 samples by the Kjeldahl method. No significant difference between the 2 methods was found (Kjeldahl mean protein ± SE, 67.49± -.74; while the calculated result was ± SE, 68.85 ± 0.47).

Typical data for dry matter, ash, fat, and protein levels in rat carcasses is given in Addendum Table 8.2

ADDENDUM TABLE 8.2

Percentage constituents of rat carcasses*

		Constituents as % dry matter		
No.	Dry matter %	Fat %	Ash %	Protein %
11	30.76 ± 0.14	5.12 ± 0.2	5.4 ± 0.08	20.1 ± 0.13

*Female rats of the Massey Colony. Data are means ± standard errors

DISCUSSION

The fat extraction devised by Atkinson *et al*. is rapid, accurate, and safe compared to the traditional methods based on the reflux of petroleum ether (such as the Soxhlet method). The modifications of the present method enable the use of a smaller disintergration bottle which is inexpensive and can be purchased in large quantities which enables several runs to be made up in advance. The precision of the original method seems to be retained even though half volumes of reagents were used in the McCartney bottles (compared to the volumes used by Atkinson *et al*.). Fat extractions were usually run in batches of 72 bottles which could be processed in a working day.

The present results confirm that protein can be accurately calculated from the dry matter, fat, and ash percentages. This avoids the tedious Kjeldahl method.

CHAPTER NINE

THE CONTROL OF BODY GROWTH IN MAMMALS

Hypotheses abound as to how growth is regulated in organs and tissues. None, however, is universally accepted. Most do not deserve to be.

(Goss, 1972; p.1).

INTRODUCTION

The aim of the experimental chapters of this thesis was to elucidate some environmental, nutritional, genetic, and hormonal influences on mammalian growth in size and form. Some of these experiments are not novel, indeed some involve a re-analysis of previous experiments. But novelty certainly resides in the methods used to analyse the results. Most workers use biometrical techniques that assume growth to be an additive process. But growth is a multiplicative process and should be analysed as such.

The experimental results will not be discussed, per se, as the relevant chapters are considered adequate in this regard. What follows is an attempt at synthesising the experimental results with what are currently believed to be the mechanisms that control temporal (chronological or physiological) changes in mammalian size and form.

To interpret the experimentally induced changes shown in this thesis the mechanisms controlling the 'normal' changes in size and form must be explained. Therefore a general review of the mechanisms thought to control mammalian body growth is pertinent.

Obviously the most satisfying description of mammalian whole body growth, or of tissue or organ growth, would be a single 'master controlling mechanism' represented mathematically by one equation. Many attemps have been made to discover such a mechanism but no unitary explanation of growth has gained universal support. None may deserve it. Such hypothetical exercises may, on biological grounds, be unnecessarily complicated and misdirected.

Huxley clearly perceived this problem and proposed the following solution,

'...there appear to be two successive and quite distinct phases of growth. In the first, the general form of the part is laid down, and this process is accompanied by very rapid alterations in form, and by marked histological changes; in the second, histological changes are absent or of an entirely secondary nature, and the form-changes are confined to quantitative alterations in the proportions of the definitive structural plan.' Huxley called the first phase '....histo-differentiation since histological change would appear to be the most decisive factor;'

and he called the second phase '...auxano-differentiation, since quantitative growth-changes are now the most significant.'

(Huxley, 1932; pp 118-20)

Zucker *et al.*, (1941a) amalgamated the idea that weaning represents a critical nutritional transition point, which greatly affects rat growth, and Huxley's anatomical analysis, to fit separate mathematical functions to the two phases of growth. Zucker *et al.* (1941a, p426) postulate '....that the effects of these two processes upon weight increase are sufficiently independent to suggest separate treatment.' At the same time Brody (1945) postulated that growth involved two phases, an initial self-accelerating phase and a second self-inhibiting phase. But Brody's analysis is not multiplicative (compared to that proposed by Zucker *et al.*, 1941, 1942), assumes an inflection at puberty (which is not shown by adequate growth curves), and is a mathematically poorer description of live weight changes in the rat (see Chapter Six).

In a massive assay, entitled 'Growth - Principles and Theory', von Bertalanffy (1960, p.175) dismisses all previous growth equations,

'....as it can be shown that none of them is physiologically well-founded.....and it is sufficient to show that these functions, for mathematical reasons, are ill-suited to represent growth curves as empirically found.' (von Bertalanffy does not consider the Zuckers' equations). von Bertalanffy applies his set of equations to the rat live weight data of Donaldson. Although the deficiencies of the Donaldson data, as described by Zucker *et al.*, (1941, 1942), Dunn *et al.*, (1947) and Mayer (1948), are referred to by von Bertalanffy, he makes the curious statement (p.222) that '.....each of the mentioned groups of investigators proposed a different empirical equation for the growth of the rat which, however, does not fit data of other observers.' (As was noted in Chapter 6 these authors agreed with the Zucker equation!) von Bertalanffy attempts to justify his use of the Donaldson data (p.222) when he states that 'If Donaldson's data presumably represents a "suboptimal" course of growth, it has to be considered, on the other hand, whether a synthetic diet yielding maximum weight increase, does not lead to "supernormal growth", particularly with respect to deposition of fat.'.

Such an argument is clearly out of order, if one starved an animal (for example, to the degree practised by McCance and Widdowson (1974) in pigs) the course of growth would obviously not comply with the uninhibited growth of an untreated animal. This digression aside; von Bertalanffy notes (p.219) 'Growth curves of mammals show segments or cycles.', but his use of the Donaldson data causes him to conclude that 'The break in the growth curve coincides with sexual maturation...'. The 'break', using the von Bertalanffy equations, in more acceptable rat data, may be nearer the earlier age proposed herein.

A firmer basis to the Huxley-Zucker analysis of growth was provided by Enesco and Leblond (1962) and by Winick and Nobel (1966) who showed, in the rat, that before three weeks of age tissues grow by cell division. Between three and six weeks of age cell division wanes and cell hypertrophy starts to dominate so that after six weeks size changes are mainly by cell enlargement.

The present analyses clearly confirm this separation of post-natal rat live weight growth into two phases. But frequent measurements show the rate of tissue formation to be variable, in both growth phases; the growth rate clearly oscillates rhythmically. The oscillations could represent changes in the rates of cell division (or cell hypertrophy) occurring contemporaneously in all tissues or different tissues beginning and ending their growth at different times. The regularity and similar size of the oscillations, especially in the first phase of growth, seems to indicate that the former explanation is correct.

Evidence supporting this explanation was found in the data of Kvinnsland and Kvinnsland (1975). They incubated cartilage, taken from the craniofacial region of rats on 9 occasions from birth to 28 days of age, studied radioactive thymidine uptake, and concluded (p.313) that '....a great increase in weight (live weight) between the 10th and the 14th day....was parallelled by high cpm/DNA values, a weight increase between the 24th and the 28th day was parallelled by low cpm/DNA values.' (Vaughan's (1975) warning against extrapolating from nasal cartilage to all cartilage should be borne in mind; all cartilage may not show these growth phases.)

Rat brain growth has recently been the subject of a tremendous amount of research. Gottlieb, Keyder and Epstein (1977) review the evidence that, 'Brain growth in both rats and mice occurs mainly during days 0-6, 8-12, and 17-23 after birth. During much of the other periods (days 6-8, 12-17, and after 23) there is little increase in brain weight.' (p.166). However they also quote Kobayashi (1963) who '...gave mouse brain/body weight ratios and found a large increase starting between days 8 and 9 and later an abrupt reduction at about 14 days. Since the body weight increases steadily at all pre-weaning ages, these results may be taken as showing a very large brain weight increase starting around 8-9 days and later a very slow or zero weight increase beginning around age 14 days.' (p.168). The present results question their conclusion that body weight increases steadily at all pre-weaning ages, the changing brain/body weight ratio could be due to the presently observed changes in body weight growth. However as the brain is growing at a faster rate than the rest of the body, over this range of ages, the oscillations in its growth rate would be large compared to those in body weight. The results seem compatible with the conclusion that the growth rates of both the brain and the body oscillate before weaning.

These results support the author's finding that during the early multiplicative phase of growth live weight growth oscillates rhythmically. The early peak in live weight growth rate may be due to a burst of cell division in all tissues of the body while the second cycle of growth, seen following weaning, could be due to an increase in the rate of cell hypertrophy. The factors causing, or the mechanisms controlling, such changes remain to be identified.

MECHANISMS REGULATING GROWTH BY HYPERPLASIA

The evidence of the previous section suggests growth in mammals occurs in two distinct, but temporally overlapping, phases. It also seems reasonable that the mechanisms controlling, and therefore the factors affecting, total body size should differ in these two phases. Most growth theories were formulated before this property of mammalian growth was

recognised but some recent reviewers (Goss, 1972) also seem to ignore this basic dichotomy of growth.

Although one can separate whole body growth into two phases it should be realised that cells of different tissues differ in their capacity to undergo mitosis. Any theory of body growth regulation must take this into account. The importance of this property to whole body growth will become clearer later in this chapter. Leblond (1972) classified cell populations as being either,

1) Static - growth only by cell enlargement - (neurons)
2) Expanding - growth by random proliferation of parenchymal cells
   There is no cell loss - (kidney)
3) Renewing - stem cells give stem cells plus differentiating cells.
   There is cell turnover and loss - (small intestine)
4) Intermediate - combinations of 2) and 3)
   i.e. skeletal muscle has no cell loss but contains stem cells.

Leblond continues, (1972, pp36-37).

'It is likely that much of the growth control of the various cell populations is exerted by chalones, that is, substances inhibiting cell proliferation. This view implies that the cells of an organ would divide indefinitely unless prevented from doing so by an inhibitory substance, the chalone, which they release to the circulation....as long as the chalone is within the cell which produces it, no inhibitory effect is exerted, but, once the chalone has passed to the circulation in sufficient amount, it does inhibit the proliferation of the cell population...as the number of cells increase with age, the amount of circulating chalone also increases gradually, and the rate of cell proliferation is correspondingly reduced. Eventually, growth becomes negligible.'

Bullough is an advocate and originator of the chalone theory but in the following passage (1973, pp 4-5) he forcefully argues against the 'functional demand' theory (proposed by Goss (1972) and others),

'When part of the liver is removed the remnant grows rapidly until its mass is again normal, when growth ceases....These responses are strictly organ specific: partial hepatectomy stimulates only liver growth....In the case of a liver remnant or of a single remaining kidney it has been postulated that, because the cells work harder, growth by mitosis will continue until the work load per cell is once more normal. This idea of increased metabolic load leading to increased tissue mass is evidently derived from the common observation that increased muscular work leads to the development of increased muscular mass. However, this is a false analogy since increased muscular work leads only to increased muscle cell size; the non-mitotic muscles cannot increase their cell number...there is still no positive evidence that an increased workload can lead to increased mitotic activity....the question of positive feedback mechanisms of one kind or another still remains open.'

The 'functional demand' theory seems a particularly inappropriate explanation of the control of cell division in the majority of mammals since hyperplasia occurs almost exclusively *in utero* when most organs are largely non-functional. As we shall see the functional demand theory seems more important during the second, hypertrophic, phase of growth.

Bullough (1975) does not discount the presence of substances which stimulate cell division; Goss (1972, p.3) interprets the chalone theory differently,

'They imply that cellular proliferation is not subject to exogenous stimulators, but that tissues have an innate tendency to grow and can therefore be controlled solely by inhibitory compounds - like controlling the velocity of an automobile by using the brakes instead of the accelerator.'

There is, however, good evidence for substances which stimulate cell division (see Lobue and Gordon, 1973). It is almost a tenet of endocrinology that regulated systems, that are hormone sensitive, are influenced by both inhibitory and stimulatory molecules.

Bullough (1973) describes oscillations, of short phase length, in the mitotic rate of epidermal cells which he believes can be explained by a mechanism depending solely on negative feedback. But the evidence reviewed in the first part of this chapter indicates that prolonged periods of enhanced cell division occur in the suckling rat. If the mitotic rate were controlled solely by negative feedback such large oscillations in the mitotic rate would not be expected to occur. The results suggest that positive feedback on the mitotic rate is occurring. It is well established that cartilage cells (cells of the proliferative zone are called chondroblasts by Vaughan (1975) - but the terminology differs) can be stimulated, *in vitro*, to take up radio-labelled thymidine. The serum factors responsible are largely dependent on pituitary growth hormone for their generation and have been therefore called somatomedins (Van Wyk *et al.*, 1974). Although there is much debate on the physiological, *in vivo*, role of these factors (see the discussion following the paper by Van Wyk *et al.*,) it is clear that the division of chrondroblasts is stimulated by true hormones (i.e. a stimulant that has an origin in one organ and its target in another). The division of chrondroblasts will be discussed, in a different context, later in this chapter.

It is postulated that cell division is sensitive to

1) substances that are produced by cell division (chalones) which feedback to inhibit further cell division in their organ of origin, and 2) hormones that stimulate cell division.

Leblond (1972, p.16) emphasises that '....cell number is the main factor in growth.'. This means that size is primarily determined by the number of cells in the body. If growth in size is viewed logarithmically the period of cell hyperplasia assumes its proper importance. Cell hypertrophy therefore represents only a 'fine tuning' of body size.

EFFECTS OF NUTRITION ON CELL HYPERPLASIA AND COMPENSATORY GROWTH

The effects of nutrition on body growth have fascinated generations of scientists. For example, Hammond hoped that by altering the plane of nutrition of an animal one could, by differentially affecting the 'early' and 'late' maturing parts of the carcass, change the shape of an animal to give a more desirable carcass, in terms of meat production (Elsley, 1976). Some reasons why this hope has been unfulfilled will be clarified later.

Our understanding of the effects of nutrition on growth has advanced with the realisation that there are what McCance (1976) calls 'critical periods' during development. For example, there is, neonatally, a critical period for the stimulatory effect of microgram doses of testosterone on the subsequent growth of female rats (Tarttelin *et al.*, 1975). Another example is that a nutritional stress during cell hypertrophy can be overcome but a similar stress during cell hyperplasia can permanently retard growth. Critical periods are clearly of both theoretical and practical importance.

In most mammalian tissues cell hyperplasia occurs almost exclusively *in utero* making the experimental manipulation of growth difficult. For example, Wallace (1948) fed ewes on a low plane of nutrition from conception but could show no adverse effect on foetal growth up to 90 days of gestation. But Everitt (1968) found that ruminant foetal growth, in the first half of pregnancy, was not inviolate if the nutritional stress was large. As the rat is born with cell hyperplasia largely uncompleted (Leblond, 1972) it is amendable to experimentation since post-natal dietry restriction, instead of pre-natal restriction can be used to study cell hyperplasia.

TECHNIQUES FOR INDUCING UNDER-NUTRITION DURING CELL HYPERPLASIA

The following description of techniques used to induce under-nutrition in foetal and suckling rats suggests that discrepancies in the

the results obtained by different workers, in apparently comparable experiments, could be due to the different methods used to induce under-nutrition.

There are two major experimental techniques used to induce under-nutrition during suckling. The technique, developed by the Cambridge group of Widdowson, McCance, and Kennedy, of cross-fostering neonates to give large and small litters, is commonly employed (Winick and Nobel, 1966; Williams and Hughes, 1975). A different technique was used by Adlard, Dobbing, and Smart (1973) who fed dams 50% of the same ration eaten *ad libitum* by control dams on the same day of pregnancy or lactation (here the number of pups/litter is held constant). Pre-natal under-nutrition by ligating some of the umbilical spiral arterioles has been described by Marthens *et al*. (1975).

Within these methods other differences have arisen. Williams and Hughes rehabilitated their large-litter animals in litters of eight, the size of their control groups, while Widdowson, McCance and Kennedy have commonly used litters of three for both control and rehabilitated animals. Within the restrictive feeding technique differences have also evolved, for example feeding restricted dams a diet low in protein. Winick *et al*. (1972) describe other variations within this technique. Dietary restriction of the dam and alteration of the litter size do not seem to have been directly compared within the same experiment for their subsequent effect on growth. Although Winick *et al*. (1972, p.71) conclude that '...these methods have produced comparable results on brain growth, and therefore we shall examine them together.' this seems a questionable conclusion.

There is some evidence that the cross-fostering method may be inferior to the caloric restriction method. Williams and Hughes cross-fostered at birth to give litters of eight and sixteen pups then reduced the large litters to eight at eight days of age. There was no permanent difference in live weight between the animals that were initially in the large or small litters. But if the large litters were maintained to 14 or 21 days before being reduced permanent effects in adulthood resulted. Thus Williams and Hughes (1975, p.191) conclude that for post-natal cell number '....the period of maximum sensitivity of the

mechanism of determination occurs between 9 and 15 days.' This view is at variance with the classical view of McCance and Widdowson (1974, p.6), 'We concluded that the earlier in the life of the animal a short period of under- or over-nutrition fell the more likely it was to have permanent effects on stature.'

This difference is explained by the live weight data supplied by Williams and Hughes (1975, p.182) which reveals that at day eight there was a similar difference between the control group and the group selected for reduction at day 15 as there was between the group selected for reduction at day 8 and the day 15 group. This suggests that a between litter effect is confounded with the treatment effect. That no real under-nutrition was present in Williams and Hughes' study, at 8 days of age, is indicated by the study of Merat and Dickerson (1974). They raised rats in smaller litters (3/litter) and litters of a similar size (15/litter) to those used by Williams and Hughes. But at 7 days of age, despite the greater disparity in litter size than in the study of William and Hughes, Merat and Dickinson (1974, p.166) found '....no difference in body weight at 7 days, and it may be presumed that up to this age maternal reserves were able to supply the additional nutrients required by the larger litter.'

Another possible shortcoming of the cross-fostering technique is also apparent from the results of Williams and Hughes (1975). Although the authors do not comment upon the effect, it is apparent that the variation in body weight, body length, and tail length was large immediately following retardation, compared to the variation in control animals of a similar average weight (but different age). Unfortunately in the original papers by McCance, Widdowson, and Kennedy means and standard deviations were not given; the data was usually plotted. But the work of Park and Nowosielski-Slepowron (1971) confirms the increased variability of large litters. These workers mixed litters at birth to give litters of 6 or 18 pups; 32 small and 21 large litters were produced, litters were killed daily until weaning and lengths and weights were measured at slaughter. They concluded (1971, p.24), 'Growth is therefore more variable in the large under-fed litters than in the small well-fed litters.'. These results could be due to either the individual animals within the large litters showing a variable resistance to nutritional stress, or being

subjected to variable nutritional stress. The possibility of genetic differences in response to under-nutrition was suggested by Park and Nowosielski-Slepowron. In Chapter 5 of the present work differences between breeds of cattle in their response to nutritional stress were alluded to. But within large litters of rats between animal differences in milk supply might be expected since the number of pups exceeded the number of nipples available (12 to 14). It has been shown by Hall *et al.*(1977) that rat pups will not leave a nipple which produces no milk in search of a productive nipple until they are 14 to 16 days old. Hall and Rosenblatt (1977) describe milk let-down in the rat as not continuous, but brief, intermittent and controlled by the dam.

Therefore cross-fostering to large or small litters:

1) may produce relatively greater under-nutrition during the second and third weeks of lactation compared to that of the first week
2) all pups may not be equally under-nourished, especially during the second week of lactation.

Conclusions reached by cross-fostering techniques may therefore be open to re-interpretation. Some of the difficulties of the cross-fostering method can be surmounted by restricting the dam to a set proportion of the diet consumed by a control female during lactation or pregnancy (as described by Adlard *et al* ., 1973). But the differences between animals in their response to under-nutrition cannot, using the designs and analyses of the studies reviewed, be removed.

As we have seen, litter size and within litter variation seem to be positively correlated. This creates statistical problems. For example comparisons of animals from large litters, as a group, with those from small litters, is complicated since the variances in the two populations are clearly unequal. The large variation within the large litters could also mask any between treatment variation. These problems suggest a different approach; the regression techniques of the present work, which are well suited to deal with these situations.

Most workers test for an effect of under-nutrition on growth using a group approach, comparing the treatment groups' weights. But using regressions one would estimate the weaning weights (logarithmic) from the pre-weaning weights, estimate the post-weaning growth rates (logarithmic)

and, initially, compare the regressions of growth rates on weaning weights. One might expect these regressions to differ if a permanent effect had been produced. Using this method one could also detect animals, as outliers, that had escaped under-nutrition. An analysis of covariance would be of use if the weaning weights were not different between the treatment groups. For example, testing for an effect of under-nutrition on cell number could be made by regressing cell number (Y) on the calculated weaning weight (X) and then comparing the within treatment regressions.

Smart, Adlard, and Dobbing (1974) and Williams and Hughes (1975) induced foetal and neo-natal under-nutrition, measured body weights & lengths but did not use allometry to test for effects of treatment on body form. The interpretation of differences in mean weights and mean lengths is difficult without allometry. For example, Williams and Hughes concluded (1975, pp.190-1) that 'The weight deficit is apparently much greater than the length deficit.' But one would expect such a numerical effect on an arithmetic scale. The important question, whether or not the relationship between length and weight (the shape of the body) has been changed by treatment, can be best answered by allometry, as was seen in Chapter Six .

Thus more refined methods of inducing under-nutrition and of analysing the results of under-nutrition may lead to a better understanding of growth itself.

EFFECT OF NUTRITION ON ADIPOSE CELL NUMBER

Referring to adipocytes Czajka-Nairns and Hirsch (1974, p.177) wrote 'Pre-weaning diet is the only factor found thus far which alters cell number.' But the different methods used to alter pre-weaning nutrition have produced conflicting results.

In 1968 Knittle and Hirsch reported that the number of cells in the epididymal fat pads of rats raised in litters of 4 contained 40% more cells than the fat pads of rats raised in litters of 22. This caused many authors to extrapolate across species and age scales to

conclude that over-feeding infant humans following birth will increase adipocyte numbers. Knittle and Hirsch's work indicates that under-nutrition will affect cell number but as there were no normal sized litters an effect of over-nutrition cannot be inferred. Over-nutrition was specifically studied by Czajka-Nairns and Hirsch (1974) by force-feeding suckling rats. Force-feeding increased the percentage body fat at weaning but not adipocyte number at maturity. Knittle (1972) had previously attempted to influence adipocyte cell number in the rat by restricting the food intake of dams to 25 grams/day compared to controls' 50 grams/day (of stock diet) or by feeding 50 grams/day of a protein deficient diet. A litter size of 12 combined with 50% of the dietary intake of controls would, Knittle expected, give a comparable nutritional stress to employing litters of 22. Litters of 12 were used in all treatment groups. But adipocyte number was not affected by caloric restriction although it was affected by the protein deficient diet. Knittle termed these results 'surprising'. His feeding a reduced but constant amount of food is yet another variation in technique to those already described. This method may have supplied sufficient nutrient during early lactation with a nutritional stress only developing in late lactation. But as the stress in a large litter would also be less in early lactation Knittle's results are still not explained. It is possible that the greater effect in large litters is due to the stress of over-crowding.

These results indicate that an extremely harsh nutritional stress is necessary to influence adipocyte number in the rat. More importantly there is little evidence indicating that over-nutrition affects adipocyte number. Gairdner and Dauncey (1974) took adipose samples, at autopsy or by needle biopsy, from infants and measured adipocyte cell diameters microscopically. They conclude (1974, p.121) that in humans 'While overf-eeding a baby certainly leads to an abnormally large gain in his total body fat, we are not yet in a position to say whether his total complement of fat cells is unduly multiplied or merely that his existing fat cells grow unduly large.' After reviewing the literature Widdowson (1976, p.361) decided that '...there seems no real evidence that there is any increase in the number of fat cells in the human baby after birth.' Therefore

although over-feeding infants may lead to obesity later in life this may not be because over-fed infants have more fat cells.

Of major importance is the lack of consistency in the results obtained using different methods of achieving under-nutrition. But increasing litter size to impose nutritional stress may cause stresses of a nature unrelated to nutrition. Thus Czajka-Nairns and Hirsch (1974) perhaps should have written that pre-natal stress, rather than a pre-natal diet, is the only factor that affects adipocyte number. The stress of increasing the number of rats per cage has been studied by Hughes and Nowak (1973). There seems to be a critical number above which, if rats are housed together, the size attained declines. The cause of this phenomenon is unknown. To the author's knowledge the increase in height and size of humans, over the last century, usually attributed to better nutrition, does not seem to have been related to a possible parallel decline in the average family size.

The effect of realimenting rats of varying weaning weights in different numbers per cage may be of interest. Is 'catch-up' inhibited?

Obviously inducing under-nutrition by increasing litter size provides a far from simple experimental model.

COMPENSATORY GROWTH

An elucidation of compensatory growth is crucial to our understanding of growth itself. During growth environmental, nutritional, and hormonal stresses are inevitable. Compensatory growth following a prolonged stress reveals, in an exaggerated form, the mechanisms controlling, or the homeorhesis of, growth in motion and therefore opens its processes to study.

Age at onset

From what age, in the rat, can compensatory growth occur?

The data given in Chapters 1 to 7 indicates compensation does not occur before weaning. The high positive correlation between birth weight

and day 28 weight (in Chapter 7) shows that differences at birth are maintained to weaning. But Winick *et al.*, (1968) and Williams and Hughes (1975) maintained large litters of rats to 8 or 9 days of age then rehabilitated them, which left no differences in cell number or body size at weaning. One could challenge these findings, which were based on cross-fostering, for the reasons outlined previously. But another explanation of this 'compensatory growth' is possible. As we have seen, before 14 to 16 days of age rat growth is primarily determined by the amount of milk supplied to each pup, a function of litter size, as internal control mechanisms are not functional at this time (Hall *et al.*, 1977). Using caloric restriction of the dam through pregnancy to 5 days following birth, then realimentation, Smart, Adlard, and Dobbing (1974) found incomplete recovery in weanling and adult rats. If pre-weaning compensation does occur, and this is debatable, it is probably passively controlled by the dam's ability to supply sufficient milk rather than by the pup's ability to actively 'catch-up'.

Mechanisms

This section illustrates how compensatory growth allows one to investigate the mechanisms controlling body size. The question is a basic one; are the average cell sizes in the body's organs controlled? We have seen that; unless an exceptional stress is imposed, cell division appears relatively inviolate; but is cell size as rigidly fixed?

To recapitulate: before weaning, in the rat, growth in weight is primarily by cell division, cell size remains relatively constant. Therefore pre- or post-natal under-nutrition slows live weight increase primarily because cell multiplication is inhibited. This effect, if under-nutrition is prolonged, can result in a permanently reduced cell number in adulthood (Leblond, 1972, Winick *et al.*, 1972). But do the reduced number of cells in such a rat grow to be of similar size to those of a control animal? This result has been inferred from the transient effect of post-weaning under-nutrition on body size (reviewed by McCance, 1976). A method of investigating this problem is as follows.

Williams and Hughes (1975) found that while rats from litters of 16 averaged at weaning 24 g and at 120 days 320 g, animals from small litters of eight weighed 42 g and 360 g respectively. Assuming that the cell numbers in most of the organs were fixed at weaning, and the cell sizes were similar in both groups, then the size difference between the groups should be due to a difference in cell number. McCance (1976, p.311) suggests this is so as such retarded animals '...showed no sign of 'catch up' growth; they behaved as though they were genetically small, slow growing, but perfectly proportioned animals.' Therefore, following McCance, the percentage difference in live weight at weaning, in William and Hughes' animals, should be preserved at maturity. So, 24/42 should equal 320/360; it plainly does not (0.57 vs 0.88). Even if there were some increase in the cell number in the retarded group this would not account for the large change in relative weight observed; there must be an increased cell size in the retarded group.

This result implies that body size is not controlled by cell size being tightly controlled during the phase of cell hypertrophy. Cell size like cell number appears to be variable.

Why did the cells of the retarded rats increase in size, giving compensatory growth?

CARTILAGE GROWTH

Arguments are to be presented linking compensatory growth to cartilage growth. But first it is necessary to discuss the mechanisms of cartilage growth and the direct effect of cartilage growth on bone growth and its indirect effect on muscle growth.

Leblond (1972) described tissues (of an 'intermediate' type) that contain stem cells which continue dividing well into life. Cartilage is such a tissue. Moss (1972, p.133) argued that '....biochemical factors play a significant role in regulating skeletal growth with respect to location as well as to duration and magnitude.' Moss (1972, p.132) described hormonal influences especially pituitary growth hormone, as '....a general stimulus to the growth of all other tissues.'

Without experimental evidence being quoted Moss writes (p.132) '...the growth plates of the long bones characteristically exhibit differential growth rates, yet presumably equal titres of STH (pituitary growth hormone) reach the chondrocytes at both sites.' The differential growth rates of cartilage cells in different body sites is obviously crucial for Moss to ascribe mechanical forces a primary role in cartilage growth.

The cellular kinetics and histology of cartilage growth have been recently studied in rats by Kember (1972,1973) and Kember and Walker (1971, 1972) by injecting tritiated thymidine and measuring both the size of the zone of cell proliferation and its rate of cell division. Kember and Walker (1971, p.428) wrote that the 'Rate of bone growth is equal to the product of two factors, the rate of production of new cells per column and the average size of hypertrophic cells.' But Kember and Walker show that the hypertrophic cells are of similar sizes at different ages so that 'The principal influence on growth rate must therefore be the rate of cell production.' Therefore, they concluded, the growth rate of bone is determined by two factors, the length of the proliferation zone and the rate of cell division in the zone. Kember (1973, p.450) found that '...proliferation zone length and proliferation zone division rate...are practically independent and that, further, while control of proliferation zone length is specific to each cartilage plate, the division rate is chiefly dependent on overall body controls such as nutritional state and hormonal levels.' Put more plainly Kember's findings show that cartilage plates in different bones have a specific number of dividing stem cells (determined genetically) but the cells in all sites divide at the same rate. Therefore mechanical stress seems to be of little importance compared to hormonal influences in the control of cartilage growth rate.

MUSCLE GROWTH

Hammond (1932) proposed that bone was an early developing tissue followed by muscle then fat. In size this may be so, but by growth in

cell number this is not so; muscle cell number being fixed while bone cell number is still increasing. The suggestion that muscle development follows bone development was indicated by Haines' law of muscle growth, '....muscle fibres grow along their whole length in response to traction set up by the growth of the bones to which the muscle is attached...' (Haines, 1932). In her recent review Stewart (1972) states that this theory has not yet been disproved. Stewart concludes (1972, p.86) that 'Although it is not suggested that passive tension is the sole factor or even the principle factor in stimulating muscle growth, it appears very likely that it does play a role, perhaps quite an important one in early development.'

Therefore one can see that by cartilage growth, and therefore bone length, being controlled the growth of the muscle mass will also be controlled. Much follows from this cascade. In the rat, muscle and skeleton comprise almost 60% of the body mass so an increase in their size could, by the functional demand (Goss, 1972) of the increased mass of tissue, increase the size of the 'supporting' organs of the body (heart, kidneys, lungs, etc.)

GROWTH IN CELL SIZE AND CARTILAGE

Compensatory growth, it was deduced, seems to involve an increase in cell size since cell number does not greatly increase in the rat following weaning. There have been no direct studies of the cellular kinetics of cartilage growth following a stress (although Mosier *et al.* (1978) review some ultrastructural studies). In the absence of firm direct evidence it must be hypothesised that the size of the zone of cell proliferation and its rate of cell division are affected to a minor degree, compared to other tissues, by pre-weaning stress.

As cartilage stem cells continue dividing for longer than most stem cells a nutritional stress could have a minor effect on them compared to tissues where hyperplasia is concentrated into a shorter period and, in many tissues, only occurs during a critical period. The long time span of cartilage cell division may therefore bestow on it a resistance to stress.

An explanation of the question posed after the analysis of Williams and Hughes' data (why did the average cell size in the body increase?) now seems possible. If cartilage cell proliferation, for the above reasons, is little affected by stress then during recovery cartilage growth will tend to be normal, even following a harsh stress. Bone growth would therefore occur and even though the number of muscle cells may be sub-normal the passive tension of the bones would increase their cell sizes. The increased muscle mass causes, by functional demand, growth in many other organs by an increase in cell size. This hypothesis can explain several properties of compensatory growth accounted for with difficulty by other growth theories.

The length of a period of under-nutrition has been shown to be negatively related to the growth response following realimentation. For example, Widdowson and McCance (1963) maintained, by under-feeding, the body weights of weanling male rats from three to twelve weeks of age. Realimentation failed to cause complete compensation. This reduced response, with age, can be explained by the present 'cartilage hypothesis'. Talwar *et al.*, (1975) have shown that the binding of growth hormone to cells declines with age as does the response of cartilage to sulphation factor. But Talwar's experiment used intact animals of various ages where the change in responsiveness may have been due to growth induced changes in cartilage. Repeating these experiments, using cartilage from long-term hypophysectomised rats, would show if cartilage did lose its responsiveness independently of the effect of growth.

Opinions vary on the contribution of body length changes to compensatory body growth. This may be due to a lack of data and, especially in the weanling rat, to the various methods used to induce under-nutrition. For example it has been recently stated (Raye *et al.*, 1977) that following foetal growth restriction two patterns of body growth result; an asymmetrical pattern (a relative weight deficit) and a symmetrical retardation. But the majority of evidence, especially in farm animals (Wallace, 1948), suggests that growth failure is largely symmetrical.

Widdowson and McCance (1960) measured body lengths and weights in rats raised in large and small litters. They concluded that the relationship between length and weight was similar in both groups. But as the variation in the large litters could have been great this may have obscured any changes in form that were present. There seems to be no detailed longitudinal study in the literature where the allometry of the weights and lengths of individual animals have been studied following under-nutrition.

Williams and Hughes (1975) using rats from large and small litters found that the catch-up in length was almost complete compared to that in body weight (as we have seen allometry was not used). They concluded (1975, p.192) 'The results indicate that growth of the axial skeleton may be of greater significance in our understanding of catch-up than changes in body weight.

Smart, Adlard, and Dobbing (1974) measured body weights, lengths, and bone maturity indices (using Hughes and Tanner's (1970) criteria) in animals retarded from five to twenty-five days of age. They state (p.145) 'The most remarkable finding to emerge from the length measurements was the degree of catch-up...' The initially reduced lengths of the retarded rats were associated with a more juvenile skeleton (gauged by the bone maturity index); aging in the skeleton seemed to have been halted by the under-nutrition. These authors surmised (p.145) that in retarded rats '...skeletal maturity and catch-up potential were associated.' and that '....the opportunity for enhanced bone growth was still open to them.'

The reduction in bone maturity due to under-nutrition found by Dobbing's group clearly supports the present argument; the resistance of the skeleton to under-nutrition being demonstrated. In the present work the size of a rat at weaning was found to be negatively related to the subsequent growth rate. The results from Dobbing's group suggest that skeletal maturity may be positively related to size at weaning. If the growth response of cartilage depends more on physiological age than on chronological age the cartilage of large more mature animals would be less responsive to growth stimulators than the cartilage of small, and therefore more juvenile, animals. Compensatory growth would

ensue. The small animals would, in terms of chronological age, also grow faster than the larger animals.

In Chapter Seven the correlations between the lengths and weights of a group of rats were much superior to the correlations between these variables and age. The body parts grew more uniformly with respect to one another than with respect to time. The cartilage hypothesis also seems to supply an explanation of the relative growth (allometry) seen between body organs.

An interesting test of this hypothesis would be to feed papain, which selectively disrupts cartilage growth, and measure the changes in body form of young rats. One would expect weight gain to be reduced, yet adipose reserves could accumulate. But would the allometric relations between other body constituents, and weight and length be maintained?

HORMONAL CONTROL OF GROWTH

The previous sections suggest that epiphyseal cartilage growth can induce a great deal of whole body growth and Kember's (1973) experiments have shown cartilage growth to depend primarily on 'overall body controls' of a hormonal nature. Therefore the stimuli controlling overall body growth would also seem to be those controlling cartilage growth. The 'cartilage hypothesis' seems to explain how body growth occurs but the endocrinology of this mechanism remains to be explained.

Although the hormonal control of cartilage growth appears from some reviews (Tonna, 1973, Vaughan, 1975) to be fairly well understood Kennedy (1970, p.324) states that '...there is no general agreement about the location or mechanism of the control of growth.' The highest level of direct integrated control of the endocrine system is from the hypothalamus where the electrical activity of neurons results in the production of hypothalamic hormones (releasing factors) which are transferred, via direct vascular connections, to the pituitary to influence its hormone production. The pituitary hormones circulate systemically to, in turn, control the production of the peripheral

hormones that directly affect tissues. The links in this system of 'hormonal amplification' that control pituitary growth hormone secretion will be discussed. Hormones of the thyroid, adrenal, gonads, and pancreas also influence body growth (see Tonna, 1973; Vaughan, 1975) but these hormones have little effect in the absence of pituitary growth hormone (GH), which can by itself stimulate whole body growth.

THE HYPOTHALAMUS AND GROWTH

The medial basal region of the hypothalamus is involved in the control of body growth, as seen by lesions or knife cuts made in this region (Rice *et al.*, 1976). As the hypothalamus also controls food intake and energy balance (Kennedy, 1967; 1969; 1970) it is obvious that the integration of these functions with the control of growth must be considered in this review. Kennedy (1969, p.1057) refers to '...the close integration of food intake with size and growth.' Although these functions are integrated they possess a high degree of autonomy. The failure to recognise this autonomy has been the source of much confusion.

Many authors fail to differentiate between the mechanisms controlling body size and those controlling adipose tissue mass. Body weight is taken to indicate a change in body fat by some authors and a change in body growth by others. But, as described in Chapter 8, ascribing a change in body weight to a change in body fat is difficult to justify, without chemically analysing body composition, even in the adult 'plateaued rat'. Due to this confusion of growth with adiposity their regulatory mechanisms have, not surprisingly, also become confused. In the rat, lesions placed in the region of the ventro-medial hypothalamus (VMH) lead to hyperphagia and obesity. The VMH has therefore been described as a 'satiety centre' which is sensitive to blood borne factor(s) which are reflective of the body's adipose reserves. Thus the VMH has been said to contain a 'body weight set-point' mechanism which adjusts food intake so as to preserve the adipose reserves. As

described in Chapter 8 the use of the term body weight set-point should be avoided; body fat rather than body weight appears to be the variable that is controlled by the VMH.

It should be mentioned here that the simple classical view, of a hypothalamic nucleus (the VMH) inhibiting food intake and another nucleus (in the lateral hypothalamus) stimulating food intake, is losing credence. Gold (1973) found that discrete lesions limited to the VMH did not affect feeding or adiposity. Sclafani and Berner (1977) using knife cuts, obtained evidence that fibre tracts passing the VMH mediate feeding. Theadipsia and aphagia resulting from lesions to the lateral hypothalamus have been interpreted as indicating that this area of the brain is a centre that specifically initiates feeding. But some recent work (Davis, 1977) has suggested that lateral hypothalamic lesions primarily affect thyroid function; similar adipsia and aphagia follow thyroidectomy. The lateral hypothalamus seems to be the site of thyroid releasing hormone production (Winokur and Utiger, 1974). The evidence for two anatomically separate feeding centres, obtained by stimulating or lesioning the hypothalamus, has been recently questioned by Woods and Porte (1978, p.298) who review the evidence that 'Whereas stimulation or lesions of the hypothalamus have been thought to influence mainly the nuclei located near the tip of the electrode, recent evidence suggests that specific tracts passing through or near these nuclei are actually the critical structures and that manipulation of their activity at any point along their pathways will lead to the same syndrome of effects as manipulations of the nuclei themselves.' Woods and Porte discuss the evidence that a ventral nor-adrenergic tract originating in the reticular formation and terminating in the septum and hypothalamus is damaged by VMH lesions. Woods and Porte conclude that '....it is the tract rather than the nucleus....' that regulates feeding.

