A STUDY OF THE RESPONSES OF FOUR STRAINS OF MICE TO THREE DIFFERENT ENVIRONMENTS

BY

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Chapter I.

SCOPE OF THE INVESTIGATION.

Over the last few years a great deal of information has been obtained on the different mechanisims whereby small mammals maintain thermal balance in cold environments. The physiological adjustments that take place to acclimation (exposure to a constant temperature in the laboratory) and acclimatization to cold have recently been reviewed at the International Symposium on Cold Acclimation Fed. Proc. 22 No. 3 1964. These studies have been confined mainly to the white rat and the Norway rat exposed in the laboratory and outdoors to cold temperatures. Few attempts have been made to investigate genetic differences in the possible adjustments that take place on exposure to cold within any one species of mammal.

The amount of data concerning the mechanisms whereby small mammals adjust to high temperatures is small. Again, no attempts have been made using small mammals to investigate genetic differences in the response to high temperatures within any one species.

The present study was designed to investigate the possibility of differences in response of four strains of mice to high and low temperatures. The factors studied were:-

1. Body Temperatures.
2. Body weight and tail length and the relationships between these two factors.
3. Tail weight.
4. Hair weight.
5. Pelt weight.
6. Total body fat.
7. Abdominal fat.
8. Food intake.

Differences in the adjustments to temperature treatments in the four strains were considered likely to show as differences in some or all of these more easily measured factors.

If such differences were found to occur between the four strains this should then provide a basis for more detailed studies of their genetic and physiological basis.

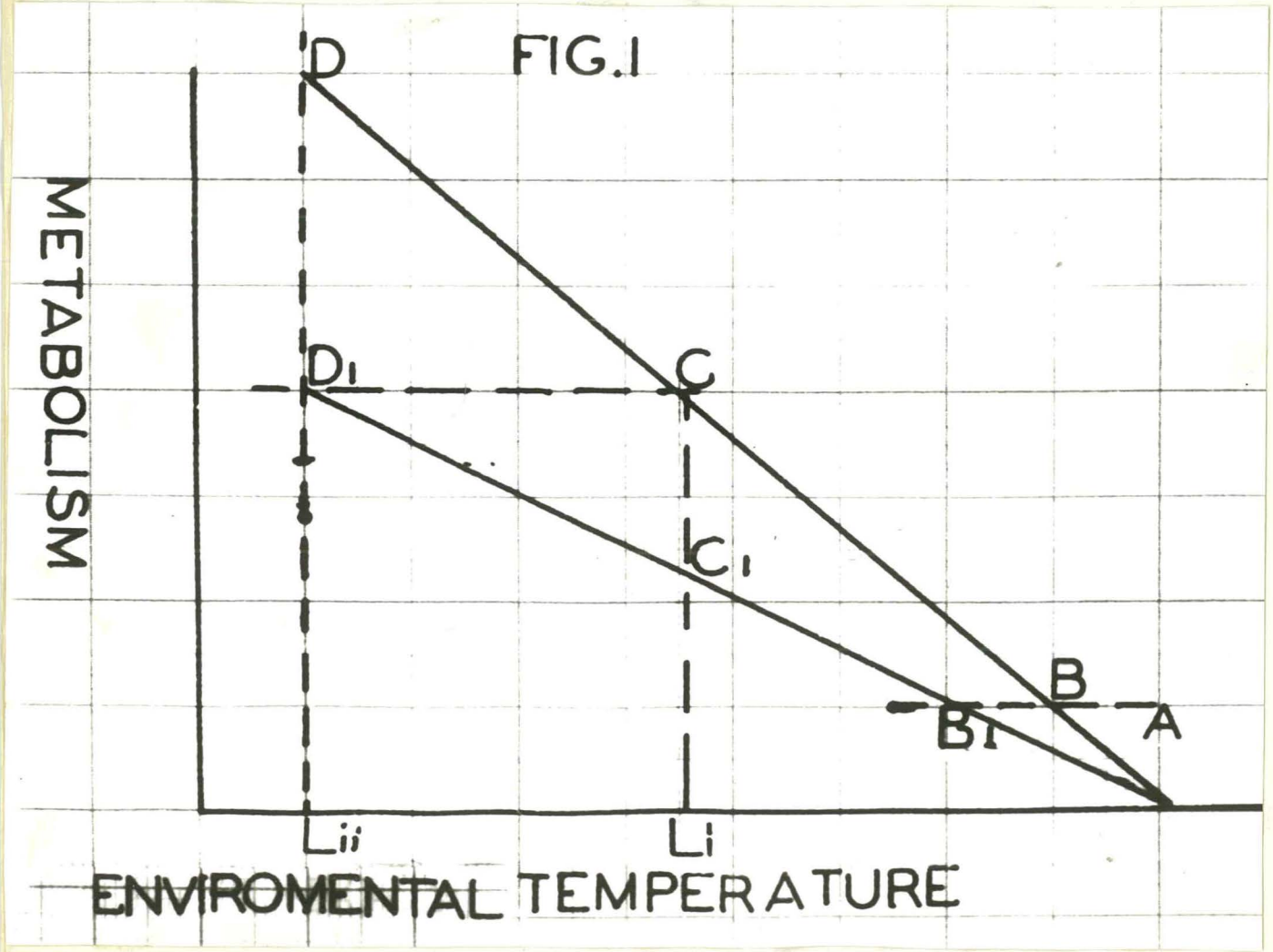


Figure 1: Metabolic VS. insulative acclimatization to cold.

Chapter II.

REVIEW OF LITERATURE.

A. INTRODUCTION.

Scholander etal. (1950) has shown that it is technically possible to have several types of adjustment to cold. This, Scholander etal. have illustrated by reference to Figure I.

In this figure the level of metabolism of an animal is plotted against the temperature of the environment. The body temperature is T at which temperature the metabolism is A. Initially, as the environmental temperature is decreased, the metabolism remains constant. The increased tendency to loose heat being compensated for by an increase in the effective insulation of the animal. At B the limit to this increase is reached and if the environmental temperature is lowered below this critical value, the body temperature can only be maintained by an increased rate of metabolism which then rises along line BC. At C the maximum rate of heat production is reached and so the temperature Li represents the lowest temperature tolerated by this individual.

If the maximum insulation that an animal is able to bring into operation is increased, perhaps as a consequence of acclimation to cold, then the body temperature can be successfully maintained, without an increase in the metabolic rate, down to the environmental temperature of Bi instead of only B. As the temperature of the environment is further lowered, the metabolism rises along the line BiCi.

In this example the maximum metabolic rate of the warm acclimated animal is taken to be the same as the maximum metabolic rate of the cold acclimated one. However, because of the greater insulation in the cold acclimated animal, it is able to tolerate a lower environmental temperature, Lii, than does the warm acclimated animal. Alternatively acclimation to cold could lower the minimum temperature tolerated without change in insulation by raising the maximum metabolic rate which is possible, that is, by extending the line BC to D. The latter adjustment, metabolic acclimation, does not involve a change in the slope of the line relating metabolism to environmental temperature nor does it affect the critical temperature. When insulative acclimation occurs, both the slope of the line and the critical temperature are altered.

The theoretical considerations of Scholander etal. (1950) show two possible ways that acclimation to cold can occur. In the review to follow, evidence will be cited that shows that both metabolic acclimation and insulative adjustments to cold can occur but that the type of acclimation depends to a large degree on the climatic condition prevailing.

The theoretical considerations of Scholander etal. (1950) for acclimation to cold can be extended to include the theoretical

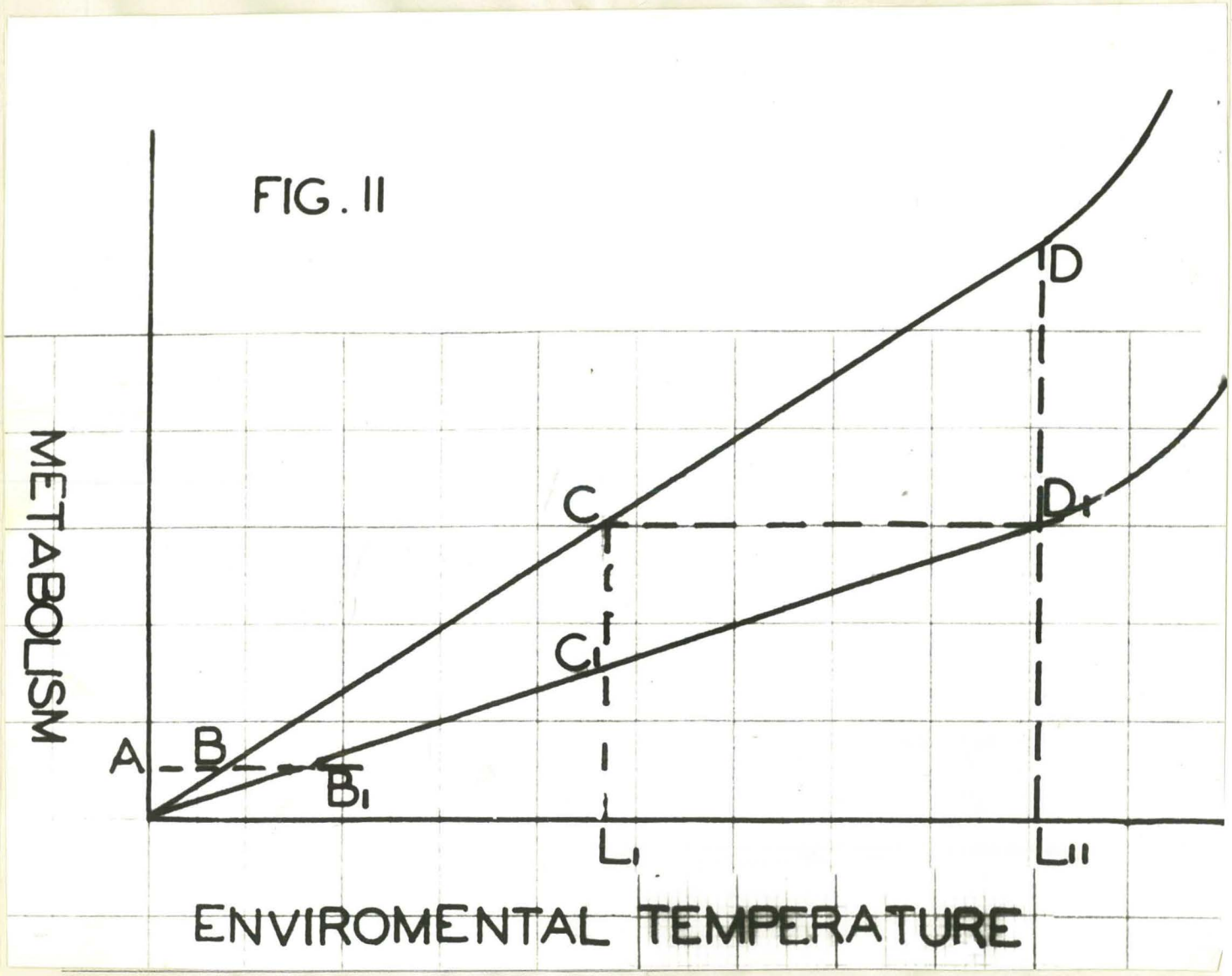


Figure II: Theoretical acclimation to high temperatures.

adjustment that may occur in a hot environment. This can be shown by reference to Figure II.

The line AB represents the thermal neutral zone for the animal at which the body temperature is T.Q at C the upper limit at which body temperature can be maintained at a normal level is reached and if the environmental temperature is raised above C then death will result as the animal cannot maintain a normal body temperature. Below the point C heat loss is sufficient to compensate for the additional heat produced by the increased metabolic rate. However, at temperatures above point C the avenues of heat loss are not sufficient to dissipate the additional heat and so body temperatures begin to rise causing a larger increase in metabolic rate and then this process is accelerated until death results. Therefore Li represents the maximum environmental temperature tolerated by this individual.

If the animal is able to extend the upper limit of the thermal neutral zone to Bi perhaps as a consequence of acclimation to heat then the metabolic rate will not start to increase until after temperature Bi is reached.

In this example the maximum metabolic rate tolerated by the unacclimated individual is taken to be the same as the maximum metabolic rate tolerated by the acclimated individual. Because the upper limit of the thermal neutral zone has been extended the warm acclimated animal can efficiently dissipate the increased heat produced up to a maximum environmental temperature of Lii.i.e. The metabolic rate rises along line Bi Ci to Di. If the avenues of heat loss in the unacclimated animal can be increased then it would be possible for the animal to tolerate the metabolic rate at D and therefore tolerate the environmental temperature Lii also.

Hart (1957) has pointed out that the amount of data concerning the responses, including acclimation to heat in small mammals is not very great. However, the experimental data concerning the adjustments made by small mammals to hot environments will be discussed in the review.

B. Physiological Aspects of Adaption to Cold.

I. Temperature Induced Changes.

Acclimation to cold has been demonstrated by Hart (1953), Sellers etal. (1951) and others, who have found that chronic exposure to cold led to a marked increase in the ability to survive at low temperatures. The energetics of this phenomenon were clarified when the oxygen consumption of the deer mice acclimated to temperatures of 10°C, 20°C, and 30°C were measured at-11°C and -22°C. (Hart 1953). Mice acclimated to 30°C, while showing an initial oxygen consumption equal to those acclimated at 20°C and 10°C, soon showed a fall in oxygen consumption and died. Those acclimated to 20°C maintained a high oxygen consumption for a longer period but ultimately died. Mice acclimated to 10°C maintained a higher metabolism for the duration of the test. When the oxygen consumption was examined at

different temperatures (-30°C to +30°C) absolutely no differences were observed in oxygen consumption in warm and cold-acclimated animals. The cold-acclimated mice, however, were able to respire at higher rates and at lower temperatures than intermediate or warm-acclimated mice.

Examination of the temperature metabolism curves for the white rat revealed an additional phenomenon not seen for deer mice. These curves showed that the heat production at 30°C as well as that at any lower temperature is greater for cold-acclimated animals (Heroux etal. 1959). Since the curve for the cold-acclimated rats is higher than and parallel to that for warm-acclimated rats the additional heat produced cannot be considered as contributing to the capabilities of the organism to increase heat production in response to a cold environment. It is the energy cost of acclimation that does not aid directly in survival in the cold, but does aid in maintaining the periphery at a warmer level (Heroux 1959).

The development of cold-acclimation requires a period of time, usually estimated to be from two to six weeks by various authors. During this time there is a gradual increase in cold resistance (Hart, 1953), an increase in food consumption (Cottle and Carlson, 1954, Sellers etal. 1954), and a reduced rate of growth (Cottle and Carlson, 1954, Heroux and Hart, 1954, and Sellers etal. 1954). There is also an elevation of peripheral temperatures of rats and rabbits (Heroux, 1959) a decrease in shivering thermogenesis and an increase in non shivering thermongenesis (Sellers etal. 1954, Hart etal. 1956 and Cottle and Carlson, 1956). Concomitant increases of vascularity of ears of rats (Heroux and St. Pierre 1957, Heroux, 1961), reduction in subcutaneous fat (Page and Babineau, 1953) and the general reduction of overall insulation in most species (Hart, 1957), signifying the wasteful aspects of metabolic acclimation in which metabolic energy is used in the establishment of enhanced peripheral heating.

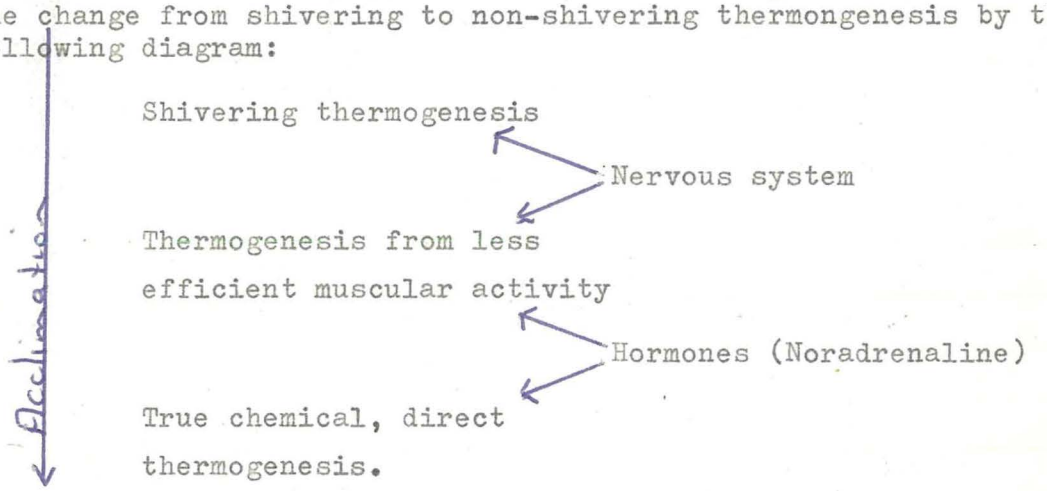
It is now well established that chemical thermogenesis (non-shivering thermogenesis) replaces the physical thermogenesis (shivering thermogenesis) during the process of acclimation to cold. According to Davies (1963), in the unacclimated rat exposed to cold, total cold-induced heat production consists of 50% shivering and 50% non-shivering thermogenesis. Throughout the period of acclimation to cold, total heat production remains unchanged but the 50% shivering heat production is gradually replaced by non-shivering heat production. Therefore by the end of the acclimation period total heat production is by non-shivering thermogenesis.

The site of origin of heat production by non-shivering thermogenesis in the acclimated animal is still a matter of contention. Sellers etal. (1954) observed a decrease in the electrical activity of the muscle of cold acclimated rats exposed to cold as compared to warm acclimated rats similarly treated. These observations were further extended by Heroux etal. (1956) who made simultaneous measurements of the total oxygen consumption and electrical activity of the muscle in anaesthetized cold-and warm-

acclimated rats exposed to cold. The cold-acclimated rats gave a greater metabolic response with hardly any muscle action potential being recorded. Cottle and Carlson (1956) showed that curarized (causing blockage of the nervous system) cold-acclimated rats not only could increase their oxygen consumption but could maintain almost perfect thermal balance when the environmental temperature was slowly lowered from 30°C to 6°C.

Much emphasis was placed by many workers on the observation of You and Sellers (1951) who showed that liver slices of rats acclimated to cold showed a greater in vitro oxygen consumption than did liver slices of rats acclimated to a warmer temperature. Weiss (1954) later confirmed this increase in oxygen consumption but also demonstrated that other tissues, the muscles in particular, showed this same response.

The work of Depocas (1958) on the eviscerated rat and Davis (1963) on denervated muscle in the dog suggests that muscle in its non-contracting state is an important contributor to non-shivering thermogenesis. Denervation experiments with rats (Hart and Jansky 1963) and dogs (Davis 1963) suggest that non-shivering thermogenesis is primarily mediated at the local site by humoral means. This, according to Davis (1963) does not exclude the neurohumoral mechanisms, which may be operative at the more proximal sites. Chatonnet (1963) has illustrated the processes involved in the change from shivering to non-shivering thermongenesis by the following diagram:



A lot of evidence has accumulated over the last few years which tends to confirm that noradrenaline is the mediator of non-shivering thermogenesis. Hseih and Carlson (1957) showed that cold-acclimated rats were remarkably sensitive to the calorigenic action of noradrenaline. This report was confirmed and extended by Depacos (1960), Hannon and Larson (1961), Johnson and Sellers (1962) and Schonbaum etal. (1962). Despite the rather obvious implication of this noradrenaline action in the cold-acclimated animal, relatively little is known concerning the anatomical site or the biochemical mechanism which supports the increased rate of energy metabolism. The only information concerning the possible site of the added heat production is found in a report by Depacos (1960), which showed that noradrenaline calorogenesis is accompanied by a very rapid rise in liver temperature. Indirectly

the data on normal rats reported by Schoty and Page (1959) would suggest that noradrenaline might exert its calorogenic action through a mobilization and subsequent oxidation of non-esterified fatty acids. Reports by Hannon and Larsen (1961) and Schonbaum etal. (1962) appear to confirm this report. Hannon etal. (1963) presents evidence which suggests that noradrenaline is probably not the sole mediator of non-shivering thermogenesis, although they suggest that it would seem reasonable to expect it to act in conjunction with the other neurohumoral factors to affect such mediation. Their work further suggests that the calorigenic action of noradrenaline predominates during the transition stage from one metabolic state to another. Thus it might serve as an emergency mechanism to raise the metabolic rate quickly under conditions of severe cold stress.

II. Seasonal Induced Changes.

Most of the studies on physiological adaption to cold have been carried out on the laboratory white rat, which has been shown to be much more sensitive to stressful conditions than its ancestor, the wild Norway rat. The studies have also been carried out under very severe conditions, i.e. under continuous individual exposure to a constant low temperature without protection.

Heroux and his group have over the last few years been conducting experiments to see whether the metabolic type of cold-acclimation found under laboratory conditions really are a universal mechanism and whether under more normal conditions such as those found outdoors in nature and in animals more resistant to stress (e.g. the wild Norway rat), the same type of structural and endocrine adjustments accompany cold-acclimation.

Heroux (1963) has recently reviewed their results. It was shown that under all conditions (outdoor exposure and climate chamber exposure in groups and as individuals) in both wild rats and white rats, cold adaption was achieved by:-

I. the ability to maintain heat production at a higher rate and therefore survive a longer time than the controls under extremely cold conditions.

II. the ability to produce heat without shivering or at least with a reduced degree of shivering.

III. a greater sensitivity to noradrenaline, as shown by greater metabolic reactions to a single intramuscular injection of noradrenaline.

These adjustments took place whether the pelt insulation was increased or not. In both individually or group exposed white rats, the pelt insulation remained unchanged after three months exposure to a constant low temperature in a cold room. During the winter, however, in both white rats and wild rats exposed to the natural fluctuating environmental condition prevailing outdoors, the pelt had a greater insulative value, than during the summer.

Protein depots, as revealed by total body growth and muscle mass, and fat depots (abdominal and subcutaneous) were always reduced and visceral organs always enlarged, to different degrees, in the white rats, whether exposed to cold indoors or outdoors, individually or in groups. Outdoors the white rats showed some exceptions. The fat depots were not significantly reduced and the heart and intestinal tract were not significantly hypertrophied. None of these adjustments took place in wild rats during the winter except the heart which was heavier than during the summer. Indeed, fat depots were even larger in winter in wild rats than in summer.

From this work Heroux (1963) concludes that these responses were therefore specific to the white laboratory rat or they were related to the special caging conditions under which they were kept. In any case their absence in cold-acclimated wild rats and their partial absence in white rats acclimated outdoors reveals that they are not essential for non-shivering thermogenesis.

III. Behavioural Adaption in the Cold.

Barnett (1959) has stressed the importance of behavioural changes in the adaption of mice to low temperatures. He showed that mice kept in the cold (-3°C) built much better nests than mice kept in the warm. Also the importance of experience of the cold was illustrated by the behaviour of mice taken from their parents at 21 days of age. Those mice brought up in the cold immediately burrowed into the cotton supplied as nesting material in their new cage, but those reared in the warm room often remained on the surface of the cotton wool for sometime.

Heroux (1963) in the discussion on Chevillard etal. (1963) paper stresses that in the cold the rat curls up its tail and sits on it, whereas in the hot room the tail is kept extended. Heroux suggests that this might be a behavioural adaption by which the animal conserves heat in the cold.

C. Effects of Cold on Growth and Metabolism.

I. Body Growth.

(a) Temperature Induced Changes.

Rats and mice individually exposed to temperatures below 0°C steadily lose weight but the loss is greater in animals previously acclimated to low temperatures than it is in warm acclimated controls. (Hart and Heroux 1956). These authors showed that white mice acclimated to 6°C had a body water content that was higher on an absolute basis and a fat free basis than that of mice acclimated to 25°C. The greater loss in water of cold-acclimated rats on exposure to freezing temperatures -7°C was shown to be due to the greater loss of water in these mice than in the warm acclimated animals. On the other hand, mice exposed in groups, on nesting material, gain weight at -3°C although more slowly than the controls kept in a warm environment (Barnett and Manly 1956). At temperatures varying from 0°C to 5°C for rats and above 5°C for guinea pigs, there is usually an initial loss in weight during the first 10 days of exposure, but as food intake is increased the animals begin to gain weight but at a slower rate than their controls (Sellers etal. 1954, Baker and Ashworth 1957, Young and

Cook 1955, Baker and Sellers 1957, and Cockrem 1964).

Cottle and Carlson (1954) have shown that the extent of this initial effect of cold on body weight depends upon the age of the animals when exposed. They showed that young rats placed in the cold (5°C) were apparently able to increase their food intake to a level sufficiently high to meet their increased needs without immediate change in their growth rate. A drop in growth rate occurred after 1 week in the cold. Older rats did not increase their food intake sufficiently to prevent weight loss until after approximately 7 days in the cold. The calorimetric studies made by these authors showed that during this period the energy production of these rats increased considerably. At temperatures above 5°C but below 20°C, rats and mice do not lose weight initially but grow at a slower rate then their controls. (Barnett and Manly 1956, Heroux and Hart 1954, Biggers etal. 1958). On at least two different occasions it has been reported that small homeotherms grew at a faster rate at low than at high temperatures. In one case rats were in groups of 20 at 18°C and the controls at 28°C. (Herrington and Melbock 1942). In the second case Deer mice were apparently in individual cages but they were maintained in complete darkness. The cold mice were maintained in the laboratory and were signigicantly heavier than those maintained under high temperatures. (Sealander 1951). According to Sealander mice exposed to cold will gain weight faster under constant darkness than when exposed to normal photoperiod.

At least in white mice exposed to sub-zero temperatures, the drastic loss in weight has been shown to be mainly due to the utilization of the fat reserves with some protein utilization (Hart and Heroux 1956). On the other hand the reduction of growth rate in rats exposed to above zero temperatures is mainly due to a reduced protein disposition in muscle (Heroux 1958). There is also a reduction in fat reserves but as Page and Babineau's (1953) showed, this accounts for less than 30% of the total body weight difference between controls and cold exposed rats.

(b) Seasonal Changes.

When exposed to outdoor environment conditions in groups of ten, white rats grew at a slower rate during the winter than during the summer (Heroux and Campbell 1959). Sealander (1951) found just the reverse in wild deer mice acclimated outdoors during summer and winter: the winter animals averaging 2.5gms. heavier in body weight than summer animals. Whereas Heroux and Campbell (1959) exposed their mice to the natural photoperiod prevailing outdoors during both seasons, the wild mice were kept in almost complete darkness at all times. Because of this fundamental difference in environmental conditions these results are not really comparable.

The mean weight of the wild mice (Pl. nove-baracensis) captured at random during the summer and winter did not differ significantly (Sealander 1951). Heroux (1961a) has found a similar result with wild Norway rats captured in summer and winter. It is possible that an age difference may exist between the wild

rats and mice captured during the summer and winter months and this may have influenced body weights in the two groups.

II. Metabolism and Carcass Composition.

Fowler (1958, 1962) has made a complete study of growth, carcass composition and food utilization from birth to maturity in mice selected for large and small body size. She used both the N strain (Falconer, 1953) and the C strain (Falconer and Robertson, 1956). The N strain was derived from a four way cross of inbred lines; CBA females were crossed to R111 males and C57 females to A males, and reciprocal crosses were made of the F1 progeny. This foundation population (generation 0) was regarded as random bred, and two lines were selected from it. Selection for large and small body size was based on body weight at 6 weeks of age. Individuals were selected on deviations from their litter means, selection therefore being entirely within litters. Litter size was standardized as far as possible to eight mice. Six to nine pair matings within each line represented one generation, and the rate of inbreeding was kept to a minimum by the choice of least related pairs.

The C strain was obtained by crosses between Whitehead's high lactation line, a line selected for large size after a cross of MacArthurs with Goodale's large lines, and two stocks carrying a variety of mutant genes and having the inbred line C57Bl/Fa in their ancestory. Selection was then carried out and a large line and a small line were selected. Selection was again within litters, but was based on weight gain from three to six weeks of age. Twelve pair matings constituted one generation to generation 15, the number being reduced to 6 pairs after this.

Fowler's (1958) results for the N strain have shown that selection for large and small body weight at 6 weeks of age, has altered both the total amounts of protein, water and fat in the carcass and also the percentage composition of the carcass. The percentage composition of mice of large and small size was fairly similar during growth up to 35 days of age i.e. when most of the increases in weight in both lines could be attributed to protein deposition and bone growth. There was considerable increase in the amount of carcass fat in mice of the large line from 35 days onwards however, which resulted in a lower percentage of protein and water and a higher percentage of carcass fat in the large line than in the small line after this age.

In most animals a period of protein deposition and skeletal growth is followed by a further increase in weight which can mainly be attributed to deposition of fat. Mice of the small line apparently fail to reach the stage of fat deposition but continue protein depositions and skeletal growth to advanced ages.

Fowler (1958) compared mice of the large and small lines during the period of protein deposition and skeletal growth and found that when body weights are similar the proportion of fat is slightly higher and protein and associated water slightly lower in the small than in the large line. Some of the differences in

carcass composition can therefore be attributed to different growth rates, so that at any absolute age each line being at a relatively different stage in development (i.e. different physiological age) will have a different carcass composition.

In the C strain body composition of the large and small lines were the same at 6 weeks of age while at 12 weeks of age the differences in percentage of fat and water in the carcasses remained small (Fowler 1958). Thus, whereas in the N strain selection has altered the carcass composition, selection in the C strain appears to have altered the rate of deposition of protein and fat and had little effect on the fat percentage composition of the carcass.

Fowler (1960) found that mice of the large line (N strain) consumed more food and utilized it more efficiently during the period of most rapid growth than do mice of the small line. From this Fowler has concluded that the efficiency of food utilization is evidently capable of modification by selection, changes in efficiency and appetite being associated with changes in growth rate. Similar associations have been found when selecting for efficiency of food utilization in rats (Morris etal. 1933, Palmer etal. 1946) and for rats and economy of gain in pigs (Dickenson 1947). The greater efficiency of large mice up to approximately 5 weeks of age indicated that the energy required for maintenance was low per unit food consumed when the growth rate was high. With increased size and decreased growth rate gross efficiency would be expected to decline for maintenance costs will increase in comparison to weight gain per unit time.

Fowler (1960) showed that the large line absorbed a greater proportion of protein (5%) from the food but that this increase was not associated with the increased efficiency of food utilization. The maximum increase in weight of the small mice which would result from a 5% increase in digestibility of protein was calculated by Fowler to be 3.1grms. and this was insufficient to account for the large weight differences between the two lines. Fowler has concluded that the main difference between the two lines was in the amount of food consumed.

In the N strain the energy expenditure of the large mice was greater than that of the small mice. This is to be expected since increased size is associated with increased maintenance costs. When animals of similar weight were compared energy expenditure of the large line was similar to that of the small line (Fowler 1960). The greatest energy metabolism of the large mice must have caused an increase in their growth rate since energy metabolism and growth rate are known to be correlated (Brody 1945). Fowler (1960) also found a positive correlation between energy metabolism and growth rate when metabolism was expressed as surface area ($W^{0.72}$).

Barnett etal. (1959) has shown that rearing mice in the cold increases the heat production by as much as 300 to 400 percent above that of controls but that the response depended to some extent on strains.

Barnett's mice in the cold had been born and reared in the cold from stock that had been maintained in the cold for many generations and were therefore fully adapted to the experimental conditions. These cold adapted mice were compared to mice which were born from stock maintained in the warm. The data on heat production and body fat therefore, relates to a permanent state and not as in most investigations, to a response to a short period of cold stress.

Barnett etal. (1959) results can be compared with those of Hart (1950 and 1952). Hart's results are expressed in terms of calories/mouse but the mean body weight is given as 27gms. From this data it can be shown that the heat production of Barnett etal. mice and Hart's mice at 25°C is very similar. At 3°C, however, Harts estimate of heat production is lower than that of Barnett etal. Using this comparison Barnett etal. (1959) have concluded that exposure of mice to cold causes a similar increase in metabolism in both mice born and reared in the cold and mice born and reared in the warm and then exposed to cold.

The maintenance of the enhanced metabolism of mice and rats in the cold, is usually accompanied by a lower body weight at all ages from 3 to 6 weeks and there is also a reduction in body length (Barnett etal. 1959); but the main source of the decrement is the failure to store fat. In their low fat content, mice and rats in the cold resemble domestic animals on a low plane of nutrition. (Hammond 1947). Widdowson and McCance (1960) and Dickerson and Widdowson (1960) have investigated some of the effects of accelerating and retarding growth in rats. These authors were able to establish a divergence in growth rate as early as one week of age by varying the number of young suckled by the dam (i.e. 3 pups for high growth rate and 15-20 pups for low growth rate). The divergence in growth rate was present all through the life of the animals. However, the percentage of fat in the body was the same whether growth had been accelerated or retarded by the time the animals reached an age of 170 days. This was not so at lower body weights however, as the high plane rats put on much more fat early in life than did the low plane rats. When the percentage of fat was related to chronological age however, the rats growing rapidly had much higher percentage of fat in their bodies than the slow growing ones.

The situation with cold exposure however, is somewhat different than the results of varying the plane of nutrition. Most rats in the cold consume much more food than do mice and rats in the warm, but are unable to utilize the extra food to lay down adipose tissue because of the large demands for heat to maintain their body temperature constant.

Both Hull (1960) and Falconer (1960) have shown a large positive correlation between the amount of abdominal fat (fatty tissue deposited in the mesenteries of the abdomen and attached to the testes or uterus) and total ether extractable fat in the mouse carcass. The weight of abdominal fat, has been used by Hull (1960) as an index of carcass composition in a study of genetic relations between carcass fat and body weight in mice.