The nature of feedback, to the hypothalamic 'lipostat' which indicates the state of the body fat stores is unknown. It has been suggested that the feedback is; via a substance that is partitioned between the lipid and water of the body, via free fatty acids, or via combinations of hormones. These theories have been based upon

the assumption that the actual concentration or amount of fat in the body is being controlled as a whole. The obvious method of testing whether there is autoregulation of the mass of body fat is by surgically removing fat and following the subsequent depot growth for evidence of compensatory growth. Although several workers have attempted such studies their results have been inconclusive, possibly due to their use of obese rats. But, recently, Kral (1976) and Faust *et al.*, (1976) have reached similar conclusions following the removal of adipose tissue form normal rats and mice. Faust *et al.*, (1976,p.538) concluded that '....surgical removal of fat does not lead to compensatory growth of fat. Autoregulation of adipose tissue mass, if is occurs, most likely operates through detection of adipocyte size rather than adipocyte number or total fat mass.' It might be concluded that genetic factors inherent to adipocytes greatly influence their size. But Ashwell *et al.*, (1977) cross-transplanted, to the kidney capsule, adipose tissue from hereditarily obese (ob/ob) mice to lean mice, and *vice versa* . They concluded (p.343) 'The fat cells of obese donor fat decrease in size in a 'lean environment' to the size typical of 'lean fat' while the cells of lean donor fat transplanted into an 'obese environment' increase to the size typical of 'obese fat'.' The evidence clearly indicates that the size of individual adipocytes is regulated rather than the total fat mass. How, or if, the hypothalamus senses the size of adipocytes, especially since the mean size of adipocytes in different body depots varies (Gairdner and Dauncey, 1974; Clark, unpublished observations), remains to be determined.

This evidence questions the theories of body fat regulation based upon the premise that the actual amount of body fat is regulated. Woods and Porte (1978, p.299) assume that '....the brain receives some signal of total body adiposity...' and construct a control mechanism for adipose reserves based on CSF insulin as an '....accurate messenger of the amount of adipose stores in the body.' (p.302). However their earlier conclusion (p.300) on adipose mass that 'What is not known is the nature of the signal that provides the CNS with the information needed to accomplish regulation, or the area of the brain where such

regulation occurs.' is a fair description of the current state of knowledge.

If one removes the effect of food intake, by pair-feeding VMH lesioned to unlesioned controls, VMH rats still show an increased deposition of adipose tissue (Han. 1967; Goldman *et al.*, 1974). A variety of evidence lead Goldman *et al.*, (1974, p.91) to conclude that '...obesity produced by hyperphagia is fundamentally different from that seen after hypothalamic destruction.' and that (p.90) '..there is a basic shift in the metabolic pattern toward lipogenesis.' In a recent paper Cox and Powley (1977) found that genetically obese diabetic mice accumulated five times more lipid than pair fed non-obese siblings. Cox and Powley (1977, p.347) review the evidence that 'Restriction of food intake to normal levels does not preclude an excessive accumulation of fat in rats made obese by hypothalamic lesions (VMH rat), in the genetically obese fatty (fa/fa) rat, or in the obese hyperglycemic (ob/ob) mouse.' Clearly the regulation of body fat stores is to a degree independent of the modulation of feeding behaviour. The hypothalamus appears to affect the partition of nutrient between the fat stores and the lean body mass. Goldman *et al.* (1974, p.91) state "The partial growth hormone deficiency produced by VMH lesions may well contribute to this phenomenon, but the possibility that other pathogenetic mechanisms exist, clearly warrants further investigation'. Other pituitary hormones, for example the little investigated lipotrophic hormone, are likely candidates for involvement

The practical importance of these studies is considerable, especiall regarding human obesity. Their importance to the agriculturalist is seen by Zucker's (1975) demonstration that the Zucker hereditarily obese rat, with food intake restricted to approximately normal levels, retained over 20 percent of the energy supplied while control rats retained less than 10 percent.

The cause of the VMH syndrome is still largely unknown. This preparation has served as the model for much of the experimental work concerning the control of energy balance. The lack of understanding of the VMH rat is therefore reflected in our lack of knowedge concerning the control of energy balance.

Using more refined techniques, the production of medial hypothalamic islands (MHA) with a Halasz-Pupp knife, Rice *et al.* (1976) studied food intake, body weight and body length. They found no 'static phase' of obesity (usually reached following VMH lesions) as food intake was still elevated 28 weeks following surgery and the body weight curves of controls and operated animals continued to diverge up to this age. These findings are difficult to account for by Kennedy's lipostatic theory of energy balance which predicts that intake is restrained by the lipostatic mechanism once the adipose reserves reach a certain size.

Attempts have been made, notably by Wade (1972, 1976) , to incorporate the hypothalamic control of energy balance and growth into a single theory. For example, based upon the work of Goldman *et al.*(1970), Wade (1976) attempts to equate the rapid gain in live weight, that occurs following the placement of VMH lesions in hypophysectomised weanling rats, with the gain in live weight following GH injections. But, as Goldman *et al.* show, hypophysectomised rats increase in weight following VMH lesions by becoming obese while growth hormone treated rats display linear growth and weight gain without obesity. Wade (1976, p.239) describes a theory of energy balance where growth hormone inhibits VMH activity since 'These effects of ventromedial hypothalamic lesions and growth hormone in immature rats are not additive,'. But, clearly, one would expect obesity (VMH lesions) and the stimulation of linear growth (GH treatment) to be additive. This is what Goldman *et al* . (1970) found; body weights and lengths were greater in hypophysectomised-VMH-GH treated weanlings than in hypophysectomised-VMH weanlings.

Wade therefore ascribes to GH a crucial role in the regulation of food intake and energy balance and implies that VMH lesions give a GH deficiency which causes obesity. This has not been established. Halasz (1964), Han *et al.* (1965), and Bernardis and Bellinger (1976) have shown hypothalamic lesions can produce obesity, obesity and stunting, or solely stunted growth. Dunn and Arimura (1974, p.198), using rats with MHA islands, concluded that 'The observation that rats with MHA were shorter as well as grossly obese indicates that linear growth and body weight may be dissociated.'

The hypothalamic control of pituitary growth hormone appears to be primarily due to an inhibitory factor (called somatostatin by its discoverers, Brazeau *et al.*, 1973). Therefore it is not surprising that complete deafferentation of the hypothalamus in rats, by producing MHA islands, should leave serum GH levels greatly elevated (Rice *et al.*, 1976). The amount of somatostatin in the hypothalamus, measured by radioimmunoassay, of animals possessing MHA islands was found to be decreased by about 80% by Brownstein *et al.* (1977). Efendic, Hokfelt, and Luft (1978) review the localisation and effects of somatostatin. Since GH appears to be subject chiefly to inhibition by the hypothalamus it is surprising that some hypothalamic lesions produce growth inhibition. This dwarfing, as it is sometimes accompanied by obesity, cannot be due solely to a depressed food intake. Perhaps the the hypothalamus, especially its dorso-medial area, produces factors that stimulate pituitary GH production. There is some support for this finding as VMH lesions, that produce linear growth retardation, have been reported to result in depressed GH levels in plasma (Goldman *et al.*, 1970).

GROWTH HORMONE AND THE SOMATOMEDINS

Like all other pituitary hormones, the majority of growth hormone's effects appear to be indirectly mediated by hormones that growth hormone, in turn, controls. The growth of cartilage appears to be governed by a family of growth hormone dependent serum factors called somatomedins (reviews by van Wyk *et al.*, 1974; and Hall *et al.*, 1975b). The somatomedins are present in very low concentrations in serum so they have proved difficult to purify and their chemical structure is therefore unknown. Whether or not somatomedins are metabolites of pituitary GH is therefore still disputed. But in perfused livers Daughaday *et al.* (1976) and Phillips *et al.* (1976) found that insulin and growth hormone produced somatomedins and Francis and Hill (1975) obtained somatomedins from prolactin perfused livers. Therefore somatomedins do not seem to be portions of the

GH molecule. This evidence also indicates that somatomedin is produced by the liver. Although the sulphate incorporation of cartilage is stimulated by somatomedin (hence the original name for somatomedins - sulphation factors) there is as yet little evidence that, *in vivo*, somatomedin actually stimulates linear growth. For example the stimulation of cartilage growth in the hypophysectomised rat (the classical rat tibia test for growth hormone-like activity) by somatomedin has yet to be demonstrated (see the discussion following the paper by van Wyk *et al.*, 1974).

Classically, hypothalamic and pituitary hormone production is controlled by the feedback of peripherally produced hormones. Feedback inhibition, of GH production and release, by the somatomedins has not been demonstrated. It may be that the feedback control of GH is via a substance produced by, rather than producing, cartilage growth. This is an area of endocrinology that has received little attention but it appears to be an area of great potential for the practial manipulation of growth. One becomes aware of how little is known about the control of body growth when one observes that we still do not know how the brain perceives the status of body growth. Furthermore we do not even know if the brain perceives body size.

OESTROGENS AND GROWTH

The profound effects of oestrogen on growth and energy balance have been used as the experimental evidence for some of the current theories concerning the control of energy balance and growth. The present study, and some of the author's related work that is not included in this thesis, has shown effects that cannot be readily explained by the regulatory mechanisms that have been proposed by others.

In Chapter Eight oestrogen treatment of ovariectomised rats was seen to increase the percentage body fat and decrease linear growth; a result that is consistent with the older literature data. If bone growth and energy balance are controlled by separate but interacting

mechanisms the results are explicable, but, as explained in Chapter Eight, they are inexplicable from the viewpoint expressed by Wade (1972, 1976). The effect on bone growth was maintained through five weeks of oestrogen treatment, growth remained inhibited. Although body fat at first did not increase (following two weeks treatment) it did increase after five weeks. The transient effect of oestrogen on food intake (Tarttelin and Gorski, 1973; Wade, 1976) could explain the changes in body fat observed. As food intake rebounds during prolonged oestrogen treatment body fat may also rise; the temporal coincidence of these two changes requires further study. This result strongly suggests that oestrogen is acting at two separate sites as its effect on growth is non-adaptive, but its effect on energy balance is adaptive. The differential effects of oestrogen on tail and body lengths (Chapter Seven) suggests a peripheral site of action for oestrogen. It is possible that the effects of oestrogen on body fat and food intake are also controlled independently. This independence could be tested by feeding oestrogen treated rats a constant amount of food and sequentially killing the animals to study changes in body fat. It may be that the presently observed decline and recovery of body fat is independent of the level of food intake. Such an experiment would be an interesting test for Kennedy's lipostat.

Gale and Sclafani (1977) either placed bilateral parasagittal knife cuts between the medial and lateral hypothalamus and/or ovariectomised rats. The ovariectomised rats showed normal post-operative weight gain on a quinine adulterated diet whilst the 'cut' animals' hyperphagia and weight gain were blocked. A high fat diet had little effect in ovariectomised rats but potentiated the weight gain of 'cut'rats. The effects of ovariectomy and'cuts' on weight gain were additive, the ovariectomised-'cut' rats weighed more than 'cut' rats. The marked effect of diet palatibility in 'cut' animals, Gale and Sclafani point out, argues against the set-point hypothesis; weight was not being defended. As Gale and Sclafani observe (1977, p.392) their results '...lend little support to the view that estrogenic modulation of feeding is mediated by the same fibres responsible for hypothalamic hyperphagia.' Gale and Sclafani speak

of 'ovarian obesity', but if one views the response to ovariectomy as true growth the additivity of the weight response and the differences due to diet palatibility between their treatments become explicable. Oestrogen or ovariectomy could act on the mechanisms controlling growth while the knife cuts affect feeding and nutrient partition. These results suggest that oestrogen may not act in the region of the VMH, so where in the hypothalamus does oestrogen act? The evidence amassed herein suggests that the control of growth and adipose tissue are anatomically separate. Separate controls of intake, subserving these functions, may also be present. Oestrogen may act directly on the mechanism controlling intake for growth. The effect on food intake of lesions in the dorsomedial hypothalamus (an area controlling growth) combined with oestrogen treatment or ovariectomy may clarify this issue.

MHA islands, produced by Rice *et al.* (1976) in female rats, increased body length and body weight and produced obesity, but in male rats they produced a decreased body length and obesity. A similar growth response of females has been reported by others. Despite the sex difference in growth similar elevations of serum GH were recorded in both sexes. The gonads in both sexes were atrophic, uterine weights were ten times smaller. The occurrence of a sex difference in growth, despite similar serum GH levels, suggests a peripheral effect caused by functional gonadectomy. MHA obesity occurs in spite of increased serum GH levels while VMH obesity occurs with depressed serum GH levels. Clearly GH and hypothalamic obesity can be separated. The stimulation of growth due directly to hypothalamic surgical manipulation is yet to be demonstrated but MHA islands have yet to be produced in previously ovariectomised rats.

The site of oestrogen's inhibition of growth was investigated by determining, by radioimmunoassay, the serum GH levels in the intact, ovariectomised, and oestrogen treated animals described in Chapters One and Eight (Clark and Tarttelin, 1975). The oestrogen treated rats showed elevated serum GH levels, despite growth inhibition, while linear growth stimulation in ovariectomised rats was accompanied by lowered serum GH. Again, as was seen following MHA island

production (Rice *et al.*, 1976), serum GH levels were not proportional to the growth response displayed by the animals.

The mechanism of oestrogen's elevation of plasma GH levels, accompanied by increased pituitary weight, is still debatable. Lisk (1969) implanted oestrogen in subcutaneous, hypothalamic, and pituitary sites and weighed pituitarys, ovarys, and uteri to conclude (p.369) '...that weight increase in the pituitary is a function of direct action of estrogen on the pituitary gland.' One cannot conclude from Lisk's results that oestrogen acts directly on the pituitary to affect GH levels, but the measurement of serum GH and oestrogen levels, in a similar experiment, may allow this conclusion. It is possible that the peripheral inhibition of growth by oestrogen produces a feedback to the pituitary which contributes to the elevation of serum GH.

As oestrogen stimulates pituitary GH secretion its inhibition of growth would appear to be a peripheral effect. This idea is supported by the results of Josimovich *et al.* who found (1967, p.1428) '...that the partial inhibitory effect of pharmacologic doses of estradiol valerate (3.3 - 10 µg total dose) on growth hormone-induced widening of the tibial epiphyseal cartilage of hypophysectomised rats occurs independently of the effects of the estrogen on dietary intake in these rats.'

Reports that oestrogen reduces serum sulphation factor activity (Wiedeman and Schwartz, 1972; Phillips *et al.*, 1973) suggested to the author an experiment which would test, *in vivo*, if decreased sulphation factor levels were responsible for the growth inhibition caused by oestrogen. Somatomedin is reported to be produced by the liver, and oestrogen should therefore inhibit liver somatomedin production. If an ovary is transplanted to the spleen the oestrogen produced by the transplanted ovary travels via the hepatic portal system to the liver where, in the rat, it is completely inactivated. Therefore, if the other ovary has been removed, the pituitary receives no steroid feedback and the resultant high gonadotrophin levels cause the grafted ovary to hypertrophy and to secrete large amounts of steroids into the liver. It was proposed that the oestrogen produced by the grafted

ovary should inhibit liver somatomedin production and therefore inhibit the body growth of grafted rats despite the lack of systemic oestrogen (Clark and Tarttelin, 1977). However grafted rats showed no inhibition of growth, they were identical, in length and weight, to ovariectomised controls. It was concluded that oestrogen does not inhibit body growth by inhibiting the liver's production of somatomedin (Clark and Tarttelin, 1977). As radio-receptor (Hall *et al.*, 1975) and radioimmunoassays (Yalow *et al.*, 1975) for somatomedins are now available the level of somatomedin in the plasma of ovarian-splenic transplanted rats could be tested directly.

Oestrogen does not appear to directly inhibit cartilage growth as it has no effect on the sulphate incorporation of cartilage when added *in vitro* (Priest *et al.*, 1960; Phillips *et al.*, 1973). But if animals are treated with oestrogen their cartilage, tested *in vitro* incorporates less radio-active sulphate (Priest *et al.*, 1960). Oestrogen treated rats also show depressed sulphate uptake compared to pair-fed controls, indicating that the suppression of sulphate incorporation is independent of food intake (Priest and Koplitz, 1962). Priest and Koplitz also found that oestrogen suppressed sulphate incorporation in hypophysectomised rats. This agrees with the suppression of linear growth by oestrogen in hypophysectomised weanling rats found by Paesi and de Jong (1954).

Phillips and Young (1976) fasted male rats for 24, 48 and 72 hours and obtained progressive disappearances of somatomedin activity and cartilage responsiveness to somatomedin. These effects could not be reversed by 500 µg of GH injected daily. Therefore it appears that nutrition can influence cartilage growth independently of possible effects on GH secretion. As Phillips and Young used only male rats these results may not be comparable to those obtained from female rats. For example the effect of sex hormones has been shown by Widdowson (1976) to extend to the response of the sexes to under-nutrition. During a 6 day fast male rats derived 33% of their energy from protein and 67% from their body fat while in females only 8% of the energy came from protein and 92% from fat. Widdowson ascribes these effects to a direct effect of androgen in the male. A similar study involving

gonadectomised and intact rats could clarify this point. It is possible that the factors affecting the partition of energy between lean and adipose tissues, seen in the VMH rat, could be operating. But clearly, when starvation is investigated such large differences between the sexes must be heeded.

The control of cartilage growth is further complicated by reports of factors in serum that inhibit somatomedin action on cartilage *in vitro* (Salmon, 1973, 1975; Phillips and Young, 1976). Salmon proposes that 'The action of the inhibitor provides a faster, and potentially greater, restrain on glycoprotein synthesis than that resulting from a decrease in the level of somatomedin alone. An increase of inhibitor and decrease of somatomedin are complementary mechanisms for limiting anabolic events under conditions of restricted nutrient intake. The physiological gain is conservation of substrate for vital processes.' (Salmon, 1975; p.198). It is pertinent to recall the earlier sections of this chapter where Bullough's chalone theory, of tissue specific growth inhibitors, was discussed. Cartilage growth may also be subject to chalone inhibition. But as it is said that chalones result from cell division it is unlikely that oestrogen, which inhibits cartilage cell division, could act via the chalones. But cartilage growth does appear to be subject to inhibitory substances, for example the effect of nutrition on growth described by Phillips and Young could be hormonally mediated. Oestrogen could inhibit growth by such a mechanism.

The words of LoBue and Gordon (1973, p.XIV) are apt,

'Finally, we believe the reader will be impressed by the fact that for all the diversity of material presented, a common and recurrent theme will emerge. This theme will be a stimulator - inhibitor, humorally based, feedback regulation of growth and differentiation.'

One can therefore envisage a controlling mechanism for growth, but is there a mechanism in the body, like the 'time - tally' hypothesised by Tanner (1963), against which growth is constantly being compared? Is there a 'growth - stat' akin to Kennedy's 'lipostat'? McCance doubts the whole concept of a 'stat' when he argues that

'....the hypothalamus is only one of the exchange centres for the whole of the behavioural activities of feeding. There can be no such thing as a localised 'stat' in it or anywhere.' (McCance, 1972; p.1271).

It seems sufficient to say that growth is subject to a multiplicity of influences; that it manages to maintain order among the chaos of the external milieu reflects the denial of entropy that Claude Bernard's idea of homeostatis entails.

CHAPTER TEN

NOTES

ONE - THE DEFINITION OF COMPENSATORY GROWTH

TWO - THE USE OF AVERAGE DAILY GAIN

THREE- SIGMOID GROWTH CURVES

FOUR - TIME SCALES IN LONGITUDINAL EXPERIMENTS

FIVE - ANALYSES OF VARIANCE: FOR REPLICATED LINES IN A CROSS CLASSIFICATION

SIX - COMPUTATION OF ORTHOGONAL COEFFICIENTS FOR THE SUBDIVISION OF MEAN SQUARES FOLLOWING AN ANALYSIS OF VARIANCE FOR UNEQUAL SUB-CLASSES

SEVEN- DO RATS PLATEAU IN LIVE WEIGHT?

EIGHT- THE BIOLOGICAL INTERPRETATION OF THE TRANSFORMATION USED FOR LIVE WEIGHTS

NOTE ONE

THE DEFINITION OF COMPENSATORY GROWTH

The term 'compensatory growth' first appeared in agricultural literature (Bohman, 1955), 'catch-up growth' being the synonymous term in medical literature. Despite compensatory growth being a widely used term, for example the title of a review by Wilson and Osbourn (1960), its meaning is sometimes questioned. This could be due to the term being somewhat ill-defined.

Bohman (1955) defined compensatory growth as the property of growth which allows an animal to show an abnormally rapid growth rate relative to age. Others define compensatory growth as occurring in animals "...which have been subject to a period of undernutrition... exhibit compensatory growth during the period of realimentation. This phenomenon is characterised by faster than average growth when liberal feed supplies become available..." (Preston & Willis, 1970; p. 307).

These definitions involve the terms 'abnormally rapid', or 'faster than average' growth. However normal growth would seem to be particularly difficult to define or measure. Compensatory growth is also defined or described in terms of under-nutrition. But the restriction of growth may be due to a variety of influences. In addition a nutritional stress may be one of over-nutrition (Widdowson, 1976), which is followed by a period of slow growth. The compensation following weaning in the rats of this study involved small animals having fast growth rates and larger rats with slower growth rates. Thus the literature definitions would seem on one hand to be vague and on the other too specific.

A simpler description of compensatory growth would be, the property of growth enabling the growth curves of individuals to converge. As live weights of animals follow a lognormal distribution, and growth is multiplicative, the convergence should be shown on a logarithmic scale. This later qualification is important as on an arithmetic scale two growth curves could be said to be parallel, but the percentage difference between the curves would decline giving convergence on a logarithmic scale, or compensatory growth.

NOTE TWO

THE USE OF AVERAGE DAILY GAIN

Wishart (1939) presented several methods for analysing a time series experiment investigating the effect of diet on pig live weights. Using the average daily live weight gains Wishart could show no significant treatment effects, but when the mean average daily gains were adjusted for initial weight the treatment effects became statistically significant. Further analyses revealed that fitting quadratic and cubic terms by orthogonal polynomials allowed hitherto undetected treatment effects to become apparent. The transformation of live weight to logarithms, followed by the fitting of polynomials, showed only the linear and parabolic terms to be statistically significant which '...represents a distinct advantage'. In addition the logarithmic analysis revealed differences between treatments that were previously non-significant.

Despite Wishart's demonstration that superior methods of analysis are available, the average daily gain has retained its place as the main method of analysing time series growth data, especially in agriculture. Many agricultural researchers have noted that estimates of average daily gains can be highly variable. Attempts to reduce the within group or between animal variation have largely centred around the belief that weighing errors cause the high variability. Weighing errors are taken to include the biological variation due to variable alimentary tract and bladder contents. For example there is a large body of literature describing the use of weighing on three consecutive days, fasting before weighing, and the optimal hour of the day to weigh grazing animals so as to reduce the variability of the live weights. The actuality of the high variability of live weights in ruminants is clear, but the author suggests that systematic variation is incorporated into the "error variance" by the statistical techniques usually used to analyse live weights. Systematic variation due to short-term and long-term growth cycles is described in Chapters Six and Seven and is separated in an analysis of variance in Note Five of the present chapter.

The rejection of average daily gains can be based on several arguments. Some of the biologically based reasons for analysing growth

on a multiplicative metameter are considered in Note Eight of this chapter.

A statistical disadvantage of average daily gains is that since successive increments (daily gains) are negatively correlated, due to the same measurement being used twice to estimate adjacent rates of gain, the correlations between adjacent rates of gain are difficult to interpret. Since two measurements are involved in calculating an increment the standard error of the difference between the measurements is $\sqrt{2}$ times that of the error of the individual measurements. Another problem with the method of increments is that increasing the number of measurements does not increase the precision of the estimates for individual velocities. When measurements are made more frequently smaller differences result between the successive measurements, yet the errors of measurement remain the same. This, perhaps unexpectedly, gives a poorer estimate of the increments. A situation where increasing the number of observations does not increase the precision of an estimate suggests, intuitively, that the techniques used are inefficient.

An obvious solution is to choose observations as far apart as possible, the first and last measurements made, to estimate the daily gain. But the intermediate observations are then not used, a terrible waste of information. Another common approach to the estimation of average daily gains, fitting a least squares regression to the untransformed live weights, neglects the obvious non-linearity of the majority of growth curves. This gives a considerable amount of unexplained variation, wasting information and increasing the errors of estimation. Fitting a least squares regression to untransformed live weights ignores an assumption of the least squares model; that the variability of live weight at each age should be constant (the live weights are then homeoscedastic about the regression). This can be easily checked for grouped data by showing that the means and standard deviations at each value of the independent variable are unrelated. But earlier chapters have shown, without exception, that the variation in live weight increases with increasing size. A transformation of live weight, to allow regression, is obviously required.

An outcome of this heterogeneity of live weights' variances on an arithmetic scale is that the difference between two live weights, the greater weight having a larger error than the smaller and neither being

normally distributed, has a distribution that is non-normal. In a recent thesis D Clark (1974) shows a frequency plot for the average daily gains of two populations of bulls; one population was skewed to the left, the other to the right, but neither resembled either a normal or a log-normal distribution. The author is unaware of frequency plots of average daily gains in other publications or of Probits or Rankits being employed to test the normality of a series of average daily gains. If the use of average daily gains is to persist its underlying frequency distribution should be established so that transformations to give normality can be used.

Multiple weighing on three consecutive days has been said to reduce the variability of live weight measurements, especially in ruminants. However in his recent review Hughes concludes that singly taken weights can be as accurate as multiple weighings. Hughes (1976, p. 117) recommends that 'It is better to increase the number of animals weighed to reduce the experimental error to an acceptable level than to increase the number of weighings'. This, as we have seen, pertains if average daily gains are to be estimated as the number of measurements does not increase the precision of the estimated increments. The opposite to Hughes' recommendation holds if linearised curves are fitted: '...but if growth curves were to be fitted the accuracy of each individual weight was not so important as the number which could be obtained.' (Wishart, 1939; p. 22). Since the curve fitting approach requires less animals, but more frequent measurements, it would seem to be the most economical form of experimentation. Also as the accuracy of each measurement is not so critical it would seem especially suitable in experiments involving pasture grazing where day to day variations in live weight tend to be large.

As Fisher noted in 'Statistical Methods for Research Workers' (1946, p.29) "...it is desirable to so manipulate the variables that the law to be tested will be represented by a straight line." But the transformation of variables is carried out with reticence by biologists; they inquire as to the biological meaning of the transformations. However the understanding of the biological significance of a transformation, although of interest and importance, need not necessarily concern the experimenter. His primary aim is to use the transformation as a statistical tool in comparison or prediction.

In defence of his use of the average daily gain the agriculturalist may state that he is not interested in the logarithm of live weight only in the absolute weight. On the logarithmic scale a difference of 10kg at 10kg live weight is equivalent to a difference of 100kg at 100kg live weight so that the logarithm is 'not what the farmer is interested in'. However if a linear relationship on transformed scales is established estimates of weights at required ages can be made and transformed back to the arithmetic scale, or a predicted initial weight can act as a covariate (giving increased precision - Chapters Two to Five) and the adjusted means can then by transformed (using the appropriate correction factors) back to the arithmetic scale for interpretation.

In the following passage Kleiber (1947, p526-7) vigorously defends the use of logarithms, which Benedict had previously criticised,

Benedict extends his accusation, stating that logarithmic interpolation "distorts or obscures striking differences between species." Since, however, a logarithmic chart in a scientific paper is presented to readers who are presumably familiar with logarithms, the accusation of distorting or obscuring can be discarded.

There is nothing obscure about the fact that a logarithmic regression line of a given set of data looks different, in general, from the corresponding arithmetic line, and if this difference in the appearance of the two regression lines be termed distortion, one could call the arithmetic line a distortion of the logarithmic just as well as vice versa."

Finally, to again quote the most quotable Kleiber, what is needed

"...in biology, however, is not less mathematics, but good mathematics."

NOTE THREE

SIGMOID GROWTH CURVES

In the present work lines are fitted to live weight-age data solely by using transformations of the variates to give linearity. Although it would be possible to review and to test the many growth equations that have been proposed the words of Snedecor (1968) are pertinent, "A stupendous amount of time has been wasted by ill-advised curve fitting."

The equations used by Brody were discussed in Chapter Six due to their being used recently by Bliss (1970). Live weight growth is frequently referred to as being sigmoid so it follows that curves of this form should be advocated and used. For example the Gompertz equation was employed by Laird & Howard (1967) to describe the live weight growth of the mouse from 14 to 70 days of age. Weighings at birth and at seven days were discarded as the '...rate of exponential decay characteristic of post-natal growth starts at about 14 days of age.' Similarly, 'As the Gompertz growth process slows at approaching maturity, a second, linear process becomes evident in mice and many other species.' Not only did the Gompertz equation not cover the extremes of growth but using '...the fitted growth curves with their standard errors....the parameters differ only slightly from one another, and their standard errors overlap.' Thus the Gompertz equation would appear to cover a smaller range in the mouse than does the log-reciprocal equation in the rat. Also in the rat the log-log equation covers the immediate post-natal period for which the Gompertz equation does not seem to give a good explanation. It seems that using the Gompertz equation post-natal live weight growth could be divided into three phases, birth to 14 days, 14 to 70 days, and from 70 days onwards. In the rat the use of the simpler log-log and log-reciprocal equations gives the more simple division of growth into two overlapping phases whose zone of overlap can be associated with the transition from hyperplastic to hypertrophic growth in most tissues.

Barton & Laird (1969) found that Gompertzian growth curves fitted to skull bone lengths gave '...systematic departures, and these growth

processes are not adequately represented by the simple Gompertz function.' So the residuals about the Gompertz equation describing live weight growth would probably also show serial correlation; giving no improvement in the explanation compared to simpler growth models, for example those proposed in this thesis. Neither Howard & Laird nor Barton & Laird give the percentage of the variation explained by the Gompertz equation; comparison with the present methods is therefore difficult without further re-analysis using both methods.

Bliss (1970) discusses '...a limitation...' of the alternative form of the Gompertz, the log-log transformation. This is the same limitation described in Section One of Chapter Six for the Brody-type equations, 'A small discrepancy near the upper live weight asymptote ...was magnified excessively...' (Bliss, 1970; p.204).

NOTE FOUR

TIME SCALES IN LONGITUDINAL EXPERIMENTS

The organism, it is argued, dispenses a Time of its own making...

Medawar, 1945

The linearisation of weight-age curves in a variety of species, subject to a range of influences, suggests a fundamental change in the experimental design of growth studies. It is usual in longitudinal experiments to use a linear time scale by obtaining measurements at daily, weekly, or monthly intervals. This practice appears to spring nore from convenience than from utility. It has long been recognised that physiological time observes a scale of its own.

The transformation of time to a reciprocal, logarithm, or other non-linear scale causes the measurements, obtained on a linear scale, to be given unequal weight in the calculation of the slope of the least squares estimate of the growth equation. For example, following a logarithmic or reciprocal transformation the early weighings exert a greater influence on the slope than the later weighings.

In a bioassay where one intends to use the logarithm of dose it is common practice to employ a multiplicative scale of dilutions or concentrations. If, *a priori*, a certain transformation of time is intended, to enable linearisation of the measured variable's relationship to age, then the choice of equal intervals between measurements on the transformed time scale leads to a more balanced regression.

From the age of four weeks a reciprocal transformation of age linearises the log live weight-age growth curve of the rat. An experimental design, based on the reciprocal transformation, to give roughly equally spaced observations is shown in Table 10.1. Although the spacing intervals on the proposed scale still decrease with age the decline is small (extreme values being .010 and .018) compared to that of a conventional weekly scale (extreme values being .05 and .004). This proposed scale should be regarded as illustrative, rather than definitive, as superior, more equally spaced scales can probably be calculated.

TABLE 10.1

SUGGESTED SCHEDULE OF WEIGHINGS FOR A LONGITUDINAL GROWTH STUDY IN THE RAT.

AGE AT WEIGHING			
USUAL	RECIPROCAL	1.0 / AGE (weeks)	SPACING INTERVAL
28	28	.25	
			.0167
35	30	.2333	
			.0146
42	32	.2187	
			.0187
49	35	.2000	
			.0158
56	38	.1842	
			.0175
63	42	.1666	
			.0177
70	47	.1489	
			.0168
77	53	.1321	
			.0154
84	60	.1166	
			.0137
91	68	.1029	
			.0132
98	78	.0897	
			.0128
105	91	.0769	
			.0102
	105	.0667	

However it has been stated by Chatfield (1970) that '...if the experimenter has convincing evidence that the relationship is linear, then ... it is better to take more observations at the ends and "starve" the middle.' It is possible that both the efficiency of experiments, in terms of the number of measurements required, and the precision of regressions, could be improved by this even more radical departure from the current experimental design of longitudinal growth experiments.

Different experimenters and experiments require different designs, but if linearising techniques are to be adopted then the proposed designs deserve consideration.

NOTE FIVE

ANALYSES OF VARIANCE: FOR REPLICATED LINES IN A CROSS CLASSIFICATION

The advice of Fisher (1939), that the slopes and constants from regression lines should be analysed as raw data, was followed in Chapter One. But analyses of variance for replicated regressions are more imformative if, as Bliss (1967) shows, they are based upon the complete data set with the variables manipulated by simple transformations to normalise the dependent variable and to give a linear relationship between the two variables.

Therefore, retrospectively, the comparison of the growth curves used in Chapter One was crude. The more refined methods described by Bliss (1967, p.451-470) allow an analysis of variance to be constructed which for (f) rats, at (k) ages, in a single treatment group, partitions the variation into five terms. The variation,

1) in position of the f regression equations,
2) of a single combined slope based upon the means at each age,
3) of the scatter of the k means about the combined regression,
4) between rats in slope,
5) between rats in scatter, the remainder or error variance.

More terms are added if the variation of term 3) is significant. The isolation of the source of this scatter is critical to the determination of the error variance of the combined regression. Bliss attributes a significant scatter to,

a) systematic departures from the growth equation or systematic environmental effects influencing all rats equally,

or b) a random component caused by serial correlation between successive values in each individual.

If all rats have essentially the same systematic pattern in their residuals then the scatter in row 3) of the analysis of variance should not be included in the variation ascribed to the error of the combined slope. If the variation due to 3) is significant the remainder (term 5) is partitioned by regressing the residuals for individuals upon the mean residuals and comparing the resultant slopes, giving two further terms,

6) the variation due to non-additivity between rats,

and 7) the new remainder (term 5) minus term 6)).

Bliss (1967, p.467-470) presents such an analysis for the live weights, from hatching, of turkey poults with age and weight transformed to logarithms. Live weights collected at Massey (used in Chapter Six), from individual rats treated with 90μg of testosterone propionate three days following birth, were also subjected to such an analysis.

Table 10.2 gives the transformed live weights and ages of the eleven rats. Column totals at each age, and row totals for each animal, are also given. Table 10.3 gives the residuals (e) about the individual growth curves, the total residuals at each age (T_Δ), and the sum of the products of the residuals and their column totals $\Sigma(T_\Delta e)$. These two tables form the basis of the analysis of variance presented in Table 10.4. The equations for deriving the variances are given by Bliss. Most calculations were made by hand since a computer program for such an analysis was not available at Massey University. The residuals about each individual's line were obtained by using the statistical program BAR3, as outlined in Chapter Six.

Table 10.4 shows that individual growth curves differed in their positions (Row 1) and their slopes (Row 4). But, as Bliss found for turkey poults, Table 10.4 shows the scatter of the means (Row 3) about the combined curve to be highly significant ($F = 8.68$, 9 by 80 DF, $P < .001$). The variances of Rows 6) and 7) were therefore calculated from the residuals of Table 10.3. The highly significant term for non-additivity ($F = 11.21$, 10 by 80 DF, $P < .001$), indicates that the residuals display different patterns in individual rats. If the reasoning used by Bliss were followed this last result would exclude the possibility that the scatter of the means was due to systematic departures from the growth equation. However in Chapter Six the residuals about the log-reciprocal lines were seen to be out of phase when plotted against arithmetic age but in phase when plotted against log body weight. Therefore the above analysis of variance statistically confirms the former subjective impression that a rat's growth pattern is not related to its chronological age. The present example therefore questions the rigid interpretation of such an analysis that was proposed by Bliss.

A proper procedure for analysing differences between treatments, in a longitudinal growth experiment, would be to subdivide each group's variances, as proposed by Bliss. Then, using the best estimate of the scatter about the combined slope for each group, compare the combined slopes and constants of the groups. The more complicated analysis, where

TABLE 10.2

ANALYSES OF VARIANCE: DATA OF TARTTELIN AND CLARK FROM FEMALE RATS WEIGHED WEEKLY (IN GRAMS), COLUMN TOTALS (T_t), ROW TOTALS FOR EACH ANIMAL (T_g) and RECIPROCALS (1 / X).