It is well known that the proportion of fat in the mouse carcass varies according to the age of the animal and that there is an absolute and relative increase in fat with age. Fenton (1956) has shown that the fat free weight (chiefly protein, water and ash) increased sharply in young mice and that this sharp increase continued up to 2 months of age when the increase was at a much slower rate. The fat content of the carcass increased linearly with the age of the animals. Consequently the total carcass weight rose sharply during the first 2 months of life, owing largely to the rapid increase in protein, water and ash. Between 2 and 5 months of age, total body weight continued to increase markedly. This was due principally to the gain in fat. At 3 weeks of age, fat accounted for 12% of the body weight while at 5 months it represented 28% of the total.

When carcass fat is plotted against fat free weight the data for the I strain and young C57 mice fell on a straight line of slope of 0.1 and 0.13 respectively. The data for older, heavier C57 animals fell on a straight line of much greater slope (0.8), corresponding to the rapid increase in carcass fat and the relatively slow increase in the fat free component.

III. Strain Differences in Responses to Cold.

Barnett etal. (1959) have shown that the heat production of mice kept in the cold is increased but that the size of this response depends on the strain of the mice. Thus exposure to -3°C with nesting material cause heat production to be increased by 432% over control in the A2G males and 322% in the C57BL males. This strain difference in heat production in the cold was also shown to be present in the control environment but in this case the C57BL males had a mean heat production some 20% above that of the A2G males. These results are shown in Table I.

	21°C.	-3°C.
A2G	1.03*	4.47*
C57BL	1.24*	4.00*

* Cals/100grms./hr.

Metabolic responses of two strains of mice to two different environments.

Barnett etal. (1959) were also able to show differences between strains in the body weight response to cold. They showed that the A2G males reared in the cold were substantially lighter than the controls whereas the C57BL males reared in the cold had a body weight very similar to the controls. The C57BL mice were lighter than the A2G strain in the control environment but heavier than the A2G males in the cold environment.

With adipose tissue Barnett etal. (1959) showed that in the cold the A2G strain has less adipose tissue than the controls whereas the C57BL strain showed no difference in the amount of

adipose tissue between the cold reared and control reared mice. There was also a significant reduction in extractable fat in the A2G strain when exposed to -3°C but no differences were found in the C57BL strain exposed to -3°C and 21°C. Thus in the C57BL males there was little or no change in total growth on cold exposure and total body fat remained unaltered when compared to control mice reared at 21°C. Their heat production in the warm environment was higher than that of the A2G males but in the cold it was lower. From these results Barnett etal. (1959) has concluded that the ability to lay down substantial amounts of fat is irrelevant to the ability to adjust metabolically to -3°C provided that there is an uninterrupted food supply and a supply of bedding materials.

D. Body Growth and Metabolism in a Hot Environment.

While there is a large amount of literature dealing with the responses, including acclimation of humans to heat, data concerning the smaller mammals are scanty as Hart (1957) notes.

It is now well established for man that the ability to withstand high temperatures (temperature above the thermally neutral temperature) is determined in part by previous experience at high temperatures. Thus acclimation to heat in man does occur. (Prosser 1958). This acclimation is manifested for example by the maintenance of lower body temperatures, lower pulse rates, and readier sweating under standard exposures, conditions and activities of heat stress.

As far as heat production is concerned, Bazzett (1949) was satisfied that acclimation to high temperatures in humans results in a reduction of the amount of heat produced. This is difficult to establish as Bazett points out, because total heat production includes that from muscular exercise and an individual transferred to a hot environment typically lowers his total heat production by reducing voluntary movement to a minimum.

According to Beattie and Chambers (1953) the basal heat production of rats exposed to heat is reduced and the reductions may persist for several months. Prosser (1958) has observed that both deer mice and lemmings greatly decrease voluntary activity at high temperatures which decreases the heat load of the individual.

Barnett (1956) has shown that in a warm environment mice minimize the use of such heat conserving devices as huddling and thick walled, well built nests.

Fleischner and Sargent (1959) showed that heat (35°C) retards the growth rates of male rats compared to controls at 24°C. They showed that growth was retarded for about the first 20 days of exposure and then proceeded at a rate comparable for controls up to at least 50 days of exposure.

Young and Cook (1955) exposed female and male rats to 35°C for a period of 4 to 5 months respectively and at the end of the exposure period no difference was found in bodyweight between the control

animals at 24°C and the heat exposed animals.

Factors responsible for the differences between the results of Fleischner and Sargent (1959) and Young and Cook (1959) could be:

1. Length of exposure period.
2. Age of rats when first exposed.
3. Strain differences.

Fleischner and Sargent (1959) exposed young (7 - 8 weeks of age) female Holtzman rats to 35°C for 30 days whereas Young and Cook (1959) exposed rats of the Long Evans strain at 5 months of age to a temperature of 35°C for a period of 120 to 150 days.

At 5 months of age the rat is nearly at its mature weight and thus protein deposition and skeletal formation are nearly completed, whereas in the young rat of 7 - 8 weeks of age these processes have yet to take place. This is illustrated by the percentage weight gain of the two lots of rats in the hot i.e. Fleischner and Sargent (1959) 67%, Young and Cook (1959) 13%.

Harrison etal. (1959) has shown that mice in the heat grow more rapidly than controls mice when the animals are small, i.e. less than 4 weeks of age. This is contradictory to the results of Fleischner and Sargent (1959) who have shown that in young rats the growth rate is retarded at least during the first 20 days of exposure. However, Harrison etal. (1959) have shown that the magnitude and direction of the response to heat depended upon the genotype of the animals, but that rearing of both inbreds and hybrids at a high temperature always promoted early growth. During the period of sexual maturity, the growth rate of all the heat reared inbreds and hybrids was slower than that of the control animals and by 8 weeks of age their weights were nearly equal to, or smaller than, those of the control animals.

Both Fleischner and Sargent (rats)(1959) and Young and Cook (1959) (rats and mice) have shown that exposure of rats and mice to high temperatures (35°C) will cause feed intake to be reduced by as much as 25% below that of the control animals.

Young and Cook (1959) made body lipid measurements on their mice and rats and found that exposure to heat caused little change in the total amount of fat in the carcass and these differences were not significant when compared with the controls.

3. Skin and Hair of Mice in Cold and Hot Environments.

I. Cold Induced Changes.

Great emphasis has been laid on the importance of the insulation provided by the skin and hair of homeotherms, in enabling them to withstand a cold environment. Scholander etal. (1950) have shown that mammals and birds of the Arctic have a body

temperatures and metabolic rate similar to those found in tropical species. The main adaption to cold in Arctic species is the additional insulation conferred by fur, skin and blubber. The ability of these species to maintain a constant body temperature in a wide range of environmental temperatures, including quite high ones, is attributed to vascular mechanisms which can bring about great variations in heat loss from the surface. Barnett (1959) has described experiments which refer to adaption of individuals,(in this case all very similar genetically within each of Barnett's three strains of mice) to a particular environment during their own life time. A further difference between the work of Barnett 1959) and Scholander (1950) lies in the size of the animals studied. Mice (Barnett (1959)) are much smaller than the Arctic animals studied by Scholander etal. (1950) and body size has an important influence on the possibilities of developing a thick fur or skin. The insulating qualities of the mammalian integuments are proportioned to their thickness, regardless of the size of the animal (Barnett 1959). Kleiber (1961) pointed out that a mouse weighing 60gms. with the same metabolic rate as that of a steer would need a covering 20cm. thick to maintain its body temperature in an environment at about 3°C.

Barnetts (1959) work showed that even with the rather meagre covering that they have, mice carry around some 14% of their body weight in the skin. Barnett (1959) found that the skin itself, as distinct from the hair, was thinner in mice born and reared in the cold (-3°C) than in mice born and reared in the control environment (21°C). However, the weight of the hair was significantly heavier in the mice kept in the cold than in the controls. From these results Barnett concluded that the skin, excluding the hair, provided less efficient thermal insulation in the cold than in the warm environment. The hair in the cold, however, evidently provided a slightly improved cover in all three strains in the cold. The results of Barnetts were confirmed by measuring the thermal insulation on skins of mice of the three strains by placing the skins over a hot plate and measuring the rate of heat flow through the skin and hair. These studies showed that the insulation provided by the skin plus hair was significantly greater in mice kept in the cold. Barnett also showed that ruffling the hair significantly increases the effective insulation of the skin and hair.

Barnett (1959) has concluded that in the control environment there is appreciable heat loss from the skin due either to convection resulting from the vascularization of the dermis or to evaporation of water, but that in the cold these effects are negligible.

These conclusions of Barnetts (1959) conflict with the results obtained by Heroux (1959b). Heroux (1959) showed that exposure of rats to cold for 4 weeks increased the number of capillaries in the cars by a factor of 12 over that of controls kept in warmth. Heroux (1959) suggests that the increase in vascularization was due to the need to keep the peripheral tissues warm to stop cold injury. Heroux (1959) has also shown that peripheral temperatures are elevated when rats are exposed to continuous cold, at least during the period of acclimation.

Barnetts (1959) results, however, are for mice born and reared in the cold and this could account for these differences.

Pelt weights have been reported by Heroux and Bridgeman (1958), Hale etal. (1959), Hale etal. (1959), Barnett (1959), and Scholander (1951). When expressed as a regression of pelt weight and body weight or as a percentage of the total body weight, no change was found in the pelt weight of cold exposed rats or mice. The interest attached to the pelt in thermoregulation is in its insulative properties, which can hardly be deduced from its weight. The weight of hair, which provides most of the insulation (Heroux 1961a) represents only 10% of the total pelt weight at least in mice (Barnett 1959). Moreover, an increase in the weight of hair may be counterbalanced by a loss in weight of subcutaneous fat.

Hair weights have been shown to be increased in prolonged cold exposed animals when compared to control animals in both rats (Heroux 1961) and mice (Barnett (1959)). In rats the increase was of the order of 18% and in mice it was 9%, 18% and 29% in three different strains.

The number of hairs has been shown to be greater in mice (Scolander 1951) and rats (Heroux 1961a) acclimated to cold than in controls. An increase in insulation would be expected from an increase in the number, if not in the weight of the hairs, but Barnett (1959) results as discussed above are the only results which suggest an improvement in insulation properties. Hart (1953) using mice and Heroux etal. (1959a) using rats, have been unable to show any increase in insulative properties. Barnett's mice have been kept in the cold for many generation now and it appears likely that selection may have taken place for mice of greater pelt/ge insulation.

One means by which the total number of hairs could be increased is by a decrease in the amount of fibre shedding during the hair cycle with the club hair remaining in the follicles during the growth of the new hair.

The weight of hair grown by animals such as rats and mice which have regular hair cycles must be influenced to some degree by factors that influence the hair cycle and should be considered in discussions on hair growth in these animals. Cockrem (1959) has shown that methoxamine hydrochloride (a sympathomimetic agent) caused the start of the 2nd hair cycle to be delayed and the cycle to be prolonged. The treatment also decreased the weight of hair grown by the mice when compared to controls.

Cold is considered to be a vasoconstriction agent and what effect this has upon the hair cycle is not known. However, exposure of mice to cold will, in the short term at least, probably cause the particular hair cycle to be prolonged and thus the weight of hair grown within a certain period of time would be reduced. With prolonged exposure to cold Heroux (1959) has shown that peripheral temperatures are raised and thus may have an effect on the hair cycle.

Heroux (1960) has investigated the effect of environmental temperature on the mitotic activity of the skin of warm and cold acclimated rats using the colchicine method. He was able to demonstrate that cold caused a reduction in the number of cells entering mitosis and that the duration of mitosis was increased. Heroux (1959) has shown that after 3 weeks cold exposure, mitotic activity of the ear skin of rats is resumed after having been stopped almost completely. From these two studies Heroux (1960) has concluded that the adaption of mitotic activity to cold that takes place in this tissue is one that allows the cells to overcome the mitotic blocking effect of cold. There is no evidence as to the possible nature of this mitotic blocking effect of cold. The blocking of mitotic activity in the skin may be due to vasoconstriction in the skin. Once this vasoconstriction has been overcome then mitotic activity can then start again. Heroux (1959) has shown that peripheral temperatures are higher in the skin and the skin has a higher number of capillaries in cold acclimated rats than in warm acclimated animals. Therefore the development of these effects could cause the mitotic activity to be re-commenced.

II. Seasonal Induced Changes.

Acclimatization to winter conditions in small mammals is often associated with an increase in pelt age insulation. White footed mice (Sealander 1951) have been shown to have a greater pelt weight and deer mice have dense fur and better pelt age insulation during winter than summer. (Sealander (1951) and Hart and Heroux (1955)). A similar increase in pelt age insulation, as measured by the hot plate technique, has also been found in white laboratory rats exposed in groups to the outdoor winter environmental conditions (Heroux etal. 1959) although pelt weights of summer and winter rats were not different (Heroux and Campbell 1959).

Sealander (1951) has shown that the number of hair follicles per unit area of white footed mice increases in winter. This was not due to an effect of body weight as weights were similar in the winter and summer mice. It is likely then an age effect could be operating here in that the summer mice could possibly be younger than the winter rats.

Hart (1956) has carried out a survey of seasonal changes in fur insulation in various Canadian mammals. This study showed substantial changes in insulation and thickness of fur in the larger mammals such as red fox, wolverine, wolf, bear. In smaller mammals, such as mice, lemmings, red squirrels, muskrat and hare, winter changes were much smaller. Hart concluded that unless these smaller species entirely escaped exposure to winter cold, seasonal acclimatization must largely rely on their ability to change heat production.

From the above discussion it seems reasonably clear that when laboratory rats and mice are exposed to continuous cold temperatures there is little evidence to suggest that there is an increase in hair weight. When exposed out of doors, however, there does appear to be an increase in hair weight and effective insulation but this does not appear to be sufficient to maintain normal body temperatures unless metabolic rate is substantially increased.

III. Heat Induced Changes.

Although there is a lot of literature dealing with the responses of the skin of humans to heat, including vascular responses, the amount of literature dealing with the responses of the skin and hair of small homeotherms is scanty.

Harrison (1959) has shown that mice reared at 90°F from 3 to 21 weeks of age had a significantly smaller weight of hair than mice reared for the same period at 70°F. Body weights were similar in the two groups and therefore this could not be the cause of the differences.

Harrison (1959) also transferred some mice from one temperature to another to investigate whether environmental determination of hair growth occurs after most of the growth in surface area is completed. The results showed that all mice in the temperate environment, whether they were reared only in this environment or transferred to it 5 weeks before killing have very similar amounts of hair. These mice not only had significantly more fur than the exclusively heat reared animals but also more than those transferred to the heat.

Thus Harrison (1959) has concluded, that throughout much if not all of a mouses life, hair growth responds in the appropriate direction and relatively quickly to a temperature change.

F. Body Temperatures in Hot and Cold Environments.

Young rats (Hill 1947) and also young mice (Barnett 1953) are poikilothermic and are able to withstand fluctuations of body temperatures which would prove fatal in older animals. In mice Barnett (1956) observed that animals less than 3 weeks of age have lower and more variable body temperatures than adults. Barnett (1956) has shown that adult mice of the A strain, bred at an environmental temperature of ~3°C had slightly lower colonic temperatures than controls bred at 21°C. No differences were found between the sexes.

McLaren (1961) has investigated some causes of variation in body temperatures in mice. She has shown that the C3H strain had a smaller individual degree of variation of body temperature over a period of 10 days than did mice of the C57BL strain. In both strains there were significant differences between the sexes in body temperatures. In the C3H strain, the temperature of the males was on an average 0.48°C higher than that of the females, but in the C57BL strain the temperature of the females was 0.27°C higher than that of the males. From her work McLaren (1961) concluded that the precise control of body temperature in the C3H strain seems to depend on long term physiological factors (sex, age, lactation), whereas in the C57BL strain it is influenced more by short term variations in internal and external factors.

Hart (1957) has estimated the average body temperature of mice by a technique of placing the mouse, immediately after killing,

in a Dewar flask containing water and recording the temperature rise. He showed that in the normal environment (20°C) the average body temperature is usually about 2°C lower than the colonic temperature. During lethal cooling, however, the average body temperature is frequently higher than colonic temperature.

Hart (1952) has determined the colonic body temperature of mice acclimated to either 6°C or 20°C over a range of environmental temperatures. He showed that the colonic temperature of the 6°C acclimated mice remained constant over the whole environmental range (10° - 30°C) during minimal activity. Mice acclimated to 20°C had a more variable temperature during minimal activity, than those of the 6°C mice and there was a slight fall in colonic temperature with decreased environmental temperature.

During activity, however, Hart (1952) showed that colonic temperatures were more dependant upon environmental temperature. At 32°C colonic temperature of the 6°C acclimated mice during work averaged about 3.5°C higher than similar inactive mice and were about 1°C higher at 10°C. At 30°C, colonic temperatures for 20°C acclimated mice during work were higher than those of similar inactive mice, but at 10°C the colonic temperatures were lower than those for inactive mice. Hart (1951) has also observed similar results for average body temperature. These results are summarized in Table II.

Table II. Responses of Body Temperatures in Mice during Activity as Compared to Body Temperatures of Inactive Mice.

Acclimation Temperature	Environmental Temperature for Test.	
	32°C	10°C
6°C	+3.5°C	+1.0°C
20°C	+	-

+ increase
- decrease
} in body temperatures.

Fleischner and Burgent (1959) have carried out an experiment to test whether or not cold acclimated rats adjusted better to heat and heat acclimated rats adjusted better to cold than rats not previously exposed to either environmental extremes.

Cold acclimated rats were maintained at between 0.6°C to 4.4°C for a period of 50 days and then suddenly transferred to a temperature of 34.7° to 35.8°C. Rats acclimated to the warm were transferred suddenly to the cold after 50 days also. A control group was maintained at 24.4° - 25.6°C for 50 days and then divided into three groups. One group was placed in the cold, one group placed in the heat and the other being maintained in the control environment. Rectal temperatures were measured at least

twice a week and the results showed that in the cold the rats exhibited a marked hypothermia for approximately the first 30 days then their rectal temperatures rose to levels only slightly below control levels. In the hot environment the rats continuously maintained rectal temperatures higher than the controls. After the cross over to the different environments cold rats transferred to the hot environment showed a progressive rise in rectal temperatures. The control rats transferred to the heat maintained a constant rectal temperature intermediate between that of the cold rats in the heat and control rats in the control environment. Hot rats transferred to the cold exhibited a marked hypothermia of 20 days duration and the thermal equilibrium was established at temperatures close to those for cold rats prior to cross.

From these results Fleischner and Sargent (1959) have concluded that cold acclimation sensitizes the rat to heat, whereas heat acclimation neither seriously impedes nor accelerates the development of cold acclimation.

G. Tail Length and Body Weight Relationships.

Many workers have now shown that both Body Weight and Tail Length are influenced by the environmental temperature at which the mice and rats are reared. (Harrison etal. (1959), Harrison 1963, Chevillard etal. (1963), Cockrem (1964).) Harrison (1959) has shown that the tail apparently acts as an efficient heat radiator, and suggests that a mouse reared at a high temperature will develop a long tail as an adapting mechanism. Harrison (1958) showed that mice from which the tails had been removed at 21 days of age were less heat tolerant than normal mice. From this work it was suggested that the longer tail of the heat reared mice with their larger surface area, absence of hair, rich vascular supply and multiple arteriovenous anastomoses, functions as an important heat regulatory structure.

At any one temperature and within a single strain there is a close positive correlation between body weight and tail length (Falconer 1954). According to Harrison etal. (1959) the functional reason for this correlation is that a larger animal required both a longer balancing organ and a larger heat radiator. Harrison etal. (1959) further suggests that the dependence of tail length and body weight at different temperatures could well be due to a difference in the relative importance of these two functions of the tail.

There is no evidence in the literature to suggest that a large animal would require a longer balancing organ. Indeed it is more likely that the weight of this balancing organ is the important factor, not its length although a longer, if not necessarily a heavier tail would give the tail better leverage. Whether or not the heavier weight of the longer tail is sufficient to compensate for the heavier body weight is not known.

The importance of the tail as a heat radiator would depend upon the relative efficiency of the total surface area of both skin and tail as a heat radiator though it is likely that the tail would dissipate more heat in unit time per unit area than does the skin surface of say the back per unit time per unit area. The mouse of high body weight would be producing less heat/unit surface area than a mouse of low body weight. (Fowler 1962). Therefore it appears unlikely that a mouse of high body weight would need a longer tail for dissipation of extra heat.

Cockrem (1964) exposed two strains of mice, one of high body weight with a short tail and the other low body weight with a long tail, to either 7°C, 21°C or 32°C for the period from 3 weeks to 6 weeks of age. Results show that the high body weight-short tail strain showed the lesser tail length response to heat in both males and females. This result differs from that of Harrison etal. (1959) who showed that the short tailed strains showed the greater tail length response to heat.

In the cold environment a long tail would presumably be a disadvantage unless there were compensating associated physiological mechanism such as extreme vasoconstriction. Cockrem (1964) showed that both the long tailed and short tailed strains showed a similar increase in tail length in the cold and the slightly longer absolute length of the long tailed strain was the result of differences at the start of the experiment.

Body weight was less in both the high-body weight-short-tail and low-body weight-long-tail strains in the cold but was not affected by exposure to a hot environment. Cockrem (1964) has concluded that in terms of adaption to the different environments, tail length does not appear to have been of importance. Harrison etal. (1959) have shown that the weight of young heat reared mice usually increases more rapidly than that of their control-reared litters mates but at later ages, particularly during the period of sexual maturity the latter typically grow more rapidly. Harrison etal. (1959) further showed that the magnitude and direction of the environmentally determined responses was dependent upon the genotype of the animals.

Chevillard etal. (1963) has also showed that rats reared in the hot environment show an early acceleration of growth. After 60-70 days of age the growth rate, as measuredby body weight, proceeds more rapidly in rats at 30°C than in those at 5°C. This also applied to tail length growth with an immediate acceleration of tail growth rate in rats at 30°C and a slowing down in those at 5°C in comparison to controls at 20°C.

H. Conclusion Drawn from the Survey of Literature.

I. Adjustments to Cold.

It appears that different methods of adjustment to cold take place when small mammals are exposed to a constant low temperature

in the laboratory and the natural cold environment prevailing outdoors. When exposed to experimental treatments in the laboratory the response to cold would appear to be purely metabolic with little change in thermal insulation. When exposed outdoors to the natural fluctuating environment however, both metabolic and insulative changes take place.

Associated with the metabolic adjustments under experimental cold treatments are:-

1. A reduced rate of body growth,
2. a reduction in the amount of total body fat,
3. a reduction in the rate of growth in length of tail,
4. a possible increase in the weight of hair grown but no change in the weight of the pelt and little change in the insulative value of the pelt and hair,
5. a large increase in food consumption.

II. Adjustments to Heat.

The evidence for the responses of rats and mice to heat appear contradictory. Basal metabolic rate may be decreased but this may be the result of a general reduction in voluntary activity.

Other associated changes may be:-

1. a change in growth rate but results are contradictory,
2. an increase in the length of the total, (tail)?
3. little or no effect on the amount of body fat,
4. hair weight may be decreased,
5. food consumption may be decreased.

The data suggests that possible genetic differences are present in the responses of rats and mice to cold temperatures, especially for body weight and body fat adjustments. However, there is no evidence in the literature that genetic differences in the response of small mammals to heat exist.

Chapter III

MATERIALS AND METHODS

A. Experimental Animals

Four strains of mice were used in the study. These were:-

1. LCA. High body weight, short tail.
2. LCB. Low body weight, long tail.
3. HMTL. Long tail Himalayan.
4. HMTS. Short tail Himalayan.

These four strains of mice are maintained by Dr. Cockrem at Massey University of Manawatu.

1. LC Strains

Cockrem (1959) has described the establishment of the LC strains. These were the result of intercrossing the inbred lines 101, C57BL and CBA obtained from the Animal Genetics Section, Commonwealth, Scientific and Industrial Research Organisation, Sydney. All possible reciprocal crosses were made and the resulting progeny were intercrossed. Selection was started on the mice from the second intercrossing, and was made from all mice of the first litter in each generation with the only restriction being the avoidance of sib matings. Males and females were selected separately and two litters were bred from each mating.

Selection was made on the deviation of the actual body weight at six weeks of age from the expected value. The expected body weight was estimated from the observed length of the tail by use of the regression of body weight on length of tail. Lines for positive andnegative deviations were selected and for the first three generations the regression used was that calculated from the litters of previous generations. Subsequent selection has been by use of the regression within the first three generations of the positive and negative lines. Regressions for males and females were calculated separately (Cockrem 1959).

Mice used in the experiment were obtained from mice not needed for breeding in generation 18. These mice were mated at random and their male progeny used in the study.

2. HMT Strains

These two strains were obtained by crossing Himalayan mice (ch) obtained from Jackson Memorial Laboratory, Bar Harbour, Maine, with each of the two L.C. strains. The progeny of each cross were then intercrossed within each cross and selected for tail length.

Mice not required for breeding in generation 10 were mated at random to supply male mice for the experiment.

B. Methods

I Feed Intake.

During the duration of the experiments (21 days) the mice were fed once each day on a diet of one part Butter milk powder and two parts whole meal flour. The food was given to the mice in a small jar placed in a large flat dish to collect spillage. At each feed time the food not eaten was separated from the faeces and sawdust which had accumulated in the dish and jar and weighed to the nearest 0.2 gr. This feed was then discarded and replaced with fresh food. Mice of 24 days of age were originally fed 2.5gms of food each per day but that was found to be insufficient and the amount was increased to 3.5gms per day.

II Body Temperature Measurements

Body temperatures were taken with a Copper-Constartan thermocouple, inserted per rectum to a depth of 3cm into the colon. The temperatures were recorded by means of a Phillips Automatic Compensator Recorder (Model PR.3210 A/00). A reference couple was kept in a vacuum flask containing ice and water. All couples were made by soldering the ends of the two wires together for a length of approximately 1 millimeter. For body temperature measurements two channels of the recorder were interconnected to give two recordings of body temperatures 2 seconds apart. Temperatures were recorded to the nearest 0.1°C.

The mice were held by the tail with the fore-feet resting on a wire grid and the hind feet in the air. According to McLaren (1959) mice in this position maintain their colonic temperatures constant for several minutes, but if they are held by the tail and the scruff of the neck in an immobilized position, their temperatures tend to fall.

The couple was inserted per rectum to a depth of 3cm. Approximately 8 seconds alapsed before the temperature was recorded by the recorder and this recording was followed 2 seconds later by a second recording. The mouse was then returned to the cage and another one taken by the tail and the above procedure repeated.

III Environments

The mice on the experiment were reared in one of three environmental temperatures. These were:-

1. Hot 30°C.
2. Control 21°C.
3. Cold 7°C.

The cold environment was obtained in a converted milk cooler refrigerator which had a nearly even temperature throughout, the variation being ± 1°C. It was found necessary to supply

the mice living in the cold environment with bedding and cotton wool was supplied for the first seven days of exposure. The cotton wool was removed from all strains after the seventh day except the HMTS strain which would not survive unless cotton wool was present at all times.

the 21°C. is the normal temperature at which the mice are reared, while 30°C. was obtained by placing the cages on a thermostatically controlled hot plate which had a variation of ±3°C.

All experimental mice were kept in steel cages and supplied with water ad lib.

IV Carcass Analysis

The mice were killed with chloroform and the pelt removed from the body, but not the head, the pelt being cut off behind the ears. Once removed from the body the pelt was stretched, with the hair uppermost, on a board where the hair was clipped off. The hair and pelt were then weighed separately to the nearest milligram. The tail was then removed from the body and this was also weighed to the nearest milligram.

The gut was then excised and all fat was dissected free from the gut and weighed to the nearest milligram. Food was then washed from the gut. The pelt, tail, gut and abdominal fat was then returned to the carcass and the whole was frozen.

To determine total body fat the frozen carcass was cut into small pieces and rolled in filter paper (Flux. per. com.). This was then freeze dried. The ether soluble material of the whole carcass was extracted in a Soxhlet extraction for a period of six hours using petroleum ether (B.P. 40 - 60°C) as solvent. After evaporation of the ether the residual was weighed and this fraction was taken to represent total body fat. Small quantities of non fatty acids e.g. cholesterol, which may be present in the residue as well as fat have been ignored in the analysis.

C. Experimental Design

Two major trials were carried out. In the first trial body temperatures were measured every second day for the duration of the treatments while in the second trial carcass component studies were made.

As the mice became available, they were weaned at 21 days of age and placed in cages for three to four days, to allow them to become adjusted to any effects of weaning. On the third or fourth day after weaning, the mice were earmarked, weighed and the tail was measured for length.

Trial 1

The mice were then randomly allocated into one of the three treatment groups. Strains were kept separate and a minimum of two mice and a maximum of five mice were placed in each cage. The mice were then reared in metal cages on sawdust for the twenty-one days of the treatments. Cotton wool was supplied to the cold treatment groups for nesting. This was removed after the seventh day of treatment for all strains except the HMTS strain which would not survive the cold treatment unless cotton wool was present at all times.

Food and water was supplied ad lib.

Body temperature of all mice were measured every second day for the duration of the treatments and body weight and tail length was measured at the finish of the treatment.

Trial 2

The mice were randomly allocated to one of four groups.

a) Hot
b) Medium
c) Cold
d) Control

The control mice were killed at twenty-four days of age, and the following weights recorded for each mouse: live weight, tail length, tail weight, pelt weight, hair weight and abdominal fat weight. Total body fat was determined also.

The other three groups were placed on their respective treatments for twenty-one days. During this period feed intakes were measured once per day, and body weights were measured on days 1,2,3,6,9,15 and 21. At the end of the treatments the mice were killed and the weights as above were recorded.

D. Statistical Methods:

The methods of analysis used were the analysis of variance and the analysis of cavariance.

I Analysis of Variance

Even subclass numbers were present in the carcass analysis trial, and therefore for each characteristic studied the usual methods of analysis of variance were used. That is:-

$$X_{ij} = \alpha + \beta_j + e_{ij}$$

where X_{ij} = the individual observation
α = the general mean
β_i = the treatment effect
e_{ij} = the error term (independent and normally distributed with zero man and constant variance assumed).
i = 1 to 3 and j = 1 to 4.

For body weights, tail lengths, and body temperatures in the first main trial however, uneven subclass numbers were present. Therefore the method of analysis used was that of fitting constants described by Snedicon (1956). Fitting constants leads to an unbiased estimate and test of interaction if it is present. If interaction is not present in the population, the estimates and tests of the main effects are unbiased. The constants are the valves A and B in the model.

$$Xijk = \mu + \alpha i + \beta j + eijk$$

where Xijk = the individual observation
μ = the general mean
αi = treatment effect.
β = Strain effect.
eijk = random error (assumed to be independant and normally distributed with zero mean and constant variance).
i = 1 to 3
j = 1 to 4

II Covariance Analysis:

(a) Introduction

Because of the differences in body weight between the subgroups both at the start and finish of the treatment covariance analyses were used to adjust the weight of the characteristic for body weight. Heroux (1958) has discussed the use of this and other techniques in the analysis of weight of an organ or other part of an animal body in relation to total body weight. Quoting from his discussion.

'When the weight of organs or other parts of an animals body are studied in relation to the weight of the body itself, the use of fractional weights (organ weight expression per unit of total body weight) involves the assumption that, normally, the part is directly proportional to the whole. If the part is large, such as a muscle mass, the assumption is satisfactory, but if it is small the assumption is weak. For instance, it would appear that, in a pair of rats, one of which is twice as big as the other, the heart can be expected to exhibit the same 2:1 ratio. But this violates elementary principles of biological growth. Perhaps the best assumption is that organ and body weight have a constant differential growth rate, i.e. if Y is organ weight and X is body weight, the allometric equation

$$Y = CX^b \quad \text{or} \quad \log Y = a + b\log X$$

holds. In many situations in which the range of values is fairly narrow, no information is lost, and computational time saved, if the logorithmic weights are replaced, by the absolute weights in the second, linear form of the equation, i.e.

$$Y = a + bX$$

This leads to the following procedure: Estimate the constant b for each homogeneous group of animals, and use it to adjust the group mean organ weight to allow for differences in

group mean body weight. In effect, this partitions the observed organ weight differences into (i) a component directly attributable to intergroup treatment differences and (ii), a component associated with body weight differences". Unquote.

The form of the covariance analysis used was:-

$$Yij = \mu + a_i + \beta\, xij + eij$$

where Yij = the individual observation of the dependent variable.
μ = the general mean.
a_i = the treatment effect
β = the slope of the common regression line in the population.
Xij = the deviation of any independent variable from the total mean.
eij = the error term (assumed to be independent and normally distributed with mean zero and constant variance).
i = 1 to 3
j = 1 to 4.

(b) Procedure

A separate covariance analysis was firstly carried out for each strain. Then if the slopes of the treatment regression lines showed no heterogeneity a more complete set of analyses were carried out as follows:-

i) An analysis of strain differences with treatments pooled, was carried out to test the slopes of the strain regression lines. If the slopes showed no heterogeneity then the adjusted means for the strains were tested for significance.

ii) An analysis of treatment differences with strain pooled, were then carried out to test the slopes of the treatment regression lines. If the slopes of the treatment regression lines showed no heterogeneity then the adjusted means were tested for significance.

iii) An analysis of within strain and treatment differences was carried out to test the slope of the within strain and treatment regression lines. If these showed no heterogeneity then the adjusted means were tested for significance.

This set of analyses allowed the estimation of the strain X treatment interaction term between the adjusted means. However, this term could only be estimated if the slopes of the strains, treatments and within strain and treatment regression lines showed no heterogeneity.