LOG LIVE WEIGHT OF EACH RAT (Y) IN EACH WEEK (X)

AGE (weeks)	4	5	6	7	8	9	10	11	12	13	14	(T_g)
6	1.5911	1.7924	1.9542	2.0864	2.1818	2.2553	2.3054	2.3464	2.3784	2.3892	2.4116	23.6920
11	1.7482	1.9494	2.0792	2.1987	2.2765	2.3444	2.4014	2.4409	2.4639	2.4786	2.4871	24.8681
17	1.8129	1.9912	2.1139	2.1987	2.2833	2.3617	2.4133	2.4502	2.4728	2.4871	2.5024	25.0876
18	1.6628	1.8573	1.9868	2.1301	2.2175	2.2788	2.3160	2.3560	2.3927	2.4099	2.4232	24.0313
22	1.6902	1.8865	2.0531	2.1523	2.2430	2.3260	2.3617	2.3945	2.4166	2.4298	2.4425	24.3861
RAT 23	1.7482	1.9243	2.0755	2.1703	2.2430	2.3096	2.3365	2.3729	2.3979	2.4133	2.4232	24.4147
NO. 27	1.7853	1.9638	2.1072	2.1959	2.2648	2.3222	2.3598	2.3874	2.4048	2.4166	2.4346	24.6425
28	1.7076	1.9243	2.0719	2.1584	2.2455	2.3139	2.3424	2.3892	2.4099	2.4216	2.4472	24.4317
30	1.6435	1.8062	1.9243	2.0719	2.1584	2.2405	2.3075	2.3560	2.3856	2.4048	2.4116	23.7102
31	1.6990	1.8261	1.9395	2.0828	2.1790	2.2577	2.3032	2.3464	2.3766	2.4133	2.4265	23.8499
32	1.7243	1.8451	1.9956	2.1303	2.2122	2.2695	2.3201	2.3636	2.3909	2.4183	2.4150	24.0850
(T_t)	18.8131	20.7666	22.3012	23.5760	24.5050	25.2696	25.7673	26.2035	26.4901	26.6825	26.8249	
1 / X	.2500	.2000	.1666	.1428	.1250	.1111	.1000	.0909	.0833	.0769	.0714	

TABLE 10.3

ANALYSES OF VARIANCE: FOR THE DATA OF TABLE 10.2

RESIDUALS (e), COLUMN TOTALS (T_Δ), and THE SUM OF THEIR PRODUCTS $\Sigma(T_\Delta\ e)$

		AGE (weeks)										
		4	5	6	7	8	9	10	11	12	13	14
RAT NO.	6	.0163	-.0215	-.0190	-.0008	.0092	.0162	.0131	.0106	.0064	-.0134	-.0172
	11	.0089	-.0052	-.0190	-.0021	-.0012	.0069	.0160	.0163	-.0067	-.0061	-.0213
	17	.0183	-.0041	-.0152	-.0261	-.0131	.0094	.0164	.0168	.0089	-.0023	-.0091
	18	.0137	-.0129	-.0309	.0072	.0154	.0152	.0033	.0031	.0063	-.0048	-.0158
	22	.0035	-.0191	.0014	-.0036	.0089	.0210	.0181	.0110	.0000	-.0149	-.0262
	23	.0011	-.0179	.0032	.0050	.0081	.0205	.0040	.0050	.0004	-.0091	-.0206
	27	-.0057	-.0128	.0069	.0073	.0100	.0158	.0122	.0061	-.0045	-.0165	-.0189
	28	-.0090	-.0006	.0080	-.0046	.0080	.0185	.0008	.0096	-.0011	-.0161	-.0134
	30	.0490	-.0199	-.0563	-.0190	-.0153	.0024	.0179	.0243	.0188	.0083	-.0103
	31	.0612	-.0306	-.0633	-.0243	-.0064	.0114	.0082	.0115	.0086	.0172	.0063
	32	.0446	-.0451	-.0349	-.0005	.0061	.0049	.0087	.0139	.0093	.0097	-.0167
	T_Δ	.2020	-.1900	-.2191	-.0616	.0297	.1427	.1191	.1288	.0601	-.0482	-.1635
	$\Sigma(T_\Delta e)$	.04075	.03596	.04802	.00378	.00089	.0203	.01407	.01642	.00358	-.00229	.02663

the within group and between group variances are analysed together would be relatively simplified if all the treatments contained equal numbers of animals. But as this is frequently not the case the separate analyses may be of more general use.

An obvious benefit of this method of analysis is that the systematic variation about the line can be tested for and its probable cause deduced. An obvious use in live weight growth experiments is the ability to remove a systematic trend, that occurs in all animals, so that the error around a combined slope is reduced. In long-term experiments this could be the effect of season, in short-term experiments it could be the effect of an inadequate water or food supply upon a few weighings or the effect of systematic short-term growth cycles, as shown herein for the daily live weights of rats.

TABLE 10.4

ANALYSIS OF VARIANCE FOR THE RAT GROWTH CURVES OF TABLES 10.2 & 10.3, DEGREES OF FREEDOM (DF), SUMS OF SQUARES (SS), MEAN SQUARES (MS), AND F RATIOS FOR THE TERMS DESCRIBED IN THE TEXT.

ROW	TERM	DF	SS	MS	F
1	BETWEEN RATS	10	0.1942	0.01942	78.49***
2	COMBINED SLOPE	1	6.4096	6.4096	25907.8 ***
3	SCATTER	9	0.0193	.00215	8.68***
4	RATS x SLOPE	10	0.0309	.00309	12.36***
5	RATS x SCATTER	90	0.0222	.00025	
6	RATS x N-A	10	0.0130	.00130	11.21***
7	REMAINDER	80	0.0092	.00012	
8	CORRECTION	1	590.0442		
9	TOTAL	120	6.6763		

*** P < .001

'F' values for rows 1,2,3,4 based on the error of row 5.

'F' value of row 6 based on the error of row 7.

NOTE SIX

COMPUTATION OF ORTHOGONAL COEFFICIENTS FOR THE SUBDIVISION OF MEAN SQUARES FOLLOWING AN ANALYSIS OF VARIANCE FOR UNEQUAL SUB-CLASSES.

The body composition analyses of Chapter Eight involved the use of a two way analysis of variance (with interaction) for unequal sub-classes and follow-up 'F' tests, of one degree of freedom (DF), between treatments and ages. For these analyses of variance the same computer programs, and therefore the mathematical models, that were described by Wilson (1977) were used. The error term of the follow-up comparisons was that from the two way analysis of variance. But as the age and treatment groups contained unequal numbers of animals simple un-weighted comparisons would be non-orthogonal. Therefore to balance the analyses weighted coefficients were calculated, by hand. To the author lucid accounts of the calculation of these coefficients appear to be lacking, the following example is therefore presented.

The analyses of the variables measured upon rats killed at weeks 12 and 15 (Analysis A) were unbalanced (see Tables 8.1 or 10.5) as there were group sizes of 8, 7, 6, 9, and 12.

Firstly the lowest common factor was calculated as

$2 \times 2 \times 2 \times 3 \times 3 \times 7 = 504.$

Therefore the group sizes and their weightings were

8 : 63, 7 : 72, 6 : 84, 9 : 56, and 12 : 42.

These weightings were then multiplied by the coefficients of comparison and their associated signs (these coefficients are all that is required if the experimental design is balanced) giving the coefficients of the four comparisons between the five treatments shown in Table 10.5.

The comparisons were made using the treatment group totals so that the sums of squares between groups (SS_b) were calculated by squaring the sum of the product of the individual coefficients (c) and their associated totals (T), giving $(\Sigma\ cT)^2$, and dividing this by the sum of the product of the squared individual coefficients (c^2) and their associated group number (n) giving $\Sigma\ (\ c^2\ n\ )$, or

$$SS_b = (\Sigma\ c\ T\ )^2\ /\ \Sigma(\ c^2\ n\ )$$

The error term (MS_w) was that of the two way analysis of variance.

The divisors, $\Sigma(c^2n)$, were also calculated by hand. For example, for comparison 1) of Table 10.5 the divisor was,

$$(-126^2 \times 8) + (-126^2 \times 8) + (-144^2 \times 7) + ... (216^2 \times 7) = 1980216$$

The coefficients, divisors, and the treatment group totals were punched onto cards (the error term was inputted manually via the console typewriter) and processed by the Massey University IBM 1620 computer using a program written in FORTRAN by Professor R. E. Munford.

TABLE 10.5

WEIGHTING COEFFICIENTS AND COMPARISONS FOR ANALYSIS A OF CHAPTER EIGHT

	WEEK 12					WEEK 15				
NO. OF RATS	8	8	7	8	7	6	9	6	12	7
WEIGHTING	63	63	72	63	72	84	56	84	42	72
COMPARISONS										
1) OvX vs C + EB	-2	-2	-2	+3	+3	-2	-2	-2	+3	+3
	-126	-126	-144	+189	+216	-168	-112	-168	+126	+216
2) C vs EB				-1	+1				-1	+1
				-63	+72				-42	+72
3) OvX D2 vs OvX W7	-1		+1			-1		+1		
	-63		+72			-84		+84		
4) OvX W4 vs OvX (D2+W7)	-1	+2	-1			-1	+2	-1		
	-63	+126	-72			-84	+112	-84		

Interactions: Comparisons 5), 6), 7), and 8) obtained by multiplying all week 12 coefficients by -1 and multiplying all week 15 coefficients by +1.

NOTE SEVEN

DO RATS 'PLATEAU' IN LIVE WEIGHT?

The term 'plateaued rat' is widely used to indicate that a rat has reached a stable, static, mature body weight. But do rats actually reach a static live weight which they maintain during adulthood?

Zucker *et al.* (1941b) showed that for a group of rats log live weight followed a linear relationship to the reciprocal of age upto 70 weeks of age, suggesting that rats do not plateau but increase in live weight through adulthood. But as their results, obtained from a mass curve, cannot necessarily be extended to the growth of individual animals it was considered important to establish the linear relationship for individual animals upto a similar age. For example, the continual increase in live weight from the mass curve could be due to individuals reaching their plateaux at different weights or ages. Therefore despite the appearance of the mass curve individual animals might still plateau.

The constant (ultimate weight) of the log-reciprocal line has been used (see Chapter One) as an estimate of the mature live weight of individual rats. If the relationship between log live weight and the reciprocal of age were shown to remain linear approaching infinite age one would be justified in estimating the ultimate weight of individuals, from several post-weaning weighings, by extrapolation.

THE LIVE WEIGHT DATA

One should be aware of, and heed, the warning of H. H. Mitchell (1962, p.382) that '...the term "normal growth" as applied to the albino rat is an equivocal one that the meticulous author will avoid.' The data to be analysed was obtained from "normal female rats" of the U.C.L.A. colony by Dr. M. F. Tarttelin during 1969 and 1970. The three rats were intact untreated females that were weighed almost daily from 56 to about 300, 340, and 380 days of age respectively.

RESULTS AND DISCUSSION

The live weight growth curves for the three rats are shown for the untransformed data in Figures 10.1a, 10.1b, & 10.1c, and for the

FIGURES 10.1A, B, & C.
UNTRANSFORMED PLOTS OF LIVE WEIGHT
VS AGE
LACK OF "PLATEAUING" IN 3 MATURE RATS.
UNPUBLISHED DATA OF TARTTELIN (1970)
U.C.L.A. COLONY.

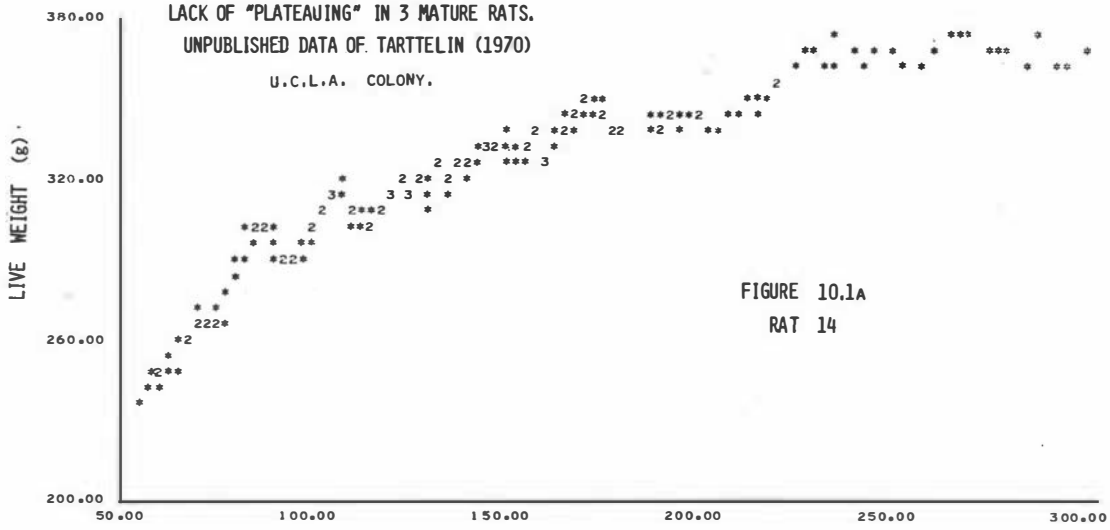


FIGURE 10.1A
RAT 14

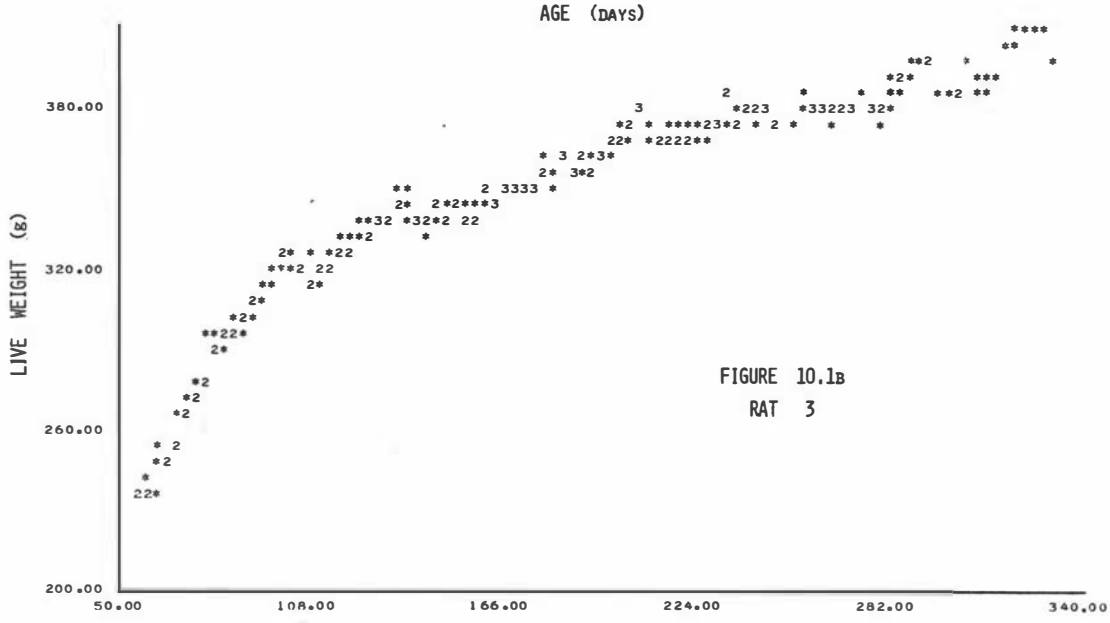


FIGURE 10.1B
RAT 3

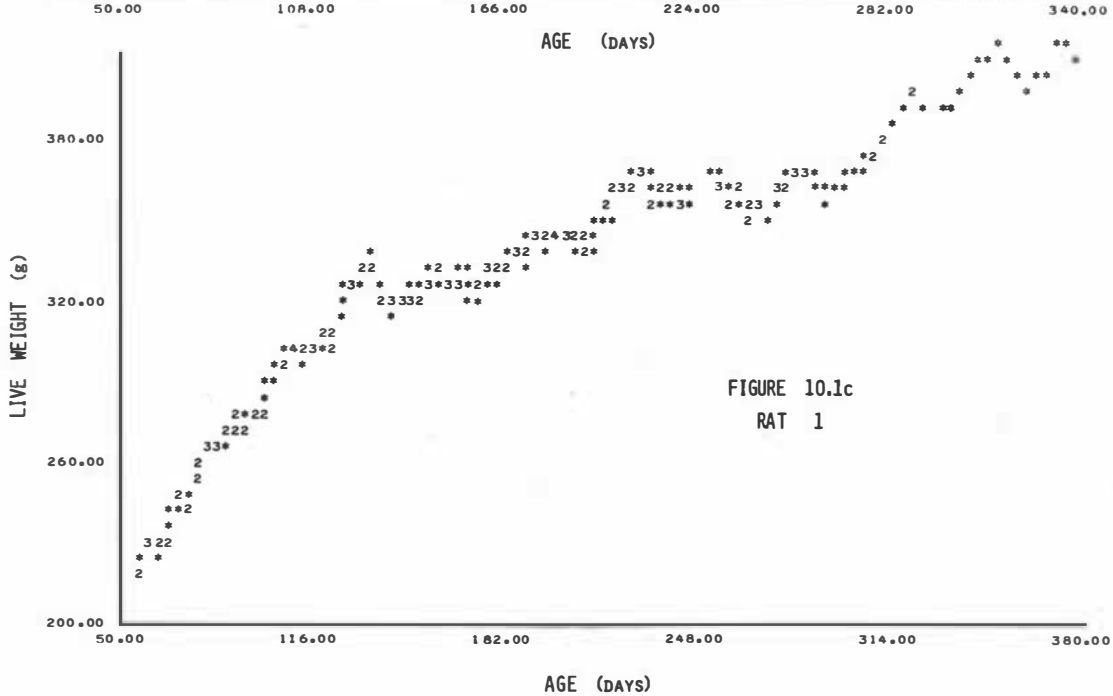
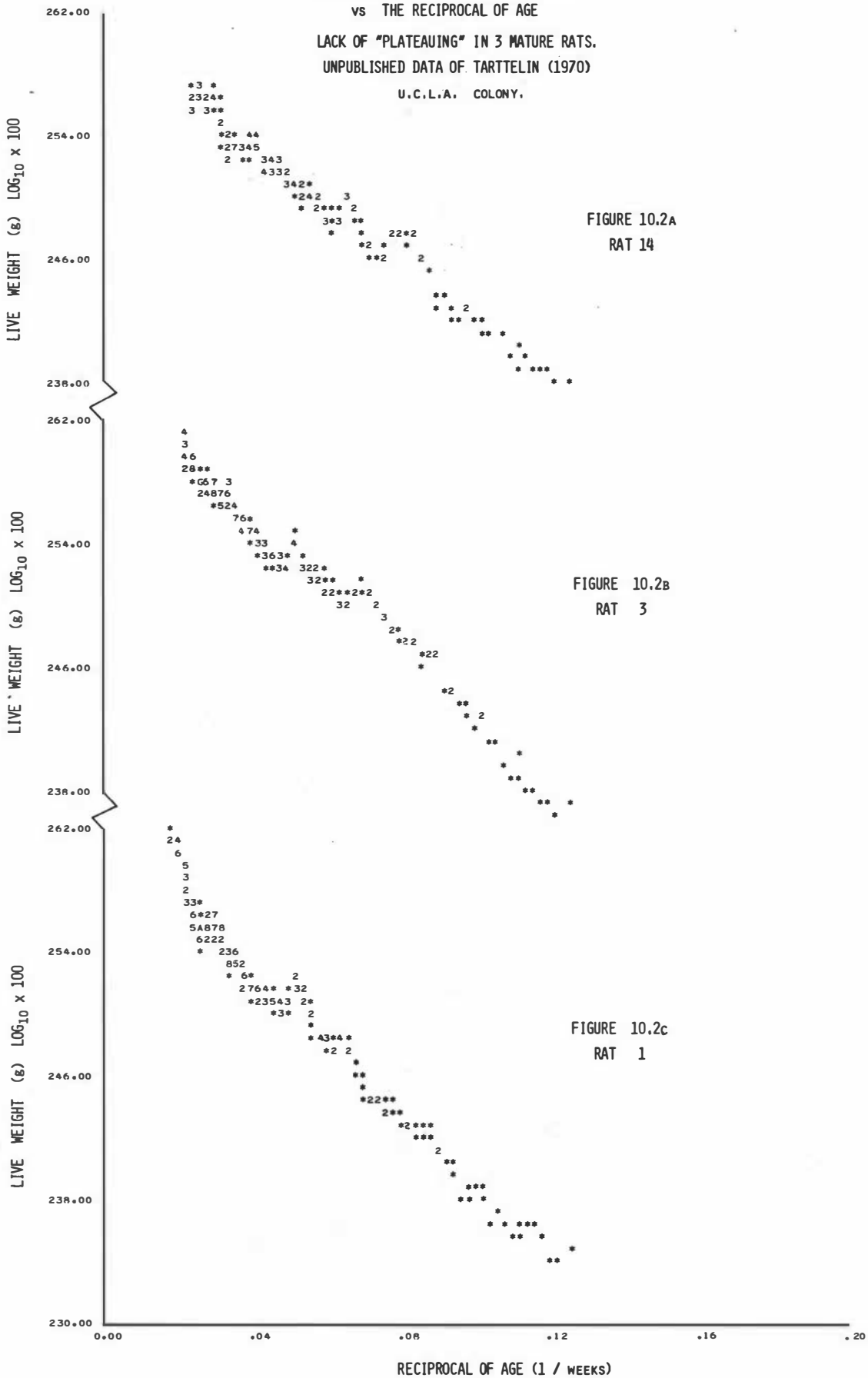


FIGURE 10.1C
RAT 1

FIGURES 10.2A, B, & C.
TRANSFORMED PLOTS OF LOG LIVE WEIGHT
vs THE RECIPROCAL OF AGE
LACK OF "PLATEAUING" IN 3 MATURE RATS.
UNPUBLISHED DATA OF TARTTELIN (1970)
U.C.L.A. COLONY.



transformed data in Figures 10.2a,b, & c. The slopes and constants for these transformed growth curves (Table 10.6) were estimated over three different periods, to illustrate the linear nature of the growth on transformed axes. These periods were

1) upto 130 days of age,
2) from 130 days of age onwards,
and 3) over the complete range of ages.

The division at 130 days was chosen since Bliss (1970) had stated that the log-reciprocal equation gave a linear fit to rat live weight data upto 130 days but '...then curved systematically about the regression.'

The untransformed growth curves (Figs 10.1a, b, & c) illustrate the steady increase in live weight in all three individuals. On transformed axes (Figs 10.2a, b, & c), over the range of ages studied, log live weight was linearly related to the reciprocal of age, confirming the findings of Zucker *et al.* (1941b).

Table 10.6 shows that the estimates of ultimate weight obtained from 56 to 130 and from 130 days onwards were comparable within animals. This result convincingly confirms the adherence of rat live weight growth to the log-reciprocal equation and questions Bliss's statement that after 130 days rat live weight growth departs from the log-reciprocal relationship. In addition the use of within-animal comparisons for estimated ultimate weights, before and after the imposition of treatment, is shown to be feasible. Also the ultimate weight, obtained by extrapolation from several post-weaning weighings, may approximate the actual maximum weight attained by a rat.

The origin of the belief that rats 'plateau' is difficult to trace. It may have been derived from Brody's (1945) general growth equations, where a maximum live weight was assumed to be approached asymptotically. If rats possess a static mature weight then the asymptotic regression would be the form of the relationship between a rat's weight and age. As discussed in Chapter Six (Section One) Bliss fitted an asymptotic equation to rat live weights from 50 to 210 days of age by estimating the upper asymptote from weights taken between 220 and 330 days of age (when the maximum weight was assumed to have been attained). That rats do not plateau supports, on biological grounds, the author's previous rejection, on statistical grounds, of the asymptotic equation.

Data from several other colonies shows that no true weight plateau is reached in the rat. Maximum weights at 500 days (Bogart *et al.*, 1940) and at 800 days (Berg and Harmison, 1957) have been reported for the female laboratory rat. But in both these studies senescence then caused a steady fall in weight. Ross (1959, p.1196) also concluded that 'Maximum weights were reached at the average time of 85 weeks of age. Beyond this time there was a general weight loss until death,'. Widdowson and Kennedy (1962) give graphs showing steady increases in live weight, of both male and female rats, through one year of age. In their study maximum weights were generally reached at about eighteen to twenty months of age.

Clearly, contrary to popular belief, the laboratory rat does not plateau in live weight.

TABLE 10.6

LOG-RECIPROCAL EQUATIONS FOR RAT LIVE WEIGHT GROWTH TO 380 DAYS OF AGE.

RAT NO.	AGES (days)	EQUATION	(n)	(r)
1	56 - 130	Y = -2.422 X + 2.627	72	-0.9857
1	130 - 380	Y = -2.865 X + 2.640	182	-0.8978
1	56 - 380	Y = -2.439 X + 2.629	254	-0.9811
3	56 - 130	Y = -2.357 X + 2.660	73	-0.9790
3	130 - 340	Y = -2.418 X + 2.647	167	-0.9361
3	56 - 340	Y = -2.129 X + 2.638	238	-0.9853
14	56 - 130	Y = -1.813 X + 2.603	71	-0.9622
14	130 - 300	Y = -2.114 X + 2.616	98	-0.9002
14	56 - 300	Y = -1.908 X + 2.608	169	-0.9652

NOTE EIGHT

THE BIOLOGICAL INTERPRETATION OF THE TRANSFORMATIONS USED FOR LIVE WEIGHTS.

Nature delights in transformations.

- Issac Newton.

The dearth of theory concerning the analysis of mammalian growth curves has not been followed by the practical application of this theory to experimental analysis. Therefore the importance of statistical theory to the analyis of growth curves has been slow to be realised. Earlier workers (ie. Brody, 1945) fitted equations to growth curves largely on the basis of their particular 'theory of growth' rather than using the best mathematical fit or allowing the variability associated with the dependent variable to determine its scale. Once normality is established for the dependent variable the independent variable, as it is assumed free from error, can be transformed to a scale that gives linearity to the relationship. This approach to linearisation is based solely on mathematical considerations; to quote Medawar (1945), '...no biological significance can be read into the exact analytical form it takes. The growth equations chief function is to facilitate the analysis of the curve of growth'. The opposite point of view, epitomised by Brody (1945, p.559), declares that 'The equations we employed represent regularities,...and the equation constants have definite, rational, physical meaning.' However, in support of Medawar, the post-weaning live weight changes in the rat have been fitted in Chapter One by orthogonal polynomials to the third power while in Chapter Six both log-reciprocal and asymptotic regressions achieved the same end. To ascribe different and strict biological meaning to the different constants and parameters of these different equations would be fallacious.

Huxley (1932, p.6) recognised two intrinsic properties of growth, 'One essential fact about growth is that it is a process of self-multiplication of living substance - ie. that the rate of growth of an organism growing equally in all its parts is at any moment proportional to the size of the organism. A second fundamental fact about growth is that the rate of self-multiplication slows down with increasing age (size)...'

The first of Huxley's properties of growth is, in essence, restated by the law of proportionate effect (due to Kapteyn), as defined by Aitchison and Brown (1966), 'A variate subject to a process of change is said to obey to law of proportionate effect if the change in the variate at any step of the process is a random proportion of the previous value of the variate.' This law forms the theoretical basis of the lognormal distribution; its

importance, derivation, and application being described by Aitchison and Brown (1966, p.1-2) in the following way, 'We may go further and state our belief that the lognormal is as fundamental a distribution in statistics as is the normal, despite the derivative nature of its name. It arises from a theory of elementary errors combined by a multiplicative process, just as the normal distribution arises from a theory of elementary errors combined by addition. There are, as Galton long since pointed out, many situations in nature where it is reasonable to suggest that the process underlying change or growth is multiplicative rather than additive...'

Medawar (1945, p.163) defines a plot of log size against age as '...the curve of specific growth...' which he states '...provides a record of the multiplication of living substance...'

The multiplicative nature of growth may even have a genetic basis as, according to Needham (1964, p.386-7), '...the various genes affecting growth prove to have a multiplicative rather than an additive effect... It is possibly a reflection of the multiplicative nature of growth itself.'

A statistical reason for applying the logarithmic transformation to live weights is that values of a normally distributed variable are assumed to be distributed between minus infinity and plus infinity. As live weights are greater than zero their transformation to logarithms is indicated.

Therefore the use of the logarithm of weight can be justified on both statistical and biological grounds; why the logarithmic transformation is so seldom used in statistics is partially explained by Aitchison and Brown (1966, p.2). '... Man has found addition an easier operation than multiplication, and so it is not surprising that an additive law of errors was the first to be formulated. Had man been more adept at multiplication the "exponential-lognormal" or normal might have been the derivative distribution.'

Pearson, using measurements he made, even challenged the lognormal distribution on the grounds that it did not occur in nature. In a similar vein Aitchison and Brown give an example where 'We have recently heard of an American candidate whose thesis was objected to on the grounds that the examiners were not interested in the logarithm of income.' Such an attitude is, unfortunately, common. The literature abounds with examples where variables obviously require transformation yet the authors are either unaware of, or reticent to use, a scale other than that upon which the variables were measured. But in the present study no weight or length was truly normally distributed. The lognormal appears to be a common distribution in nature.

A glance at published means and standard deviations commonly shows them to be correlated, and thus not independent estimates, indicating that a transformation is required. The following is a somewhat extreme example. Krulich *et al.* (1974) commendably plotted graphs of the frequency distributions of serum concentrations of pituitary hormones but they failed in the interpretation of the plots. The authors recognised that the frequency distributions were non-normal due to skewing so they analysed the data using non-parametric tests; an approach much less likely to detect real differences. Their frequency plots could be described as definitively illustrating lognormality. The authors conlude their discussion with the misleading suggestion that 'Our results suggest, then, that nonparametric procedures should always be used for LH, prolactin, and GH analysis.' This advice should not be followed, the counsel of Antipholus of Syracuse being more apposite (Transform me then, and to your power I'll yield).

The second of Huxley's properties of growth was rephrased by Medawar (1945, p.163);'...the curve of specific growth...' which '...suggests that the energies of growth decline progressively; that living matter progressively loses a power to multiply itself at the rate which it was formed.' Reciprocal or logarithmic transformations of time are mathematical expressions of this property of growth. Therefore the transformations used in this work can be rationalised by the above properties of growth.

Thus the present techniques appear to the author to be overwhelmingly justified on both biological and statistical grounds. But I feel the words of Macauley are apt,

> If I am in the wrong, my errors may set the minds of others at work, and may be the means of bringing both them and me to a knowledge of the truth.

REFERENCES CITED

Abrams, J.T. (1966). Some nutritional aspects of the growth of the laboratory rat. In Recent advances in animal nutrition. Ed. Abrams, J.T., London, Churchill.

Acheson, R.M., McIntyre, M.N., & E. Oldham (1959). Techniques in longitudinal studies of the skeletal development of the rat. Brit. J. Nutr. 13: 283-295

Adlard, B.P.F., Dobbing, J. & J.L. Smart (1973). An alternative animal model for the full-term small-for-dates human baby. Biol. Neonate 23 : 95-108

Aitchison, J. & J.A.C. Brown (1966). The log-normal distribution, with special reference to its uses in economics. 2d. Ed. Cambridge, Cambridge University Press.

Allden, W.G. (1970). The effects of nutritional deprivation on the subsequent productivity of sheep and cattle. Nutr. Abstr. Rev. 40 : 1167 - 1184.

Ashton, G.C., Lucas, H.L., & F.W. Sherwood (1955). Control of error variance in swine feeding trials. I. By covariance adjustment for pre-experimental measures. (Abstr.) J. Anim. Sci. 14 : 1174 - 1175.

Ashwell, M., Meade, C.J., Medawar, P.B., & C. Sowter (1977). Adipose tissue: contributions of nature and nurture to the obesity of an obese mutant mouse (ob/ob). Proc. Roy. Soc. Lond. (Ser B) 195 : 343-353.

Atkinson, T., Fowler, V.R., Garton, G.A., & A.K. Lough (1972). A rapid method for the accurate determination of lipid in animal tissues. Analyst 97 : 562-568.

Bailey, G.L., Broster, W.H., & A.W.A. Burt (1958). Experiments on the nutrition of the dairy heifer. J. Agric. Sci. (Camb) 50 : 1-7.

Baker, G.A. & H.R. Guilbert (1942). Non-randomness of variations in daily weights of cattle. J. Anim. Sci. 1 : 293-299.

Barnicoat, C.R. & F.B. Shorland (1952). New Zealand lamb and mutton. Part II. Chemical composition of edible tissues. N.Z. J. Sci. Tech. 33 : 17-23.

Barton, A.D. & A.K. Laird (1969). Analysis of allometric and non-allometric differential growth. Growth 33 : 1-16.

Berg, B.N. & C.R. Harmison (1957). Growth, disease, and aging in the rat. J. Geront. 12 : 370-377.

Berkson, J. (1950). Are there two regressions ?
J. Am. Stat. Assoc. 45 : 164-180.

Bernard, C. (1961). An introduction to the study of experimental medicine. Translated by H. C. Greene, New York, Collier Books.

Bernardis, L.L. & L.L. Bellinger (1976). Production of weanling rat ventromedial and dorsomedial hypothalamic syndromes by electrolytic lesions with platinium-iridium electrodes. Neuroendocrinology 22 : 97-106.

Bertalanffy, L. von (1960). Growth - Principles and theory. In Fundamental aspects of normal and malignant growth. Ed. W.W. Nowinski, Amsterdam, Elsevier.

Billewicz, W.Z. (1965). The efficiency of matched samples: an empirical investigation. Biometrics 21 : 623-644.

Bliss, C.I. (1967). Statistics in biology. Vol I. New York, McGraw-Hill.

Bliss, C.I. (1970). Statistics in biology. Vol II. New York, McGraw-Hill.

Bogart, R., Lasley, J.F., & D.T. Mayer (1944). Influence of reproductive hormones upon growth in ovariectomised and normal female rats.
Endocrinology 35 : 173-181.

Bohman, V.R. (1955). Compensatory growth of beef cattle: the effect of hay maturity. J. Anim. Sci. 14 : 249-255.

Bondari, K., & R.L. Wilham (1977). A comparison of carcass characteristics of straightbred and crossbred steers. Growth 41 : 33-40.

Bowman, J.C. (1968). Genetic variation of body weight in sheep.
In Growth and development of mammals. Eds. Lodge, G.A. & G.E. Lamming. London, Butterworths, pp.291-308.

Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., & R. Guillemin (1973). Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179 : 77-79.

Brody, S. (1928). An analysis of growth and senescence. In Growth. Eds. Robbins, W.G. *et al.*, New Haven, Yale University Press, pp. 31-64.

Brody, S. (1945). Bioenergetics and growth. New York, Reinhold.

Brown, J.E., Fitzhugh, H.A., & T.C. Cartwright (1971). Comparison of weight-age models for cattle. J. Anim. Sci. 32 : 372 (Abstr.).

Brownstein, M.J., Arimura, A., Fernandez-Durango, R., Schally, A.V., Palkovits, M., & J.S. Kizer (1977). The effect of hypothalamic deafferentation on somatostatin-like activity in the rat brain. Endocrinology 100 : 246-249.

Brumby, P.J. & J. Hancock (1956). A preliminary report of growth and milk production in identical and fraternal-twin dairy cattle. N.Z. J. Sci. Tech. A38 : 184-193.

Bryden, M.M. (1969). Regulation of relative growth by functional demand: its importance in animal production. Growth 33 : 143-156.

Bullough, W.S. (1973). Chalone control systems. In Humoral control of growth and differentiation. Vol I. Eds. Lobue, J. & A.S Gordon. New York, Academic Press.

Bullough, W.S. (1975). Mitotic control in adult mammalian tissues. Biological Reviews 50 : 99-127.

Butterfield, R.M. & R.T Berg (1976). New concepts of cattle growth. Sydney, Sydney University Press.

Carlson, G.L. (1977). Regulatory phenomena and the data of growth. Growth 41 : 25-32.

Chatfield, C. (1970). Statistics for technology. Harmondsworth, Penguin Books.

Chevillard, L., Portel, R., & M. Cadot (1963). Growth rate of rats born at 5°c and 30°c. Fed. Proc. 22 : 699-703.

Clark, D.A. (1974). A study of the efficiency of feed utilisation in young Fresian bulls fed cut pasture. Thesis, M.Agr.Sc. Massey University, pp. 165.

Clark, R.G., & M.F. Tarttelin (1975). Oestrogen stimulates rat growth hormone release but inhibits growth. (Abstr.) N.Z. Med. J. 82 : 241.

Clark, R.G., & M.F. Tarttelin (1977). In Proceedings of the Physiological Society of N.Z. (Abstr.) N.Z. Med. J. 86 : 487.

Clark, R.G., & M.F. Tarttelin (1978). The linear regression of body weight and age in intact, ovariectomised and estrogen treated rats - some applications and implications. Growth 42 : 113-128.

Cox, J.E. & T.L. Powley (1977). Development of obesity in diabetic mice pair-fed with lean siblings. J. Comp. Physiol. Psych. 91 : 347-358.

Cumming, M.C. & S.D. Morrison (1960). The total metabolism of rats during fasting and re-feeding. J. Physiol. 154 : 219-243.

Czajka-Nairns, D.M. & J. Hirsch (1974). Supplementary feeding during the preweaning period: effect on carcass composition and adipose cellularity of the rat. Biol. Neonate 25 : 176-185.

Daughaday, W.H., Phillips, L.S., & M.C. Mueller (1976). The effects of insulin and growth hormone on the release of somatomedin by the isolated rat liver. Endocrinology 98 : 1214-1219.

Davey, A.W.F. (1974). Nutrition of the pre-ruminant calf. Proc. N.Z. Soc. Anim. Prod. 34 : 133-144.

Davis, J.R. (1977). Decreased metabolic rates contingent upon lateral hypothalamic lesion-induced body weight losses in male rats. J. Comp. Physiol. Psych. 91 : 1019-1031.

De Smet, J. (1953). Lipid concentration in different tissues of the castrated rat. C.R. de la Soc de Biol. 147 : 726-729.

Dick, I.D. & P. Whittle (1951). Contributions to the statistical design of identical twin experiments. N.Z. J. Sci. Tech. B33 ; 145-172.

Dickerson, G.E. (1954). Hereditary mechanisms in animal growth. In Dynamics of growth processes. Princeton, Princeton University Press, pp. 242-276.

Dickerson, G.E., Glimp,H.A., Tuma, H.J., & K.E. Gregory (1972). Genetic resources for efficient meat production in sheep. Growth and carcass characteristics of seven breeds. J. Anim. Sci. 34 : 940-951.

Donald, H.P. (1960). Evidence from twins on variation in growth and production of cattle. Proc. Internatl. Congr. Genet. 10 : 225-235.

Donovan, B.T. (1974). Concluding comments In Control of onset of puberty. Eds. Grumbach, M.M., Grave, G.D., & F.E. Mayer. New York, J. Wiley & Sons.

Dunn, J. & A. Arimura (1974). Serum growth hormone levels following ablation of medial basal hypothalamus. Neuroendocrinology 15 : 189-199.

Dunn,M.S., Murphy, E.A., & L.B. Rockland (1947). Optimal growth of the rat. Physiol. Rev. 27 : 72-94.

Durbin, J., & J.S. Watson (1950). Testing for serial correlation in least squares regression. Biometrika 37 : 409.

Ebling, F.J. & P.A. Hale (1966). The composition of female rat skin in relation to region, age, hair growth cycle and hormones. J. Endocrinol. 36 : 177-201.

Efendic, S., Hokfelt, T., & R. Luft (1978). Somatostatin. Advan. Metab. Disord. 9 : 367-424.

Elsley, F.W.H., McDonald, I., & V.R. Fowler (1964). The effect of plane of nutrition on the carcasses of pigs and lambs when the variations in fat content are excluded. Animal Production 6 : 141-154.

Elsley, F.W.H. (1976). Limitations to the manipulation of growth. Proc. Nutr. Soc. 35 : 323-337.

Enesco, M. & C.P. Leblond (1962). Increase in cell number as a factor in the growth of the organs and tissues in the young male rat. J. Embryol. Exp. Morphol. 10 : 530.

Everitt, G.C. & K.E. Jury (1966). Effects of sex and gonadectomy on the growth and development of Southdown x Romney lambs. J. Agric. Sci. 66: 15-27.

Everitt, G.C. (1968). Prenatal development of uniparous animals, with particular reference to the influence of maternal nutrition in sheep. In Growth and development of mammals. Eds. Lodge, G.A. & G.E. Lamming London, Butterworths. pp. 131-157.