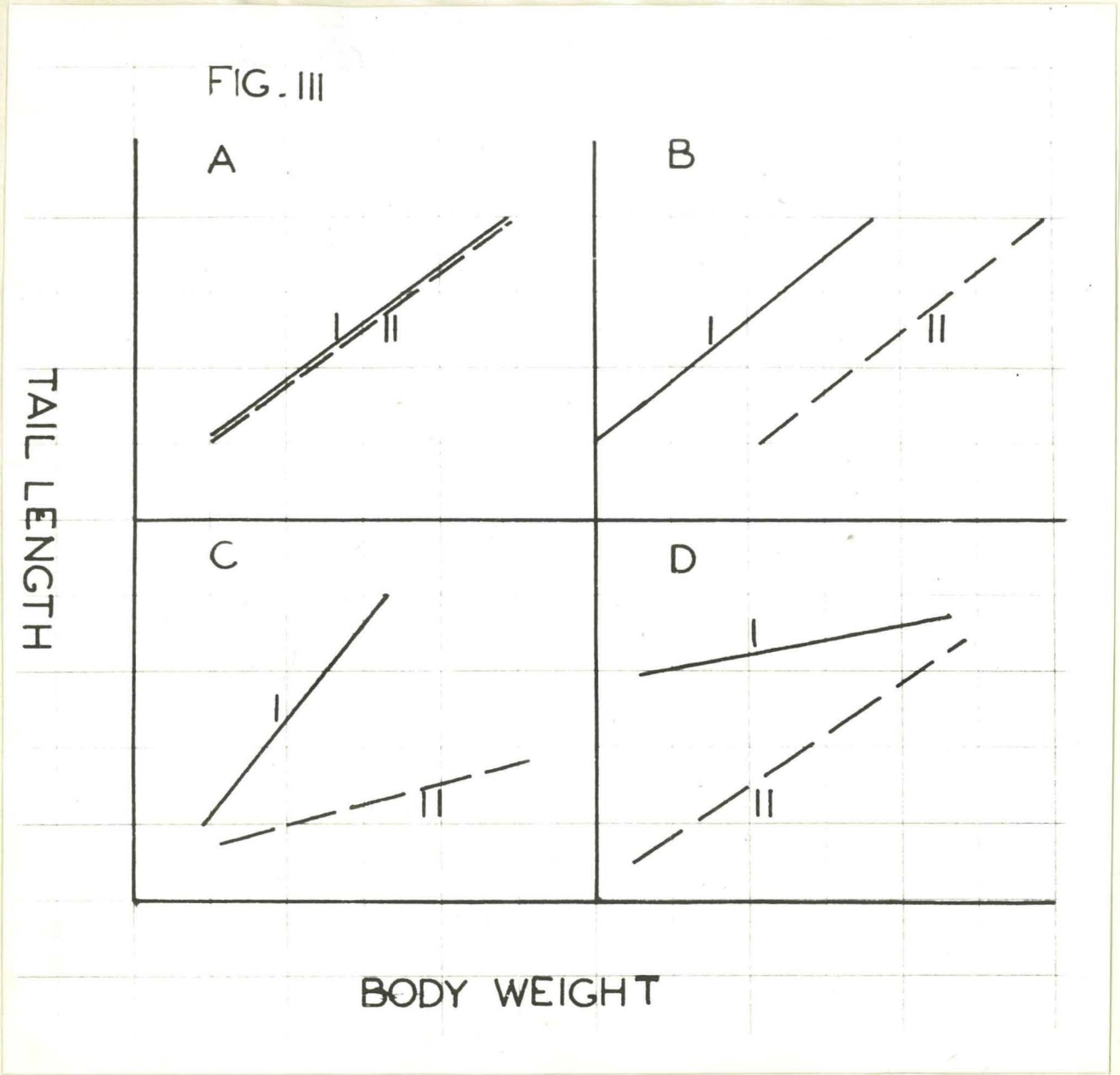


Figure III: Four possible regression settings for body weight and tail length relationships.

The interaction term therefore was estimated as follows:-

Interaction SS = Strain and Treatment SS - (Strain SS + treatment SS)

6 df = 11 df - (2 df + 3 df)

This interaction term was then tested against the within strain and treatment residual mean square for significance.

(c) Interpretation of Results of the Covariance Analysis

The interpretation of the results from the covariance analyses can be illustrated by references to Figure III in which two compared sets of hypothetical body weights and tail lengths under two different treatments are shown in four possible regression settings. In graph (a) no differences were present between either the slopes of the regression lines or the adjusted means. Therefore, the two sets have the same regression line and no treatment differences were present. In graph (b) no differences are present in the slopes of the regression lines but the adjusted means are significantly different. Therefore, the regression lines are parallel. Treatment differences were present between the two sets and the animals within each set have reacted similarly to the treatments.

In graph (c) and (d) two situations are shown in which the slopes of the regression lines show heterogeneity; that is, the regression lines have different slopes. Therefore treatment differences are again present but these differences have been arrived at in a different manner than in graph (b). In graph (c) mice of higher body weight in set (I) have made a greater tail length response to the treatment than have mice of lower body weight in comparison to set (II), considered here to be the control set. In graph (d) however, mice of lower body weight in set (I) have made a greater tail length response to the treatment than have mice of higher body weight in comparison to those in set (II).

(d) Presentation of Results of the Covariance Analyses

The manner in which the results of the covariance analyses are presented depended on the results obtained. The results are presented in three ways.

i) If in the analysis of the treatments within each of the strains the slopes of the regression lines showed heterogeneity then the residual mean squares for each strain are presented separately in the results.

ii) If no heterogeneity was present within each strain but was present between strains or between treatments (i.e. see Procedure ii), viii).) then the residual mean squares for regression coeffecients, and adjusted means were presented for strains, treatment, and within strains and treatments.

iii) If no heterogeneity was present between the slopes of either the strains, treatment or within strain and treatment regression lines, then only the residual mean squares for adjusted means are presented in the results the interaction term was also included in these results.

Unless otherwise stated, all significant differences are $p \triangleright 1\%$.

Chapter IV

PRELIMINARY TRIALS

A. Introduction

Two preliminary trials were carried out to find the most suitable time of the day at which to measure body temperatures of mice and to establish how often it was necessary to measure body temperatures over a period of days to obtain repeatible results.

Hart (1950) has shown that there is a definite diurnal metabolic cycle. He was able to show that the peak of metabolic activity occurred at or about midnight while the metabolic activity was at its lowest at midday. It is now well established that body temperatures of many animals show a diurnal rhythm and that this rhymth follows closely the metabolic cycle of the animals. Hart (1950) showed that there was considerable variations in the metabolic cycle between mice but that the metabolic activity was always greater during the hours of darkness than during the daylight hours. For this reason it was decided to investigate the body temperature cycle of the A B and L strains from 9.00 a.m. to 4.00 p.m. (N.Z. time) to establish the period when body temperatures were least variable.

McLaren (1961) has shown that the repeatibility of body temperatures of mice over a period of days depends to a very large degree on the strain of the mice. She showed that mice of the C3H strain had a greater degree of individual consistency of body temperatures over a period of ten days than did mice of the C57BL strain. For this reason it was decided to investigate the degree of variability of body temperature of mice of the four strains A, B, L and S over a period of 16 days.

B. Experimental Design

As the mice became available they were weaned at 21 days of age and left in cages for 3-4 days to allow them to become adjusted to any effects of weaning. On the third or fourth day after weaning the mice were ear marked and placed in metal cages. Food and water was supplied *ad lib*. Strains were kept separate and a minimum of 2 mice and a maximum of 5 mice were placed in each cage.

Trial 1:

Twenty male mice of each strain (A, B & L) were used in this trial. Body temperatures of these mice were measured every second day for a period of 8 days. The time that any one mouse being measured was randomly selected from times of 9.00 a.m. 10.00 a.m., 11.00 a.m., 12.00, 1.00 p.m., 2.00 p.m., 3.00 p.m. and 4.00 p.m.

FIG. IV

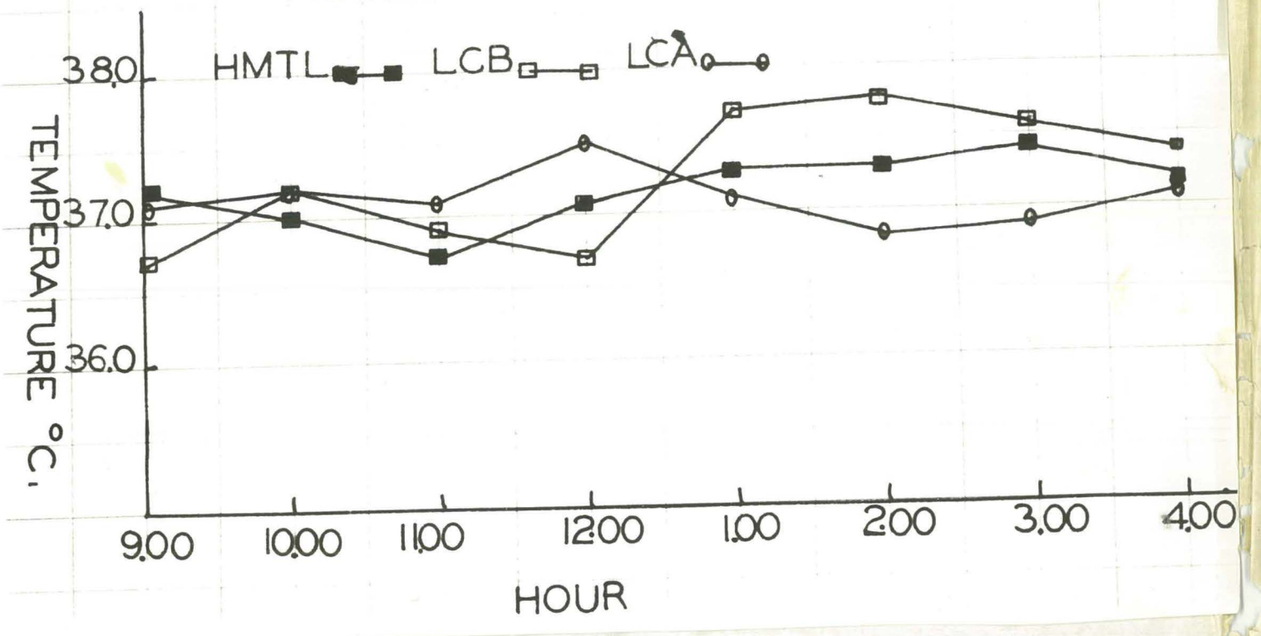


Figure IV: Body temperatures for three strains of mice at hourly intervals.

FIG. V

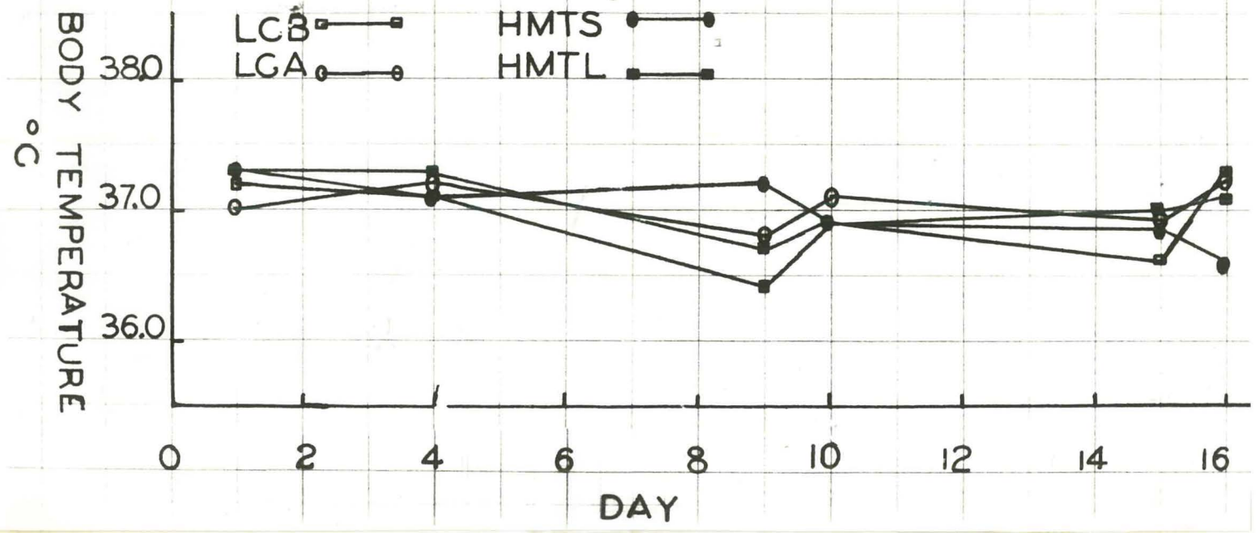


Figure V: Body temperatures for four strains of mice on days 1, 4, 9, 10, 15 & 16.

Table III.

Means & Variances for Body Temperatures.

	LCA		LCB		HNTL	
Time	Mean	Variance	Mean	Variance	Mean	Variance
9.00 a.m.	37.1	.911	36.7	.753	37.2	.258
10.00 a.m.	37.2	.181	37.2	.529	37.0	.560
11.00 a.m.	37.1	.378	36.9	.233	36.7	.784
12.00	37.5	.173	36.7	.131	37.1	.396
1.00 p.m.	37.1	.498	37.7	.451	37.3	.356
2.00 p.m.	36.8	.437	37.8	.154	37.3	.531
3.00 p.m.	36.9	.580	37.6	.219	37.4	.624
4.00 p.m.	37.1	.080	37.4	.434	37.2	.514

Table IV. Analysis of Variance of Body temperatures.

Source	df	MS
Strains	2	0.50
Times	7	0.79
S x T	14	0.98 **
Error	217	0.43

Preliminary Trial.

Table V. Means & Variance for Body Temperatures on Day 1, 4, 9, 10, 15 & 16.

	LCA		LCB		HMTL		HMTS	
Day	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
1	37.0	.480	37.2	.298	37.3	.396	37.3	.244
4	37.2	.272	37.1	.219	37.3	.153	37.1	.235
9	36.4	.207	36.4	.224	36.7	.185	37.2	.394
10	37.1	.274	36.9	.214	36.9	.254	36.9	.161
15	36.9	.237	36.6	.212	37.0	.314	36.8	.110
16	37.2	.412	37.3	.411	37.1	.204	36.6	.151

Table VI. Analysis of Variance for Body Temperatures on Days 1, 4, 9, 10, 15 & 16.

Source	df	MS
Strains	3	0.27
Days	5	3.40
S x D	15	1.13 **
Mice within S x D	53	0.07
Days within mice	380	0.29

Trial II:

Twenty mice of the three strains A, B and S were used but one mouse in each strain died. Twenty two mice of the L strain were used as three died. Body temperatures of all mice were measured on days 1, 4, 9, 10 15 and 16 at a time each day that depended on the results of trial I. Results for all mice dying were discarded.

C. Results

Trial I:

Table III shows the means and variances of body temperatures of the three strains at hourly intervals. Figure IV shows that there is no regular pattern in any of the three strains or between strains. In the B strain body temperatures appear to rise during the morning and decrease during the afternoon while in the L strians body temperatures appear to rise slowly during the day. In the A Strain, however, there was a large rise in body temperature between 12.00 and 1.00 p.m. The body temperature of the B strain do not then appear to follow the decline in metabolic rate during the hours of daylight as shown by Hart (1950). In the L. and A. strains, however, body temperatures may be following the metabolic cycle as shown by Hart (1950).

The variances for body temperatures show a very similar pattern in the three strains. The variances decrease during the morning and then increase again in the afternoon. Because variances were lowest at 12.00 all subsequent measurements of body temperature were taken between 12.00 and 12.30 p.m. (N.Z. time).

Table IV shows the analysis of variance for the body temperature. This shows a significant strains x, time interaction which would suggest that the strains are reacting differently during the day in body temperature and this is shown in Figure I.

Trial II:

Table V shows the means and variance of body temperatures for the four strains on days 1, 4, 9, 10, 15 and 16. These are shown in Figure V. This shows that quite large fluctuations in body temperature occurs within a strain over a period of time. This was less so in the S strain which showed a slow decline in body temperature over the 16 days. In the other three strains (A, B and L) there was a decline in body temperature over days 1, 4 and 9 and then body temperatures rose again. The extent of the decline was similar in these three strains, i.e. 0.8°C. for A strain, 0.6°C. for the B strain and 0.6°C. for the L Strain, while between day 1 and day 16 the S strain reduced body temperature by 0.7°C.

Table VI shows the analysis of variance for body temperature on the 6 days. This shows a significant strain X days interaction which would suggest that body temperatures in the four strains have not followed any regular pattern between the strains.

From these results, body temperatures in the following experiments were measured at least once every second day between 12.00 and 12.30 p.m.

Table VII. Number of Mice Completing the Treatments and Number of Mice Dying in each Subgroup for the First Main Trial.

		HMTL	HMTS	LCA	LCB
Hot	Living	19	13	27	23
	Dying	2	3	2	3
Med.	Living	15	10	20	19
	Dying	0	2	2	1
Cold	Living	20	14	24	20
	Dying	10	10	16	36

Table VIII. Means and Variances for Body Weight and Tail Length at the Start of the Treatments for all Mice Completing the Treatments.

		HMTL	HMTS	LCA	LCB
Body Weight (grams)					
Hot	Mean	12.6	10.4	13.6	11.8
	Variances	6.051	4.133	4.960	5.431
Med.	Mean	11.6	11.3	12.1	11.0
	Variance	3.006	4.364	4.475	3.194
Cold	Mean	13.9	10.8	14.3	12.7
	Variance	3.784	5.102	5.717	2.242
Tail Length (cms)					
Hot	Mean	7.2	5.2	6.6	7.5
	Variance	.359	.121	.135	.301
Med.	Mean	6.6	5.4	6.0	6.8
	Variance	.178	.111	.190	.403
Cold	Mean	7.6	5.4	6.5	7.5
	Variance	.271	0079	.213	1270

Table IX. Analysis of Variance for Body Weight and Tail Length at the Start of the Treatments for all Mice that Completed the Treatments.

Source	df.	Body Weight Mean Square	Tail Length Mean Square
Strains	3	65.353**	36.843
Treatment	2	49.195**	5.300
Interaction	6	6.245	4.380**
Individuals	212	4.402	.229

Table X. Mean Body Weight of all Mice Dying.

	HMTL	HMTS	LCA	LCB
Hot	14.3	10.6	12.5	10.7
Med.	-	8.4	11.0	11.0
Cold	11.8	10.5	12.5	11.8

Chapter V.

RESULTS

A) INTRODUCTION

Harrison (1958) has suggested that the length of the tail of a mouse is related to its ability to loose heat, as a longer tail could be a more efficient radiator of heat than a shorter tail. On this hypothysis, therefore, a long tail should be an advantage in a hot environment and a disadvantage in a cold environment unless associated physiological changes take place on exposure to these extremes of environment.

It was therefore of interest to study body weight and tail length and their relationships in three different environments in mice strains known to differ in these respects in the normal environment. In addition possible acclimation and heat loss effects were examined by the measurement of body temperatures (colon temperatures). Other physiological changes that may have resulted from the exposure to extremes of environmental temperature or that may have assisted in the adaption to the extremes of environment to be studied were growth rate, total body fat, abdominal fat, pelt weight, hair weight, tail weight and food intake.

B) Number of Mice Completing Treatments.

(i) The first main Trial.

The number of mice for each subgroup that completed the treatments, together with the number of mice dying, are shown in Table VII.

(ii) The Carcass Trial.

This experiment was continued until 5 mice in each treatment group had completed the treatments. One mouse in the HMTS cold treatment group died a few days before the finish of the treatments and final weights for this mouse were estimated using the method described by Snedecor pg.310 (1956).

C) Results of Main Trial.

(i) Body Weights & Tail Lengths at the Start of the Treatments.

The means and variances of body weight and tail length at the start for all mice completing the treatments are shown in Table VIII. The results of the analysis of variance (fitting constants) for both body weight and tail length are shown in Table IX. A significant strain plus treatment interaction was present for tail length, while both strain and treatment differences were present for body weight. The t/test showed that within the HMTS strain no significant differences for body weight were present between the treatments groups. Significant differences, however, were present between the treatment groups within the other three strains.

Table XI. Chi Square Analysis of Number of Mice Dying in the Cold Treatments.

Strain	Chi Square	Probability of Difference +++
HMTL	.419	> .75 < .50
HMTS	.784	> .5 < .25
LCA	9.260	> .005
LCB	3.469	> .10 < .05

+++ Probability of mice dying had body weights below the mean body weight for all mice in the cold treatment groups.

Table XII. Means and Variance for Body Weight and Tail Length at the Finish of the Treatments.

Body Weight (gms)		HMTL	HMTS	LCA	LCB
Hot	Mean	21.8	17.6	21.1	17.9
	Variance	6.982	7.403	9.327	2.652
Med.	Mean	20.8	19.4	22.1	19.8
	Variance	2.794	4.111	13.716	4.022
Cold	Mean	19.0	15.7	20.4	17.1
	Variance	5.594	5.853	8.736	4.048
Tail Length (cms)					
Hot	Mean	9.6	7.3	8.1	9.1
	Variance	.107	.163	.296	.197
Med.	Mean	8.3	6.5	7.4	8.2
	Variance	.146	.071	.155	.174
Cold	Mean	8.2	5.9	7.1	8.1
	Variance	.347	.193	.295	.276

Table XIII. Analysis of Variance for Body Weight and Tail Length at the Finish of the Treatments.

Source	df	Body Weight Mean Square	Tail Length Mean Square
Strains	3	168.12**	42.930
Treatment	2	87.90**	30.180
Interaction	6	10,792	.562**
Individuals	212	6.532	.221

A possible cause of these initial differences between treatment groups in body weight was that the mice that died tended to be the mice of lower initial body weight. Table X shows the mean initial body weight of all mice dying in each subgroup. The Chi Square analyses shown in Table XI shows that within each of the LC strains on the cold treatment a greater number of mice with body weights below the mean body weight died than did mice with body weights above the mean.

These results for mice dying would suggest that within the LC cold treatment groups some selection for mice of higher initial body weight may be taking place. However, an analysis of variance (fitting constants) for all mice starting the treatments still showed that significant differences were present between the treatment groups.

One other possible cause of these differences between treatment groups in initial body weight was that mice used to replace the mice dying were from second, third and fourth litters. It has been shown that the body weight of mice at 3 weeks of age in the first litter is lower than are mice of latter litters. As a greater number of replacement mice were used in the cold treatments a greater number of mice of higher initial body weight would be entering these groups.

This, together with selection against mice of low body weight in the cold would probably be the main reasons for the differences between treatment groups in initial body weight.

(ii) Body Weights & Tail Lengths at the Finish of the Treatments.

(a) Analysis of Variance.

Table XII shows the means and variances of body weight and tail length for all possible subgroups at the end of the treatments. The analyses of variance (fitting constants) of both body weight and tail length are presented in Table XIII. A significant strain plus treatment interaction was present for tail length while both strain and treatment differences were present for body weight. The lack of a strain plus treatment interaction for body weight would suggest that the strain and treatment effects were additive; that is the treatments affected all four strains similarly. For tail length, however, the presence of the interaction would suggest that the treatment effect on the four strains were not additive.

Table XIV shows the mean body weight for all subgroups, estimated by fitting constants.

(b) Analysis of Covariance Adjusting for Initial Body Weight & Tail Length Respectively.

It was possible that strain and treatment differences in both body weight and tail length at the finish of the treatments resulted from those present at the start of the treatments. To examine this possibility, analyses of covariance of final body

Table XIV. Mean Body Weight at the Finish of the Treatments Estimated by Fitting Constants.

	b / a	HMTL	HMTS	LCA	LCB
		1.18	-1.87	1.81	-1.14
Hot	.18	20.8	17.7	21.4	18.4
Med.	-1.07	21.61	18.6	22.3	19.3
Cold	1.26	19.3	16.3	19.9	17.0

Table XV. Covariance Analysis for Final Body Weight adjusted for Initial Body Weight.

Source	df	Residual M.S.
Strains		
Error	216	6.2858
Reg. Coefficient	3	19.5903**
Within Strains	219	6.4680
Adjusted Means	3	76.0302
Treatments		
Error	218	5.4984
Regression Coefficient	2	2.5932
Within Treatments	220	5.4720
Adjusted Means	2	220.3733**
Strains & Treatments		
Error	200	4.5490
Regression Coefficient	11	6.5146
Within S & T.	211	4.6515
Adjusted Means	11	60.2844**

Table XVI. Covariance Analysis for Final Tail Length Adjusted for Initial Tail Length. (Residual Mean Squared.) (Between Treatments for each Strain.

		HMTL		HMTS		LCA		LCB
Source	df	R.M.S.	df	R.M.S.	df	R.M.S.	df	R.M.S.
Error	48	.1152	31	.1001	65	.1387	56	.1027
Reg. Coef.	2	.9746**	2	.2494	2	.3055	2	.4196*
Within	50	.1496	33	.1100	67	.1437	58	.1136
Adj. Means	2	12.5051	2	6.7691**	2	5.5312**	2	5.7668

FIG. VI

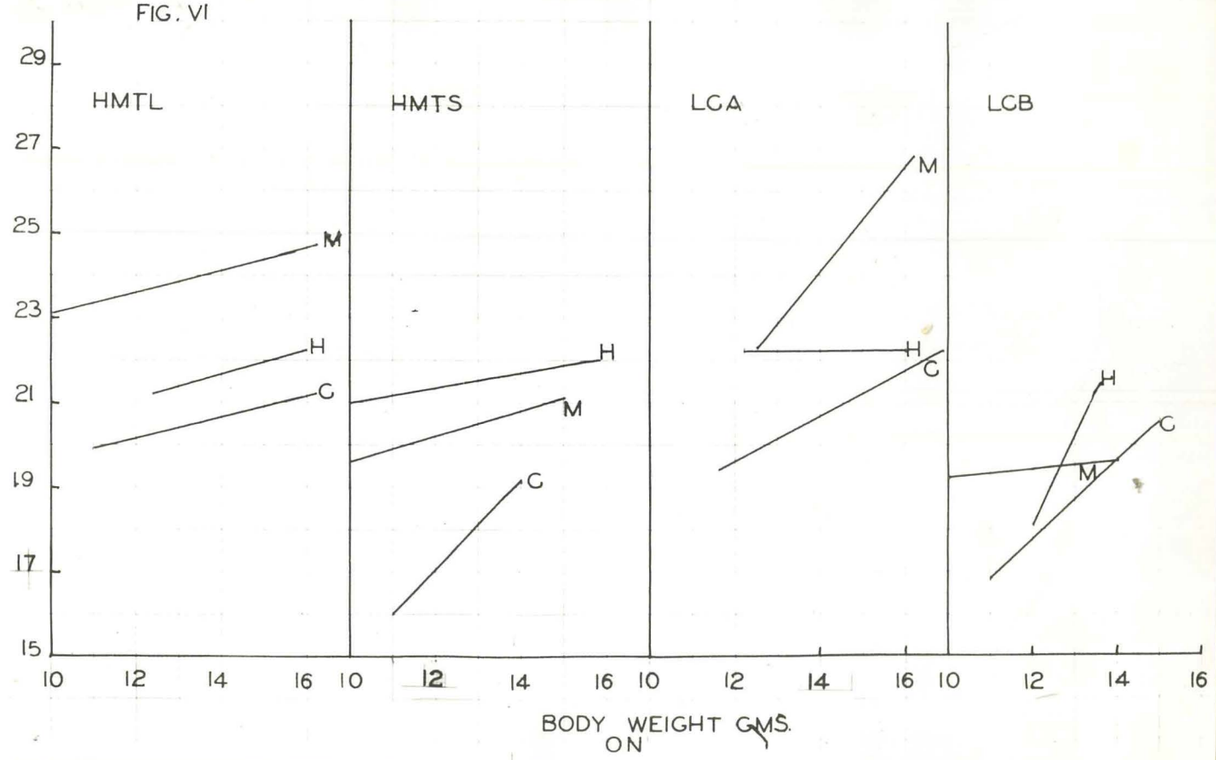


Figure VI: The relationship between final bodyweight and initial bodyweight, H, Hot; M, Med; C, Cold.

FIG. VII

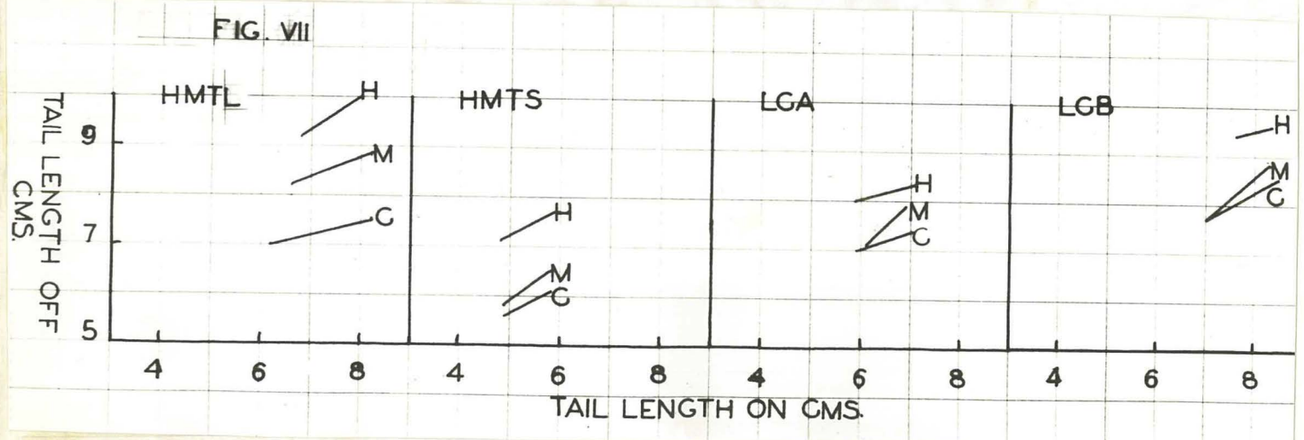


Figure VII: The relationship between final tail length and initial tail length. H, Hot; M, Med; C, Cold.

weight and tail length adjusted for initial body weight and tail length respectively were carried out. The covariance methods used have been discussed on pgs.

(i) Body Weight.

The results of the covariance analyses for final body weight adjusted for initial body weight is shown in Table XV. The regression lines are shown in Figure VI. The slopes of the regression lines showed heterogeneity between strains but not between treatments or strains and treatments. The two long-tail strains (HMTL and LCB) have regression lines of similar slopes which differed from the slope of the two short-tail strains (HMTS and LCA) which also had similar slopes. These results would suggest that mice within each of the short-tail strains have made different body weight responses than have the long-tail strains during the treatment period.

The analysis of the treatment effects with strains pooled showed that no differences were present between the slopes of the treatment regression lines, but that the adjusted means were significantly different. These results have shown that the medium treatment group always had a higher final body weight after adjusting for initial body weight than did the hot and cold treatment groups. Also, the hot treatment group always had a higher final body weight than did the cold treatment group.

The strain and treatment analysis showed that the subgoup regression lines showed no heterogeneity but the adjusted means were significantly different.

(ii) Tail Length.

The results of the covariance analysis of final tail length adjusted for initial tail length is shown in Table XVI. The treatment regression lines for each strain are shown in Figure VII. Within the two long-tail strains (HMTL and LCB) the slopes of the treatment regression lines showed heterogeneity. This would suggest that mice in the treatment groups for these two strains had reacted differently to the treatments. However, within the two short-tail strains (HMTS and LCA), no heterogeneity of slope was present, but the adjusted means were significantly different. This would suggest that the mice in each of the treatment groups for these two strains had reacted in a similar manner to the treatments.

(iii) Covariance analysis of Tail Length at the Start & Finish of the Treatments Adjusted for Initial & Final Body Weight Respectively.

(a) Start of Treatments.

Table XVII shows the results of the covariance analysis of initial tail length adjusted for initial body weight. The regression lines for the individual strains, strains with treatments pooled, and treatments with strains pooled are shown in Figure VIII.

Table XVII. Covariance Analysis for Tail Length at the Start and Finish of the Treatments adjusted for Body Weight at the Start and Finish of the Treatments Respectively.

		START	FINISH
Source	df	Residual M.S.	Residual M.S.
Strains within Treatment Pooled.			
Reg. Coef.	3	1.3614**	1.0174
Error	216	.1298	.3876
Adj. Means	3	32.7106	37.4952
Within Strains	219	.1467	.3962
Treatments with Strains Pooled.			
Reg. Coef.	2	.5187	.2034
Error	218	.5752	.7142
Adj. Means	2	1.9171*	21.5774
Within Treatments	220	.5747	.7096
Strains & Treatments.			
Reg. Coef.	11	3.3561**	.0509
Error	200	.1056	.1579
Adj. Means	11	9.5852	15.1926
Within S. & T.	211	.1176	.1523
Interaction	6		1.9130**

Table XVIII. Tail Length at the Start and Finish of the Treatments Adjusted to Initial Body Weight of 12gms. and Final Body Weight of 20gms. respectively.