Everitt, G.C. (1972). Calf growth and lifetime performance of beef cattle. Proc. N.Z. Soc. Anim. Prod. 32 : 20-25.

Faust, I.M., Johnson, P.R., & J. Hirsch (1976). Noncompensation of adipose mass in partially lipectomised mice and rats. Am. J. Physiol. 231: 538-544.

Fisher, R.A. (1939). In Discussion of animal experiments. Suppl. J. Roy. Stat. Soc. 6 : 12-22.

Fisher, R.A. (1946). Statistical methods for research workers. Edinburgh, Oliver & Boyd.

Fitzhugh, H.A., & St. C.S. Taylor (1971). Genetic analysis of degree of maturity. J. Anim. Sci. 33 : 717-725.

Ford, E.H.R. & G. Horn (1959). Some problems in the evaluation of differential growth in the rat skull. Growth 23 : 191-204.

Francis, M.J.N., & D.J. Hill (1975). Prolactin-stimulated production of somatomedin by rat liver. Nature 255 : 167-168.

Freudenberger, C.B. (1932). A comparison of the Wistar albino rat and the Long-Evans hybrid strain of the Norway rat. Am. J. Anat. 50 : 293-349.

Freudenberger, C.B. & O.A. Billeter (1935). The effect of spaying on body growth and the organ weights of the albino rat. Endocrinology 19 : 347-355.

Frisch, R.E. (1974). Critical weight at menarche, initiation of adolescent growth spurt, and the control of puberty. In Control of onset of puberty. Eds. Grumbach, M.M., Grave, G.D., & F.F. Mayer. New York, J. Wiley & Sons.

Gairdner, D., & J. Dauncey (1974). The effect of diet on the development of the adipose organ. Proc. Nutr. Soc. 33 : 119-121.

Gale, S.K. & A. Sclafani (1977). Comparison of the ovarian and hypothalamic obesity syndromes in the female rat: effects of diet palatibility on food intake and body weight regulation. J. Comp. Physiol. Psychol. 91 : 381-392.

Galletti, F., & A. Klopper (1964). The effect of progesterone on the quantity and distribution of the body fat in the female rat. Acta. Endo. (Kbh.) 46 : 379-386.

Gilmour, K.E. & K.W. McKerns (1966). Insulin and estrogen regulation of lipid synthesis in adipose tissue. Biochim. Biophys. Acta. 116 : 220-228.

Glasser, S.R. (1957). Influence of a protein-free diet on the immediate and latent effects of stilbestrol. Am. J. Physiol. 189 : 441-446.

Gold, R.M. (1973). Hypothalamic obesity: the myth of the ventromedial nucleus. Science 182 : 488-490.

Goldman, J.K., Schnatz, J.D., Bernardis, L.L., & L.A. Frohman (1970). Adipose tissue metabolism of weanling rats after destruction of ventromedial nuclei: effect of hypophysectomy and growth hormone. Metabolism 19 : 995-1005.

Goldman, J.K., Bernardis, L.L., & L.A. Frohman (1974). Food intake in hypothalamic obesity. Am. J. Physiol. 227 : 88-91.

Goss, R.J. (1964). Adaptive growth. London, Logos Press.

Goss, R.J. (1972). Theories of growth regulation. In Regulation of organ and tissue growth. Ed. Goss, R.J. New York, Academic Press.

Gottlieb, A., Keydar, I., & H.T. Epstein (1977). Rodent brain growth stages: An analytical review. Biol. Neonate 32 : 166-176.

Gray, H. & T. Addis (1948). Rat colony testing by Zucker's weight-age relation. Am. J. Physiol. 153 : 35-40.

Grossman, M. & G.A.E. Gall (1968). Covariance analysis with unequal subclass numbers: component estimation in quantitative genetics. Biometrics 24 : 49-59.

Grunt, J.A. (1964). Effects of adrenalectomy and gonadectomy on growth and development in the rat. Endocrinology 75 : 446-451.

Grunt, J.A. (1964). Effects of neonatal gonadectomy on skeletal growth and development in the rat. Endocrinology 75 : 805-808.

Haines, R.W. (1932). The laws of muscle and tendon growth. J. Anat. 66 : 578-589.

Halasz, B. (1964). Neural control of growth hormone secretion. In Proc. 2d. Intern. Cong. of Endocrinology. Part I. Intern. Congr. Ser. No. 83. Amsterdam, Exercerpta. Medica. Foundation. pp.517-521.

Hall, K., Takano, K., Fryklund, L., & H. Sievertsson (1975a). The measurement of somatomedin A by radioreceptor assay. Advan. Metab. Disord. 8: 61-71.

Hall, K., Takano, K., Fryklund, L., & H. Sievertsson (1975b). Somatomedins. Advan. Metab. Disord. 8 : 19-46.

Hall, W.G., Cramer, C.P. & E.M. Blass (1977). Ontogeny of suckling in rats: Transitions toward adult ingestion. J. Comp. Physiol. Pyschol. 91: 1141-1155.

Hall, W.G. & J.S. Rosenblatt (1977). Suckling behavior and intake control in the developing rat pup. J. Comp. Physiol. Pyschol. 91: 1232-1247.

Hammond, J. (1932). Growth and development of mutton qualities in the sheep. Edinburgh, Oliver & Boyd.

Hammond, J. (1944). Physiological factors affecting birth weight. Proc. Nutr. Soc. 2: 8-14.

Han, P.W., Lin, C.H., Chu, K.C., Mu, J.Y. & A.C. Liu (1965). Hypothalamic obesity in weanling rats. Am. J. Physiol. 209: 627-631.

Han, P.W. (1967). Hypothalamic obesity in rats without hyperphagia. Trans. N.Y. Acad. Sci. 30: 229-238.

Hancock, J. (1951). Studies in monozygotic cattle twins. V. Uniformity trials - growth. N.Z. J. Sci. Tech. A33: 17-29.

Hansson, A. & O. Claesson (1960). Report on research with monozygotic cattle twins. In Research work with monozygotic twins. Proc. Europ. Ass. An. Prod. Publ. No. 9: 21-75.

Harrison, G.A. (1963). Temperature adaptation as evidenced by growth of mice. Fed. Proc. 22: 691-698.

Holt, H., Keeton, R.W. & B. Vennesland (1936). The effect of gonadectomy on body structure and body weight in albino rats. Am. J. Physiol. 114: 515-525.

Houpt, R. (1970). Transfer of urea and ammonia in the rumen. In Physiology of digestion and metabolism in the ruminant. Ed. A.T. Phillipson. Newcastle-upon-Tyne, Oriel Press.

Hughes, J.G. (1976). Short-term variation in animal live weight and the reduction of its effect on weighing. Anim. Breeding Abstr. 44: 111-118.

Hughes, P.C.R. & J.M. Tanner (1970). A longitudinal study of the growth of the black-hooded rat: methods of measurementand rates of growth for skull, limbs, pelvis, nose-rump, and tail length. J. Anat. 106: 349-370.

Hughes, P.C.R. & N. Nowak (1973). The effect of the number of animals per cage on the growth of the rat. Laboratory Animals 7: 293-296.

Husian, S.M. & G. Pincus (1968). Effects of oral administration of a Norethynodrel-mestranol combination (Enovid) in rats. Rev. Can. Biol. 27 : 217-224.

Huxley, J.S. (1932). Problems of relative growth. London. Methuen & Co.

Ingle, D.J. (1949). A simple means of producing obesity in the rat. Proc. Soc. Exp. Biol. Med. 72 : 604-605.

Jinks, J.L. & P.L. Broadhurst (1963). Diallel analysis of litter size and body weight in rats. Heredity 18 : 319-336.

Johns, J.T. & W.G. Bergen (1976). Growth in sheep, pre- and post-weaning hormone changes and muscle and liver development. J. Anim. Sci. 43: 192-200.

Josimovich, J.B., Mintz, D.M., & J.L. Finster (1967). Estrogenic inhibition of growth hormone-induced tibial epiphyseal growth in hypophysectomised rats. Endocrinology 81 : 1428-1430.

Kassab, S. (1964). On maternal and other influences on birth weight, growth and hair coat colour in two Dutch cattle breeds. Wageningen, H. Veenman & Zonen.

Kendall, M. (1973). Time-Series. London, Griffin Books.

Kennedy, G.C. (1950). Hypothalamic control of food intake in the rat. Proc. Roy. Soc. Lond. (Ser B) 137: 535-549.

Kennedy, G.C. (1957). The development with age of hypothalamic restraint upon the appetite of the rat. J. Endocrinol. 16 : 9-17.

Kennedy, G.C. (1967). Ontogeny of mechanisms controlling food and water intake. In Handbook of physiology, Section 6. Alimentary Canal, Vol I. Control of food and water intake, Ed. Code, C.F. Washington, D.C. American Physiological Society.

Kennedy, G.C. (1969). Interactions between feeding behavior and hormones during growth. Ann. N.Y. Acad. Sci. 157 : 1049-1061.

Kennedy, G.C. (1970). The relation between the central control of appetite, growth and sexual maturation. Guy Hosp. Rep (Lond.) 118 : 315-327.

Kidwell, J.F., Howard, A., 7 A.K. Laird (1969). The inheritance of growth and form in the mouse. II. The Gompertz growth equation. Growth 33: 339-52.

Kincaid, C.M., Litton, G.W., & R.E. Hunt (1945). Some factors that influence the production of steers from pasture. J. Anim. Sci. 4: 164.

Kember, N.F. (1972). Comparative patterns of cell division in epiphyseal cartilage plates in the rat. J. Anat. 111 : 137-142.

Kember, N.F. (1973). Patterns of cell division in the growth plates of the rat pelvis. J. Anat. 116 : 445-452.

Kember, N.F., & K.V.R. Walker (1971). Control of bone growth in rats. Nature 229 : 428-429.

Kember, N.F., & K.V.R. Walker (1972). Cell kinetics of growth cartilage in the rat tibia. II Measurements during ageing.
Cell. Tissue Kinet. 5 : 409-419.

Kleiber, M. (1947). Body size and metabolic rate. Physiol. Rev. 27; 511-541.

Kleiber, M. (1969). Further consideration of the relation between metabolic rate and body size. In Energy metabolism of farm animals. Eds. Blaxter, K.L., Kielanowski, K., & G. Thorbek. Newcastle-upon-Tyne, Oriel Press, pp. 505-511.

Knapp, B., & R.T. Clark (1947). Genetic and environmental correlations between growth rates of beef cattle at different ages. J. Anim. Sci. 6: 174

Knittle, J., & J. Hirsch (1968). Effect of early nutrition on the development of rat epididymal fat pads: cellularity and metabolism.
J. Clin. Invest. 47 : 2091-2098.

Knittle, J. (1972). Maternal diet as a factor in adipose tissue cellularity and metabolism in the young rat. J. Nutr. 102 : 427-434.

Koch, R.M., Schleicher, E.W., & V.T. Arthaud (1958). The accuracy of weights and gains in beef cattle. J. Anim. Sci. 17 : 604-611.

Kral, J.G. (1976). Surgical reduction of adipose tissue in the male Sprague-Dawley rat. Am. J. Physiol. 231: 1090-1096.

Krulich, L., Hefco, E., Illner, P., & C.B. Read (1974). The effects of acute stress on the secretion of LH, FSH, prolactin, and GH in the normal male rat, with commentson their statistical evaluation. Neuroendocrinology 16 : 293-311.

Kvinnsland, S., & S. Kvinnsland (1975). Growth in craniofacial cartilages studied by ^{3}H-Thymidine incorporation. Growth 39 : 305-314.

Laird, A.K., & A. Howard (1967). Growth curves in inbred mice. Nature 213 : 768-788.

Laird, A.K., Barton, A.D., & S.A. Tyler (1968). Growth and time: an interpretation of allometry. Growth 32 : 347-354.

Leathem, J.H. (1952). quoted by Gaunt, R. (1954). Chemical control of growth in animals. In Dynamics of growth processes. Ed. Boell, E.J. Princeton, Princeton University Press. pp. 183-211.

Leblond, C.P. (1972). Growth and renewal. In Regulation of organ and tissue growth. Ed. Goss, R.J. New York, Academic Press.

Leshner, A.I., Litwin, V.A., & R.L. Squibb (1972). A simple method for carcass analysis. Physiol. Behav. 9 : 281-282.

Leshner, A.I., & G. Collier (1973). The effects of gonadectomy on the sex differences in dietary self-selection patterns and carcass composition in rats. Physiol. Behav. 11 : 671-676.

Lindley, D.V. (1953). Estimation of a functional relationship. Biometrika 40: 47

Lisk, R.D. (1969). Estrogen: direct effects on hypothalamus in relation to pituitary weight changes. Neuroendocrinology 4 : 368-373.

Lobue, J., & A.S. Gordon (1973). General summary. In Humoral control of growth and differentiation. Vol I. Eds. Lobue, J., & A.S Gordon. New York, Academic Press.

Loeb, H.G. (1942). Influence of estradiol benzoate on fat storage. Proc. Soc. Exp. Biol. Med. 51 : 330-332.

Mayer, J. (1948). Growth characteristics of rats fed a synthetic diet. Growth 12 : 341

McCance, R.A. (1972). The composition of the body: its maintenance and regulation. Nutr. Abstr. Rev. 42 : 1269-1279.

McCance, R.A., & E.M. Widdowson (1974). The determinants of growth and form. Proc. Roy. Soc. (Ser B.) 185 : 1-17.

McCance, R.A. (1976). Critical periods of growth. Proc. Nutr. Soc. 35 : 309-313.

McCarthy, J.C. (1968). Discussion. In Growth and development of mammals. Eds. Lodge, G.A. & G.E. Lamming. London, Butterworths. pp. 325-326.

McCarthy, J.C. (1974). Insights into genetic variation in the deposition of fat from selection experiments in mice. 1st. World Cong. Genetics. Appl. to Livestock Prod. Vol III. pp. 529-531.

McDonald, P., Edwards, R.A., & J.F.D. Greenhalgh (1973). Animal Nutrition. 2d. Ed. Edinburgh, Oliver & Boyd.

Medawar, P.B. (1945). Size, shape, and age. In Essays on growth and form. Eds. Le Gros Clark, W.E., & P.B. Medawar. Oxford, Clarendon. pp. 157-187.

Meites, J. (1950). Relation of food intake to growth-depressing action of natural and artifical estrogens. Am. J. Physiol. 159 : 281-286.

Merat, A., & J.W.T. Dickerson (1974). The effect of severity and timing of malnutrition on brain gangliosides in the rat. Biol. Neonate 25: 158-70.

Meredith, H.V., & E.M. Meredith (1958). Concomitant variation of measures of body size on boys 5 years of age and measures of growth rate for the ensuing sexennium. Growth 22 : 1-8.

Meyer, J.H., & W.J. Clawson (1964). Undernutrition and subsequent realimentation in rats and sheep. J. Anim. Sci. 23 : 214-224.

Mickelsen, O., & A. Anderson (1959). A method of preparing intact animals for carcase analysis. J. Lab. clin. Med. 53 : 282-290.

Minot, C. (1908) quoted by Medawar, P.B. (1945) above ref.

Mitchell, H.H. (1962). Comparative nutrition of man and domestic animals. Vol I. New York, Academic Press.

Monteiro, L.S., & D.S. Falconer (1966). Compensatory growth and sexual maturity in mice. Animal Production 8 : 179-192.

Morris, H.P., Palmer, L.S., and C. Kennedy (1933). Fundamental food requirements for growth in the rat. VII. An experimental study of inheritance as a factor influencing food utilisation in the rat. Minn. Agr. Expt. Stat. Tech. Bul. 92.

Morrison, F.B. (1956). Feeds and feeding; a handbook for student and stockman. Ithaca, N.Y., Morrison Publishing Co.

Mosier, H.D. (1969). Allometry of body weight and tail length in studies of catch-up growth in rats. Growth 33 : 319-330.

Mosier, H.D. (1971). Failure of compensatory (catch-up) growth in the rat. Pediat. Res. 5 : 59-63.

Mosier, H.D., Drearden, L.C., Jansons, R.A., & R.R. Hill (1978). Cartilage sulphation during catch-up growth after fasting in rats. Endocrinology 102 : 386-392.

Moss, M.L., & M.J. Baer (1956). Differential growth of the rat skull. Growth 20 : 107-120.

Moss, M.L. (1972). The regulation of skeletal growth. In Regulation of organ and tissue growth. Ed. Goss, R.J. New York, Academic Press.

Moulton, C.R. (1923). Age and chemical development in mammals. J. Biol. Chem. 57: 79-97.

National Academy of Sciences: Committee on Animal Nutrition, Nutrient requirements of laboratory animals. (1962). Nutrient requirements of the laboratory rat. Nat. Acad. Sci. Publ. No. 990 : 51-95.

Needham, A.E. (1964). The growth process in animals. London, Plenum.

Nicoll, G.B. (1975). A study of adjustment factors for the weaning weights of Hereford and Angus calves; Thesis, M.Agr.Sci., Massey University, p.121.

Nilson, H.W., Palmer, L.S., & C. Kennedy (1935). Physiological effects of pituitary growth hormone: growth and efficiency of food utilisation. Am. J. Physiol. 111 : 341-351.

Nyda, M.J., de Majo, S.F., & R.A. Lewis (1948). The effect of ovariectomy and physiologic doses of estradiol upon body weight, linear growth, and fat content of the female albino rat. Bull. Johns Hopkins Hosp. 83 : 279-287.

Osborne, T.B. & L.B. Mendel (1926). The relation of growth to diet. J. Biol. Chem. 69 : 661.

Oscai, L.B., Mole, P.A., Krusack, L.M., & J.O. Holloszy (1973). Detailed body composition analysis on female rats subject to a program of swimming. J. Nutr. 103 : 412-418.

Paesi, F.J.A., & S.E. de Jongh (1954). Growth inhibition by oestrogen in hypophysectomised immature rats. Acta. Physiol. Pharmacol. Neerl. 3 : 227-231.

Palmer, L.S., Kennedy, C., Calverley, C.F., Lohn, C., & P.H. Weswig (1946). Genetic differences in the biochemistry and physiology influencing food utilisation for growth in rats. Minn. Agr. Exp. Stat. Tech. Bull. 176.

Park, A.W., & B.A.J. Nowosielski-Slepowron (1971). The effects of litter size on rat body growth. Acta. Anat. 79 : 15-26.

Phillips, L.S., Herington, A.C., & W.H. Daughaday (1973). Hormone effects on somatomedin action and somatomedin generation. In Advances in human growth hormone research. Ed. Raiti, S. DHEW Publ. No. (NIH) 74-612.

Phillips, L.S., & H.S. Young (1976). Nutrition and somatomedin. I. Effect of fasting & re-feeding on serum sulfation activity and cartilage growth activity in rats. Endocrinology 99 : 304-314.

Phillips, L.S., Herington, A.C., Karl, I.E., & W.H. Daughaday (1976). Comparison of somatomedin activity in perfusates of normal and hypophysectomised rat livers with and without added growth hormone. Endocrinology 98 :606-614;

Preston, T.R., & M.B. Willis (1970). Intensive beef production. Oxford, Pergamon.

Priest, R.E., Koplitz, R.M., & E.P. Benditt (1960). Estradiol reduces incorporation of radioactive sulphate into cartilage and aortas of rats. J. Exptl. Med. 112 : 225-236.

Priest, R.E., & R.M. Koplitz (1962). Inhibition of synthesis of sulphated mucopolysaccharides by estradiol. J. Exptl. Med. 116 : 565-574.

Rabii, J., & Gnaong, W.F. (1976). Response of plasma 'estradiol' and plasma LH to ovariectomy, ovariectomy plus adrenalectomy and estrogen injection at various ages. Neuroendocrinology 20 : 270-281.

Raye, J.R., Dubin, J.W., & J.N. Belchner (1977). Fetal growth retardation following maternal morphine administration: Nutritional or drug effect. Biol. Neonate 32 : 222-228.

Redick, J.H., Nussbaum, A.I., & D.G. Mook (1973). Estradiol induced suppression of feeding in the female rat; dependence on body weight. Physiol. Behav. 10 : 543-547.

Reed, L.L., Anderson, W.E., & W.B. Mendel (1932). Factors influencing the distribution of adipose tissue in the rat. II. The effect of ovariectomy and of feeding thyroxine. J. Biol. Chem. 96 : 313-323.

Reeve, E.C.R., & J.S. Huxley (1945). Some problems in the study of relative growth. In Essays on growth and form. Eds. Le Gros Clark, W.E., & P.B. Medawar. Oxford, Clarendon. pp. 121-156.

Reid, J.T., *et al.* (1967). Some pecularities in the body composition of animals. In Body composition in man and animals. Washington, D.C., National Academy of Sciences.

Reuter, J. (1976). Some aspects of the growth of rats. Growth 40:379-384.

Rice, R.W., Kroning, J., & V. Critchlow (1976). Sex differences in the effects of surgical isolation of the medial basal hypothalamus on linear growth and plasma growth hormone levels in the rat. Endocrinology 98 : 982-990.

Richards, O.W., & A.J. Kavanagh (1945). The analysis of growing form. In Essays on growth and form. Eds. Le gros Clark, W.E., & P.B. Medawar. Oxford, Clarendon, pp. 188-229.

Robinson, R. (1965). The genetics of the Norway Rat. Oxford, Pergamon.

Ross, M.H. (1959). Protein, calories, and life expectancy. Fed. Proc. 18 : 1190-1207.

Roy, J.H.B. (1970). The calf. Vol I. London, Iliffe Books.

Russell, W.S. (1976). Effect of twin birth on growth of cattle. Anim. Prod. 22 : 167-173.

Salmon, W.D. (1973). Effects of somatomedin on cartilage metabolism: further observations on an inhibitory factor. In Advances in human growth hormone research. Ed. Raiti, S., DHEW Publ. No. (NIH) 74-612

Salmon, W.D. (1975). Interaction of somatomedin and a peptide in serum of hypophysectomised and starved pituitary intact rats. Advan. Metab. Disord. 8 : 183-199.

Saubidet, C.P., & L.S. Verde (1976). Relationship between live weight, age, and dry matter intake for beef cattle and different levels of food restriction. Anim. Prod. 22 : 61-69.

Schemmel, R., Mickelsen, O., & Z. Tolgay (1969). Dietary obesity in rats: Influence of diet, weight, and sex on body composition. Am. J. Physiol. 216 : 373-379.

Sclafani, A., & C.N. Berner (1977). Hyperphagia and obesity produced by parasagittal and coronal hypothalamic knife cuts: Further evidence for a longitudinal feeding inhibitory pathway. J. Comp. Physiol. Psych. 91 : 1000-1018.

Sholl, D.A. (1950). The theory of differential growth analysis. Proc. Roy. Soc. Lond. (Ser B.) 137 : 470-474.

Sholl, D.A. (1954). Regularities in growth curves, including rhythms and allometry. In Dynamics of growth processes. Ed. Boell, E.J., Princeton, Princeton Universtiy Press.

Simpson, M.E., Asling, C.W., & H.M. Evans (1950). Some endocrine influences on skeletal growth and differentiation. Yale J. Biol. Med. 23: 1-27.

Sinha, Y.N., Wilkins, J.N., Selby, F., & W.P. Van der Laan (1973). Pituitary and serum growth hormone during undernutrition and catch-up growth in young rats. Endocrinology 92 : 1768.

Slonaker, J.R. (1930). The effect of excision of the different sexual organs on the development, growth, and longevity, of the albino rat. Am. J. Physiol. 93 : 307-317.

Smart, J.L, Adlard, B.P.F., & J. Dobbing (1974). Further studies of body growth and brain development in "small-for-dates" rats. Biol. Neonate 25 : 135-150.

Smith, A.H., & F.C. Bing (1928). Improved rate of growth of stock albino rats. J. Nutr. 1 : 179.

Smith, A.L. (1973). Maize silage; a study of the nutritive value of ensiled Zea Mays L. for growth in young cattle. Thesis, M.Agr.Sci. Massey University, p. 141.

Smith, G.M., Fitzhugh, H.A., Cundiff, L.V., Cartwright, T.C. & K.E. Gregory (1976). A genetic analysis of maturing patterns in straightbred and cross-bred Hereford, Angus and Shorthorn cattle. J. Anim. Sci. 43 : 389-395.

Smith, G.M., Laster, D.B., Cundiff, L.V., & K.E. Gregory (1976). Characterisation of biological types of cattle. II. Post-weaning growth and feed efficiency of steers. J. Anim. Sci. 43 : 32-47.

Snedecor, G.W., & W.G. Cochran (1968) Statistical Methods. 6th. Ed. Ames, Iowa, Iowa State University Press.

Sorensen, P.H. (1962). Influence of climatic environment on pig perf-ormance. In Nutrition of pigs and poultry. Eds. Morgan, J.T. & D. Lewis. London, Butterworths. pp. 88-102.

Stewart, D.M. (1972). The role of tension in muscle growth. In Regulation of organ and tissue growth. Ed. Goss, R.J. New York, Academic Press.

Stotsenberg, J.M. (1913). The effect of spaying and semi-spaying young albino rats (Mus Norvegicus Albinus) on the growth in body weight and body length. Anat. Rec. 7 : 183-194.

Sullivan, L.W., & T.C. Smith (1957). Influence of estrogens on body growth and food intake. Proc. Soc. Exp. Biol. Med. 96 : 60.

Talwar, G.P., Pandian, M.R., Kumar, N., Hanjan, S.N.S., Saxena, R.K., Krishnaraja, R., & R.L. Gupta (1975). Mechanism of action of pituitary growth hormone. Rec. Prog. Horm. Res. 31: 141-174.

Tam, C.S., Reed, R., Campbell, J.E., & B Cruickshank (1974). Bone growth kinetics. II. Short-term observations on bone growth in Sprague-Dawley rats. J. Path. 113 : 39-46.

Tam, C.S., Reed, R., Swinson, D.R., Little, A.H., & B. Cruickshank (1974). Bone growth kinetics III. A biorhythm in bone growth in the rabbit. J. Path. 114 : 127-133.

Tang, Y.Z. (1941). Sex differences in growth in gonadectomised albino rats. Anat. Rec. 80 : 13-32.

Tanner, J.M. (1963). Regulation of growth in size in mammals. Nature 199 : 645-650.

Tanner, J.M. (1974). Sequence and tempo in the somatic changes in puberty. In Control of the onset of puberty. Eds. Grumbach, M.M., Grave, G.D., & F.F. Mayer. New York, J. Wiley & Sons.

Tanner, J.M. (1976). Growth as a monitor of nutritional status. Proc. Nutr. Soc. 35 : 315-322.

Tarttelin, M.F., & R.A. Gorski (1971). Variations in food and water intake in the normal and acyclic female rat. Physiol. Behav. 7: 847-852.

Tarttelin, M.F., & R.A. Gorski (1973). The effects of ovarian steriods on food and water intake and body weight in the female rat. Acta. Endocr. (Kbh.) 72 : 551-568.

Tarttelin, M.F., Shyrne, J.E., & R.A. Gorski (1975). Patterns of body weight change in rats following neonatal hormone manipulation: a critical period for androgen-induced growth increases. Acta. Endocr. 79 : 177-191.

Tarttelin, M.F., & R.G. Clark (1975). Further studies of the effect of androgen in the neonatal rat. N.Z. Med. J. 82 : 241 (Abstr.).

Taylor, St. C.S., & G.B. Young (1967). Variations in growth and efficiency of twin cattle on constant feeding levels. Anim. Prod. 9 : 295-311.

Taylor, St. C.S. (1968). Genetic variation in growth and development of cattle. In Growth and development of mammals. Eds. Lodge, G.A. & G.E. Lamming. London, Butterworths. pp. 267-290.

Taylor, St. C.S., & H.A. Fitzhugh (1971). Genetic relationships between mature weight and time taken to mature within a breed, J. Anim. Sci. 33 : 726-731.

Tonna, E. A. (1973). Hormonal influences on skeletal growth and regeneration. In Hormonal control of growth and differentiation. Eds. Lobue, J., & A.S. Gordon. New York, Academic Press.

Urick, J.J., Knapp, B.W., Brinks, J.S., Pahnish, O.F., & T.M. Riley (1971). Relationships between cow weights and calf weaning weights in Angus, Charolais, and Hereford breeds. J. Anim. Sci. 33 : 343-348.

Valenstein, E.S., Kakolewski, J.W., & V.C. Cox (1969). Sex differences in body weight change following gonadectomy of rats. Psychol. Rep. 22 : 547-554

van't Hof, M.A., Machteld, J.R., & C.J. Kowalski (1976). Estimation of growth velocities from individual longitudinal data. Growth 40 : 217-240.

van Wyk, J.J., Underwood, L.E., Hintz, R.L., Clemmons, D.R., Voina, S.J., & R.P. Weaver (1974). The somatomedins: a family of insulin-like hormones under growth hormone control. Rec. Prog. Horm. Res. 30 : 259-318.

van Marthens, E., Grauel, L., & S. Zamenhof (1975). Enhancement of prenatal development in the rat by operative restriction of litter size. Biol. Neonate 25 : 53-56.

Vaughan, J.M. (1975). The physiology of bone. 2d. Ed. Oxford, Clarendon.

Vercoe, J.E., & J.E. Frisch (1974). Fasting metabolism, live weight, and voluntary feed intake of different breeds of cattle. In Energy metabolism of farm animals. Europ. Ass. for An. Prod. Publ. No. 14 : 131-134.

Wade, G.N., & I. Zucker (1970). Development of hormonal control over food intake and body weight in female rats. J. Comp. Physiol. Psychol. 70: 213-20.

Wade, G.N. (1972). Gonadal hormones and behavioral regulation of body weight. Physiol. Behav. 8 : 523-534.

Wage, G.N. (1976). Sex hormones, regulatory behavior and body weight. In Advances in the study of behavior. Eds. Rosenblatt, J.S., & Hinde, R.A., & C. Beer. New York, Academic Press. 6 : 201-279.

Wallace, L.R. (1948). Growth of lambs before and after birth in relation to the level of nutrition. J. Agric. Sci. (Camb) 38 : 93-153, 243-302, 367-401.

Watkins, M.L., Fizette, N., & M. Heimberg (1972). Sexual influences on hepatic secretion of triglyceride. Biochimica et Biophysica Acta 280: 82-5.

Webb, A.J., & J.W.B. King (1976). Development of a synthetic pig sire line by selection with immigration. Anim. Prod. 22 : 231-244.

Weideman, E., & E. Schwartz (1972). Suppression of growth hormone-dependent human serum sulphation factor by estrogen.
J. Clin. Endocr. 34 : 51-58.

Widdowson, E.M., & R.A. McCance (1960). Some effects of accelerating growth. I. General somatic development. Proc. Roy. Soc. Lond. 152:188-206.

Widdowson, E.M., & G.C. Kennedy (1962). Rate of growth, mature weight and life span. Proc. Roy. Soc. Lond. (Ser B.) 156 : 96-108.

Widdowson, E.M., & R.A. McCance (1963). The effect of finite periods of undernutrition at different ages on the composition and subsequent development of the rat. Proc. Roy. Soc. LOnd. (Ser B.) 158 : 329-342.

Widdowson, E.M., & R.A. McCance (1975). A review: new thoughts on growth. Pediat. Res. 9 : 154-156.

Widdowson, E.M. (1976a). The response of the sexes to nutritional stress. Proc. Nutr. Soc. 35 : 175-180.

Widdowson, E.M. (1976b). Cellular growth and function.
Proc. Nutr. Soc. 35 : 357-362.

Williams, J.P.G., & P.C.R. Hughes (1975). Catch-up growth in rats undernourished for different periods during the suckling period.
Growth 39 : 179-193.

Wilson, P.N., & D.F. Osbourn (1960). Compensatory growth after undernutrition in mammals and birds. Biol. Rev. 35 : 324-363.

Wilson, P.R. (1977). Studies of testicular and reproductive endocrine parameters in the ram with particular reference to sexual maturation. Thesis, PHd., Massey University, pp.234.

Winick, M., & A. Noble (1966). Cellular response in rats during undernutrition at various ages. J. Nutr. 89 : 300-306.

Winick, M., Brasel, J.A., & P. Rosso (1972). Nutrition and cell growth. In Nutrition and development. Ed. Winick, M.,New York, J. Wiley & Sons.

Winokur, A., & R.D. Utiger (1974). Thyrotrophin-releasing hormone: regional distribution in rat brain. Science 185 : 265-267.

Wirtshafter, D., & J.D. Davis (1977). Set points, settling points, and the control of body weight. Physiol. Behav. 19 : 75-78.

Wishart, J. (1939). Statistical treatment of animal experiments. Suppl. J. Roy. Stat. Soc. 6 : 1-12.

Woods, S.C. (1974). Metabolic hormones and the regulation of body weight. Physiol. Rev. 81 : 26-43.

Woods, S.C., & D. Porte (1978). The central nervous system, pancreatic hormones, feeding, and obesity. Advan. Metab. Disord. 9 : 283-312.

Yalow, R.S., Hall, K., & R.Luft (1975). Radioimmunoassay of Somatomedin B. Advan. Metab. Disord. 8 : 73-83.

Zucker, L., & T.F. Zucker (1942). A simple time-weight relation observed in well nourished rats. J. Gen. Physiol. 25 : 445-463.

Zucker, L.M. (1975). Efficiency of energy utilisation by the Zucker hereditarily obese rat 'fatty'. Proc. Soc. Exp. Biol. Med. 148 : 498-500.

Zucker, T.F., Hall, L., Young, M., & L. Zucker (1941a). Quantatitive formulation of rat growth. Growth 5 : 415-436.

Zucker, T.F., Hall, L., Young, M., & L. Zucker (1941b). The growth curve of the albino rat in relation to diet. J. Nutr. 22 : 123-138.

Zucker, T.F., & L.M. Zucker (1963). Fat accretion and growth in the rat. J. Nutr. 80 : 6-19.

MEASUREMENT, MATHEMATICS, AND MECHANISMS OF MAMMALIAN GROWTH

A thesis presented in partial fulfilment of
the requirements for the
Degree of Doctor of Philosophy
at Massey University

Ross Graham Clark

1978

APPENDIX TABLES

(VOLUME TWO)

Time goes, you say? Ah, no!

Alas, Time stays, we go.

H.A. Dobson

The Paradox of Time, stanza 1.

LIST OF APPENDIX TABLES

A LIST OF APPENDIX TABLES 8.1 to 8.36

APPEARS ON PAGE 25

APPENDIX TABLE 2.1,

Slopes, constants and correlation coefficients for the linear regressions of log live weight times 100.0 on log age times 100.0 (with 1.0 added to the age, in weeks, before taking logs) and calculated with live weight transformed to a) two parameter , and b) three parameter (Y + 7.0) logarithmic metameters. Lambs of Wallace (1948).

	a) TWO PARAMETER				b) THREE PARAMETER		
Lamb No.	Slope	Constant	Correlation coefficient	Lamb No.	Slope	Constant	Correlation
3	90.30	85.18	.9977	3	71.84	109.17	.9917
5	83.79	80.16	.9904	5	64.63	106.37	.9797
6	91.27	74.98	.9873	6	70.56	102.56	.9739
9	77.77	100.78	.9905	9	64.57	119.09	.9830
10	78.20	104.03	.9909	10	65.73	121.22	.9844
11	90.59	83.03	.9903	11	72.34	107.13	.9798
12	80.69	95.92	.9952	12	65.88	116.05	.9882
13	84.50	93.64	.9950	13	69.03	114.29	.9876
14	88.77	74.48	.9931	14	67.43	103.03	.9808
15	92.80	75.48	.9923	15	71.85	102.98	.9804
16	82.65	78.30	.9619	16	64.24	104.41	.9431
19	87.70	81.77	.9853	19	69.33	106.51	.9720
21	72.21	105.20	.9882	21	60.25	122.34	.9808
22	81.37	95.23	.9963	22	64.50	116.67	.9900
23	83.43	86.39	.9977	23	65.53	110.43	.9911
26	78.45	106.70	.9961	26	66.26	123.28	.9911
27	94.08	74.56	.9879	27	73.01	102.20	.9766
28	82.23	77.08	.9874	28	62.29	104.79	.9737
31	74.07	89.43	.9900	31	57.97	112.52	.9799
32	78.43	84.59	.9957	32	60.41	109.67	.9871
33	84.98	78.52	.9991	33	64.18	106.32	.9930
34	87.46	70.09	.9953	34	63.93	101.63	.9866
36	83.52	75.15	.9949	36	61.52	104.88	.9886
37	91.69	67.08	.9949	37	67.21	99.47	.9840
40	77.19	93.53	.9958	40	61.59	115.11	.9904
41	74.05	95.22	.9961	41	59.03	116.38	.9910
42	69.11	100.10	.9969	42	54.79	120.43	.9974
43	66.89	101.52	.9967	43	53.16	121.36	.9962
44	70.90	103.39	.9979	44	57.31	122.38	.9983
45	75.58	107.72	.9955	45	63.59	124.26	.9917
46	69.82	102.65	.9965	46	56.63	121.50	.9937
47	68.28	101.44	.9942	47	54.67	121.02	.9925
50	79.90	91.51	.9960	50	63.51	113.80	.9910
51	76.70	99.62	.9959	51	62.75	118.86	.9909
48	70.94	81.85	.9938	48	52.11	109.27	.9857
49	75.39	74.64	.9964	49	54.04	105.15	.9880

APPENDIX TABLE 3.1.

Slopes, constants, and correlation coefficients for the linear regressions of log live weight times 100.0 on log age times 100.0 (with 1.0 added to age, in weeks, before taking logs). Lambs of Tartellin and Munford (1974-75).

	FEMALES			MALES			
Lamb No	Slope	Constant	Correlation coefficient	Lamb No.	Slope	Constant	Correlation
Treated Singles							
4	62.63	69.75	.9955	3	71.47	64.35	.9955
10	57.81	63.96	.9900	5	65.14	72.83	.9970
11	66.85	65.14	.9943	7	79.00	50.88	.9838
13	65.64	61.53	.9954	12	67.33	68.65	.9962
16	69.64	54.11	.9949	14	71.64	67.39	.9968
17	63.70	64.36	.9942	24	72.83	67.75	.9948
21	65.06	66.69	.9941	25	65.39	64.98	.9888
22	68.84	56.65	.9962	26	80.73	52.90	.9993
23	77.28	43.85	.9899	38	72.56	63.55	.9975
27	67.83	59.43	.9964	42	89.36	42.39	.9988
33	71.99	58.52	.9980				
Treated Twins							
6	63.58	70.19	.9893	20	70.16	49.55	.9933
15	73.24	45.15	.9945	45	63.68	57.64	.9935
18	66.45	48.40	.9912	35	56.31	57.83	.9916
37	71.81	48.84	.9926				
40	66.16	62.15	.9977				
41	78.50	41.87	.9921				
135	64.17	65.52	.9941				
140	69.42	59.96	.9974				
141	80.50	38.74	.9932				
Control Singles							
1	68.86	59.49	.9949	4	75.44	57.16	.9984
16	84.08	42.41	.9873	7	71.09	67.34	.9955
20	74.11	35.22	.9792	17	76.16	58.15	.9947
				18	70.29	68.29	.9923
				22	69.51	60.09	.9758
				23	70.19	56.35	.9763
Control Twins							
2	65.38	46.62	.9923	3	68.11	51.59	.9869
6	67.92	48.84	.9762	5	68.72	55.73	.9759
9	64.42	62.16	.9886	12	62.16	63.55	.9873
10	65.48	47.10	.9674	13	77.21	45.82	.9864
11	67.64	52.17	.9889	14	77.10	48.03	.9861
21	64.33	64.82	.9965				
Treated Castrate Twins							
				1	62.16	62.28	.9808
				9	76.78	30.71	.9906
				19	63.02	60.20	.9966
				30	69.01	55.57	.9921
				36	86.13	29.16	.9995
				43	67.82	51.18	.9910
				44	65.93	58.26	.9807
				130	73.33	50.46	.9894

APPENDIX TABLE 3.2.