Tail Length adjusted to 12gms Body Weight					Tail length adjusted to 20gms Body Weight.			
	HMTC	HMTS	LCA	LCB	HMTL	HMTS	LCA	LCB
Hot.	7.0	5.4	6.4	7.6	9.4	7.5	8.0	9.3
Med.	6.7	5.5	6.0	7.1	8.1	6.6	7.2	8.2
Cold.	7.2	5.4	6.2	7.3	8.3	6.3	7.1	8.3

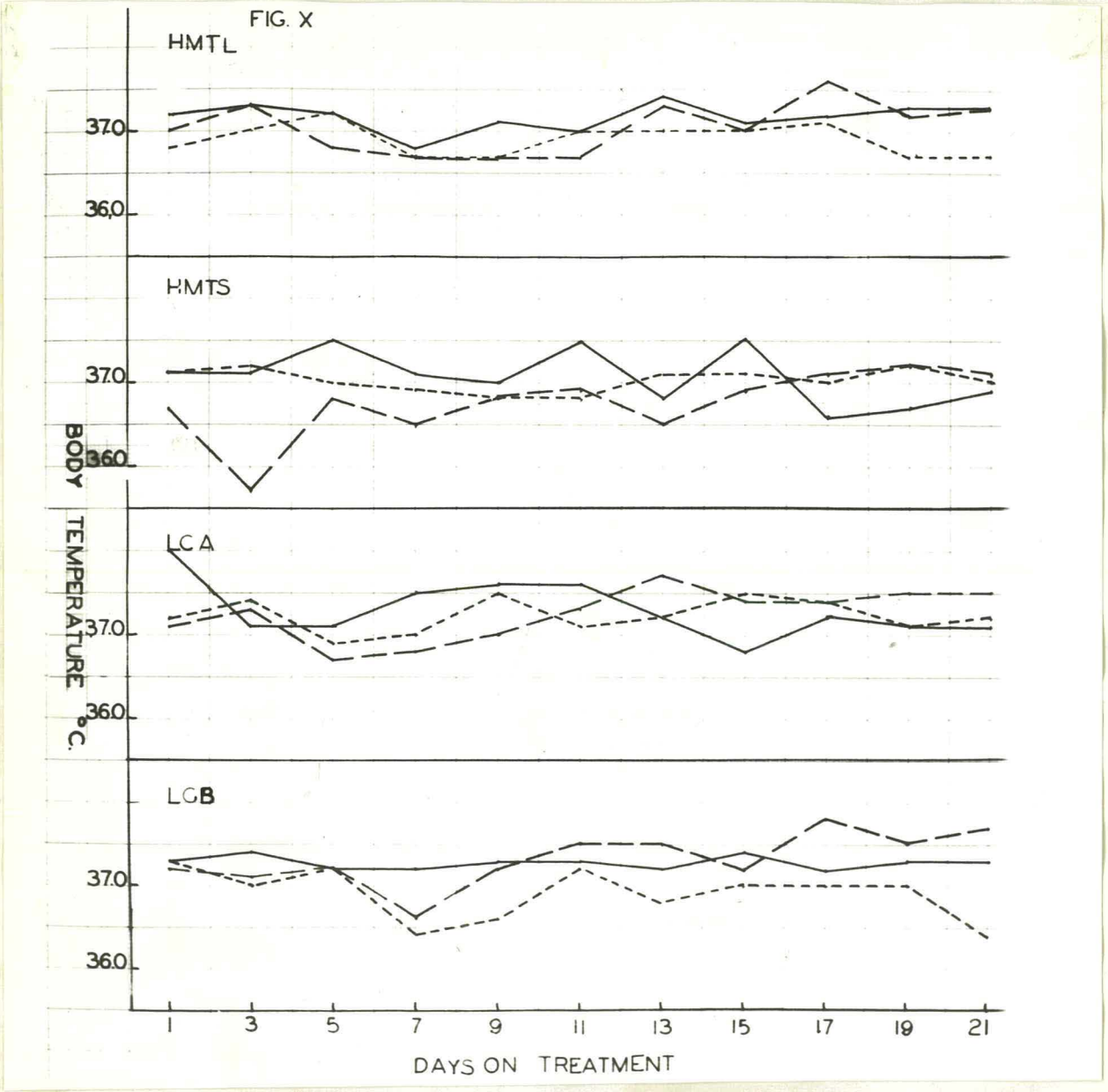


Figure X: Body temperatures for four strains of mice during the treatments. ———— Hot, -- - - - - - Med, —— —— Cold.

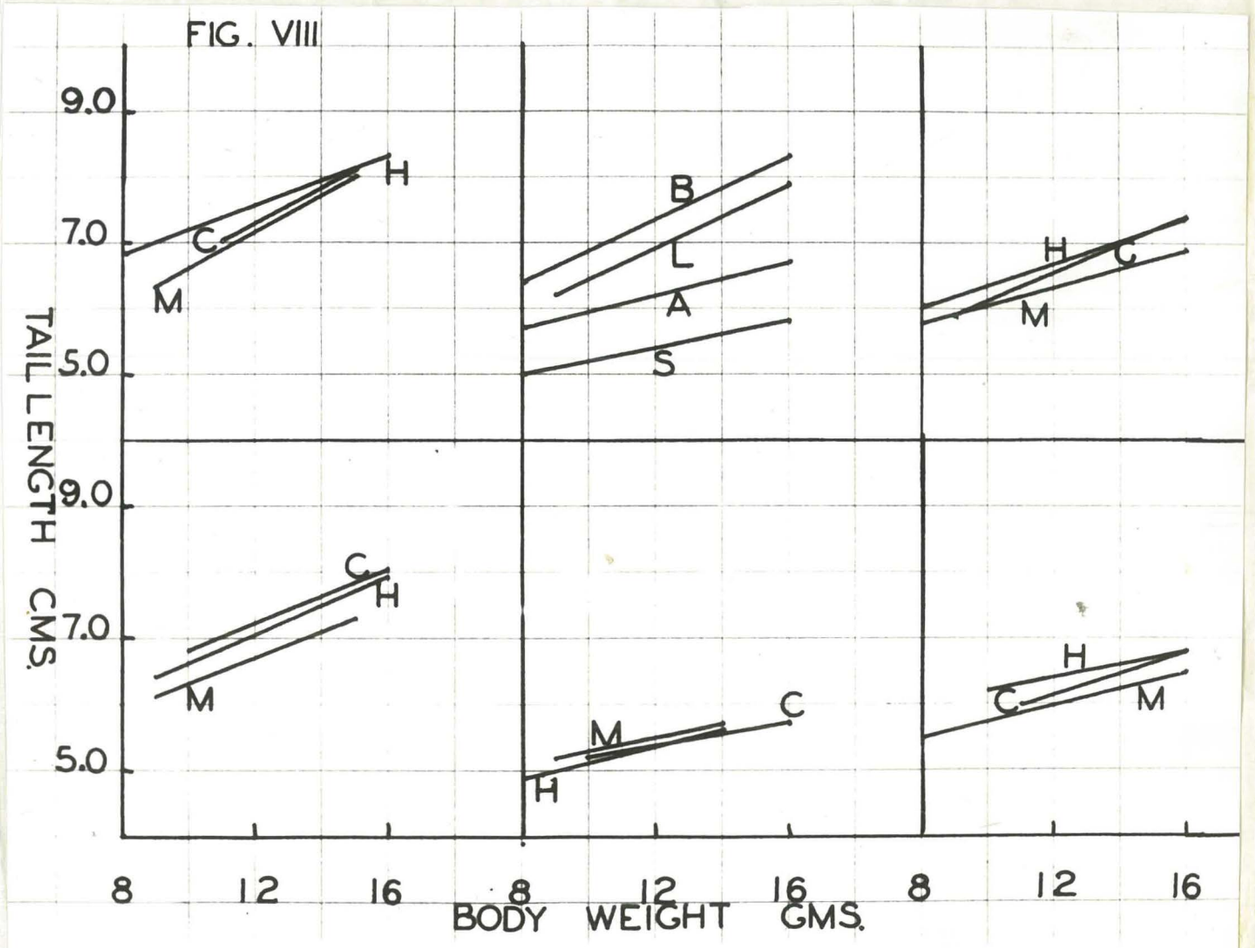


Figure VIII: The relationship between initial tail length and initial bodyweight. H, Hot; M, Med; C. Cold; B,LCB; A, LCA; L, HMTL; S, HMTS.

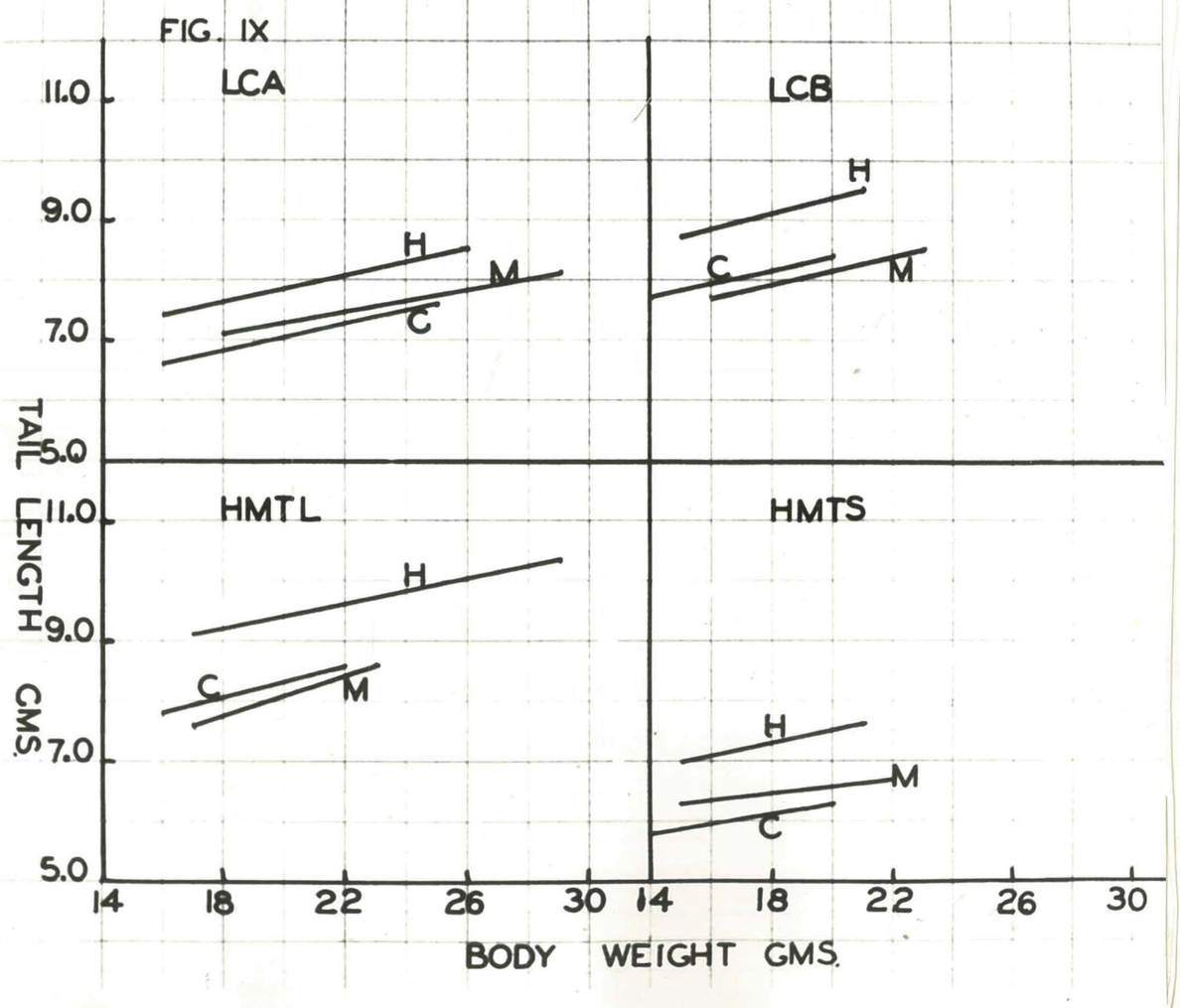


Figure IX: The relationship between final tail length and final bodyweight. H, Ho M, Med; C, Cold.

The analysis of strains with treatments pooled showed that no heterogeneity was present in the slopes of the regression lines but that the adjusted means were significantly different. This indicates that differences in tail length adjusted to constant body weight were present between the four strains.

The analysis of treatments with strains pooled showed that no differences were present either in the slopes of the regression lines or in the adjusted means. Therefore at the start of the treatments no differences were present in the relationships of tail length and body weight although mean body weight differed between the treatments.

(b) Finish of Treatments.

Table XVII also shows the results of the covariance analysis of final tail length adjusted for final body weight. The treatment regression lines for each strain are shown in Figure IX. A significant strain and treatment interaction was present. An examination of the individual strains analyses showed that within each strain the slopes of the regression lines showed no heterogeneity but the adjusted means were significantly different. The hot treatment groups for all strains had a longer tail after body weight was adjusted for than had the medium groups, but in the medium and cold groups differences between the strains are present. In the HMTS strain the cold treatment group had a significantly shorter tail after body weight was adjusted for than did the medium group. In the other three strains, however, little difference was present between the medium and cold groups.

Table XVIII shows the mean tail length of all possible subgroups after adjusting tail length to an initial and final body weight of 12.0gms. and 20.0gms. respectively. These results show that the HM strains have shown the greatest response at these weights to the hot treatment than have the LC strains, i.e. 2.4 and 2.1cms. as against 1.6 and 1.7cms. In the medium and cold treatment groups, however, the four strains have made similar response to the treatments except for the HMTL strain in the medium treatment groups which has increased tail length by 0.3cms. above that of the other three strains.

III. BODY TEMPERATURE RESULTS.

Table XIX shows the means and variances for body temperatures for all possible subgroups during the treatments. The means are shown in Figure X. This shows that quite large fluctuations are present between the measurements of body temperatures in the medium treatment groups. The degree of variation between measurements of body temperatures appears to differ between the four strains in the medium treatment groups however. The LC strain would appear to have a greater degree of variation between measurements than the HM strains. The HMTS strain has the lowest degree of variation while the LCA strain has the greatest degree of variation.

Because of the large amount of variation between measurements it is likely that the measurement of body temperatures every second day would be measuring fluctuation of body temperature about the mean rather than the true mean body temperature. Therefore, to get consistency in the measurement of body temperature

FIG. XI

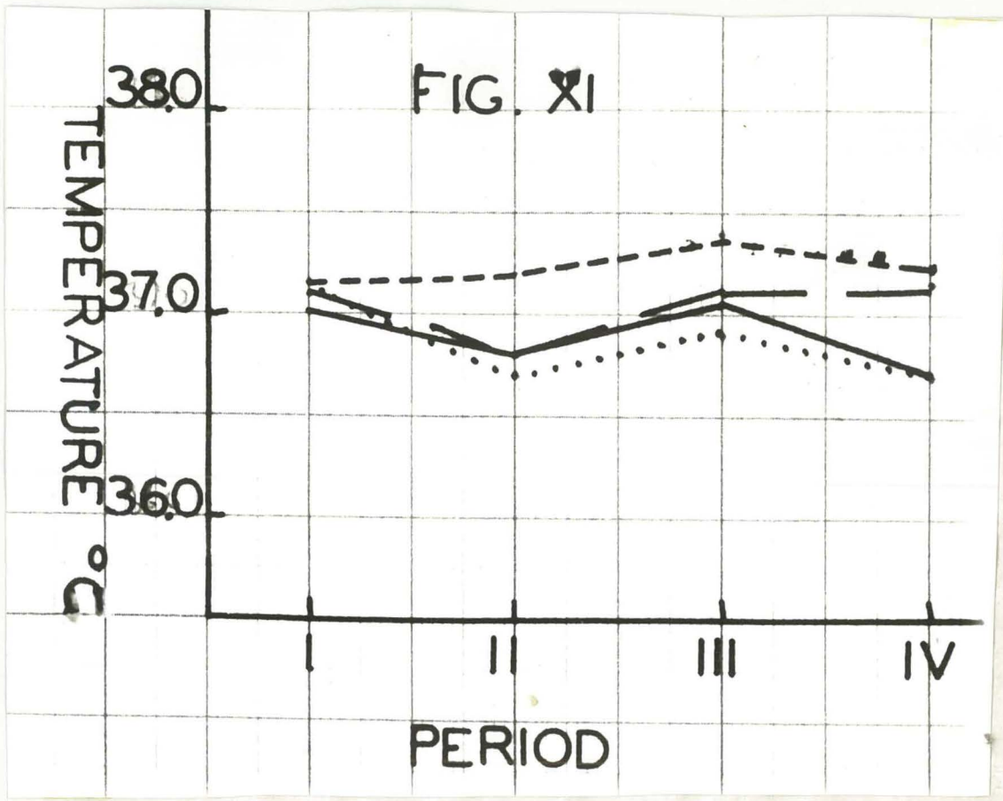


Figure XI: Body temperatures for mice in the medium treatment groups for the four periods.
- - - - LCA, —— —— HMTS
———— HMTL · ····· LCB.