Slopes, constants, and correlation coefficients for the linear regressions of log live weight times 100.00 on log age times 100.0 (with 1 added to age, in weeks, before taking logs) with live weight transformed to a three parameter logarithmic metameter (Y + 2.5).

	FEMALES			MALES			
Lamb No	Slope	Constant	Correlation coefficient	Lamb No	Slope	Constant	Correlation coefficient
Treated Singles							
4	52.42	85.05	.9922	3	59.91	80.71	.9914
10	46.50	81.59	.9887	5	55.37	87.11	.9942
11	55.60	81.56	.9898	7	64.56	70.92	.9774
13	53.69	79.20	.9909	12	58.22	82.49	.9963
16	55.58	74.41	.9892	14	60.57	82.92	.9939
17	52.41	81.25	.9908	24	62.08	82.84	.9900
21	53.70	83.18	.9930	25	54.50	81.25	.9833
22	55.48	75.95	.9920	26	65.59	73.09	.9969
23	60.05	67.70	.9868	38	59.99	80.81	.9982
27	55.23	77.75	.9921	42	71.10	66.00	.9946
33	58.82	77.10	.9954				
Treated Twins							
6	53.64	85.11	.9855	20	55.22	71.26	.9864
15	56.72	68.63	.9878	35	43.95	77.62	.9878
18	51.58	70.74	.9834	45	51.07	76.73	.9875
37	56.73	70.59	.9846				
40	53.80	80.04	.9967				
41	60.97	66.09	.9843				
135	53.17	81.94	.9902				
140	56.64	78.19	.9944				
141	62.06	64.14	.9858				
Control Singles							
1	55.21	78.63	.9981	4	61.91	75.83	.9943
16	66.50	66.01	.9835	7	59.38	83.29	.9935
20	55.60	62.31	.9687	17	63.18	76.14	.9890
				18	59.73	83.37	.9879
				22	57.70	77.46	.9703
				23	57.60	74.89	.9679
Control Twins							
2	49.77	70.10	.9851	3	54.12	72.36	.9776
6	53.64	70.40	.9652	5	55.95	74.69	.9680
9	52.78	79.61	.9831	12	51.08	80.55	.9821
10	59.62	61.26	.9695	13	61.38	68.09	.9784
11	53.68	72.94	.9819	14	61.95	69.35	.9778
21	52.93	81.66	.9936				
Treated Castrate Twins				1	50.94	79.60	.9739
				9	55.75	60.54	.9804
				19	50.82	78.59	.9924
				30	55.74	74.94	.9861
				36	63.43	59.38	.9939
				43	53.57	72.29	.9830
				44	53.81	76.56	.9731
				130	58.54	71.50	.9837

APPENDIX TABLE 4.1

Ruakura Monozygotic Twins, data of Hancock (1951): Analyses of variance and covariance for the slopes (Y) and constants (X), from the linear equations relating 100 times log live weight to log age.

TWIN PAIRS	X MEAN	Y MEAN	ADJ. Y. MEAN	± SEM
1	125.05	81.10	74.54	.477
2	128.84	79.37	74.94	.396
3	131.74	78.60	75.78	.347
4	128.00	79.27	74.36	.412
5	141.62	74.45	77.16	.345
6	150.51	72.24	79.92	.525
7	149.86	70.60	77.92	.509
8	140.03	73.10	74.92	.327
9	134.71	78.11	76.96	.317
10	137.40	75.13	75.48	.311

Average within group regression, correlation = -.9863

Total estimates, ignoring groups, correlation = -.9602

MEAN SQUARE FOR EACH VARIABLE

SOURCE	DF	SLOPES	CONSTANTS
TOTAL	19	15.406	84.575
BETWEEN	9	25.254	155.913
WITHIN	10	6.544	20.371
F RATIO		3.859	7.653

ANALYSIS OF WITHIN GROUP VARIANCE OF Y

SOURCE OF VARIATION	DF	SUMS OF SQUARES	MEAN SQUARE	F RATIO
DUE TO AV. REGR.	1	63.658	63.658	322.88
DEV. FROM AV. REGR.	9	1.774	.197	

ANALYSIS OF VARIANCE OF Y AFTER FITTING REGRESSION ON X

SOURCE OF VARIATION	DF	MEAN SQUARE	F RATIO
TOTAL	18	1.268	
BETWEEN GROUPS	9	2.343	12.092
WITHIN GROUPS	9	.193	

ORIGINAL ERROR MEAN SQUARE = 6.544 PERCENT REDUCTION OF ERROR = 97.03

APPENDIX TABLE 4.2

Ruakura Monozygotic Twins, Data of Hancock (1951): Analyses of variance and covariance for the slopes (Y) and constants (X), from the linear equations relating 100 x log live weight (on a 3 parameter log scale) to log age.

TWIN PAIRS	X MEAN	Y MEAN	ADJ. Y. MEAN	± SEM
1	158.11	64.99	60.65	.472
2	162.91	62.50	60.83	.315
3	163.41	63.03	61.64	.304
4	162.30	62.35	60.34	.330
5	168.62	61.41	62.93	.308
6	172.01	62.13	65.53	.409
7	173.10	59.67	63.68	.449
8	168.67	59.38	60.92	.310
9	164.08	63.76	62.74	.292
10	165.85	61.50	61.47	.278

Average within group regression, correlation = -.9667

Total estimates, ignoring groups, correlation = -.8103

MEAN SQUARE FOR EACH VARIABLE

SOURCE	DF	SLOPES	CONSTANTS
TOTAL	19	3.894	24.090
BETWEEN	9	5.841	43.671
WITHIN	10	2.142	6.468
F RATIO		2.726	6.751

ANALYSIS OF WITHIN GROUP VARIANCE OF Y

SOURCE OF VARIATION	DF	SUMS OF SQUARES	MEAN SQUARE	F RATIO
DUE TO AV. REGR.	1	20.025	20.025	128.46
DEV. FROM AV. REGR.	9	1.402	.155	

ANALYSIS OF VARIANCE OF Y AFTER FITTING REGRESSION ON X

SOURCE OF VARIATION	DF	MEAN SQUARE	F RATIO
TOTAL	18	1.411	
BETWEEN GROUPS	9	2.667	17.160
WITHIN GROUPS	9	.155	

ORIGINAL ERROR MEAN SQUARE = 2.142 PERCENT REDUCTION OF ERROR = 92.74

APPENDIX TABLE 4.3

RUAKURA MONOZYGOTIC TWINS, data from Hancock (1951).

Slopes, constants, and correlation coefficients for the linear equations relating live weight on (a), a two parameter lognormal, and on (b) a three parameter lognormal metameter, to log age (weeks + 1.1).

	(a) TWO PARAMETER LOGNORMAL			(b) THREE PARAMETER LOGNORMAL		
Twin pair	Slope	Constant	Correlation coefficient	Slope	Constant	Correlation coefficient
1	77.04	132.25	.9956	62.85	162.00	.9924
2	85.16	117.84	.9965	67.13	154.22	.9940
3	76.38	134.64	.9910	60.94	166.17	.9937
4	82.36	123.03	.9926	64.06	159.65	.9958
7	77.54	133.71	.9968	62.60	164.29	.9981
8	79.65	129.78	.9945	63.46	162.52	.9968
11	78.42	128.52	.9930	61.62	162.74	.9948
12	80.11	127.48	.9918	63.08	161.86	.9941
13	73.48	142.75	.9958	60.60	169.52	.9968
14	75.44	140.49	.9965	62.23	167.73	.9978
17	72.22	150.34	.9979	62.16	171.79	.9965
18	72.27	150.68	.9981	62.11	172.23	.9973
19	69.69	151.40	.9964	58.90	174.39	.9965
20	71.52	148.32	.9982	60.44	171.82	.9977
23	74.25	139.05	.9961	60.41	167.79	.9974
24	71.94	141.00	.9972	58.35	169.55	.9986
25	78.10	134.82	.9953	63.75	164.17	.9955
26	78.13	134.60	.9961	63.77	163.99	.9963
29	73.62	140.12	.9969	60.69	167.36	.9952
30	76.65	134.68	.9970	62.32	164.33	.9961

APPENDIX TABLE 5.1

Analyses of variance and covariance for the slopes (Y) and constants (X), from the linear equations relating log live weight to log tan age (weeks/2), Massey Monozygotic Twin Heifers, Jerseys compared to Non-Jerseys.

GROUP	X MEAN	Y MEAN	ADJ.Y. MEAN	± SEM	CORRELATION
Non-Jerseys	148.26	29.19	29.68	.285	-.8671
Jerseys	148.80	28.63	28.48	.156	-.8792

Average within group regression, correlation = -.8740

Total estimates, ignoring groups, correlation = -.8602

MEAN SQUARE FOR EACH VARIABLE

SOURCE	DF	SLOPES	CONSTANTS
TOTAL	129	10.200	114.931
BETWEEN	1	7.220	140.000
WITHIN	128	10.223	114.735
F RATIO		.706	1.220

ANALYSIS OF WITHIN GROUP VARIANCE OF Y

SOURCE OF VARIATION	DF	SUMS OF SQUARES	MEAN SQUARE	F RATIO
TOTAL WITHIN GROUP	128	1308.622	10.223	
DUE TO AV. REGR.	1	999.624	999.624	410.851
DEV. FROM. AV. REGR.	127	308.998	2.433	
BETWN. IND. REGRS.	1	4.357	4.347	1.802
DEV. FROM. IND. REGRS	126	304.640	2.417	

ANALYSIS OF VARIANCE OF Y, AFTER FITTING REGRESSION ON X

SOURCE OF VARIATION	DF	MEAN SQUARE	F RATIO
TOTAL	128	2.671	
BETWEEN GROUPS	1	33.017	13.570
WITHIN GROUPS	127	2.433	

ORIGINAL ERROR MEAN SQUARE = 10.223 PERCENT REDUCTION OF ERROR = 76.200

APPENDIX TABLE 5.2

Analyses of variance and covariance of the slopes and constants from the linear equations relating log live weight to log tan age (weeks/2), for Massey Monozygotic Twins; comparison of years

Y = slope X = constant

Year	X mean	Y mean	Adj.Y mean	St.error Adj.Y	Correlation
1964	148.93	28.12	29.01	.235	-.9043
1965	144.36	29.86	29.45	.233	-.9202
1966	148.17	28.75	29.42	.318	-.9866
1967	140.50	29.19	27.69	.348	-.9455
1968	145.18	27.41	27.23	.252	-.9131

Average withing group regression correlation = -.9205

Total estimates, ignoring groups regression correlation= -.8796

Source	D.F	Mean square for each variable Slope	Constant
Total	99	9.584	99.413
between	4	20.728	183.300
Within	95	9.114	95.881
F. ratio		2.274	1.911
Std deviation		3.019	9.791
General mean		28.637	145.804

Analysis of within group variance of Y

Source of variation	D.F	Sums of squares	Mean Square	F. ratio
Due to average regression	1	733.761	733.761	521.907
Deviations from average regression	94	132.156	1.405	
Between individual group regs.	4	12.098	3.024	2.267
Deviations from individual regs	90	120.057	1.333	

Analysis of variance of Y after fitting regression on X

Total	98	2.190	
Between groups	4	20.631	14.674
Within groups	94	1.405	

Original mean sq.= 9.114 percent reduction = 84.57

APPENDIX TABLE 5.3

Analyses of variance and covariance of the slopes and constants, from the linear equations relating log live weight to log tan age (weeks/2), for Massey Monozygotic Twins; Twins compared, ignoring years

Analyses of Variance

Mean square for each variable.

Source	D.F.	Slope	Constant
Total	99	9.568	99.448
Between	49	18.378	193.191
Within	50	.934	7.580
F. Ratio		19.675	25.487
Std deviation		.966	2.753
General mean		28.641	145.788

Average within group regression correlation = -.8428

Total estimates, ignoring groups correlation= -.8791

Analysis of within group variance of Y

Source of variation	D.F	Sums of squares	Mean square	F. ratio
Due to average regression	1	33.170	33.1709	120.092
Deviations from average regression	49	13.534	.2762	

Analysis of variance of Y after fitting regression on X

Source of variation	D.F	Mean square	F. ratio
Total	98	2.194	
Between groups	49	4.112	14.895
Within groups	49	.276	

Original mean Sq. = .934 Percent reduction = 70.44

APPENDIX TABLE 5.4

Analyses of variance and covariance of the slopes and constants from the linear equations relating log live weight to log tan age (weeks/2), for Massey Monozygotic Twins; twins compared for 1964

Y = slope X = constant

Twin pairs	X mean	Y mean	Adj.Y mean	Std.Error Adj.Y
1	162.25	23.49	27.61	.521
2	149.40	28.01	28.12	.272
3	148.17	26.44	26.17	.273
4	145.16	31.57	30.35	.302
5	132.23	36.25	30.99	.627
6	141.42	29.74	27.36	.374
7	139.52	29.51	26.53	.420
8	158.16	26.58	29.43	.410
9	144.95	29.96	28.68	.305
10	150.73	27.19	27.72	.278
11	152.41	26.55	27.60	.294
12	152.33	25.21	26.23	.293
13	160.88	24.37	28.06	.482

Average within group regression correlation = -.936

Total estimates, ignoring groups regression correlation = -.907

Mean square for each variable

Source	D.F	Slope	Constant
Total	25	11.650	76.161
Between	12	23.058	147.766
Within	13	1.119	10.064
F. ratio		20.600	14.681
Std. deviation		1.057	3.172
General Mean		28.071	149.052

Analysis of within group variance of Y

Source of variation	D.F	Sums of squares	Mean Square	F. ratio
Due to average regression	1	12.764	12.764	85.726
Deviations from average regression	12	1.786	.148	

Analysis of variance of Y after fitting regression on X

Source of variation	D.F	Mean square	F. ratio
Total	24	2.130	
Between Groups	12	4.113	27.756
Within groups	12	.148	

Original mean sq. = 1.119 Percent reduction = 86.76

APPENDIX TABLE 5.5

Analyses of variance and covariance of the slopes and constants from the linear equations relating log live weight to log tan age (weeks/2), for Massey Monozygotic Twins: Twins compared for 1965

Twin Pairs	X mean	Y = slope Y mean	X = constant Adj.Y. mean	St.Error Adj.Y
1	160.50	24.560	29.19	1.021
2	143.30	32.310	32.00	.353
3	155.82	27.84	31.14	.765
4	151.82	27.32	29.47	.564
5	131.86	33.39	29.79	.821
6	132.29	31.72	28.25	.798
7	141.68	30.34	29.57	.382
8	148.82	28.79	30.08	.437
9	132.25	33.88	30.40	.800
10	139.64	30.10	28.74	.447
11	140.96	29.92	28.94	.402
12	152.66	27.58	29.97	.604
13	145.14	30.43	30.65	.350

Average within group regression correlation = -.8126

Total estimates, ignoring groups regression correlation = -.9202

Mean square for each variable

Source	D.F	Slope	Constant
Total	25	7.034	84.940
Between	12	13.941	171.268
Within	13	.657	5.253
F. ratio		21.190	32.598
Std. deviation		.811	2.292
General Mean		29.8655	144.368

Analysis of within group variance of Y

Source of variation	D.F	Sums of squares	Mean Square	F. ratio
Due to average regression	1	5.648	5.648	23.33
Deviations from average regression	12	2.904	.242	

Analysis of variance of Y after fitting regression on X

Source of variation	D.F	Mean square	F. ratio
Total	24	1.121	
Between Groups	12	2.001	8.266
Within groups	12	.242	

Original mean sq. = .657 Percent reduction = 63.19

APPENDIX TABLE 5.6

Analyses of variance and covariance of the slopes and constants from the linear equations relating log live weight to log tan age (weeks/2), for Massey Monozygotic Twins; Twins compared for 1966

	Y = slope		X = constant	
Twin pairs	X mean	Y mean	Adj.Y mean	Std.Error adj.Y
1	144.06	29.82	28.35	.339
2	159.82	25.55	29.63	.738
3	162.57	24.70	29.74	.898
4	149.24	29.06	29.41	.234
5	132.88	32.06	26.66	.858
6	130.10	33.82	27.45	1.123
7	158.96	25.60	29.37	.689

Average within group regression correlation = -.9210

Total estimates, ignoring groups regression correlation = -.9900

Mean square for each variable

Source	D.F	Slope	Constant
Total	13	11.762	161.779
Between	6	24.809	345.886
Within	7	.579	3.972
F. ratio		42.848	87.062
Std. deviation		.760	1.993
General Mean		28.663	148.238

Analysis of within group variance of Y

Source of variation	D.F	Sums of squares	Mean Square	F. ratio
Due to average regression	1	3.439	3.439	33.613
Deviations from average regression	6	.613	.102	

Analysis of variance of Y after fitting regression on X

Source of variation	D.F	Mean square	F. ratio
Total	12	.251	
Between Groups	6	.401	3.921
Within groups	6	.102	

Original mean sq. = .579 Percent reduction = 82.31

APPENDIX TABLE 5.7

Analyses of variance and covariance of the slopes and constants from the linear equations relating log live weight to log tan age (weeks/2). for Massey Monozygotic Twins; Twins compared for 1967

	Y = slope		X = constant	
Twin pairs	X mean	Y mean	Adj. Y mean	Std. error Adj.Y
1	140.70	28.18	28.26	.286
2	130.87	31.66	27.68	.678
3	136.06	30.92	29.09	.402
4	145.49	27.63	29.69	.428
5	150.39	26.85	30.94	.693
6	139.49	29.91	29.49	.293

Average within group regression correlation = -.9452

Total estimates, ignoring groups regression correlation = -.9455

Mean square for each variable

Source	D.F	Slope	Constant
Total	11	4.078	46.599
Between	5	7.428	94.460
Within	6	1.285	6.715
F. ratio		5.777	14.067
Std. deviation		1.133	2.591
General Mean		29.196	140.505

Analysis of within group variance of Y

Source of variation	D.F	Sums of squares	Mean Square	F. ratio
Due to average regression	1	6.893	6.893	41.999
Deviations from average regression	5	.820	.164	

Analysis of variance of Y after fitting regression on X

Source of variation	D.F	Mean square	F. ratio
Total	10	.475	
Between Groups	5	.786	4.791
Within groups	5	.164	

Original mean sq. = 1.285 Percent reduction = 87.22

APPENDIX TABLE 5.8

Analyses of variance and covariance of the slopes and constants from the linear equations relating log live weight to log tan age (weeks/2), for Massey Monozygotic Twins; Twins compared for 1968

	Y = slope		X = constant	
Twin pairs	X mean	Y mean	Adj.Y mean	Std.error adj.Y
1	153.33	23.00	24.73	.773
2	149.49	26.65	27.56	.601
3	133.30	29.90	27.38	.984
4	153.72	25.66	27.47	.794
5	158.87	25.32	28.22	1.095
6	154.50	23.97	25.94	.836
7	142.81	29.83	29.32	.544
8	121.49	33.50	28.48	1.747
9	146.28	27.80	28.03	.524
10	138.09	29.21	27.70	.719
11	145.05	26.68	26.66	.518

Average within group regression correlation = -.6893

Total estimates, ignoring groups regression correlation = -.9131

Mean square for each variable

Source	D.F	Slope	Constant
Total	21	9.291	118.708
Between	10	18.486	238.453
Within	11	.931	9.850
F. ratio		19.843	24.208
Std. deviation		.965	3.138
General Mean		27.415	145.180

Analysis of within group variance of Y

Source of variation	D.F	Sums of squares	Mean Square	F. ratio
Due to average regression	1	4.869	4.869	9.052
Deviations from average regression	10	5.378	.537	

Analysis of variance of Y after fitting regression on X

Source of variation	D.F	Mean square	F. ratio
Total	20	1.620	
Between Groups	10	2.703	5.027
Within groups	10	.537	

Original mean sq. = 0.931 Percent reduction = 42.27

APPENDIX TABLE 5.9

Analyses of variance and covariance of the slopes and constants from the linear equations relating live weight to age for Massey Monozygotic Twins; Twins compared for 1966.

Y = slope X = constant

Twin pairs	X mean	Y mean	Adj.Y mean	Std.error Adj.Y
1	51.33	6.89	6.61	.341
2	72.90	6.44	6.72	.341
3	67.08	6.77	6.90	.236
4	63.04	7.00	7.02	.200
5	51.43	6.08	5.81	.339
6	73.11	6.32	6.60	.345
7	55.85	6.80	6.64	.255

Average within group regression correlation = -.3768

Total estimates, ignoring groups regression correlation = -.1641

Mean square for each variable

Source	D.F	Slope	Constant
Total	13	.148	91.146
Between	6	.228	177.470
Within	7	.079	17.155
F. ratio		2.878	10.345
Std. deviation		.281	4.141
General Mean		6.619	62.111

Analysis of within group variance of Y

Source of variation	D.F	Sums of squares	Mean Square	F. ratio
Due to average regression	1	.078	.078	.992
Deviations from average regression	6	.476	.079	

Analysis of variance of Y after fitting regression on X

Source of variation	D.F	Mean square	F. ratio
Total	12	.156	
Between Groups	6	.232	2.932
Within groups	6	.079	

Original mean sq. = .079 Percent reduction = -.097

APPENDIX TABLE 5.10

Slopes, constants, and correlation coefficients for the linear regressions of log live weight on log tan age (weeks/2) for Massey Monozygotic Twin Jersey heifers.

I.D. TWINS JERSEYS

Twin No.	Slope	Constant 1964	Correlation Coefficient
11	23.49	162.41	.9747
12	23.49	162.09	.9681
37	28.06	150.28	.9773
38	27.97	148.52	.9809
39	27.72	144.69	.9700
40	25.16	151.66	.9718
41	31.57	145.45	.9924
42	31.56	144.87	.9949
45	36.40	131.02	.9950
46	36.09	133.46	.9932
49	29.86	142.48	.9893
50	29.63	140.37	.9892
57	28.50	143.31	.9787
58	30.51	135.73	.9767
77	26.24	159.34	.9788
78	26.92	156.98	.9772
79	29.30	148.22	.9909
80	30.62	141.67	.9892
89	27.19	151.27	.9786
90	27.19	150.19	.9734
91	24.57	157.00	.9626
92	28.52	147.82	.9959
97	25.58	150.96	.9853
98	24.84	153.70	.9815
99	24.37	160.88	.9671
100	25.72	157.94	.9737
		1965	
17	24.35	161.86	.9703
18	24.76	159.13	.9721
61	32.24	142.87	.9873
62	32.37	143.72	.9893
63	27.47	156.41	.9859
64	28.21	155.23	.9866
3	26.25	153.48	.9544
4	28.40	150.17	.9606
7	33.11	134.48	.9919
8	33.66	129.24	.9960
9	31.68	132.90	.9909
10	31.76	131.68	.9922
13	31.41	139.96	.9946
14	29.27	143.39	.9913
23	29.14	148.26	.9967
24	28.45	149.39	.9951
29	34.09	132.17	.9939
30	33.68	132.32	.9917
41	29.75	139.89	.9915
42	30.45	139.38	.9947
43	30.65	137.18	.9949
44	29.18	144.74	.9891
47	27.59	152.90	.9923
48	27.58	152.42	.9917
49	29.48	147.16	.9967
50	31.38	143.11	.9935

Twin No.	Slope	Constant 1966	Correlation Coefficient
9	30.14	143.45	.9941
10	29.50	144.66	.9934
27	24.80	161.47	.9874
28	26.30	158.17	.9837
33	24.27	162.52	.9858
34	25.13	162.61	.9897
39	28.82	149.26	.9836
40	29.30	149.23	.9857
79	33.09	129.66	.9921
80	31.02	136.10	.9836
89	25.41	159.60	.9860
90	25.79	158.32	.9870
93	33.82	130.10	.9911
94	35.06	129.20	.9821
		1967	
49	28.98	139.05	.9880
50	27.37	142.35	.9858
65	31.95	130.09	.9900
66	31.38	131.64	.9865
67	31.52	135.89	.9924
68	30.33	136.24	.9863
71	27.01	147.28	.9799
72	28.25	143.70	.9782
73	25.56	153.81	.9744
74	28.13	146.96	.9834
91	29.05	140.85	.9847
92	30.76	138.14	.9847
		1968	
27	23.78	152.45	.9835
28	22.22	154.21	.9817
45	25.08	153.80	.9658
46	28.21	145.19	.9823
47	29.62	138.13	.9930
48	30.18	128.46	.9950
61	25.68	152.72	.9811
62	25.64	154.73	.9877
63	25.10	158.66	.9702
64	25.53	159.09	.9732
95	24.32	155.08	.9669
96	23.62	153.92	.9657
97	29.98	140.54	.9963
98	29.72	145.07	.9966
7	33.41	122.38	.9936
8	33.60	120.59	.9931
9	27.91	145.77	.9871
10	27.68	146.79	.9891
15	30.52	136.30	.9932
16	27.89	139.88	.9882
49	26.90	144.23	.9893
50	26.47	145.87	.9896

APPENDIX TABLE 5.11

Slopes constants and correlations coefficients, for the linear regressions of log live weight on log tan age (week/2), for Non-Jersey heifers and for the linear regressions of live weight on age for heifers born in 1966: Massey Monozygotic Twins.

NON JERSEYS

Twin No.	Slope	Constant	Correlation coefficient
31	27.72	165.08	.9822
32	27.70	161.18	.9827
55	25.80	158.01	.9806
56	25.82	156.97	.9788
7	32.41	137.67	.9854
8	33.07	138.73	.9874
1	30.67	144.53	.9940
2	32.00	140.00	.9953
3	27.39	154.57	.9863
4	26.12	165.51	.9849
11	25.09	154.73	.9861
12	26.04	153.34	.9874
5	40.35	106.31	.9955
6	33.60	133.97	.9939
17	27.35	154.59	.9757
18	27.34	153.07	.9629
19	25.86	154.34	.9724
20	24.98	154.48	.9663
75	28.18	141.86	.9856
76	28.18	141.67	.9837
83	28.17	152.97	.9897
84	27.78	158.62	.9917
85	27.59	156.41	.9850
86	28.78	152.23	.9804
9	32.10	152.48	.9939
10	33.56	145.15	.9896
13	35.12	120.77	.9939
14	33.11	131.46	.9806
29	27.49	155.69	.9845
30	28.37	151.41	.9805

ARITH WT vs ARITH AGE

1966

9	6.97	51.29	.9884
10	6.80	51.37	.9893
27	6.10	76.78	.9720
28	6.79	69.03	.9803
33	6.53	65.82	.9889
34	7.02	68.34	.9907
39	6.91	61.50	.9758
40	7.09	64.59	.9749
79	6.32	46.14	.9823
80	5.84	56.72	.9777
89	6.29	73.92	.9831
90	6.34	72.30	.9822
93	6.62	52.33	.9848
94	6.98	59.38	.9684

APPENDIX TABLES 7.1a & 7.1b

LOG LIVE WEIGHT (Y) linearly regressed on LOG CONEPTION AGE (X)

(data of Acheson *et al.* (1959) from 16 -38 days)

	7.1a FEMALES			7.1b MALES		
Slope ± SEM	= 2.986	±	.081	= 3.154	±	.053
Constant ± SEM	= -324.67	±	13.61	-346.13	±	8.88
R^2	= 98.47			= 99.41		
RMS RESIDUAL	= 2.367			= 1.543		

Birth age	Obs	Exp.	Residual	Obs	Exp.	Residual
16	147.71	143.64	4.06	151.85	148.41	3.43
17	150.51	147.10	3.41	153.14	152.06	1.07
18	151.85	150.47	1.38	156.82	155.62	1.19
19	154.40	153.75	.65	159.10	159.09	.01
20	155.63	156.95	-1.32	162.32	162.47	-.15
21	154.40	160.08	-5.67	161.27	165.77	-4.49
22	157.97	163.13	-5.15	166.27	168.99	-2.72
23	163.34	166.11	-2.76	170.75	172.14	-1.38
24	167.20	169.02	-1.81	174.03	175.22	-1.18
25	171.60	171.88	-.27	178.53	178.23	.29
26	174.03	174.66	-.63	180.61	181.18	-.56
27	177.81	177.39	.41	184.50	184.06	.44
28	181.95	180.07	1.88	187.50	186.88	.61
29	184.50	182.69	1.81	190.30	189.65	.65
30	186.92	185.26	1.66	193.95	192.36	1.58
31	189.76	187.78	1.98	195.42	195.02	.39
32	190.84	190.25	.59	198.22	197.63	.59
33	192.94	192.67	.26	199.99	200.19	-.19
34	194.93	195.05	-.11	202.53	202.70	-.17
35	197.77	197.39	.38	205.30	205.17	.13
36	199.99	199.68	.31	207.91	207.60	.31
37	201.70	201.94	-.23	210.03	209.98	.05
38	203.34	204.16	-.81	212.38	212.32	.06

Durbin-Watson = .3716

$P < .01$

Durbin-Watson = .7591

$P < .01$

APPENDIX TABLES 7.2a & 7.2b

LOG TOTAL LENGTH (Y) linearly regressed on LOG CONCEPTION AGE (X)
(data of Acheson *et al.* (1959) from 16 - 38 days)

	7.2a FEMALES	7.2b MALES
Slope ± SEM	= 1.010 ± .011	= 1.082 ± .011
Constant ± SEM	= -37.49 ± 1.92	= -47.70± 1.87
R^2 %	= 99.73	= 99.78
RMS RESIDUAL	= 0.3341	= 0.3259

Birth age	Obs	Exp	Residual	Obs	Exp	Residual
16	120.95	120.96	-.01	122.27	121.96	.30
17	122.53	122.13	.39	123.80	123.21	.58
18	123.55	123.27	.27	124.79	124.43	.35
19	124.55	124.38	.16	125.52	125.62	-.09
20	125.76	125.46	.29	126.71	126.78	-.07
21	126.95	126.52	.42	127.87	127.91	-.04
22	127.41	127.55	-.14	128.33	129.02	-.69
23	127.87	128.56	-.69	129.88	130.10	-.22
24	129.22	129.55	-.32	131.17	131.16	.01
25	130.31	130.51	-.19	132.22	132.19	.02
26	130.96	131.46	-.49	132.63	133.20	-.57
27	131.80	132.38	-.57	134.04	134.19	-.14
28	133.24	133.28	-.04	134.63	135.16	-.52
29	134.43	134.17	.26	135.98	136.11	-.12
30	135.02	135.04	-.02	136.92	137.04	-.12
31	135.60	135.89	-.29	138.02	137.95	.06
32	136.92	136.73	.18	139.09	138.84	.24
33	137.47	137.55	-.07	140.14	139.72	.41
34	138.38	138.35	.02	140.99	140.59	.40
35	139.09	139.14	-.05	141.49	141.43	.06
36	139.96	139.92	.04	142.16	142.26	-.10
37	141.16	140.68	.47	143.13	143.08	.05
38	141.83	141.43	.39	144.09	143.88	.20

Durbin-Watson = .9197

P < .01

Durbin-Watson = .9746

P < .01

APPENDIX TABLES 7.3a & 7.3b

LOG TAIL LENGTH (Y) linearly regressed on LOG CONCEPTION AGE (X)
(data of Acheson *et al.*(1959) from 16 - 38 days)

	7.3a FEMALES	7.3b MALES
Slope ± SEM	= 1.250 ± .027	= 1.174 ± .020
Constant ± SEM	= -106.71 ± 4.54	= -93.83 ± 3.42
R^2 %	= 99.05	= 99.37
RMS RESIDUAL	= 0.7892	= 0.5941

Birth age	Obs	Exp	Residual	Obs	Exp	Residual
16	87.50	89.34	-1.83	88.64	90.33	-1.68
17	89.76	90.79	-1.03	90.84	91.69	- .84
18	91.90	92.20	- .29	92.94	93.01	- .07
19	92.94	93.57	- .63	94.93	94.30	.63
20	95.42	94.91	.50	96.37	95.56	.81
21	96.84	96.22	.62	97.77	96.79	.97
22	97.77	97.50	.26	98.22	97.99	.23
23	99.56	98.75	.81	99.56	99.16	.39
24	100.86	99.97	.88	100.86	100.31	.54
25	102.11	101.16	.95	101.70	101.43	.26
26	102.93	102.33	.60	102.53	102.53	-.00
27	103.74	103.47	.26	103.74	103.60	.13
28	105.69	104.59	1.09	104.54	104.64	-.12
29	106.44	105.69	.75	106.06	105.68	.38
30	107.18	106.76	.41	106.44	106.69	-.25
31	108.27	107.82	.45	107.55	107.68	-.13
32	108.63	108.85	-.22	108.63	108.65	-.02
33	108.69	109.87	-.18	108.99	109.61	-.62
34	110.38	110.86	-.48	110.03	110.54	-.51
35	111.05	111.84	-.78	111.05	111.46	-.40
36	112.05	112.80	-.75	112.71	112.37	.34
37	113.03	113.75	-.71	113.03	113.25	-.22
38	113.98	114.67	-.69	114.30	114.12	.17

Durbin-Watson = .3710

$P < .01$

Durbin-Watson = .6474

$P < .01$

APPENDIX TABLES 7.4 & 7.4b

LOG BODY LENGTH (Y) linearly regressed on LOG CONCEPTION AGE (X)

(data of Acheson *et al.* (1959) from 16 - 38 days)

	7.4a FEMALES	7.4b MALES
Slope ± SEM	= 0.768 ± .0419	= 0.9952 ± .0351
Constant ± SEM	= -28.28 ± 7.02	= -62.76 ± 5.88
R^2 %	= 94.14	= 97.46
RMS RESIDUAL	= 1.2216	= 1.0228

Birth age	Obs.	Exp.	Residual	Obs.	Exp.	Residual
16	93.95	92.29	1.65	95.42	93.30	2.12
17	94.93	93.18	1.75	96.37	94.45	1.92
18	94.93	94.05	.88	96.37	95.57	.79
19	95.90	94.89	1.00	95.90	96.67	-.76
20	95.90	95.72	.18	96.84	97.74	-.89
21	96.84	96.52	.31	97.77	98.78	-1.00
22	96.84	97.31	-.46	98.22	99.79	-1.57
23	95.90	98.08	-2.17	99.99	100.79	-.79
24	97.31	98.83	-1.51	101.28	101.76	-.48
25	98.22	99.56	-1.33	102.53	102.71	-.18
26	98.67	100.28	-1.60	102.53	103.64	-1.11
27	99.56	100.98	-1.42	104.13	104.55	-.41
28	100.43	101.67	-1.24	104.53	105.44	-.91
29	102.11	102.35	- .23	105.69	106.31	-.62
30	102.53	103.01	- .48	107.18	107.17	.01
31	102.53	103.66	-1.12	108.27	108.01	.26
32	104.92	104.29	.62	109.34	108.83	.50
33	104.92	104.92	.00	111.05	109.64	1.41
34	106.06	105.53	.53	111.72	110.43	1.29
35	106.81	106.13	.68	111.72	111.21	.51
36	107.55	106.72	.82	111.39	111.98	-.58
37	108.99	107.30	1.68	113.03	112.73	.30
38	109.34	107.87	1.46	113.67	113.47	.20

Durbin-Watson = .3737	Durbin-Watson = .4809
P <.01	P <.01

APPENDIX TABLES 7.5a & 7.5b

ALLOMETRY

LOG LIVE WEIGHT (Y) linearly regressed on LOG TOTAL LENGTH (X)

(data of Acheson *et al.* (1959) from 16 -38 days)

	7.5a FEMALES	7.5b MALES
Slope ± SEM	= 2.9497 ± .0835	= 2.9125 ± .0461
Constant ± SEM	= 213.10 ± 11.02	= -206.78 ± 6.18
R^2 %	= 98.35	= 99.48
RMS RESIDUAL	= 2.4646	= 1.4575

Birth age	Obs	Exp.	Residual	Obs	Exp.	Residual
16	147.71	143.67	4.04	151.85	149.33	2.51
17	150.51	148.33	2.18	153.14	153.80	-.65
18	151.85	151.34	.50	156.82	156.69	.12
19	154.40	154.28	.11	159.10	158.82	.28
20	155.63	157.87	-2.24	162.32	162.28	.03
21	154.40	161.36	-6.96	161.27	165.65	-4.38
22	157.97	162.73	-4.76	166.27	166.98	-.70
23	163.34	164.09	-.74	170.75	171.51	-.75
24	167.20	168.07	-.86	174.03	175.27	-1.23
25	171.60	171.30	.29	178.53	178.31	.21
26	174.03	173.20	.83	180.61	179.51	1.09
27	177.81	175.68	2.12	184.50	183.62	.88
28	181.95	179.92	2.02	187.50	185.34	2.15
29	184.50	183.45	1.05	190.30	189.27	1.03
30	186.92	185.18	1.74	193.95	192.00	1.94
31	189.76	186.88	2.87	195.42	195.20	.21
32	190.84	190.77	.07	198.22	198.33	-.10
33	192.94	192.40	.53	199.99	201.38	-1.38
34	194.93	195.08	-.14	202.53	202.86	-1.33
35	197.77	197.18	.58	205.30	205.33	-.02
36	199.99	199.76	.23	207.91	207.26	.65
37	201.70	203.28	-1.58	210.03	210.10	-.07
38	203.34	205.25	-1.91	212.38	212.88	-.50

Durbin-Watson	= .5788	= 1.3056
	$P < .01$	NS

APPENDIX TABLES 7.6a & 7.6b
ALLOMETRY

LOG LIVE WEIGHT (Y) linearly regressed on LOG TAIL LENGTH (X)

(data of Acheson *et al.* (1959) from 16 -38 days)

	7.6a. FEMALES			7.6b. MALES		
Slope ± SEM	= 2.3530	±	.0979	= 2.6575	±	.0834
Constant ± SEM	= -66.10	±	10.10	=-91.2870	±	8.62
R^2 %	= 96.49			= 97.98		
RMS RESIDUAL	= 3.5878			= 2.8650		

Birth age	Obs	Exp.	Residual	Obs	Exp.	Residual
16	147.71	139.80	7.91	151.85	144.30	7.54
17	150.51	145.11	5.40	153.14	150.14	3.00
18	151.85	150.15	1.69	156.82	155.71	1.10
19	154.40	152.59	1.81	159.10	161.01	-1.91
20	155.63	158.43	-2.80	162.32	164.84	-2.51
21	154.40	161.78	-7.37	161.27	168.54	-7.26
22	157.97	163.95	-5.97	166.27	169.75	-3.48
23	163.34	168.17	-4.82	170.75	173.30	-2.55
24	167.20	171.22	-4.01	174.03	176.75	-2.71
25	171.60	174.18	-2.58	178.53	178.99	-.46
26	174.03	176.11	-2.07	180.61	181.19	-.57
27	177.81	178.00	-.19	184.50	184.41	.09
28	181.95	182.58	-.63	187.50	186.51	.99
29	184.50	184.36	.14	190.30	190.59	-.29
30	186.92	186.11	.80	193.95	191.59	2.35
31	189.76	188.67	1.08	195.42	194.54	.87
32	190.84	189.52	1.32	198.22	197.41	.80
33	192.94	192.00	.93	199.99	198.36	1.63
34	194.93	193.62	1.31	202.53	201.14	1.38
35	197.77	195.22	2.55	205.30	203.85	1.44
36	199.99	197.57	2.42	207.91	208.24	-.32
37	201.70	199.86	1.83	210.03	209.10	.93
38	203.34	202.11	1.22	212.38	212.47	-.09

Durbin-Watson = .2813

$P < .01$

Durbin-Watson = .5528

$P < .01$

APPENDIX TABLES 7.7a & 7.7b

ALLOMETRY

LOG LIVE WEIGHT (Y) linearly regressed on LOG BODY LENGTH (X)

(data of Acheson *et al.* (1959) from 16 -38 days)

	7.7a FEMALES		7.7b MALES	
Slope ± SEM	= 3.6898	± .1958	= 3.1118 ±	.0878
Constant ± SEM	= -195.29	± 19.74	= -141.31±	9.16
R^2 %	= 94.41		= 98.36	
RMS RESIDUAL	= 4.5282		= 2.5813	

Birth age	Obs	Exp.	Residual	Obs	Exp.	Residual
16	147.71	151.37	-3.66	155.63	151.85	-3.78
17	150.51	155.01	-4.50	158.60	153.14	-5.45
18	151.85	155.01	-3.16	158.60	156.82	-1.78
19	154.40	158.57	-4.17	157.12	159.10	1.98
20	155.63	158.57	-2.94	160.06	162.32	2.26
21	154.40	162.06	-7.65	162.93	161.27	-1.66
22	157.97	162.06	-4.08	164.35	166.27	1.92
23	163.34	158.57	4.76	169.87	170.75	.88
24	167.20	163.77	3.43	173.86	174.03	.17
25	171.60	167.15	4.44	177.74	178.53	.78
26	174.03	168.81	5.22	177.74	180.61	2.87
27	177.81	172.08	5.73	182.75	184.50	1.75
28	181.95	175.28	6.66	183.97	187.50	3.53
29	184.50	181.51	2.99	187.57	190.30	2.72
30	186.92	183.03	3.89	192.23	193.95	1.71
31	189.76	183.03	6.73	195.63	195.42	-.20
32	190.84	181.85	-1.00	198.94	198.22	-.71
33	192.94	191.85	1.08	204.28	199.99	-4.28
34	194.93	196.08	-1.15	206.36	202.53	-3.83
35	197.77	198.85	-1.07	206.36	205.30	-1.05
36	199.99	201.56	-1.56	205.32	207.91	2.58
37	201.70	206.86	-5.16	210.42	210.03	-.39
38	203.34	208.16	-4.82	212.41	212.38	-.03

Durbin-Watson = .5287 — Durbin-Watson = .8435

$P < .01$ — $P < .01$

LIST OF APPENDIX TABLES 8.1 to 8.36

SUB CLASS MEANS AND MAIN CLASS MEANS - TABLES 8.X (A)

and MEAN SQUARES AND "F" VALUES - TABLES 8.X (B)

VARIABLE	APPENDIX TABLE NUMBERS ANALYSIS A	ANALYSIS B	ANALYSIS C
a) Constituent Weights			
Skin	8.1	8.4	8.7
Carcass	8.2	8.5	8.8
Whole body	8.3	8.6	8.9
b) % Wet and % Dry Weights			
Skin	8.10	8.13	8.16
Carcass	8.11	8.14	8.17
Whole body	8.12	8.15	8.18
c) % Fat-Free Weights			
Skin	8.19	8.22	8.25
Carcass	8.20	8.23	8.26
Whole body	8.21	8.24	8.27
d) Nose-Anal Length	8.28	8.29	8.30
e) Allometry	8.31	8.32	8.33
f) % Skin/Whole Body	8.34	8.35	8.36

APPENDIX TABLE 8.1A

WEIGHTS OF SKIN CONSTITUENTS

(log_{10} gms)

AGE (Weeks)	TREATMENT	NO	TOTAL	WATER	DM	FAT	ASH*	PROTEIN
				Subclass means				
12	OvX D2	8	1.763	1.548	1.355	0.967	0.2685	1.101
12	OvX W4	8	1.777	1.559	1.373	0.960	0.2806	1.134
12	OvX W7	7	1.764	1.549	1.355	0.931	0.2792	1.121
12	Control	8	1.663	1.446	1.257	0.839	0.2514	1.019
12	Oestrogen	7	1.680	1.467	1.267	0.804	0.2373	1.061
15	OvX D2	8	1.802	1.562	1.430	1.064	0.2938	1.158
15	OvX W4	9	1.860	1.617	1.491	1.136	0.2979	1.214
15	OvX W7	6	1.830	1.594	1.451	1.071	0.2914	1.187
15	Control	12	1.706	1.477	1.319	0.924	0.2595	1.067
15	Oestrogen	7	1.692	1.437	1.340	0.979	0.2699	1.060
				Main class means				
-	OvX D2	16	1.783	1.555	1.393	1.016	0.2812	1.129
-	OvX W4	17	1.821	1.589	1.435	1.053	0.2898	1.176
-	OvX W7	13	1.794	1.569	1.399	0.996	0.2848	1.152
-	Control	20	1.689	1.464	1.294	0.890	0.2562	1.048
-	Oestrogen	14	1.686	1.452	1.304	0.891	0.2536	1.061
12		38	1.730	1.514	1.322	0.902	0.2637	1.087
15		42	1.773	1.533	1.399	1.026	0.2806	1.132

* ASH $\log_{10}$ (X + 1.1)

APPENDIX TABLE 8.1B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	DM	FAT	ASH	PROTEIN
		MEAN SQUARES / F VALUES					
Treatment	4	.06590 27.513	.06532 27.858	.06798 22.404	.09608 14.102	.04665 5.609	.05522 22.445
1) All OvX vs Control + oestrogen	1	.24523 102.374	.24845 105.963	.24227 79.849	.34471 50.592	.17807 21.410	.1912 77.723
2) Control vs Oestrogen	1	.00002 0.008	.00069 0.292	.001945 0.641	.00083 0.121	.00026 0.031	.002598 1.056
3) OvX D2 vs OvX W7	1	.00135 0.565	.00185 0.788	.000696 0.229	.00147 0.215	.00121 0.145	.004495 1.827
4) OvX W4 vs OvX D2 + OvX W7	1	.00855 3.571	.00645 2.751	.01235 4.069	.01670 2.450	.00388 0.466	.01099 4.466
AGE	1	.04609 19.241	.01086 4.632	.1387 45.726	.3513 51.560	.07091 8.525	.04806 19.537
Tr. x Age (Interaction)	4	.002715 1.133	.004292 1.831	.002135 0.704	.007774 1.141	.00386 0.464	.003450 1.402
Comparison 1) W12 vs W15	1	.005543 2.314	.006924 2.952	.004034 1.329	.00027 0.040		.00907 3.686
Comparison 2) W12 vs W15	1	.001936 0.808	.007639 3.257	.00021 0.068	.01656 2.431		.00485 1.973
Comparison 3) W12 vs W15	1	.001273 0.531	.001663 0.709	.00080 0.263	.003394 0.498		.00014 0.059
Comparison 4) W12 vs W15	1	.002451 1.023	.002192 0.934	.00283 0.931	.009220 1.353		.00090 0.367
Residual	70	.002395	.002345	.003034	.006814	.008317	.002460
Total	79						

APPENDIX TABLE 8.2A

WEIGHTS OF CARCASS CONSTITUENTS

AGE (Weeks)	TREATMENT	NO.	TOTAL	WATER	DM	FAT	ASH	PROTEIN
				SUBCLASS MEANS (log_{10} gms)				
12	OvX D2	8	2.246	2.100	1.700	0.895	0.920	1.530
12	OvX W4	8	2.271	2.120	1.740	0.990	0.955	1.557
12	OvX W7	7	2.281	2.127	1.754	1.012	0.957	1.572
12	Control	8	2.207	2.048	1.695	0.985	0.917	1.499
12	Oestrogen	7	2.221	2.060	1.709	1.013	0.936	1.509
15	OvX D2	8	2.314	2.158	1.794	1.051	1.008	1.610
15	OvX W4	9	2.372	2.216	1.851	1.134	1.046	1.664
15	OvX W7	6	2.346	2.185	1.837	1.125	1.043	1.643
15	Control	12	2.257	2.091	1.758	1.051	0.987	1.558
15	Oestrogen	7	2.258	2.078	1.789	1.191	1.006	1.552
				MAIN CLASS MEANS				
	OvX D2	16	2.280	2.129	1.747	0.973	0.964	1.570
	OvX W4	17	2.325	2.171	1.799	1.066	1.003	1.614
	OvX W7	13	2.311	2.154	1.792	1.064	0.996	1.604
	Control	20	2.237	2.073	1.733	1.024	0.959	1.535
	Oestrogen	14	2.239	2.069	1.749	1.102	0.971	1.530
12		38	2.245	2.091	1.719	0.977	0.936	1.533
15		42	2.305	2.142	1.801	1.102	1.015	1.602

APPENDIX TABLE 8.2B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	DM	FAT	ASH	PROTEIN
		MEAN SQUARES / F VALUE					
TREATMENT (Tr.)	4	.02737 14.476	.03553 17.829	.01574 8.153	.03842 5.158	.007836 3.718	.02497 14.999
1) All OvX vs Control + Oestrogen	1	.09058 47.926	.12644 63.449	.03239 16.773	.01249 1.676	.01343 6.371	.08283 49.765
2) Control vs Oestrogen	1	.00044 0.213	.0000 0.000	.00401 2.076	.05712 7.668	.00285 1.354	.00002 0.011
3) OvX D2 vs OvX W7	1	.00806 4.263	.00532 2.669	.01680 8.702	.06523 8.757	.00919 4.358	.00994 5.970
4) OvX W4 vs OvX D2+OvX W7	1	.00663 3.510	.00695 3.488	.00611 3.166	.01812 2.432	.00379 1.797	.00523 3.143
AGE	1	.07998 42.298	.05765 28.930	.14316 74.138	.33514 44.991	.12733 60.420	.10076 60.537
Tr X Age Interaction	4	.002347 1.241	.003282 1.647	.001397 0.724	.008188 1.099	.000391 0.185	.002235 1.343
Comparison 1) W12 vs W15	1	.00580 3.067	.00786 3.943	.00293 1.515	.00115 0.154		.00568 3.411
Comparison 2) W12 vs W15	1	.00028 0.146	.00131 0.655	.00053 0.275	.02605 3.497		.00050 0.298
Comparison 3) W12 vs W15	1	.00002 0.008	.0000 0.000	.00023 0.120	.00325 0.435		.00013 0.077
Comparison 4) W12 vs W15	1	.00306 1.618	.00403 2.022	.00129 0.668	.00023 0.031		.00255 1.530
RESIDUAL (ERROR)	70	.001891	.001993	.001931	.007449	.002107	.001664
TOTAL	79						

APPENDIX TABLE 8.3A

WEIGHTS OF WHOLE BODY CONSTITUENTS

AGE (Weeks)	TREATMENT	NO.	TOTAL	WATER	DM	FAT	ASH	PROTEIN
				SUBCLASS MEANS (log_{10} gms)				
12	OvX D2	8	2.369	2.207	1.862	1.235	0.958	1.668
12	OvX W4	8	2.392	2.225	1.895	1.276	0.992	1.697
12	OvX W7	7	2.396	2.229	1.900	1.274	0.994	1.704
12	Control	8	2.316	2.145	1.830	1.219	0.951	1.624
12	Oestrogen	7	2.331	2.159	1.843	1.222	0.966	1.641
15	OvX D2	8	2.431	2.256	1.950	1.358	1.043	1.741
15	OvX W4	9	2.488	2.314	2.008	1.436	1.080	1.796
15	OvX W7	6	2.462	2.284	1.987	1.400	1.075	1.773
15	Control	12	2.364	2.185	1.893	1.294	1.018	1.680
15	Oestrogen	7	2.362	2.167	1.921	1.399	1.037	1.674
				MAIN CLASS MEANS				
	OvX D2	16	2.400	2.232	1.906	1.297	1.000	1.704
	OvX W4	17	2.443	2.272	1.955	1.361	1.039	1.749
	OvX W7	13	2.426	2.254	1.940	1.332	1.031	1.736
	Control	20	2.345	2.169	1.868	1.264	0.991	1.657
	Oestrogen	14	2.347	2.163	1.882	1.310	1.002	1.657
12		38	2.361	2.193	1.866	1.245	0.972	1.666
15		42	2.417	2.237	1.947	1.369	1.047	1.729

APPENDIX TABLE 8.3B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	DM	FAT	ASH	PROTEIN
		MEAN SQUARES / F VALUES					
TREATMENT (Tr.)	4	0.03432 17.790	0.04065 20.489	0.02472 11.773	0.0259 3.854	0.00846 4.117	0.0313 18.039
1) All OvX vs Control + Oestrogen	1	0.1194 61.876	0.1477 74.463	0.07227 34.417	0.04087 6.082	0.01742 8.481	0.1064 61.278
2) Control vs Oestrogen	1	0.0003 0.155	0.00002 0.011	0.00337 1.604	0.02358 3.508	0.00231 1.123	0.00027 0.153
3) OvX D2 vs OvX W7	1	0.0059 3.067	0.00439 2.214	0.00975 4.643	0.01178 1.752	0.00827 4.024	0.00829 4.774
4) OvX W4 vs OvX D2 + OvX W7	1	0.00706 3.660	0.00686 3.459	0.00765 3.642	0.01658 2.467	0.00369 1.796	0.00657 3.782
AGE	1	0.07123 36.923	0.0451 22.730	0.1418 67.538	0.3385 50.372	0.1193 58.093	0.0851 49.040
Treatment X age Interaction	4	0.00230 1.192	0.00326 1.644	0.00147 0.702	0.00668 0.994	0.00032 0.158	0.00236 1.358
Comparison 1) W12 vs W15	1	0.00561 2.911	0.0075 3.778				0.00632 3.640
Comparison 2) W12 vs W15	1	0.00052 0.270	0.0021 1.079				0.00120 0.691
Comparison 3) W12 vs W15	1	0.00003 0.016	0.00008 0.038				0.00003 0.015
Comparison 4) W12 vs W15	1	0.00294 1.522	0.00363 1.829				0.00204 1.172
RESIDUAL (ERROR)	70	0.00193	0.00198	0.00210	0.00672	0.00205	0.00174
TOTAL	79						

APPENDIX TABLE 8.4A

WEIGHTS OF SKIN CONSTITUENTS

AGE (Weeks)	TREATMENT	NO.	TOTAL	WATER	DM	FAT	ASH*	PROTEIN
				SUBCLASS MEANS (log_{10} gms)				
9	OvX D2	8	1.608	1.383	1.212	0.868	0.2140	0.922
9	OvX W4	7	1.651	1.444	1.228	0.828	0.2283	0.976
9	OvX W7	7	1.685	1.485	1.253	0.855	0.2449	1.002
9	CONTROL	8	1.555	1.338	1.149	0.747	0.2240	0.897
12	OvX D2	8	1.763	1.548	1.355	0.967	0.2685	1.101
12	OvX W4	8	1.777	1.559	1.373	0.960	0.2806	1.134
12	OvX W7	7	1.764	1.549	1.355	0.931	0.2792	1.121
12	Control	8	1.663	1.446	1.257	0.839	0.2514	1.019
15	OvX D2	8	1.802	1.562	1.430	1.064	0.2938	1.158
15	OvX W4	9	1.860	1.617	1.491	1.136	0.2980	1.214
15	OvX W7	6	1.830	1.594	1.451	1.071	0.2914	1.187
15	Control	12	1.706	1.477	1.319	0.924	0.2595	1.067
				MAIN CLASS MEANS				
	OvX D2	24	1.724	1.498	1.333	0.966	0.2588	1.060
	OvX W4	24	1.771	1.547	1.375	0.988	0.2718	1.118
	OvX W7	20	1.756	1.540	1.348	0.947	0.2708	1.099
	Control	28	1.651	1.428	1.253	0.849	0.2470	1.005
9		30	1.622	1.409	1.209	0.823	0.2272	0.946
12		31	1.741	1.524	1.334	0.924	0.2696	1.093
15		35	1.789	1.552	1.411	1.036	0.2827	1.146

* $\log_{10}(X + 1.1)$

APPENDIX TABLE 8.4B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	DM	FAT	ASH	PROTEIN
		MEAN SQUARES / F VALUE					
TREATMENT (Tr.)	3	.08020 33.326	.08115 34.323	.07889 24.467	.1083 13.408	.03607 4.972	.06928 29.738
1) All OvX vs Control	1	.2220 92.267	.2182 92.280	.2254 69.893	.3157 39.081	.08956 12.344	.1781 76.430
2) OvX D2 vs OvX W7	1	.01343 5.579	.02146 9.070	.00441 1.367	.00202 0.250	.01848 2.547	.02042 8.765
3) OvX W4 vs OvX D2 + OvX W7	1	.00643 2.672	.00602 2.540	.00681 2.112	.00364 0.451	.00203 0.280	.01045 4.485
AGE	2	.2466 102.491	.1891 79.997	.3555 110.253	.3992 49.409	.2771 38.239	.3513 150.786
Linear (W9 vs W15)	1	.4771 198.233	.3511 148.490	.7046 218.520	.7877 97.497	.5242 72.250	.6731 288.895
Quadratic (W12 vs W9 + W15)	1	.01802 7.487	.02949 12.47	.00697 2.160	.00314 0.388	.03599 4.960	.03508 15.055
Tr. X Age Interaction	6	.003780 1.571	.004423 1.871	.004232 1.312	.008729 1.080	.00933 1.286	.003487 1.496
Comparison 1) W9 vs W15	1	.00334 1.386	.00074 0.310	.01040 3.226	.01323 1.637	.02984 4.112	.00837 3.592
Comparison 1) W12 vs W9 + W15	1	.0004 0.015	.00000 0.000	.00017 0.052	.00189 0.233	.00090 .124	.00013 0.056
Comparison 2) W9 vs W15	1	.00461 1.915	.00870 3.670	.00073 0.226	.00069 0.085	.01984 2.734	.00451 1.936
Comparison 2) W12 vs W9+W15	1	.00665 2.763	.01048 4.430	.0024 0.743	.00279 0.345	.000316 0.043	.00286 1.227
Comparison 3) W9 vs W15	1	.00384 1.593	.00211 0.890	.00778 2.412	.02646 3.274	.00107 0.147	.00190 0.817
Comparison 3) W12 vs W9+W15	1	.00041 0.170	.00070 0.290	.00008 0.025	.00021 0.026	.000747 0.103	.00008 0.033
RESIDUAL	84	.002407	.002364	.003224	.008079	.007255	.002330
TOTAL	95						

APPENDIX TABLE 8.5A

WEIGHT OF CARCASS CONSTITUENTS

AGE (Weeks)	TREATMENT	NO.	TOTAL	WATER	DM	FAT	ASH	PROTEIN
				SUBCLASS MEANS (log_{10} gms)				
9	OvX D2	8	2.082	1.933	1.544	0.791	0.746	1.365
9	OvX W4	7	2.142	1.994	1.604	0.856	0.797	1.425
9	OvX W7	7	2.135	1.988	1.591	0.867	0.800	1.402
9	Control	8	2.078	1.925	1.552	0.839	0.756	1.360
12	OvX D2	8	2.246	2.100	1.700	0.895	0.920	1.530
12	OvX W4	8	2.271	2.120	1.740	0.990	0.955	1.557
12	OvX W7	7	2.281	2.127	1.754	1.012	0.957	1.572
12	Control	8	2.207	2.048	1.695	0.985	0.917	1.499
15	OvX D2	8	2.314	2.158	1.794	1.051	1.008	1.610
15	OvX W4	9	2.372	2.216	1.851	1.134	1.046	1.664
15	OvX W7	6	2.346	2.185	1.837	1.125	1.043	1.643
15	Control	12	2.257	2.091	1.758	1.051	0.987	1.558
				MAIN CLASS MEANS				
	OvX D2	24	2.214	2.064	1.679	0.912	0.891	1.502
	OvX W4	24	2.271	2.119	1.742	1.005	0.943	1.559
	OvX W7	20	2.249	2.096	1.722	0.995	0.928	1.534
	Control	28	2.192	2.031	1.681	0.972	0.901	1.485
9		30	2.107	1.958	1.571	0.837	0.773	1.386
12		31	2.250	2.098	1.721	0.969	0.937	1.539
15		35	2.315	2.154	1.804	1.085	1.017	1.612

APPENDIX TABLE 8.5B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	DM	FAT	ASH	PROTEIN
		MEAN SQUARES / F VALUES					
TREATMENT (Tr)	3	.0348 21.070	.04047 23.381	.02517 14.606	.03786 5.171	.01528 8.840	.03011 18.888
1) All OvX vs Control	1	.075012 45.415	.09490 54.834	.03780 21.938	.002108 0.287	.02002 11.587	.06254 39.239
2) OvX D2 vs OvX W7	1	.017345 10.501	.01447 8.357	.02511 14.569	.08637 11.797	.01916 11.086	.01505 9.442
3) OvX W4 vs OvX D2 + OvX W7	1	.011968 7.246	.01200 6.931	.01212 7.246	.02055 2.806	.00640 3.703	.01243 7.798
AGE	2	.3644 220.616	.3318 191.704	.4476 259.773	.4947 67.574	.4867 281.622	.4259 267.173
Linear (W9 vs W15)	1	.7097 429.691	.6415 370.653	.8827 512.243	.9900 135.217	.9489 549.070	.8336 522.992
Quadratic (W12 vs +W9 + W15)	1	.02606 15.779	.02889 16.691	.01997 11.587	.00076 0.104	.03214 18.598	.02698 16.924
Tr. X Age Interaction	6	.002047 1.239	.002543 1.469	.001382 0.802	.004213 0.575	.000683 0.395	.001593 1.000
Comparison 1) W9 vs W15	1	.007140 4.323	.008036 4.643				.006346 3.981
Comparison 1) W12 vs W9 + W15	1	.000153 0.092	.00005 0.031				.00011 0.066
Comparison 2) W9 vs W15	1	.000759 0.459	.00136 0.786				.00003 0.017
Comparison 2) W12 vs W9 + W15	1	.000129 0.078	.00046 0.267				.00010 .064
Comparison 3) W9 vs W15	1	.000168 0.101	.00038 0.222				.00003 .017
Comparison 3) W12 vs W9 + W15	1	.003066 1.856	.003697 2.136				.00366 2.297
RESIDUAL (Error)	84	.001652	.001731	.001723	.007321	.001728	.001594
TOTAL	95						

APPENDIX TABLE 8.6A

WEIGHTS OF WHOLE BODY CONSTITUENTS

AGE (Weeks)	TREATMENT	NO.	TOTAL	WATER	DM	FAT	ASH	PROTEIN
				SUBCLASS MEANS (log_{10} gms)				
9	OvX D2	8	2.208	2.041	1.710	1.132	0.786	1.499
9	OvX W4	7	2.264	2.102	1.757	1.144	0.836	1.557
9	OvX W7	7	2.267	2.107	1.755	1.163	0.843	1.548
9	Control	8	2.192	2.025	1.697	1.097	0.797	1.489
12	OvX D2	8	2.369	2.207	1.862	1.235	0.958	1.668
12	OvX W4	8	2.392	2.225	1.895	1.276	0.992	1.697
12	OvX W7	7	2.396	2.229	1.900	1.274	0.994	1.704
12	Control	8	2.316	2.145	1.830	1.219	0.951	1.624
15	OvX D2	8	2.431	2.256	1.950	1.358	1.043	1.741
15	OvX W4	9	2.488	2.314	2.008	1.436	1.080	1.796
15	OvX W7	6	2.462	2.284	1.987	1.400	1.075	1.773
15	Control	12	2.364	2.185	1.893	1.294	1.018	1.680
				MAIN CLASS MEANS				
	OvX D2	24	2.336	2.168	1.841	1.242	0.929	1.636
	OvX W4	24	2.391	2.222	1.897	1.298	0.979	1.693
	OvX W7	20	2.370	2.203	1.875	1.273	0.965	1.670
	Control	28	2.302	2.128	1.819	1.216	0.936	1.609
9		30	2.231	2.066	1.728	1.133	0.814	1.521
12		31	2.368	2.201	1.871	1.251	0.973	1.672
15		35	2.428	2.251	1.952	1.363	1.049	1.740

APPENDIX TABLE 8.6B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	DM	FAT	ASH	PROTEIN
		MEAN SQUARES / F VALUES					
TREATMENT (Tr.)	3	0.04303 24.900	0.04760 26.884	0.03435 17.615	0.03583 4.986	0.01569 9.143	0.03839 23.392
1) All OvX vs Control	1	0.1026 59.390	0.1168 65.937	0.07589 38.915	0.08281 11.524	0.02212 12.892	0.08685 52.917
2) OvX D2 vs OvX W7	1	0.01641 9.493	0.01598 9.024	0.01702 8.726	0.01497 2.082	0.01902 11.084	0.01651 10.059
3) OvX W4 vs OvX D2 + OvX W7	1	0.01038 6.005	0.01047 5.910	0.01025 5.255	0.00980 1.363	0.00581 3.387	0.01184 7.215
AGE	2	0.3336 193.052	0.2975 167.989	0.4187 214.708	0.4452 61.953	0.4575 266.675	0.4053 246.931
Linear (W9 vs W15)	1	0.6489 375.497	0.5718 322.900	0.8271 424.163	0.8873 123.484	0.8907 519.170	0.7895 480.993
Quadratic (W12 vs W9 + W15)	1	0.02390 13.827	0.02875 16.235	0.01543 7.913	0.00007 0.009	0.03157 18.401	0.02906 17.704
Treatment X Age Interaction	6	0.002145 1.241	0.00256 1.443	0.00170 0.873	0.00480 0.668	0.00084 0.490	0.00165 1.006
Comparison 1) W9 vs W15)	1	0.00588 3.403	0.00565 3.189				0.00665 4.049
Comparison 1) W12 vs W9 + W15	1	0.00013 0.072	0.00004 0.021				0.00002 0.011
Comparison 2) W9 vs W15	1	0.00141 0.814	0.00249 1.406				0.0005 0.301
Comparison 2) W12 vs W9 + W15	1	0.00082 0.474	0.00156 0.881				0.00005 0.030
Comparison 3) W9 vs W15	1	0.00064 0.373	0.00068 0.384				0.00006 0.039
Comparison 3) W12 vs W9 + W15	1	0.00220 1.271	0.00285 1.607				0.00217 1.324
RESIDUAL ERROR	84	0.001728	0.001771	0.001950	0.007186	0.001716	0.001641
TOTAL	95						

APPENDIX TABLE 8.7A

WEIGHTS OF SKIN CONSTITUTENTS

AGE (Weeks)	TREATMENT	NO.	TOTAL	WATER	DM	FAT	ASH*	PROTEIN
				SUBCLASS MEANS (log_{10} gms)				
7	OvX W4	8	1.486	1.294	1.039	0.609	0.1916	0.807
7	Control	4	1.465	1.247	1.051	0.688	0.1853	0.773
9	OvX W4	7	1.651	1.444	1.228	0.828	0.2283	0.976
9	Control	8	1.555	1.338	1.149	0.747	0.2240	0.897
12	OvX W4	8	1.777	1.559	1.373	0.960	0.2806	1.134
12	Control	8	1.663	1.446	1.257	0.839	0.2514	1.019
15	OvX W4	9	1.860	1.617	1.491	1.136	0.2980	1.214
15	Control	12	1.706	1.477	1.319	0.924	0.2595	1.067
				MAIN CLASS MEANS				
	OvX W4	32	1.700	1.484	1.291	0.893	0.2518	1.040
	Control	32	1.628	1.406	1.227	0.829	0.2393	0.976
7		12	1.479	1.278	1.043	0.635	0.1895	0.795
9		15	1.600	1.388	1.186	0.785	0.2260	0.934
12		16	1.720	1.502	1.315	0.899	0.2660	1.076
15		21	1.772	1.537	1.393	1.015	0.2760	1.130

* log_{10} (X + 1.1)

APPENDIX TABLE 8.7B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	DM	FAT	ASH	PROTEIN
		MEAN SQUARES / F VALUES					
TREATMENT (Tr)	1	0.1358 64.615	0.1518 57.018	0.1161 43.148	0.1037 16.063	0.05628 8.515	0.1287 53.519
AGE	3	0.2568 122.159	0.2106 79.109	0.3485 129.501	0.3885 60.195	0.2317 35.053	0.3389 140.915
Linear(W7+W9 vs W12+W15)	1	0.7697 366.138	0.6282 235.935	1.0448 388.178	1.1454 177.486	0.6877 104.036	1.0134 421.382
Quadratic (W7+W15 vs W9 + W12)	1	0.03782 17.990	0.04492 16.872	0.03298 12.253	0.00836 1.294	0.0507 7.663	0.05896 24.516
Cubic (W7+W12 vs W9+W15)	1	0.00064 0.306	0.000162 0.060	0.00231 0.859	0.00886 1.373	0.00024 0.036	0.00018 0.074
Tr. x Age Interaction	3	0.01042 4.957	0.005057 1.899	0.02065 7.671	0.0510 7.903	0.01144 1.731	0.00854 3.549
Tr. by Linear	1	0.02989 14.217	0.01394 5.236	0.06065 22.533	0.1461 22.638	0.02859 4.324	0.02553 10.614
Tr. by Quadratic	1	0.00177 0.842	0.00121 0.455	0.00220 0.818	0.0070 1.085	0.00016 0.023	0.00062 0.255
Tr. by Cubic	1	0.00213 1.012	0.00163 0.611	0.00232 0.861	0.01064 1.649	0.00291 0.439	0.00013 0.052
RESIDUAL (ERROR)	56	0.002102	0.002662	0.002691	0.006454	0.006610	0.002405
TOTAL	63						

APPENDIX TABLE 8.8.A

WEIGHTS OF CARCASS CONSTITUENTS

AGE (Weeks)	TREATMENT	NO.	TOTAL	WATER	DM	FAT	ASH	PROTEIN
				SUBCLASS MEANS (log_{10} gms)				
7	OvX W4	8	1.972	1.829	1.419	0.645	0.615	1.246
7	Control	4	1.969	1.823	1.422	0.669	0.614	1.244
9	OvX W4	7	2.142	1.994	1.604	0.856	0.797	1.425
9	Control	8	2.078	1.925	1.552	0.839	0.756	1.360
12	OvX W4	8	2.271	2.120	1.740	0.990	0.955	1.557
12	Control	8	2.207	2.048	1.695	0.985	0.917	1.499
15	OvX W4	9	2.372	2.216	1.851	1.134	1.046	1.664
15	Control	12	2.257	2.090	1.758	1.051	0.987	1.558
				MAIN CLASS MEANS				
	OvX W4	32	2.196	2.047	1.661	0.915	0.861	1.480
	Control	32	2.164	2.005	1.649	0.934	0.865	1.455
7		12	1.971	1.827	1.420	0.653	0.614	1.245
9		15	2.108	1.957	1.576	0.847	0.775	1.390
12		16	2.239	2.084	1.718	0.987	0.936	1.528
15		21	2.306	2.144	1.798	1.086	1.013	1.604

APPENDIX TABLE 8.8.B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	DM	FAT	ASH	PROTEIN
		MEAN SQUARES / F. VALUES					
TREATMENT (Tr.)	1	0.05558 36.386	0.06800 41.509	0.03174 20.365	0.00597 0.982	0.01756 9.218	0.04838 28.989
AGE	3	0.32201 210.809	0.2925 178.528	0.3983 255.550	0.4956 8.1469	0.4468 234.530	0.3642 218.220
Linear (W7+W9 vs W12+W15)	1	0.9655 632.085	0.8767 535.157	1.1947 766.518	1.4858 244.229	1.3379 702.242	1.0921 654.367
Quadratic (W7+W15 vs W9+W12)	1	0.04159 27.224	0.03902 23.819	0.04925 31.597	0.0646 10.618	0.06381 33.494	0.0431 25.816
Cubic W7+W12 vs W9 + W15	1	0.00089 0.581	0.00061 0.371	0.00186 1.190	0.00954 1.567	0.00022 0.116	0.00117 0.701
Tr. X Age Interaction	3	0.00766 5.017	0.00879 5.368	0.00556 3.567	0.00848 1.395	0.00199 1.042	0.00647 3.877
Tr. by Linear	1	0.02010 13.156	0.02349 14.338	0.01368 8.774	0.01755 2.884		0.01627 9.746
Tr. by Quadratic	1	0.00016 0.103	0.00019 0.113	0.00008 0.050	0.00120 0.197		0.00028 0.165
Tr. by Cubic	1	0.00319 2.086	0.00315 1.920	0.00323 2.075	0.00390 0.641		0.00367 2.197
RESIDUAL (ERROR)	56	0.001527	0.001638	0.001559	0.006083	0.001905	0.001669
TOTAL	63						

APPENDIX TABLE 8.9A.

WEIGHTS OF TOTAL BODY CONSTITUTENTS

AGE (Weeks)	TREATMENT	NO.	TOTAL	WATER	DM	FAT	ASH	PROTEIN
			SUBCLASS MEANS (log_{10}gms)					
7	OvX W4	8	2.095	1.940	1.570	0.929	0.660	1.381
7	Control	4	2.087	1.927	1.576	0.981	0.658	1.371
9	OvX W4	7	2.264	2.102	1.757	1.144	0.836	1.557
9	Control	8	2.192	2.025	1.697	1.097	0.797	1.489
12	OvX W4	8	2.392	2.225	1.895	1.276	0.992	1.697
12	Control	8	2.316	2.145	1.830	1.219	0.951	1.624
15	OvX W4	9	2.488	2.314	2.008	1.436	1.080	1.796
15	Control	12	2.364	2.185	1.893	1.294	1.018	1.680
			MAIN CLASS MEANS					
	OvX W4	32	2.317	2.152	1.815	1.206	0.899	1.615
	Control	32	2.275	2.103	1.789	1.187	0.901	1.579
7		12	2.092	1.936	1.572	0.946	0.659	1.377
9		15	2.226	2.061	1.725	1.119	0.815	1.521
12		16	2.354	2.185	1.863	1.248	0.972	1.660
15		21	2.418	2.240	1.942	1.355	1.045	1.730

APPENDIX TABLE 8.9B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	DM	FAT	ASH	PROTEIN
		MEAN SQUARES / F VALUES					
TREATMENT (Tr)	1	0.07141 45.212	0.08242 48.914	0.0504 29.835	0.03499 6.184	0.01865 10.077	0.06534 38.087
AGE	3	0.3064 194.00	0.2734 162.229	0.3841 227.380	0.4430 78.292	0.4204 227.094	0.3576 208.452
Linear (W7+W9 vs W12+W15)	1	0.9187 581.65	0.8191 486.114	1.1521 681.987	1.3235 233.923	1.2586 679.847	1.0721 624.908
Quadratic (W7+W15 vs W9+W12)	1	0.04076 25.807	0.0397 23.530	0.0442 26.167	0.0311 5.491	.06169 33.321	0.0469 27.332
Cubic (W7+W12 vs W9 + W15)	1	0.00084 0.531	0.000465 0.275	0.00200 1.186	0.0081 1.557	.00018 0.097	.00080 0.467
Tr.x Age Interaction	3	0.00810 5.128	0.00792 4.700	0.008737 5.172	0.02301 4.067	0.002072 1.119	0.006763 3.942
Tr. by Linear	1	0.0219 13.858	0.0214 12.695	0.0237 14.030	.0624 11.035	0.00567 3.065	0.01845 10.754
Tr. by Quadratic	1	0.00037 0.235	0.00036 0.211	0.00041 0.241	.00049 0.086	0.00033 0.178	0.00038 0.222
Tr. by Cubic	1	0.00291 1.843	0.00286 1.695	0.00303 1.795	.00725 1.280	0.00074 0.399	0.0023 1.324
RESIDUAL (ERROR)	56	0.001579	0.001685	0.001689	0.005658	0.001851	0.001716
TOTAL	63						

APPENDIX TABLE 8.10A

SKIN

AGE (Weeks)	TREATMENT	NO.	% WET WEIGHT WATER	FAT	ASH	% DRY WEIGHT PROTEIN	FAT	ASH	PROTEIN
					SUBCLASS MEANS				
12	OvX D2	8	60.896	16.036	1.292	21.751	40.981	3.316	55.686
12	OvX W4	8	60.526	15.337	1.349	22.770	38.762	3.430	57.794
12	OvX W7	7	60.974	14.831	1.386	22.789	37.879	3.577	58.529
12	Control	8	60.664	15.107	1.485	22.724	38.319	3.781	57.884
12	Oestrogen	7	61.280	13.302	1.303	24.079	34.403	3.376	62.209
15	OvX D2	8	57.525	18.416	1.356	22.680	43.230	3.204	53.552
15	OvX W4	9	57.196	18.948	1.237	22.601	44.244	2.899	52.837
15	OvX W7	6	58.122	17.795	1.267	22.797	42.155	3.065	54.765
15	Control	12	58.978	16.641	1.412	22.949	40.454	3.460	56.072
15	Oestrogen	7	55.567	19.543	1.540	23.330	43.806	3.471	52.704
					MAIN CLASS MEANS				
	OvX D2	16	59.211	17.226	1.324	22.216	42.106	3.260	54.619
	OvX W4	17	58.763	17.249	1.289	22.681	41.665	3.149	55.169
	OvX W7	13	59.658	16.199	1.331	22.792	39.852	3.341	56.792
	Control	20	59.652	16.027	1.441	22.859	39.600	3.588	56.796
	Oestrogen	14	58.424	16.431	1.421	23.704	39.104	3.424	57.456
12		38	60.854	14.971	1.364	22.790	38.170	3.497	58.318
15		42	57.629	18.122	1.364	22.865	42.597	3.236	54.151

APPENDIX TABLE 8.10B

SOURCE OF VARIATION	Degrees of Freedom	% WET WEIGHT (MEAN SQUARES/F VALUES) WATER	FAT	ASH	PROTEIN	% DRY WEIGHT FAT	ASH	PROTEIN
TREATMENT (Tr.)	4	4.8325 1.573	5.708 1.183	.0781 2.187	4.2772 5.265	28.037 1.927	0.5463 2.250	22.370 1.706
1) All OvX vs Control + Oestrogen	1				9.4021 11.573			
2) Control vs Oestrogen	1				6.0976 7.505			
3) OvX D2 vs OvX W7	1				2.3800 2.929			
4) OvX W4 vs OvX D2 + OvX W7	1				0.3502 0.431			
AGE	1	222.82 72.519	216.51 44.871	.00001 .0002	.0440 .054	429.84 29.541	1.4794 6.092	381.14 29.061
Tr x Age (Interaction)	4	8.3825 2.728	12.224 2.533	.08427 2.361	1.4157 1.743	33.870 2.328	0.2639 1.087	36.487 2.782
Comparison 1) W12 vs W15	1	1.2481 0.406	3.7671 0.780	0.08947 2.506	1.2640 1.555	14.730 1.012		19.639 1.497
Comparison 2) W12 vs W15	1	32.831 10.685	44.513 9.225	0.19511 5.465	1.9202 2.363	106.904 7.346		119.766 9.131
Comparison 3) W12 vs W15	1	0.4807 0.156	0.6087 0.126	0.0597 1.672	1.5149 1.864	7.3482 0.505		4.7475 0.361
Comparison 4) W12 vs W15	1	0.1273 0.041	2.3425 0.485	0.0190 0.531	1.0803 1.329	13.1001 0.900		10.728 0.818
RESIDUAL (Error)	70	3.0726	4.8251	.03570	.8124	14.551	0.2428	13.115
TOTAL	79							

APPENDIX TABLE 8.11A

CARCASS

AGE (Weeks)	TREATMENT	NO.	% WET WEIGHT WATER	FAT	ASH	PROTEIN	% DRY WEIGHT FAT	ASH	PROTEIN
					SUBCLASS MEANS				
12	OvX D2	8	71.501	4.489	4.735	19.259	15.742	16.617	67.622
12	OvX W4	8	70.545	5.270	4.832	19.330	17.865	16.420	65.699
12	OvX W7	7	70.227	5.473	4.743	19.536	18.319	15.953	65.709
12	Control	8	69.229	6.039	5.126	19.586	19.606	16.666	63.711
12	Oestrogen	7	69.156	6.206	5.186	19.433	20.139	16.837	63.007
15	OvX D2	8	69.776	5.504	4.936	19.761	18.199	16.354	65.430
15	OvX W4	9	69.889	5.786	4.719	19.587	19.214	15.690	65.082
15	OvX W7	6	69.013	6.180	4.977	19.810	19.863	16.092	64.030
15	Control	12	68.233	6.306	5.392	20.048	19.804	17.009	63.172
15	Oestrogen	7	66.044	8.646	5.603	19.686	25.401	16.537	58.046
					MAIN CLASS MEANS				
	OvX D2	16	70.639	4.996	4.836	19.510	16.971	16.486	66.526
	OvX W4	17	70.198	5.543	4.772	19.466	18.579	16.034	65.372
	OvX W7	13	69.667	5.799	4.851	19.662	19.032	16.017	64.934
	Control	20	68.631	6.199	5.286	19.863	19.725	16.872	63.388
	Oestrogen	14	67.600	7.426	5.394	19.559	22.770	16.687	60.526
12		38	70.155	5.477	4.922	19.426	18.287	16.504	65.191
15		42	68.629	6.414	5.137	19.800	20.313	16.392	63.280

APPENDIX TABLE 8.11B

SOURCE OF VARIATION	Degrees of Freedom	% WET WEIGHT (MEAN SQUARES/F VALUES) WATER	FAT	ASH	PROTEIN	% DRY WEIGHT FAT	ASH	PROTEIN
Treatment (Tr)	4	22.602 22.973	12.299 14.172	1.2356 14.811	0.3657 1.078	67.185 10.216	2.1727 2.382	79.087 17.408
1) All OvX vs Control + Oestrogen	1	74.983 76.291	34.342 39.569	4.7782 57.274		174.115 26.474		246.15 54.180
2) Control vs Oestrogen	1	10.357 10.537	12.720 14.656	0.1474 1.766		76.049 11.563		68.818 15.147
3) OvX D2 vs OvX W7	1	7.4161 7.545	4.9270 5.677	0.0042 0.049		32.140 4.887		19.628 4.320
4) OvX W4 vs OvX D2 + OvX W7	1	0.0814 0.082	0.1442 0.166	0.0551 0.661		2.7555 0.418		1.0045 0.221
AGE	1	46.020 46.775	18.959 21.845	0.783 9.385	2.3700 6.985	90.630 13.781	0.513 0.526	77.350 17.026
Tr x Age (Interaction)	4	3.4475 3.504	2.7516 3.170	0.1514 1.814	0.0605 0.178	14.034 2.134	0.7475 0.819	12.287 2.705
Comparison 1) W12 vs W15	1	3.4505 3.510	1.7423 2.007	0.2596 3.111		4.2310 0.643		7.4231 1.633
Comparison 2) W12 vs W15	1	9.0629 9.220	9.5569 11.011	0.0461 0.552		51.925 7.895		39.591 8.714
Comparison 3) W12 vs W15	1	0.4670 0.475	0.1694 0.195	0.0019 0.022		1.4849 0.225		0.4720 0.103
Comparison 4) W12 vs W15	1	1.7592 1.789	0.3175 0.365	0.2916 3.495		1.1274 0.171		4.6271 1.018
RESIDUAL (Error)	70	.9839	.8679	.08343	0.3393	6.5766	0.9121	4.5431
TOTAL	79							

APPENDIX TABLE 8.12A

WHOLE BODY

AGE (Weeks)	TREATMENT	NO.	% WET WEIGHT WATER	FAT	ASH	PROTEIN	% DRY WEIGHT FAT	ASH	PROTEIN
					SUBCLASS MEANS				
12	OvX D2	8	68.869	7.359	3.881	19.871	23.636	12.479	63.872
12	OvX W4	8	68.114	7.712	3.987	20.166	24.147	12.516	63.320
12	OvX W7	7	68.060	7.661	3.959	20.299	23.896	12.426	63.660
12	Control	8	67.330	8.052	4.316	20.281	24.611	13.222	62.151
12	Oestrogen	7	67.401	7.791	4.317	20.470	23.921	13.261	62.803
15	OvX D2	8	66.889	8.554	4.096	20.445	25.782	12.387	61.815
15	OvX W4	9	66.900	8.886	3.899	20.296	26.838	11.794	61.353
15	OvX W7	6	66.463	8.900	4.110	20.508	26.382	12.300	61.302
15	Control	12	66.196	8.583	4.517	20.684	25.337	13.390	61.259
15	Oestrogen	7	63.807	10.977	4.733	20.466	30.237	13.111	56.634
					MAIN CLASS MEANS				
	OvX D2	16	67.879	7.956	3.989	20.158	24.709	12.433	62.844
	OvX W4	17	67.471	8.334	3.941	20.235	25.572	12.134	62.279
	OvX W7	13	67.323	8.233	4.028	20.395	25.043	12.368	62.572
	Control	20	66.649	8.371	4.436	20.523	25.046	13.323	61.616
	Oestrogen	14	65.604	9.384	4.525	20.468	27.079	13.186	59.719
12		38	67.967	7.715	4.090	20.209	24.049	12.778	63.158
15		42	66.119	9.087	4.282	20.494	26.709	12.655	60.620

APPENDIX TABLE 8.12B

(MEAN SQUARES/F VALUES)

SOURCE OF VARIATION	Degrees of Freedom	% WET WEIGHT WATER	% WET WEIGHT FAT	% WET WEIGHT ASH	% WET WEIGHT PROTEIN	% DRY WEIGHT FAT	% DRY WEIGHT ASH	% DRY WEIGHT PROTEIN
Treatment (Tr.)	4	11.477 10.069	4.2891 3.033	1.1368 19.464	0.3500 1.256	12.886 1.518	4.4995 6.686	22.240 3.898
1) All OvX vs Control + Oestrogen	1	35.195 30.876	8.5349 6.034	4.3850 75.079			16.298 24.218	64.034 11.222
2) Control vs Oestrogen	1	10.8688 9.535	9.2067 6.509	0.0954 1.633			0.1162 0.172	31.954 5.600
3) OvX D2 vs OvX W7	1	2.7223 2.388	0.7526 0.532	0.0148 0.253			0.0353 0.052	0.9416 0.165
4) OvX W4 vs OvX D2 + OvX W7	1	0.0427 0.037	0.3468 0.245	0.0497 0.850			0.6264 0.930	1.1280 0.197
AGE	1	70.260 61.639	41.583 29.402	0.6198 10.612	1.333 4.783	159.97 18.849	0.658 0.978	140.12 24.557
Tr. x Age (Interaction)	4	3.7750 3.312	3.8136 2.696	0.1271 2.176	0.2027 0.727	16.461 1.939	0.4667 0.693	15.387 2.697
Comparison 1) W12 vs W15	1	2.7791 2.438	2.0311 1.436	0.2191 3.750		5.5014 0.648		9.2864 1.627
Comparison 2) W12 vs W15	1	12.250 10.747	14.267 10.087	0.0938 1.606		63.256 7.453		56.354 9.876
Comparison 3) W12 vs W15	1	0.2626 0.230	0.0034 0.002	0.0072 0.123		0.2062 0.024		0.1617 0.028
Comparison 4) W12 vs W15	1	0.8781 0.770	0.0051 0.003	0.1965 3.364		0.3724 0.043		0.1548 0.027
RESIDUAL (Error)	70	1.1400	1.4143	0.05840	0.2787	8.4869	0.6730	5.7059
Total	79							

APPENDIX TABLE 8.13A

SKIN

AGE (Weeks)	TREATMENT	NO.	% WET WEIGHT WATER	FAT	ASH	PROTEIN	% DRY WEIGHT FAT	ASH	PROTEIN
					SUBCLASS MEANS				
9	OvX D2	8	59.704	18.332	1.321	20.622	45.360	3.302	51.322
9	OvX W4	7	62.149	15.397	1.314	21.119	40.270	3.497	56.219
9	OvX W7	7	62.991	14.933	1.354	20.703	40.170	3.686	56.126
9	Control	8	60.691	15.742	1.587	21.956	39.846	4.082	56.145
12	OvX D2	8	60.896	16.036	1.292	21.751	40.981	3.316	55.686
12	OvX W4	8	60.526	15.337	1.349	22.770	38.762	3.430	57.794
12	OvX W7	7	60.974	14.831	1.386	22.789	37.879	3.577	58.529
12	Control	8	60.664	15.107	1.485	22.724	38.319	3.781	57.884
15	OvX D2	8	57.525	18.416	1.356	22.680	43.230	3.204	53.552
15	OvX W4	9	57.196	18.948	1.237	22.601	44.244	2.899	52.837
15	OvX W7	6	58.122	17.795	1.267	22.797	42.155	3.065	54.765
15	Control	12	58.978	16.641	1.412	22.949	40.454	3.460	56.072
					MAIN CLASS MEANS				
	OvX D2	24	59.375	17.595	1.323	21.685	43.190	3.274	53.520
	OvX W4	24	59.750	16.709	1.297	22.225	41.258	3.250	55.475
	OvX W7	20	60.824	15.756	1.339	22.061	39.963	3.461	56.558
	Control	28	59.949	15.946	1.483	22.601	39.670	3.730	56.610
9		30	61.305	16.164	1.398	21.113	41.491	3.645	54.872
12		31	60.758	15.344	1.378	22.499	39.021	3.524	57.439
15		35	58.041	17.838	1.329	22.772	42.355	3.189	54.440

APPENDIX TABLE 8.13B

SOURCE OF VARIATION	Degrees of Freedom	% WET WEIGHT (MEAN SQUARES / F VALUES) WATER	FAT	ASH	PROTEIN	% DRY WEIGHT FAT	ASH	PROTEIN
Treatment (Tr)	3	6.3800 1.629	16.294 2.771	0.2043 5.927	3.1413 4.133	63.110 3.553	1.4361 5.274	50.3133 3.204
1) All OvX vs Control	1		13.5666 2.307	0.5906 17.133	6.0772 7.995	70.2849 3.956	3.7940 13.934	43.1461 2.747
2) OvX D2 vs OvX W7	1		33.005 5.612	0.00162 0.047	1.8415 2.422	106.062 5.971	0.3087 1.133	94.8351 6.038
3) OvX W4 vs OvX D2+OvX W7	1		0.4094 0.069	0.01341 0.389	1.1443 1.505	4.4274 0.249	0.1060 0.389	5.8976 0.375
AGE	2	106.06 27.094	58.730 9.987	0.05379 1.561	24.296 31.967	105.040 5.913	2.0673 7.593	89.220 5.681
Linear (W9 vs W15)	1	184.069 47.021	53.516 9.100		42.976 56.543	19.2696 1.084	3.6840 13.530	6.5443 0.416
Quadratic (W12 vs W9+W15)	1	24.839 6.345	59.615 10.137		6.9602 9.157	183.856 10.350	0.3323 1.220	167.276 10.650
Tr. x Age	6	7.7033 1.968	6.3225 1.075	.02618 0.759	1.0427 1.372	15.4967 0.872	0.1704 0.626	13.075 0.832
Comparison 1) W9 vs W15	1	17.5183 4.475	5.3754 0.914		2.6221 3.449			
Comparison 1) W12 vs W9+W15	1	0.5198 0.132	2.7478 0.467		0.6974 0.917			
Comparison 2) W9 vs W15	1	12.9423 3.306	13.7965 2.346		0.0024 0.003			
Comparison 2) W12 vs W9+W15	1	8.5226 2.177	1.5918 0.270		2.1616 2.844			
Comparison 3) W9 vs W15	1	5.1831 1.324	10.9605 1.863		0.8932 1.175			
Comparison 3) W12 vs W9 + W15	1	0.8458 0.216	0.0347 0.005		0.3998 0.526			
RESIDUAL (Error)	84	3.9145	5.8805	0.03447	0.76005	17.7627	0.2723	15.7052
TOTAL	95							

APPENDIX TABLE 8.14A

CARCASS

AGE (Weeks)	TREATMENT	NO.	% WET WEIGHT WATER	FAT	ASH	PROTEIN	% DRY WEIGHT FAT	ASH	PROTEIN
			SUBCLASS MEANS						
9	OvX D2	8	71.022	5.150	4.612	19.194	17.762	15.935	66.287
9	OvX W4	7	71.030	5.279	4.509	19.161	18.129	15.590	66.263
9	OvX W7	7	71.383	5.449	4.633	19.536	19.024	16.206	64.753
9	Control	8	70.237	5.825	4.766	19.586	19.550	16.042	64.395
12	OvX D2	8	71.501	4.489	4.735	19.259	15.742	16.617	67.622
12	OvX W4	8	70.545	5.270	4.832	19.330	17.865	16.420	65.699
12	OvX W7	7	70.227	5.473	4.743	19.536	18.319	15.953	65.709
12	Control	8	69.229	6.039	5.126	19.586	19.606	16.666	63.711
15	OvX D2	8	69.776	5.504	4.936	19.761	18.199	16.354	65.430
15	OvX W4	9	69.889	5.786	4.719	19.587	19.214	15.690	65.082
15	OvX W7	6	69.013	6.180	4.977	19.810	19.863	16.092	64.030
15	Control	12	68.233	6.306	5.392	20.048	19.804	17.009	63.172
			MAIN CLASS MEANS						
	OvX D2	24	70.767	5.047	4.761	19.405	17.234	16.302	66.447
	OvX W4	24	70.440	5.466	4.695	19.377	18.448	15.904	65.632
	OvX W7	20	70.267	5.676	4.774	19.261	19.029	16.083	64.870
	Control	28	69.090	6.092	5.137	19.660	19.675	16.635	63.676
9		30	70.899	5.430	4.634	19.017	18.619	15.946	65.419
12		31	70.380	5.313	4.863	19.424	17.869	16.429	65.683
15		35	69.145	5.967	5.044	19.823	19.296	16.363	64.327

APPENDIX TABLE 8.14B

SOURCE OF VARIATION	Degrees of Freedom	% WET WEIGHT (MEAN SQUARES / F VALUES) WATER	FAT	ASH	PROTEIN	% DRY WEIGHT FAT	ASH	PROTEIN
Treatment (Tr)	3	11.550 13.298	4.5619 5.346	0.8413 11.173	0.4223 1.477	26.734 3.842	2.0997 2.596	33.807 6.837
1) All OvX vs Control	1	30.2946 34.878	8.3619 9.798	2.3723 31.506		38.728 5.565	4.3901 5.428	69.021 13.958
2) OvX D2 vs OvX W7	1	3.3977 3.911	4.6380 5.435	0.0057 0.075		36.594 5.258	0.5201 0.643	28.413 5.746
3) OvX W4 vs OvX D2+OvX W7	1	0.0000 0.000	0.0768 0.090	0.1137 1.510		0.9680 0.139	1.3167 1.627	0.0282 0.005
AGE	2	23.500 27.056	3.6237 4.246	1.1301 15.009	4.873 17.038	15.500 2.227	1.8345 2.268	14.470 2.926
Linear (W9 vs W15)	1	44.735 51.504	4.2055 4.928	2.2141 29.405	9.9209 34.686			15.529 3.140
Quadratic (W12 vs W9+W15)	1	1.8927 2.179	2.7878 3.266	0.0349 0.463	0.0120 0.042			11.908 2.408
Tr. X Age (Interaction)	6	1.3917 1.602	0.3721 0.436	0.1201 1.595	0.3952 1.382	2.6300 0.378	0.9640 1.192	2.4567 0.497
Comparison 1) W9 vs W15	1	0.5864 0.675		0.3726 4.948	0.0623 0.217			
Comparison 1) W12 vs W9 + W15	1	0.6981 0.803		0.0002 0.003	0.0100 0.035			
Comparison 2) W9 vs W15	1	2.2550 2.596		0.0007 0.009	0.9403 3.287			
Comparison 2) W12 vs W9+W15	1	2.8228 3.249		0.0012 0.016	0.8563 2.994			
Comparison 3 W9 vs W15	1	1.1288 1.299		0.0387 0.514	0.6474 2.263			
Comparison 3) W12 vs W9+W15	1	0.7930 0.912		0.2499 3.319	0.00502 0.175			
RESIDUAL	84	0.8686	0.8533	0.07530	0.2860	6.9586	0.8088	4.9446
TOTAL	95							

APPENDIX TABLE 8.15A

WHOLE BODY

AGE (Weeks)	TREATMENT	NO.	% WET WEIGHT WATER	FAT	ASH	PROTEIN	% DRY WEIGHT FAT	ASH	PROTEIN
					SUBCLASS MEANS				
9	OvX D2	8	68.166	8.482	3.785	19.546	26.585	11.916	61.482
9	OvX W4	7	68.851	7.766	3.729	19.636	24.757	12.003	63.226
9	OvX W7	7	69.186	7.933	3.771	19.090	25.690	12.264	62.033
9	Control	8	68.027	8.119	4.035	19.796	25.331	12.654	62.025
12	OvX D2	8	68.869	7.359	3.881	19.871	23.636	12.479	63.872
12	OvX W4	8	68.114	7.712	3.987	20.166	24.147	12.516	63.320
12	OvX W7	7	68.060	7.661	3.959	20.299	23.896	12.426	63.660
12	Control	8	67.330	8.052	4.316	20.281	24.611	13.222	62.151
15	OvX D2	8	66.889	8.554	4.096	20.445	25.782	12.387	61.815
15	OvX W4	9	66.900	8.886	3.899	20.296	26.838	11.794	61.353
15	OvX W7	6	66.463	8.900	4.110	20.508	26.382	12.300	61.302
15	Control	12	66.196	8.583	4.517	20.684	25.337	13.390	61.259
					MAIN CLASS MEANS				
	OvX D2	24	67.975	8.132	3.921	19.954	25.335	12.261	62.390
	OvX W4	24	67.874	8.168	3.879	20.060	25.334	12.096	62.555
	OvX W7	20	67.975	8.128	3.938	19.938	25.269	12.331	62.383
	Control	28	67.043	8.299	4.322	20.315	25.128	13.132	61.733
9		30	68.527	8.090	3.835	19.527	25.615	12.214	62.162
12		31	68.094	7.697	4.038	20.150	24.078	12.668	63.238
15		35	66.581	8.709	4.192	20.499	26.004	12.564	61.418

APPENDIX TABLE 8.15 B

SOURCE OF VARIATION	Degrees of Freedom	% WET WEIGHT (MEAN SQUARES / F VALUES) WATER	FAT	ASH	PROTEIN	% DRY WEIGHT FAT	ASH	PROTEIN
TREATMENT(Tr.)	3	3.7633 3.176	0.09183 0.058	0.9414 16.917	0.4943 2.209	0.3130 0.031	4.979 7.357	3.0733 0.446
1) All OvX vs Control	1	11.1160 9.381		2.7261 48.987			14.1419 20.896	
2) OvX D2 vs OvX W7	1	0.0557 0.047		0.0073 0.130			0.05204 0.076	
3) OvX W4 vs OvX D2 + OvX W7	1	0.0041 0.003		0.0592 1.064			0.5599 0.827	
AGE	2	32.785 27.669	8.900 5.592	0.8449 15.183	7.380 32.987	35.1965 3.519	1.5715 2.322	27.060 3.924
Linear W9 vs W15	1	59.281 50.031	6.7322 4.230	1.6585 29.802		3.8182 0.381		9.0258 1.309
Quadratic W12 vs W9 + W15	1	5.3451 4.511	10.3277 6.489	0.0386 0.692		64.460 6.445		42.846 6.214
Tr x Age (Interaction)	6	1.445 1.220	0.8494 0.534	0.06402 1.150	0.3265 1.459	4.9428 0.494	0.5320 0.786	3.493 0.507
Comparison 1) W9 vs W15	1	0.0774 0.065		0.1452 2.609				
Comparison 1) W12 vs W9 + W15	1	0.6142 0.518		0.0000 0.000				
Comparison 2) W9 vs W15	1	3.7312 3.148		0.0013 0.023				
Comparison 2) W12 vs W9 + W15	1	2.9988 2.530		0.0146 0.262				
Comparison 3) W9 vs W15	1	0.0060 0.005		0.0607 1.090				
Comparison 3) W12 vs W9 + W15	1	1.042 0.880		0.1303 2.341				
RESIDUAL (Error)	84	1.1849	1.5914	0.05565	0.2237	10.0004	0.67676	6.8950
TOTAL	95							

APPENDIX TABLE 8.16A

SKIN

AGE (Weeks)	TREATMENT	NO.	% WET WEIGHTS WATER	FAT	ASH	PROTEIN	% DRY WEIGHTS FAT	ASH	PROTEIN
					SUBCLASS MEANS				
7	OvX W4	8	64.232	13.350	1.473	20.925	37.284	4.125	58.572
7	Control	4	61.348	16.760	1.480	20.390	43.388	3.838	52.762
9	OvX W4	7	62.149	15.397	1.314	21.119	40.270	3.497	56.219
9	Control	8	60.691	15.743	1.588	21.956	39.846	4.082	56.145
12	OvX W4	8	60.526	15.338	1.349	22.770	38.762	3.430	57.794
12	Control	8	60.664	15.108	1.485	22.724	38.319	3.781	57.884
15	OvX W4	9	57.196	18.948	1.237	22.601	44.244	2.899	52.837
15	Control	12	58.978	16.641	1.412	22.949	40.454	3.460	56.072
					MAIN CLASS MEANS				
	OvX W4	32	60.871	15.869	1.341	21.900	40.264	3.469	56.250
	Control	32	60.124	16.048	1.483	22.325	40.135	3.743	56.129
7		12	63.271	14.487	1.475	20.747	39.318	4.029	56.636
9		15	61.371	15.581	1.460	21.565	40.044	3.809	56.179
12		16	60.595	15.223	1.417	22.747	38.541	3.606	57.839
15		21	58.214	17.630	1.337	22.800	42.079	3.220	54.685

APPENDIX TABLE 8.16B

		% WET WEIGHT (MEAN SQUARES / F VALUES)				% DRY WEIGHT		
SOURCE OF VARIATION	Degrees of Freedom	WATER	FAT	ASH	PROTEIN	FAT	ASH	PROTEIN
TREATMENT (Tr.)	1	5.3900 1.434	1.3660 0.274	0.3223 8.577	0.3370 0.416	1.920 0.120	1.3470 4.822	6.020 0.433
AGE	3	62.303 16.575	29.058 5.821	0.0741 1.972	14.330 17.707	44.983 2.822	1.8944 6.782	34.920 2.51
Linear W7+W9 vs W12+W15	1	165.45 44.014	47.982 9.611		40.591 50.155	18.503 1.160	5.021 17.973	
Quadratic W7+W15 vs W9+W12	1	2.125 0.565	14.833 2.971		5.260 6.499	68.152 4.275	0.0937 0.335	
Cubic W7+W12 vs W9+W15	1	4.837 1.286	8.804 1.763		0.533 0.658	21.413 1.343	0.0647 0.231	
Tr x Age (Interaction)	3	15.263 4.061	19.555 3.917	0.0378 1.005	1.103 1.363	57.313 3.595	0.5050 1.808	47.923 3.443
Tr x Linear	1	44.611 11.868	55.168 11.051			150.68 9.452	0.828 2.965	128.05 9.199
Tr x Quadratic	1	0.0966 0.025	1.581 0.316			11.207 0.703	0.3635 1.301	7.806 0.560
Tr x Cubic	1	0.0605 0.016	4.850 0.971			27.445 1.721	0.6386 2.286	20.822 1.496
ERROR	56	3.7589	4.9919	0.03758	0.8093	15.9409	0.2793	13.918
TOTAL	63							

APPENDIX TABLE 8.17A

CARCASS

AGE (Weeks)	TREATMENT	NO.	% WET WEIGHTS WATER	FAT	ASH	PROTEIN	% DRY WEIGHTS FAT	ASH	PROTEIN
			SUBCLASS MEANS						
7	OvX W4	8	71.974	4.806	4.396	18.805	17.078	15.704	67.204
7	Control	4	71.555	5.105	4.455	18.865	17.870	15.610	66.503
9	OvX W4	7	71.030	5.279	4.509	19.161	18.129	15.590	66.263
9	Control	8	70.238	5.825	4.766	19.150	19.550	16.043	64.395
12	OvX W4	8	70.545	5.270	4.833	19.330	17.865	16.420	65.699
12	Control	8	69.229	6.039	5.126	19.586	19.606	16.666	63.711
15	OvX W4	9	69.889	5.786	4.719	19.587	19.214	15.690	65.082
15	Control	12	68.233	6.306	5.393	20.048	19.804	17.009	63.173
			MAIN CLASS MEANS						
	OvX W4	32	70.824	5.301	4.621	19.234	18.105	15.854	66.025
	Control	32	69.398	5.969	5.052	19.560	19.449	16.507	64.029
7		12	71.834	4.906	4.416	18.825	17.342	15.673	66.970
9		15	70.607	5.570	4.646	19.155	18.887	15.831	65.267
12		16	69.887	5.654	4.979	19.458	18.736	16.543	64.705
15		21	68.943	6.083	5.104	19.850	19.551	16.444	63.991

APPENDIX TABLE 8.17B

(MEAN SQUARES / F VALUES)

SOURCE OF VARIATION	Degrees of Freedom	% WET WEIGHT				% DRY WEIGHT		
		WATER	FAT	ASH	PROTEIN	FAT	ASH	PROTEIN
TREATMENT (Tr)		16.100 16.662	4.189 4.917	1.516 14.273	0.540 2.109	18.997 2.802	3.406 3.582	38.440 6.448
AGE	3	18.913 19.574	2.834 3.326	1.234 11.621	2.645 10.331	9.734 1.436	2.516 2.646	18.417 3.089
Linear W7+W9 vs W12+W15	1	56.256 58.221	7.970 9.355	3.600 33.895	7.723 30.170			53.864 9.034
Quadratic W7+W15 vs W9+W12	1	1.198 1.240	0.2575 0.302	0.2040 1.921	0.0168 0.065			5.077 0.851
Cubic W7+W12 vs W9+W15	1	0.718 0.743	0.7435 0.872	0.0421 0.396	0.0357 0.139			1.840 0.308
TR x Age (Interaction)	3	1.113 1.152	0.121 0.142	0.267 2.520	0.1907 0.745	1.205 0.177	1.508 1.586	1.110 0.186
Tr x Linear	1			0.6550 6.167				
Tr x Quadratic	1			0.0201 0.188				
Tr x Cubic	1			0.0525 0.494				
ERROR	56	0.9663	0.8519	0.1062	0.2560	6.7799	0.9510	5.9618
TOTAL	63							

APPENDIX TABLE 8.18A

WHOLE BODY

AGE (Weeks)	TREATMENT	NO.	% WET WEIGHTS WATER	FAT	ASH	PROTEIN	% DRY WEIGHTS FAT	ASH	PROTEIN
			SUBCLASS MEANS						
7	Ovx W4	8	70.066	6.919	3.675	19.321	23.039	12.296	64.651
7	Control	4	69.118	7.880	3.750	19.230	25.485	12.115	62.383
9	OvX W4	7	68.851	7.766	3.729	19.636	24.757	12.003	63.226
9	Control	8	68.028	8.119	4.035	19.796	25.331	12.654	62.025
12	OvX W4	8	68.114	7.713	3.988	20.166	24.148	12.516	63.320
12	Control	8	67.330	8.053	4.316	20.281	24.611	13.223	62.151
15	OvX W4	9	66.900	8.886	3.899	20.296	26.838	11.794	61.353
15	Control	12	66.196	8.583	4.517	20.684	25.337	13.390	61.259
			MAIN CLASS MEANS						
	OvX W4	32	68.422	7.856	3.828	19.875	24.760	12.146	63.079
	Control	32	67.303	8.247	4.250	20.180	25.173	13.005	61.814
7		12	69.750	7.239	3.700	19.291	23.854	12.236	63.895
9		15	68.412	7.954	3.892	19.721	25.063	12.350	62.585
12		16	67.722	7.883	4.152	20.224	24.379	12.869	62.736
15		21	66.498	8.713	4.252	20.518	25.980	12.706	61.299

APPENDIX TABLE 8.18B

SOURCE OF VARIATION	Degrees of Freedom	% WET WEIGHT (MEAN SQUARES / F VALUES)				% DRY WEIGHT		
		Water	Fat	Ash	Protein	Fat	Ash	Protein
TREATMENT (Tr)	1	9.740 7.936	1.681 1.188	1.622 22.107	0.301 1.383	3.619 0.404	7.063 10.301	20.570 2.968
AGE	3	24.223 19.737	4.881 3.449	0.764 10.409	4.163 19.132	11.961 1.336	1.216 1.773	13.477 1.944
Linear (W7+W9 vs W12+W15)	1	70.330 57.303	11.359 8.025	2.225 30.324	12.437 57.152			
Quadratic (W7+W15 vs W9+W12)	1	0.2388 0.194	0.2690 0.190	0.1359 1.852	0.408 1.876			
Cubic (W7+W12 vs W9+W15)	1	1.351 1.100	1.862 1.315	0.0264 0.360	0.0016 0.007			
TR. x Age(Interaction)	3	0.0367 0.030	0.996 0.703	0.1897 2.586	0.1457 0.669	9.711 1.085	1.973 2.877	2.9233 0.422
Tr. x Linear	1			0.493 6.714			5.192 7.572	
Tr. x Quadratic	1			0.0009 0.012			0.0004 0.000	
Tr. x Cubic	1			0.0522 0.711			0.6217 0.906	
Error	56	1.2273	1.4154	0.07336	0.2176	8.9531	0.6857	6.9309
Total	63							

APPENDIX TABLE 8.19A

TOTAL SKIN FAT-FREE WEIGHT, AND COMPONENT PERCENTAGES

AGE (Weeks)	TREATMENT	No.	TOTAL (log_{10}gms)	WATER	ASH	PROTEIN
				SUB CLASS MEANS		
12	OvX D2	8	1.687	72.532	1.540	25.915
12	OvX W4	8	1.704	71.497	1.590	26.897
12	OvX W7	7	1.694	71.586	1.627	26.771
12	Control	8	1.591	71.462	1.752	26.770
12	Oestrogen	7	1.618	70.699	1.504	27.783
15	OvX D2	8	1.714	70.529	1.667	27.789
15	OvX W4	9	1.768	70.567	1.523	27.898
15	OvX W7	6	1.744	70.703	1.540	27.740
15	Control	12	1.627	70.763	1.694	27.530
15	Oestrogen	7	1.598	69.059	1.916	29.004
				MAIN CLASS MEANS		
	OvX D2	16	1.701	71.531	1.604	26.852
	OvX W4	17	1.738	71.005	1.555	27.427
	OvX W7	13	1.717	71.178	1.587	27.218
	Control	20	1.613	71.043	1.717	27.226
	Oestrogen	14	1.608	69.879	1.710	28.394
12		38	1.659	71.577	1.605	26.804
15		42	1.686	70.384	1.667	27.934

APPENDIX TABLE 8.19B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	ASH	PROTEIN
		(MEAN SQUARES / F VALUES)			
TREATMENT	4	0.06132 26.773	5.6325 7.723	0.09538 1.885	5.0165 6.898
1) All OvX vs Control + Oestrogen	1	0.2292 100.090	10.336 14.173		6.8718 9.449
2) Control vs Oestrogen	1	0.00002 0.008	12.336 16.914		12.521 17.217
3) OvX D2 vs OvX W7	1	0.0024 1.056	1.0657 1.461		1.1659 1.603
4) OvX W4 vs OvX D2 + OvX W7	1	0.00746 3.257	0.9928 1.361		1.2578 1.729
AGE	1	0.01888 8.243	29.390 40.300	0.0828 1.636	26.300 36.166
TREATMENT X AGE (INTERACTION)	4	0.003727 1.627	1.2675 1.738	0.1638 3.238	0.7607 1.046
Comparison 1) W12 vs W15	1	0.00712 3.107	0.0498 0.068	0.1621 3.202	
Comparison 2) W12 vs W15	1	0.00625 2.727	1.7917 2.456	0.4467 8.828	
Comparison 3) W12 vs W15	1	0.00100 0.437	2.2474 3.081	0.0823 1.627	
Comparison 4) W12 vs W15	1	0.00175 0.763	0.6978 0.956	0.0201 0.396	
RESIDUAL	70	0.002290	0.7293	0.05060	0.7272
TOTAL	79				

APPENDIX TABLE 8.20A

TOTAL CARCASS FAT-FREE WEIGHT, AND COMPONENT PERCENTAGES

AGE (Weeks)	TREATMENT	No.	TOTAL (log_{10}gms)	WATER	ASH	PROTEIN
			SUB CLASS MEANS			
12	OvX D2	8	2.226	74.864	4.956	20.165
12	OvX W4	8	2.248	74.474	5.102	20.410
12	OvX W7	7	2.256	74.297	5.019	20.669
12	Control	8	2.180	73.684	5.457	20.845
12	Oestrogen	7	2.193	73.736	5.530	20.721
15	OvX D2	8	2.289	73.847	5.222	20.912
15	OvX W4	9	2.346	74.181	5.011	20.792
15	OvX W7	6	2.318	73.567	5.303	21.113
15	Control	12	2.228	72.830	5.753	21.402
15	Oestrogen	7	2.219	72.297	6.131	21.556
			MAIN CLASS MEANS			
	OvX D2	16	2.257	74.356	5.089	20.539
	OvX W4	17	2.300	74.319	5.054	20.612
	OvX W7	13	2.285	73.960	5.150	20.874
	Control	20	2.209	73.171	5.635	21.179
	Oestrogen	14	2.206	73.016	5.831	21.139
12		38	2.220	74.221	5.210	20.555
15		42	2.276	73.330	5.492	21.162

APPENDIX TABLE 8.20B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	ASH	PROTEIN
		(MEAN SQUARES / F VALUES)			
TREATMENT	4	0.03086	6.0225	1.8731	1.3047
		16.611	11.959	20.790	3.572
1) All OvX vs Control+Oestrogen	1	0.1077	21.543	7.1551	3.8908
		57.955	42.780	79.414	10.653
2) Control vs Oestrogen	1	0.00002	0.4681	0.4110	0.00188
		0.009	0.929	4.561	0.005
3) OvX D2 vs OvX W7	1	0.00632	1.2835	0.0366	0.8868
		3.403	2.548	0.406	2.428
4) OvX W4 vs OvX D2 + OvX W7	1	0.00637	0.3589	0.0497	0.1376
		3.428	0.712	0.551	0.376
AGE	1	0.06895	14.540	1.4275	6.8200
		37.107	28.874	15.844	18.674
TREATMENT x AGE (INTERACTION)	4	0.002788	0.6725	0.2348	0.1412
		1.500	1.335	2.606	0.387
Comparison 1) W12 vs W15	1	0.00681	1.0264	0.4118	
		3.663	2.038	4.570	
Comparison 2) W12 vs W15	1	0.00097	0.6923	0.1890	
		0.524	1.374	2.098	
Comparison 3) W12 vs W15	1	0.00000	0.1459	0.0006	
		0.002	0.289	0.006	
Comparison 4) W12 vs W15	1	0.00336	0.8969	0.3580	
		1.807	1.781	3.973	
RESIDUAL	70	0.001858	0.5036	0.09010	0.3652
TOTAL	79				

APPENDIX TABLE 8.21A

TOTAL WHOLE BODY FAT-FREE WEIGHT, AND COMPONENT PERCENTAGES

AGE (Weeks)	TREATMENT	No.	TOTAL (log_{10} gms)	WATER	ASH	PROTEIN
			SUB CLASS MEANS			
12	OvX D2	8	2.336	74.342	4.191	21.452
12	OvX W4	8	2.357	73.805	4.324	21.854
12	OvX W7	7	2.361	73.710	4.289	21.984
12	Control	8	2.280	73.231	4.695	22.057
12	Oestrogen	7	2.295	73.103	4.683	22.203
15	OvX D2	8	2.392	73.151	4.480	22.356
15	OvX W4	9	2.448	73.427	4.281	22.279
15	OvX W7	6	2.421	72.963	4.512	22.508
15	Control	12	2.325	72.417	4.941	22.627
15	Oestrogen	7	2.312	71.671	5.317	22.996
			MAIN CLASS MEANS			
	OvX D2	16	2.364	73.747	4.336	21.904
	OvX W4	17	2.405	73.605	4.301	22.079
	OvX W7	13	2.389	73.365	4.392	22.226
	Control	20	2.307	72.743	4.842	22.399
	Oestrogen	14	2.304	72.387	5.000	22.599
12		38	2.326	73.651	4.434	21.901
15		42	2.376	72.727	4.713	22.545

APPENDIX TABLE 8.21B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	ASH	PROTEIN
			(MEAN SQUARES / F VALUES)		
TREATMENT	4	0.03619 19.276	4.9725 12.566	1.5581 25.169	1.0755 3.810
1) All OvX vs Control + Oestrogen	1	0.1291 68.827	17.421 44.024	5.9808 96.613	2.9952 10.612
2) Control vs Oestrogen	1	0.00000 0.003	1.5478 3.911	0.2684 4.336	0.5356 1.897
3) OvX D2 vs OvX W7	1	0.00529 2.815	1.2030 3.039	0.0297 0.480	0.8358 2.961
4) OvX W4 vs OvX D2 + OvX W7	1	0.00662 3.525	0.0584 0.147	0.0456 0.735	0.0009 0.003
AGE	1	0.05578 29.711	16.140 40.787	1.4118 22.806	8.0180 28.408
TREATMENT x AGE (INTERACTION)	4	0.00279 1.489	0.6475 1.636	0.2217 3.581	0.1560 0.553
Comparison 1) W12 vs W15	1	0.00671 3.575	0.5797 1.465	0.3797 6.133	
Comparison 2) W12 vs W15	1	0.00168 0.896	0.7722 1.951	0.3054 4.933	
Comparison 3) W12 vs W15	1	0.00003 0.015	0.3533 0.892	0.0077 0.124	
COMPARISON 4) W12 vs W15	1	0.00298 1.587	0.9278 2.344	0.2371 3.829	
RESIDUAL	70	0.001877	0.3957	0.06190	0.2822
TOTAL	79				

APPENDIX TABLE 8.22A

TOTAL SKIN FAT-FREE WEIGHT, AND COMPONENT PERCENTAGES

AGE (Weeks)	TREATMENT	No	TOTAL (log_{10}gms)	WATER	ASH	PROTEIN
			SUB CLASS MEANS			
9	OvX D2	8	1.519	73.117	1.619	25.250
9	OvX W4	7	1.578	73.447	1.559	24.981
9	OvX W7	7	1.615	74.043	1.590	24.349
9	Control	8	1.481	72.021	1.885	26.077
12	OvX D2	8	1.687	72.532	1.540	25.915
12	OvX W4	8	1.704	71.497	1.590	26.897
12	OvX W7	7	1.694	71.586	1.627	26.771
12	Control	8	1.591	71.462	1.752	26.770
15	OvX D2	8	1.714	70.529	1.667	27.789
15	OvX W4	9	1.768	70.567	1.523	27.898
15	OvX W7	6	1.744	70.703	1.540	27.740
15	Control	12	1.627	70.763	1.694	27.530
			MAIN CLASS MEANS			
	OvX D2	24	1.640	72.060	1.609	26.318
	OvX W4	24	1.691	71.717	1.556	26.714
	OvX W7	20	1.681	72.181	1.588	26.214
	Control	28	1.575	71.322	1.765	26.898
9		30	1.545	73.118	1.669	25.198
12		31	1.668	71.775	1.627	26.583
15		35	1.703	70.649	1.618	27.720

APPENDIX TABLE 8.22B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	ASH	PROTEIN
		(MEAN SQUARES / F VALUES)			
TREATMENT	3	0.07680 33.776	2.4967 3.612	0.2512 5.382	1.3990 1.967
1) All OvX vs Control	1	0.2042 89.782	6.6295 9.591	0.7195 15.414	
2) OvX D2 vs OvX W7	1	0.0212 9.323	0.02835 0.041	0.0058 0.123	
3) OvX W4 vs)vX D2 + OvX W7	1	0.00697 3.066	0.9449 1.367	0.0245 0.524	
AGE	2	0.2268 99.744	49.125 71.073	0.02681 0.574	51.501 72.397
Linear (W9 vs W15)	1	0.4260 187.344	99.173 143.481		103.80 145.919
Quadratic (W12 vs W9 + W15)	1	0.03064 13.474	0.3459 0.500		0.3868 0.543
TREATMENT x AGE (INTERACTION)	6	0.00395 1.739	2.6300 3.805	0.03718 0.797	2.1325 2.998
Comparison 1) W9 vs W15	1	0.00207 0.908	9.4295 13.642		7.4954 10.536
Comparison 1) W12 vs W9 + W15	1	0.00000 0.003	0.2908 0.420		0.2123 0.298
Comparison 2) W9 vs W15	1	0.00765 3.362	1.0074 1.457		1.2994 1.826
Comparison 2) W12 vs W9 + W15	1	0.00780 3.428	5.4944 7.949		4.3482 6.112
Comparison 3) W9 vs W15	1	0.00204 0.897	0.0178 0.025		0.00603 0.008
Comparison 3) W12 vs W9 + W15	1	0.0005 0.198	0.7619 1.102		0.5413 0.760
RESIDUAL	84	0.002274	0.6912	0.04668	0.7114
TOTAL	95				

APPENDIX TABLE 8.23A

TOTAL CARCASS FAT-FREE WEIGHT, AND COMPONENT PERCENTAGES

AGE (Weeks)	TREATMENT	No.	TOTAL (log_{10}gms)	WATER	ASH	PROTEIN
			SUB CLASS MEANS			
9	OvX D2	8	2.059	74.884	4.864	20.239
9	OvX W4	7	2.119	74.990	4.763	20.231
9	OvX W7	7	2.110	75.501	4.897	19.583
9	Control	8	2.052	74.587	5.062	20.335
12	OvX D2	8	2.226	74.864	4.956	20.165
12	OvX W4	8	2.248	74.474	5.102	20.410
12	OvX W7	7	2.256	74.297	5.019	20.669
12	Control	8	2.180	73.684	5.457	20.845
15	OvX D2	8	2.289	73.847	5.222	20.912
15	OvX W4	9	2.346	74.181	5.011	20.792
15	OvX W7	6	2.318	73.567	5.303	21.113
15	Control	12	2.228	72.830	5.753	21.402
			MAIN CLASS MEANS			
	OvX D2	24	2.191	74.532	5.014	20.439
	OvX W4	24	2.247	74.515	4.969	20.501
	OvX W7	20	2.224	74.500	5.061	20.422
	Control	28	2.164	73.576	5.471	20.938
9		30	2.083	74.974	4.901	20.110
12		31	2.226	74.331	5.137	20.517
15		35	2.288	73.536	5.364	21.084

APPENDIX TABLE 8.23 B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	ASH	PROTEIN
		(MEAN SQUARES / F VALUES)			
TREATMENT	3	0.03635 22.187	4.3133 10.250	1.1411 13.933	1.0547 3.699
1) All OvX vs Control	1	0.08246 50.334	12.674 30.115	3.2230 39.354	3.1332 10.989
2) OvX D2 vs OvX W7	1	0.01483 9.052	0.0638 0.151	0.0377 0.460	0.00284 0.009
3) OvX vs OvX D2 + OvX W7	1	0.01171 7.147	0.0463 0.110	0.1104 1.348	0.01481 0.051
AGE	2	0.3576 218.274	14.945 35.513	1.4384 17.564	7.1360 25.028
Linear (W9 vs W15)	1	0.6938 423.528	30.008 71.305	2.8417 34.699	14.368 50.393
Quadratic (W12 vs W9 + W15)	1	0.0286 17.455	0.0200 0.047	0.01206 0.147	0.0599 0.210
TREATMENT x AGE (INTERACTION)	6	0.00212 1.292	0.8267 1.964	0.1410 1.722	0.5390 1.890
Comparison 1) W9 vs W15	1	0.00707 4.318	0.8287 1.969	0.4174 5.096	0.0704 0.246
Comparison 1) W12 vs W9 + W15	1	0.00007 0.040	0.0230 0.054	0.00473 0.057	0.00679 0.023
Comparison 2) W9 vs W15	1	0.00090 0.546	1.4429 3.428	0.00402 0.049	1.3118 4.600
Comparison 2) W12 vs W9 + W15	1	0.00022 0.135	1.3250 3.148	0.00007 0.000	1.3109 4.597
Comparison 3) W9 vs W15	1	0.00018 0.108	1.1624 2.762	0.0457 0.558	0.7440 2.609
Comparison 3) W12 vs W9 + W15	1	0.00338 2.066	0.2023 0.480	0.3094 3.778	0.0111 0.038
RESIDUAL	84	0.001638	0.4208	0.08190	0.2851
TOTAL	95				

APPENDIX TABLE 8.24A

TOTAL WHOLE BODY FAT-FREE WEIGHTS, AND COMPONENT PERCENTAGES

AGE (Weeks)	TREATMENT	No.	TOTAL (log_{10}gms)	WATER	ASH	PROTEIN
			SUB CLASS MEANS			
9	OvX D2	8	2.169	74.489	4.137	21.362
9	OvX W4	7	2.229	74.646	4.046	21.293
9	OvX W7	7	2.231	75.150	4.097	20.740
9	Control	8	2.155	74.044	4.390	21.551
12	OvX D2	8	2.336	74.342	4.191	21.452
12	OvX W4	8	2.357	73.805	4.324	21.854
12	OvX W7	7	2.361	73.710	4.289	21.984
12	CONTROL	8	2.280	73.231	4.695	22.057
15	OvX D2	8	2.392	73.151	4.480	22.356
15	OvX W4	9	2.448	73.427	4.281	22.279
15	OvX W7	6	2.421	72.963	4.512	22.508
15	Control	12	2.325	72.417	4.941	22.627
			MAIN CLASS MEANS			
	OvX D2	24	2.299	73.994	4.270	21.724
	OvX W4	24	2.354	73.908	4.227	21.850
	OvX W7	20	2.334	73.990	4.288	21.706
	Control	28	2.264	73.115	4.713	22.157
9		30	2.194	74.561	4.174	21.251
12		31	2.333	73.774	4.377	21.832
15		35	2.388	72.938	4.592	22.455

APPENDIX TABLE 8.24B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	ASH	PROTEIN
		(MEAN SQUARES / F VALUES)			
TREATMENT	3	0.04351 25.720	3.5067 11.448	1.1364 18.928	0.6867 3.369
1) All OvX vs Control	1	0.1041 61.545	10.374 33.866	3.2913 54.818	1.9676 9.655
2) OvX D2 vs OvX W7	1	0.01625 9.605	0.0306 0.099	0.0095 0.158	0.0045 0.022
3) OvX W4 vs OvX D2 + OvX W7	1	0.01049 6.199	0.00111 0.003	0.0700 1.165	0.0853 0.418
AGE	2	0.3260 192.693	19.700 63.314	1.1704 19.494	11.262 55.264
Linear (W9 vs W15)	1	0.6293 371.956	39.704 129.618	2.3308 38.820	22.769 111.732
Quadratic (W12 vs W9 + W15)	1	0.02885 17.053	0.00387 0.012	0.00414 0.068	0.0001 0.000
TREATMENT x AGE (INTERACTION)	6	0.002182 1.289	0.6750 2.204	0.0774 1.289	0.4623 2.269
Comparison 1) W9 vs W15	1	0.00554 3.276	0.00683 0.022	0.1621 2.699	0.1013 0.497
Comparison 1) W12 vs W9 + W15	1	0.0004 0.021	0.00150 0.044	0.0017 0.028	0.0061 0.029
Comparison 2) W9 vs W15	1	0.00187 1.107	1.2887 4.207	0.0093 0.154	1.0723 5.261
Comparison 2) W12 vs W9 + W15	1	0.00100 0.591	1.8528 6.048	0.0253 0.422	1.4428 7.079
Comparison 3) W9 vs W15	1	0.00044 0.260	0.7487 2.444	0.0520 0.866	0.3962 1.944
Comparison 3) W12 vs W9 + W15	1	0.00249 1.470	0.3506 1.144	0.1774 2.955	0.0287 0.140
RESIDUAL	84	0.001692	0.3063	0.06004	0.2038
TOTAL	95				

APPENDIX TABLE 8.25A

TOTAL SKIN FAT-FREE WEIGHT, AND COMPONENT PERCENTAGES

AGE (Weeks)	TREATMENT	No.	TOTAL (log_{10}gms)	WATER	ASH	PROTEIN
			SUB CLASS MEANS			
7	OvX W4	8	1.424	74.135	1.697	24.149
7	Control	4	1.384	73.705	1.778	24.503
9	OvX W4	7	1.578	73.447	1.559	24.981
9	Control	8	1.481	72.021	1.885	26.077
12	OvX W4	8	1.704	71.497	1.590	26.897
12	Control	8	1.591	71.462	1.752	26.770
15	OvX W4	9	1.768	70.567	1.523	27.898
15	Control	12	1.627	70.763	1.694	27.530
			MAIN CLASS MEANS			
	OvX W4	32	1.625	72.322	1.591	26.072
	Control	32	1.551	71.620	1.767	26.598
7		12	1.411	73.992	1.724	24.267
9		15	1.526	72.687	1.733	25.566
12		16	1.648	71.480	1.671	26.834
15		21	1.688	70.679	1.621	27.688

APPENDIX TABLE 8.25B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	ASH	PROTEIN
		(MEAN SQUARES / F VALUES)			
TREATMENT	1	0.1409 6 2.345	2.640 2.691	0.5033 10.028	0.8390 0.941
AGE	3	0.2381 105.337	29.350 29.916	0.0549 1.095	31.907 35.778
Linear W7 + W9 vs W12 + W15	1	0.7109 314.520	87.748 89.441		95.249 106.807
Quadratic W7 + W15 vs W9 + W12	1	0.04654 20.592	2.201 2.243		2.034 2.280
Cubic W7 + W12 vs W9 + W15	1	0.00011 0.047	0.0175 0.017		0.0153 0.017
TREATMENT x AGE (INTERACTION)	3	0.00615 2.721	2.087 2.127	0.0356 0.708	1.712 1.919
TREATMENT W7 + W9 vs W12 + W15	1	0.01771 7.836	2.402 2.448		2.468 2.767
TREATMENT W7 + W15 vs W9 + W12	1	0.001196 0.529	0.684 0.697		0.397 0.444
TREATMENT W7 + W12 vs W9 + W15	1	0.001144 0.506	2.587 2.636		1.738 1.948
RESIDUAL	56	0.002260	0.9811	0.05019	0.8918
TOTAL	63				

APPENDIX TABLE 8.26A

TOTAL CARCASS FAT-FREE WEIGHT, AND COMPONENT PERCENTAGES

AGE (Weeks)	TREATMENT	No.	TOTAL (log_{10}gms)	WATER	ASH	PROTEIN
			SUB CLASS MEANS			
7	OvX W4	8	1.950	75.610	4.622	19.755
7	Control	4	1.946	75.405	4.700	19.880
9	OvX W4	7	2.119	74.990	4.763	20.231
9	Control	8	2.052	74.587	5.062	20.335
12	OvX W4	8	2.248	74.474	5.102	20.410
12	Control	8	2.180	73.684	5.457	20.845
15	OvX W4	9	2.346	74.181	5.011	20.792
15	Control	12	2.228	72.830	5.753	21.402
			MAIN CLASS MEANS			
	OvX W4	32	2.173	74.788	4.882	20.315
	Control	32	2.137	73.805	5.375	20.806
7		12	1.949	75.542	4.648	19.797
9		15	2.083	74.775	4.923	20.287
12		16	2.214	74.079	5.280	20.627
15		21	2.279	73.409	5.435	21.140

APPENDIX TABLE 8.26B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	ASH	PROTEIN
		(MEAN SQUARES / F VALUES)			
TREATMENT	1	0.06011 38.036	6.9500 17.812	1.9994 16.072	1.4890 5.699
AGE	3	0.3137 198.478	10.9667 28.107	1.5702 12.622	4.3427 16.620
Linear W7 + W9 vs W12 + W15	1	0.9403 595.002	32.674 83.740	4.6190 37.129	12.706 48.630
Quadratic W7 + W15 vs W9 + W12	1	0.04066 25.726	0.5318 1.362	0.2452 1.971	0.0550 0.210
Cubic W7 + W12 vs W9 + W15	1	0.00067 0.423	0.0451 0.115	0.03109 0.249	0.1467 0.561
TREATMENT X AGE (INTERACTION)	3	0.00777 4.919	1.0300 2.640	0.3059 2.459	0.2447 0.936
TREATMENT W7 + W9 vs W12 + W15	1	0.02057 13.017	2.7641 7.084	0.7749 6.228	
TREATMENT W7 + W15 vs W9 + W12	1	0.00022 0.141	0.0473 0.121	0.01457 0.117	
TREATMENT W7 + W12 vs W9 + W15	1	0.00313 1.983	0.0005 0.001	0.05362 0.430	
RESIDUAL	56	0.001580	0.3902	0.1244	0.2613
TOTAL	63				

APPENDIX TABLE 8.27A

TOTAL WHOLE BODY FAT-FREE WEIGHT, AND COMPONENT PERCENTAGES

AGE (Weeks)	TREATMENT	No.	TOTAL (log_{10} gms)	WATER	ASH	PROTEIN
			SUB CLASS MEANS			
7	OvX W4	8	2.064	75.275	3.952	20.759
7	Control	4	2.052	75.035	4.070	20.880
9	OvX W4	7	2.229	74.646	4.046	21.293
9	Control	8	2.155	74.044	4.390	21.551
12	OvX W4	8	2.357	73.805	4.324	21.854
12	Control	8	2.280	73.231	4.695	22.057
15	OvX W4	9	2.448	73.427	4.281	22.279
15	Control	12	2.325	72.417	4.941	22.627
			MAIN CLASS MEANS			
	OvX W4	32	2.281	74.250	4.158	21.577
	Control	32	2.237	73.355	4.633	21.997
7		12	2.060	75.195	3.992	20.799
9		15	2.190	74.325	4.229	21.431
12		16	2.318	73.518	4.509	21.956
15		21	2.378	72.850	4.658	22.478

APPENDIX TABLE 8.27B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	ASH	PROTEIN
		(MEAN SQUARES / F VALUES)			
TREATMENT	1	0.07427 46.528	5.390 15.631	2.050 23.304	0.797 3.753
AGE	3	0.2959 185.364	13.713 39.769	1.068 12.146	7.181 33.813
Linear W7 + W9 vs W12 + W15	1	0.8867 555.495	40.964 118.80	3.159 35.923	21.368 100.61
Quadratic W7 + W15 vs W9 + W12	1	0.04143 25.956	0.859 2.491	0.137 1.553	0.302 1.422
Cubic W7 + W12 vs W9 + W15	1	0.00048 0.301	0.0270 0.078	0.0137 0.155	0.0807 0.380
TREATMENT X AGE (INTERACTION)	3	0.00738 4.623	0.380 1.102	0.1901 2.162	0.0343 0.162
TREATMENT W7 + W9 vs W12 + W15	1	0.01999 12.524		0.495 5.629	
TREATMENT W7 + W15 vs W9 + W12	1	0.0004 0.250		0.0011 0.012	
TREATMENT W7 + W12 vs W9 + W15	1	0.00267 1.671		0.0492 0.559	
RESIDUAL	56	0.001596	0.3448	0.08795	0.2124
TOTAL	63				

APPENDIX TABLE 8.28 BODY LENGTH: MEANS AND ANALYSIS OF VARIANCE

AGE (Weeks)	TREATMENT	No.	LENGTH
	SUB CLASS MEANS		
12	OvX D2	8	21.556
12	OvX W4	8	22.281
12	OvX W7	7	22.421
12	Control	8	21.171
12	Oestrogen	7	21.500
15	OvX D2	8	22.500
15	OvX W4	9	23.411
15	OvX W7	6	22.783
15	Control	12	22.217
15	Oestrogen	7	21.879
	MAIN CLASS MEANS		
	OvX D2	16	22.028
	OvX W4	17	22.879
	OvX W7	13	22.588
	Control	20	21.799
	Oestrogen	14	21.689
12		38	21.777
15		42	22.551

ANALYSIS A

SOURCE OF VARIATION	Degrees of Freedom	F VALUE
TREATMENT	4	12.115
1) All OvX vs Control + Oestrogen	1	32.470
2) Control vs Oestrogen	1	0.000
3) OvX D2 vs OvX W7	1	6.327
4) OvX W4 vs OvX D2 + OvX W7 .	1	8.049
AGE	1	30.993
TREATMENT X AGE (INTERACTION)	4	1.388
Comparison 1) W12 vs W15	1	0.126
Comparison 2) W12 vs W15	1	2.414
Comparison 3) W12 vs W15	1	1.624
Comparison 4) W12 vs W15	1	1.624
RESIDUAL MEAN SQUARE	70	0.3726
TOTAL	79	

APPENDIX TABLE 8.29 BODY LENGTH: MEANS AND ANALYSIS OF VARIANCE ANALYSIS B

AGE (Weeks)	TREATMENT	No.	LENGTH
	SUB CLASS MEANS		
9	OvX D2	8	19.486
9	OvX W4	7	20.400
9	OvX W7	7	20.386
9	Control	8	19.493
12	OvX D2	8	21.556
12	OvX W4	8	22.281
12	OvX W7	7	22.421
12	Control	8	21.171
15	OvX D2	8	22.500
15	OvX W4	9	23.411
15	OvX W7	6	22.783
15	Control	12	22.217
	MAIN CLASS MEANS		
	Ovx D2	24	21.181
	OvX W4	24	22.156
	OvX W7	20	21.818
	Control	28	21.140
9		30	19.911
12		31	21.839
15		35	22.686

SOURCE OF VARIATION	Degrees of Freedom	F VALUE
TREATMENT	3	20.893
1) All OvX vs Control	1	33.051
2) OvX D2 vs OvX W7	1	16.275
3) OvX W4 vs OvX D2 + OvX W7	1	12.758
AGE	2	201.63
Linear (W9 vs W15)	1	390.19
Quadratic (W12 vs W9 + W15)	1	18.171
TREATMENT X AGE (INTERACTION)	6	0.780
Comparison 1) W9 vs W15	1	
Comparison 1) W12 vs W9 + W15	1	
Comparison 2) W9 vs W15	1	
Comparison 2) W12 vs W9 + W15	1	
Comparison 3) W9 vs W15	1	
Comparison 3) W12 vs W9 + W15	1	
RESIDUAL MEAN SQUARE	84	0.3117
TOTAL	95	

APPENDIX TABLE 8.30 BODY LENGTH: MEANS AND ANALYSIS OF VARIANCE ANALYSIS C

AGE (Weeks)	TREATMENT	No.	LENGTH
	SUB CLASS MEANS		
7	OvX D2	6	17.583
7	OvX W4	7	18.029
7	Control	6	17.583
9	OvX D2	7	19.486
9	OvX W4	7	20.400
9	Control	7	19.493
12	OvX D2	8	21.556
12	OvX W4	8	22.281
12	Control	7	21.171
15	OvX D2	8	22.500
15	OvX W4	9	23.411
15	Control	12	22.217
	MAIN CLASS MEANS		
	OvX D2	29	20.495
	OvX W4	31	21.224
	Control	32	20.523
7		19	17.747
9		21	19.793
12		23	21.691
15		29	22.666

SOURCE OF VARIATION	Degrees of Freedom	F VALUE
TREATMENT	2	19.231
1) OvX D2 + OvX W4 vs Control	1	15.480
2) OvX D2 vs OvX W4	1	22.218
AGE	3	284.625
Linear W7 + W9 vs W12 + W15	1	845.27
Quadratic W7 + W15 vs W9 + W12	1	39.366
Cubic W7 + W12 vs W9 + W15	1	0.728
TREATMENT X AGE (INTERACTION)	6	.655
Comparison 1) Linear	1	
Comparison 1) Quadratic	1	
Comparison 1) Cubic	1	
Comparison 2) Linear	1	
Comparison 2) Quadratic	1	
Comparison 2) Cubic	1	
RESIDUAL MEAN SQUARE	80	.3735
TOTAL	91	

APPENDIX TABLE 8.31 SKIN ALLOMETRY

EQUATIONS

Treatment	No.		Wet	Dry	Water	Fat	Ash	Protein
OvX D2	24	Slope	3.4158	3.6535	3.1828	3.4717	3.5325	3.8326
		Const	-280.064	-350.817	-271.918	-363.372	-483.504	-401.796
OvX W4	32	Slope	3.2810	3.8925	2.8921	4.3888	2.6536	3.6240
		Const	-263.895	-385.685	-234.087	-491.102	-368.634	-375.260
OvX W7	20	Slope	2.6011	3.5166	2.0083	3.7767	2.3410	3.3420
		Const	-172.467	-335.822	-114.778	-410.739	-325.293	-337.323
Control	32	Slope	2.6091	2.7904	2.4379	2.4839	2.1425	3.0501
		Const	-180.371	-244.167	-179.925	-243.718	-302.067	-303.483
Estrogen	14	Slope	3.7226	4.1050	3.4351	4.8310	4.7993	3.5449
		Const	-328.746	-418.100	-313.748	-556.300	-657.680	-367.536

ANOVA AND COMPARISONS
(F Values and Significances)

Source of Variation	DF						
Non Parallelism	4	7.264	5.461	4.143	3.808	2.761	3.831
All OvX vs Control + EB	1	* *	* *	+	*		*
Control vs EB	1						
OvX D2 vs OvX W7	1	*		*			
D2 + W7 vs OvX W4							
Slope	1	2208.07	1195.42	1108.36	303.69	284.58	2240.99
Position	4	31.465	14.015	33.877	7.184	NS	15.494
All OvX vs Control + EB	1	* *	* *		*		*
Control vs Estrogen	1						
OvX D2 vs OvX W7	1	*		* *			
D2+W7 vs OvX W4	1						
ERROR (MS)	113	7.8982	14.252	9.388	61.106	33.975	7.389

\+ P >.05 <.10
* P <.05
** P <.01

APPENDIX TABLE 8.32

CARCASS ALLOMETRY

EQUATIONS

Treatment	No.		Wet	Dry	Water	Fat	Ash	Protein
OvX D2	25	Slope	3.4633	3.7640	3.5678	4.1655	3.7932	3.6496
		Const	-237.410	-330.790	-266.354	-460.701	-413.458	-333.398
OvX W4	32	Slope	3.4434	3.6942	3.3421	4.0924	3.7383	3.5905
		Const.	-235.739	-322.443	-237.326	-449.701	-408.273	-326.784
OvX W7	20	Slope	3.9988	4.5783	3.7545	4.8427	4.3985	4.5124
		Const	-310.212	-440.499	-292.853	-548.563	-495.846	-450.491
Control	32	Slope	3.0040	3.4256	2.8226	3.7417	3.7999	3.2418
		Const	-178.610	-285.530	-170.635	-398.594	-413.105	-280.751
Oestrogen	14	Slope	3.6202	3.9482	3.4486	5.5409	4.4991	3.2217
		Const	-259.749	-352.598	-253.840	-630.109	-504.020	-277.386

ANOVA AND COMPARISONS

(F Values and Significances)

Source of Variation	DF					
Non Parallelism	4	6.440	3.642	6.544	NS	NS	5.187
All OvX vs Control + EB	1	* *		* *			*
Control vs EB	1						
OvX D2 vs OvX W7	1	+	+				+
D2 + W7 vs OvX W4	1						
Combined slope	1	4524.7	2508.1	3083.2	406.97	1878.6	2669.2
Position	4	2.507	5.162	6.897	10.314	9.736	NS
All OvX vs Control + EB	1	*		* *			
Control vs EB	1						
OvX D2 vs OvX W7	1	+	+				
D2 + W7 vs OvX W4	1						
Error (MS)	113	4.523	7.495	4.740	56.329	10.713	6.535

APPENDIX TABLE 8.33 WHOLE BODY ALLOMETRY

EQUATIONS

Treatment	No.		Wet	Dry	Water	Fat	Ash	Protein
OvX D2	24	Slope	3.4478	3.7242	3.4838	3.7970	3.7705	3.6987
		Const	-223.150	-309.330	-244.764	-378.902	-406.699	-326.475
OvX W4	32	Slope	3.4042	3.7534	3.2458	4.2365	3.6485	3.6004
		Const	-218.516	-314.826	-214.055	-439.694	-392.560	-314.641
OvX W7	20	Slope	3.6513	4.2595	3.3638	4.3341	4.2249	4.1924
		Const	-251.578	-382.488	-229.875	-452.704	-468.855	-394.055
Control	32	Slope	2.9145	3.2463	2.7453	3.1595	3.6678	3.1938
		Const	-155.757	-247.971	-150.696	-296.740	-392.136	-261.989
Oestrogen	14	Slope	3.6450	3.9922	3.4508	5.2687	4.5218	3.3166
		Const	-252.344	-345.162	-244.718	-572.882	-503.973	-277.374

ANOVA and COMPARISONS
(F Values and Significances)

Source of Variation	DF					
Non Parallelism	4	6.604	3.699	5.447	NS	NS	4.663
All OvX vs Control + EB	1	* *	+	* *			*
Control vs EB	1						
OvX D2 vs OvX W7	1						
D2 + W7 vs OvX W4	1						
CombinedSlope	1		2416.1	2986.6	390.46	1966.5	3264.1
Position	4	8.657	NS	14.894	NS	8.358	2.627
All OvX vs Control + EB	1	* *		* *			*
Control vs EB	1						
OvX D2 vs OvX W7	1						
D2 + W7 vs OvX W4	1						
ERROR (MS)	113	4.111	7.567	4.572	53.280	9.726	5.274

APPENDIX TABLE 8.34A

SKIN COMPONENTS AS % WHOLE BODY COMPONENTS

AGE (Weeks)	TREATMENT	NO	TOTAL	WATER	DM	FAT	ASH	PROTEIN
					SUB CLASS MEANS			
12	OvX D2	8	24.801	21.931	31.170	54.142	8.296	27.146
12	OvX W4	8	24.269	21.562	30.042	48.264	8.226	27.402
12	OvX W7	7	23.330	20.887	28.513	45.394	8.156	26.204
12	Control	8	22.194	19.997	26.716	41.664	7.644	24.860
12	Oestrogen	7	22.357	20.336	26.547	38.224	6.776	26.311
15	OvX D2	8	23.540	20.237	30.200	50.741	7.844	26.089
15	OvX W4	9	23.514	20.099	30.408	50.153	7.399	26.182
15	OvX W7	6	23.335	20.390	29.135	46.908	7.175	25.933
15	Control	12	21.991	19.590	26.688	42.778	6.901	24.394
15	Oestrogen	7	21.379	18.613	26.234	38.023	6.969	24.373
					MAIN CLASS MEANS			
	OvX D2	16	24.171	21.084	30.685	52.442	8.070	26.617
	OvX W4	17	23.869	20.788	30.236	49.264	7.788	26.756
	OvX W7	13	23.332	20.658	28.800	46.093	7.703	26.079
	Control	20	22.072	19.753	26.699	42.332	7.198	24.580
	Oestrogen	14	21.868	19.474	26.391	38.124	6.872	25.342
12		38	23.419	20.960	28.654	45.734	7.838	26.392
15		42	22.702	19.774	28.428	45.673	7.238	25.316

APPENDIX TABLE 8.34B

SOURCE OF VARIATION	Degrees of Freedom	(MEAN SQUARES / F VALUES) TOTAL	WATER	DM	FAT	ASH	PROTEIN
TREATMENT	4	17.572 23.234	7.5725 10.866	63.064 36.801	493.60 79.485	3.3939 3.616	14.353 10.068
1) All OvX vs Control + Oestrogen	1	62.397 82.501	27.965 40.127	213.71 124.711	1561.3 251.422	11.398 12.146	42.94 30.120
2) Control vs Oestrogen	1	0.4078 0.539	0.8263 1.185	0.7860 0.458	135.93 21.889	1.296 1.381	4.140 2.903
3) OvX D2 vs OvX W7	1	5.0218 6.639	1.4208 2.038	24.761 14.449	282.9 45.554	1.117 1.247	2.152 1.509
4) OvX W4 vs OvX D2 + OvX W7	1	0.2086 0.275	0.0100 0.014	2.357 1.375	0.0825 0.013	0.0323 0.034	2.147 1.505
AGE	1	7.9000 10.445	25.947 37.232	0.0820 0.048	0.6400 0.103	6.1251 6.527	19.023 13.344
Treatment x Age (Interaction)	4	1.0652 1.408	1.6925 2.429	1.4502 0.846	18.525 2.983	0.7651 0.815	1.5945 1.118
Comparison 1) W12 vs W15	1	0.0298 0.039	0.0110 0.158		0.9803 0.157		
Comparison 2) W12 vs W15	1	1.218 1.610	3.502 5.025		3.506 0.564		
Comparison 3) W12 vs W15	1	2.866 3.788	2.559 3.672		43.18 6.953		
Comparison 4) W12 vs W15	1	0.0423 0.055	0.3605 0.517		21.35 3.437		
RESIDUAL	70	0.7563	0.6969	1.7137	6.2100	9.3843	1.4256
TOTAL	79						

APPENDIX TABLE 8.35 A

SKIN COMPONENTS AS % WHOLE BODY COMPONENTS

AGE (Weeks)	TREATMENT	NO	TOTAL	WATER	DM	FAT	ASH	PROTEIN
					SUB CLASS MEANS			
9	OvX D2	8	25.141	22.009	31.830	54.376	8.787	26.496
9	OvX W4	7	24.420	22.023	29.659	48.393	8.610	26.244
9	OvX W7	7	26.230	23.884	31.481	49.306	9.427	28.459
9	Control	8	23.086	20.584	28.356	44.687	9.102	25.601
12	OvX D2	8	24.801	21.931	31.170	54.142	8.296	27.146
12	OvX W4	8	24.269	21.562	30.042	48.264	8.226	27.402
12	OvX W7	7	23.330	20.887	28.513	45.394	8.156	26.204
12	Control	8	22.194	19.997	26.716	41.664	7.644	24.860
15	OvX D2	8	23.540	20.237	30.200	50.741	7.844	26.089
15	OvX W4	9	23.514	20.099	30.408	50.153	7.399	26.182
15	OvX W7	6	23.335	20.390	29.135	46.908	7.175	25.933
15	Control	12	21.991	19.590	26.688	42.778	6.961	24.394
					MAIN CLASS MEANS			
	OvX D2	24	24.494	21.392	31.067	53.087	8.309	26.577
	OvX W4	24	24.030	21.148	30.067	49.010	8.028	26.607
	OvX W7	20	24.346	21.787	29.738	47.217	8.306	26.912
	Control	28	22.362	19.990	27.173	43.005	7.742	24.872
9		30	24.679	22.070	30.316	49.213	8.979	26.657
12		31	23.659	21.101	29.130	47.430	8.078	26.410
15		35	22.967	20.006	28.867	47.203	7.291	25.505

APPENDIX TABLE 8.35B

SOURCE OF VARIATION	Degrees of Freedom	TOTAL	WATER	DM	FAT	ASH	PROTEIN
		(MEAN SQUARES / F VALUES)					
TREATMENT	3	23.101 23.414	13.071 15.822	67.545 28.066	439.34 61.206	0.9236 0.925	19.451 13.162
1) All OvX vs Control	1	66.845 67.750	37.202 45.029	175.31 72.845	864.13 120.386		57.783 39.102
2) OvX D2 vs OvX W7	1	0.4172 0.422	1.170 1.416	20.028 8.321	376.59 52.464		0.904 0.611
3) OvX W4 vs OvX D2+OvXW7	1	1.658 1.680	1.657 2.005	1.903 0.790	22.422 3.123		0.191 0.129
AGE	2	20.555 20.834	31.979 38.708	14.826 6.160	29.005 4.041	21.355 21.391	8.8870 6.014
Linear (W9 vs W15)	1	41.312 41.872	65.535 79.324	23.451 9.744	37.390 5.208	42.742 42.813	17.279 11.692
Quadratic (W12 vs W9+W15)	1	1.386 1.404	0.0011 0.001	7.680 3.191	22.892 3.189	0.117 0.117	1.079 0.730
Treatment x Age (Interaction)	6	3.1063 3.148	3.9033 4.725	5.2800 2.194	19.7500 2.751	1.0114 1.013	4.3817 2.965
Comparison 1) W9 vs W15	1	1.665 1.687	6.587 7.973	1.174 0.487	0.788 0.109		0.146 0.098
Comparison 1) W12 vs W9 + W15	1	0.054 0.054	0.0491 0.059	0.283 0.117	7.561 1.053		0.979 0.662
Comparison 2) W9 vs W15	1	2.991 3.031	5.306 6.422	0.917 0.381	2.738 0.381		8.015 5.424
Comparison 2) W12 vs W9+W15	1	8.976 9.098	10.389 12.574	9.329 3.876	45.274 6.307		8.352 5.652
Comparison 3) W9 vs W15	1	4.577 4.638	1.276 1.544	19.026 7.905	57.932 8.070		5.007 3.388
Comparison 3) W12 vs W9 + W15	1	2.190 2.219	1.798 2.175	2.369 0.984	0.681 0.094		5.451 3.688
RESIDUAL	84	0.9866	0.8262	2.4067	7.1780	0.9983	1.4777
TOTAL	95						

APPENDIX TABLE 8.36A

SKIN COMPONENTS AS % WHOLE BODY COMPONENTS

AGE	TREATMENT	NO	TOTAL	WATER	DM	FAT	ASH	PROTEIN
					SUB CLASS MEANS			
7	OvX W4	8	24.662	22.610	29.456	47.902	9.887	26.670
7	Control	4	23.820	21.140	29.863	51.065	9.620	25.283
9	OvX W4	7	24.420	22.023	29.659	48.393	8.610	26.244
9	Control	8	23.086	20.584	28.356	44.687	9.102	25.601
12	OvX W4	8	24.269	21.562	30.042	48.264	8.226	27.402
12	Control	8	22.194	19.997	26.716	41.664	7.644	24.860
15	OvX W4	9	23.514	20.099	30.408	50.153	7.399	26.182
15	Control	12	21.991	19.590	26.688	42.778	6.901	24.394
					MAIN CLASS MEANS			
	OvX W4	32	24.188	21.513	29.915	48.733	8.493	26.623
	Control	32	22.559	20.146	27.509	44.013	7.977	24.923
7		12	24.382	22.120	29.592	48.957	9.798	26.207
9		15	23.709	21.255	28.964	46.417	8.873	25.901
12		16	23.231	20.780	28.379	44.964	7.935	26.131
15		21	22.644	19.808	28.282	45.939	7.114	25.160

APPENDIX TABLE 8.36B

SOURCE OF VARIATION	Degrees of Freedom	(MEAN SQUARES / F VALUES) TOTAL	WATER	DM	FAT	ASH	PROTEIN
TREATMENT	1	30.671 32.818	22.836 27.256	58.004 26.806	193.77 23.622	0.6732 0.553	37.214 25.621
AGE	3	6.146 6.576	11.628 13.879	4.218 1.949	44.017 5.362	18.662 15.326	2.5543 1.759
Linear W7 + W9 vs W12 + W15	1	18.150 19.420	32.400 38.669		71.046 8.657	55.426 45.517	
Quadratic W7 + W15 vs W9 + W12	1	0.125 0.133	0.0987 0.117		86.603 10.553	0.644 0.528	
Cubic W7 + W12 vs W9 + W15	1	0.0212 0.022	0.3205 1.827		0.0522 0.006	0.0677 0.055	
Treatment x Age (Interaction)	3	0.8607 0.921	1.127 1.346	12.602 5.824	73.067 8.900	0.9389 0.771	2.4170 1.664
Treatment at W7 + W9 vs W12+W15	1			36.915 17.059	202.29 24.651		
Treatment at W7 + W15 vs W9 + W12	1			3.368 1.556	45.707 5.570		
Treatment at W7 + W12 vs W9 + W15	1			0.117 0.054	5.550 0.676		
RESIDUAL (Error)	56	0.9346	0.8378	2.1638	8.2059	1.2177	1.4525
TOTAL	63						