FIG. XII

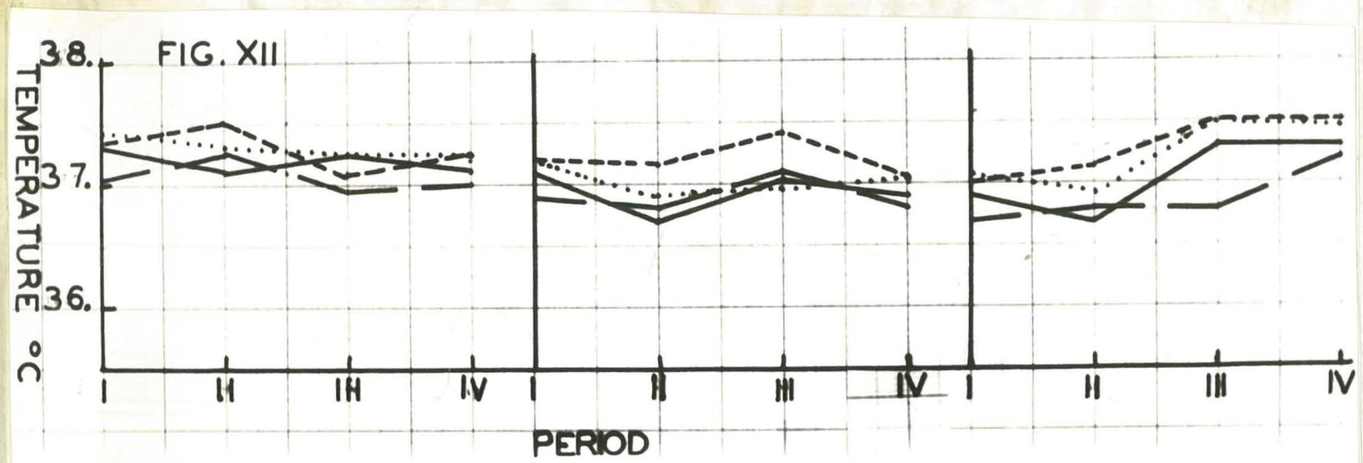


Figure XII: Mean body temperatures of all subgroups for the four periods. a) Hot, b) Med, c) Cold. - - - - LCA, —— —— HMTS, ———— HMTL, ····· LCB.

Table XIX. Means and Variances for Body Temperature for all Subgroups.

			Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
HMTL	Hot	Mean	37.2	37.3	37.2	36.8	37.1	37.0	37.4	37.1	37.2	37.3	37.3
		Variance	.065	.242	.249	.281	.184	.168	.437	.170	.221	.813	.216
	Med.	Mean	36.8	37.0	37.2	36.7	36.7	37.0	37.0	37.0	37.1	36.7	36.7
		Variance	.573	.534	.172	.477	.196	.284	.322	.350	.414	.183	.259
	Cold	Mean	37.0	37.3	36.8	36.7	36.7	36.7	37.3	37.0	37.6	37.2	37.3
		Variance	.593	.220	.889	1.137	.397	.506	.421	.242	.681	.961	.281
HMTS	Hot	Mean	37.1	37.1	37.5	37.1	37.0	37.5	36.8	37.5	36.6	36.7	36.9
		Variance	.293	.376	.816	.140	.186	1.163	.311	.434	.512	.251	.149
	Med.	Mean	37.1	37.2	37.0	36.9	36.8	36.8	37.1	37.1	37.0	37.2	37.0
		Variance	.509	.100	.264	.233	.224	.220	.081	.324	.427	.322	.156
	Cold	Mean	36.7	35.7	36.8	36.5	36.8	36.9	36.5	36.9	37.1	37.2	37.1
		Variance	1.120	4.271	.526	.175	.096	.721	1.553	.942	.531	3.220	2.471
LCA	Hot	Mean	38.0	37.1	37.1	37.5	37.6	37.6	37.2	36.8	37.2	37.1	37.1
		Variance	.335	.210	.207	.442	.337	.196	.318	.432	.226	.340	.297
	Med.	Mean	37.2	37.4	36.9	37.0	37.5	37.1	37.2	37.5	37.4	37.1	37.3
		Variance	.234	.278	.302	.458	.207	.493	.267	.196	.247	.402	.238
	Cold	Mean	37.1	37.3	36.7	36.8	37.0	37.3	37.7	37.4	37.4	37.5	37.5
		Variance	.366	.617	1.166	.161	.456	.500	.448	.647	1.156	1.163	1.077
LCB	Hot	Mean	37.3	37.4	37.2	37.2	37.3	37.3	37.2	37.4	37.2	37.3	37.3
		Variance	.639	.202	.405	.251	.635	.610	.464	.414	.126	.339	.341
	Med.	Mean	37.3	37.0	37.2	36.4	36.6	37.2	36.8	37.0	37.0	37.0	36.4
		Variance	.428	.249	.179	.614	.316	.281	.307	.457	.066	.151	.234
	Cold	Mean	37.2	37.1	37.2	36.6	37.2	37.5	37.5	37.2	37.8	37.5	37.7
		Variance	1.093	.970	.435	.670	.626	1.111	.943	.626	.317	.476	.174

Table XX. Analysis of Variance for Body Temperatures of the Four Strains in the Medium Environment (Mean Squares).

Source	df	Period I	Period II	Period III	Period IV
Between Strains	3	.083	.883**	.740**	1.060**
Within Strains	60	.124	.127	.143	.149

Table XXI. Analysis of Variance for Body Temperature in the Three Treatments (Mean Squares).

Source	df	Period I	Period II	Period III	Period IV
Strains	3	1.073**	2.320**	1.003	1.233**
Treatments	2	2.570**	4.265**	1.010	4.435**
Strains & Trtmnts.	6	.460	.238	.808**	0.927
Individuals	212	.226	.209	.219	.461

Table XXII. Mean Body Temperature estimated by Fitting Constants (Period III Unadjusted Means due to Presence of Interaction).

Period I	X=37.09	HMTL	HMTS	LCA	LCB
	a.	b .00	-.21	+.09	+.12
Hot	.18	37.3	37.1	37.4	37.4
Med.	-.01	37.1	36.9	37.2	37.2
Cold	-.17	36.9	36.7	37.0	37.0
Period II	X=37.01				
	a	b -.18	-.08	+.26	+.01
Hot	+.26	37.1	37.3	37.5	37.3
Med.	-.14	36.7	36.8	37.1	36.9
Cold	-.13	36.7	36.8	37.1	36.9
Period III					
Hot		37.2	37.0	37.1	37.3
Med.		37.0	37.1	37.4	36.9
Cold		37.3	36.8	37.5	37.5
Period IV	X=37.14				
	a	b -.05	-.15	+.11	+.08
Hot	-.01	37.1	37.0	37.2	37.2
Med.	-.22	36.9	36.8	27.0	27.0
Cold	+.23	37.3	37.2	37.5	37.5

the eleven recordings of body temperature for each mouse were divided into four periods, and the mean body temperature for each of these four periods was used in the following analysis. The periods were:-

Period I	Days 1, 3 and 5
Period II	Days 7, 9 and 11
Period III	Days 13, 15 and 17
Period IV	Days 19 and 21.

The mean body temperatures for the medium treatment groups are shown in Figure XI. The results of the analysis of variance for the medium treatments are shown in Table XX. These show that during period I there were no significant differences between the four strains. During periods II, III and IV, however, significant differences were present. The t/test showed that during periods II and III, the LCA strain differed significantly from the other three strains, but these three strains did not differ significantly amongst themselves. During period IV the LCA and HMTS did not differ significantly but did differ significantly from the LCB and HMTL strains. The LCB strain and the HMTL strains did not differ significantly.

Therefore although body temperature means of the four strains have changed over a period of time the ranking of the four strains have not. The LCA strain had a higher temperature in all the four periods but was significantly higher in periods II and III only. The HMTS strain has a higher body temperature than the HMTL strain which in turn has a higher body temperature than the LCB strain. Therefore the two short tail strains of mice have a higher body temperature than the two long tail strains of mice. The LCA strains body temperature being significantly higher during periods II and III and both the LCA and HMTS strain being significantly higher in period IV.

The analysis of variance for the strains and treatments is shown in Table XXI. This shows that for periods I, II and IV significant strain and treatment differences are present. During period III however, a significant strain and treatment interaction is present. These results would suggest that during periods I, II and IV the strain and treatment effects are additive; that is, the treatments have affected the four strains similarly. However, during period III the presence of the significant interaction would suggest that the treatment and strains effect was not additive and the treatments have not affected all strains similarly. Table XXII shows the means as estimated by fitting of the constants for periods I, II and IV and unadjusted means for period III. These are shown in Figure XII.

These means show that the ranking of the four strains in the three environments is the same within each of period I, II and IV but it differs between the three periods. During period III however, the ranking of the four strains differs in the three environments which has caused the interaction. The main cause of the interaction would appear to be that in the cold treatment

Table XXIII. Mean Body Temperature of Mice Dying (Measurement taken the Day Before the Mouse Died).

	Mean °C.	Range °C.
HMTL	35.2	33.0 - 36.5
HMTS	34.5	29.7 - 36.6
LCA	34.5	29.2 - 38.0
LCB	34.8	28.5 - 37.9

Table XXIV. Covariance Analysis of Mean Body Temperature over the last Ten Days of the Treatments Adjusted for Final Body Weight.

	HMTL		HMTS		LCA		LCB	
Source	df	R.M.S.	df	R.M.S.	df	R.M.S.	df	R.M.S.
Error	48	3.8263	31	7.422	65	7.162	56	2.936
Reg. Coef.	2	27.7100**	2	73.955**	2	75.355**	2	10.475**
Within	50	4.7820	33	11.454	67	9.196	58	3.196
Adj. Means	2	1.469	2	18.910	2	25.355	2	68.263

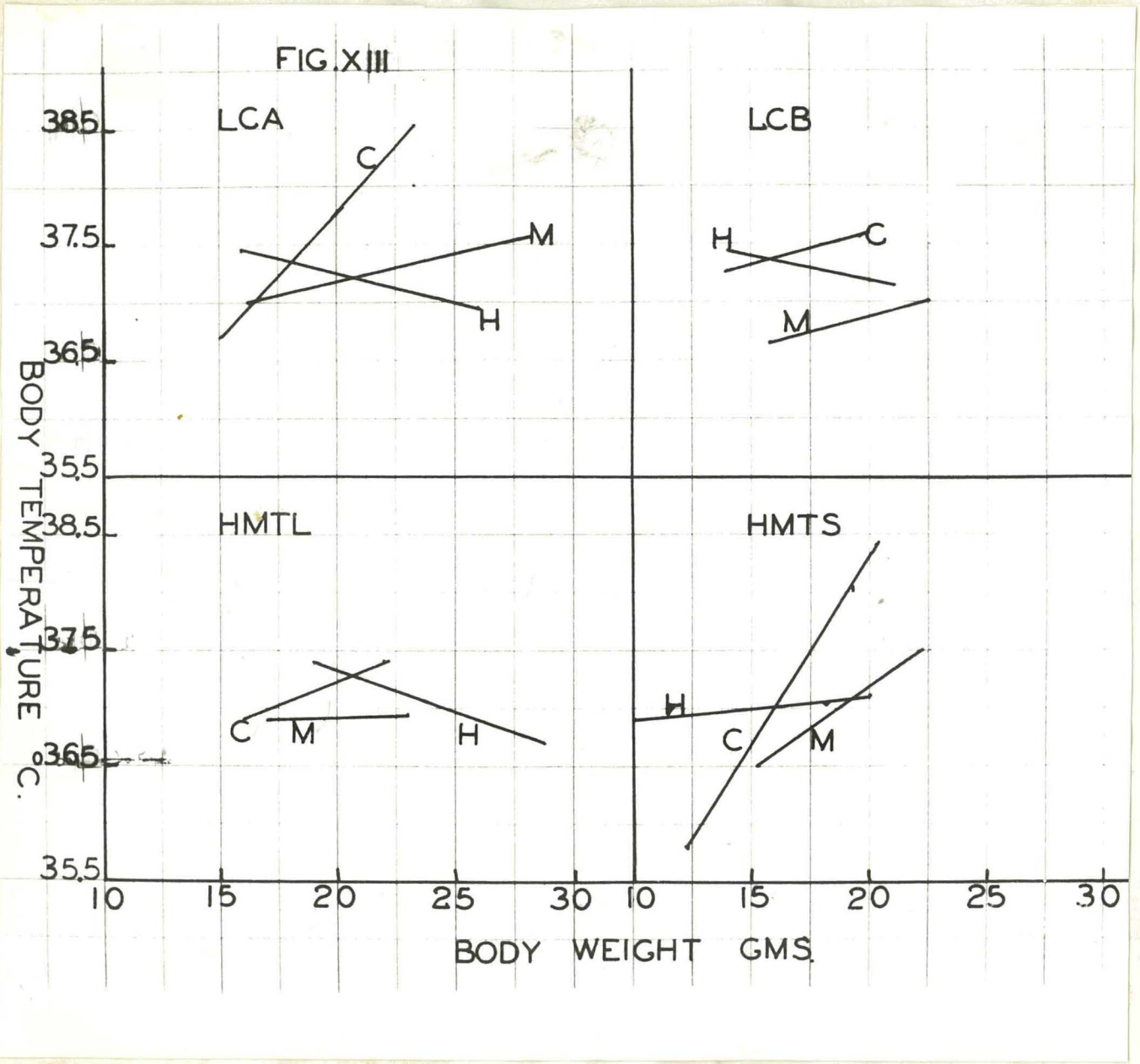


Figure XIII: The relationship between mean body temperature over the last ten days of the treatments and final bodyweight for all subgroups. H, Hot; M, Med; C, Cold.

the HMTS strain has not raised its body temperature mean during period III as the other three strains have and in the hot treatment group the LCA strain has reduced body temperature to a greater degree than has the other three strains.

In the medium treatment groups there is a gradual drop in body temperature over the four periods. This also has occurred in the hot treatment groups but the body temperatures in period I are consistently higher than temperatures in the medium treatment groups. By period IV the hot treatment groups have reduced body temperature to a level only slightly higher than the medium treatment groups. In the cold treatment groups body temperatures are substantially below those of the medium treatment group during period I but rise to the medium levels during period II and by period IV are considerably higher than either the hot or medium treatment groups.

Therefore the treatments have influenced body temperatures to a considerable degree. The treatments have caused an immediate difference in body temperatures in that the hot treatment groups have raised body temperature and the cold treatment lowered body temperature over that of the medium treatment.

The trend in body temperature during the four periods also differ. The medium and hot treatment groups show a decline in body temperature, this being more pronounced in the hot treatment, while the cold treatment groups show an increase in body temperature over the four periods.

Table XXII shows the mean body temperature for all mice dying, this measurement being the one the day before the mouse died. This has shown that mice dying tended to have a mean body temperature well below the average body temperature of all mice completing the treatments.

IV. BODY WEIGHT AND BODY TEMPERATURE ANALYSIS.

A covariance analysis of mean body temperature over the last ten days adjusted for final body weight was carried out to determine what effect body temperature may have had on final body weight.

The results of the covariance analyses are presented in Table XXIV. The results show that within each of the four strains significant differences in the slopes of the treatment regression lines were present. The individual strain regression lines are presented in Figure XII which shows that within each of the three strains LCB, HMTL and LCA, the hot treatment groups have a negatively sloped regression line, while the HMTS strain hot treatment group regression line has little slope. This would suggest that in the hot treatment groups of the three strains LCA, LCB and HMTL body weight has been influenced by body temperature and mice with a high body temperature have a lower body weight, than do mice with a low body temperature.

Table XXV. Means and Variances for Body Weight, Tail Length, Tail Weight, Pelt Weight, Abdominal Fat and Total Body Fat.

Body Weight (gms.)	HMTL	HMTS	LCA	LCB
Mean	15.2	12.00	14.7	12.4
Variance	.548	1.108	4.893	4.668
Tail Length (cms.)				
Mean	7.6	5.4	6.5	7.3
Variance	.068	.070	.110	.220
Tail Weight (gms.)				
Mean	.403	.249	.346	.312
Variance	.00243	.00085	.00142	.0096
Pelt Weight (gms.)				
Mean	1.952	1.306	2.028	1.010
Variance	.0327	.0333	.0872	.0381
Abdominal Fat (gms.)				
Mean	.198	.196	.255	.165
Variance	.000059	.000944	.000489	.000391
Total Fat (gms.)				
Mean	1.343	1.120	1.321	1.181
Variance	.1014	.0117	.0614	.0523

Table XXVI. Analysis of Variance.

Source	df	Body Weight	Tail Length	Tail Weight	Pelt Weight	Ab. Fat	Total Fat.
Between Groups	3	13.463**	4.773**	.02060**	1.2704**	.00699**	.0583
Within Groups	16	2.804	.117	.00142	0.0478	.00047	.0567

Table XXVII. Covariance Analysis of Pelt Weight, Tail Weight, Abdominal Fat, Total Body Fat and Tail Length, Adjusted for Body Weight.

Source	df	Pelt Weight	Tail Weight	Abdominal Fat	Body Fat	Tail Length
Error	12	.0473	.000883	.000497	.0454	.130
Reg.Coeff.	3	.0013	.000679	.000137	.0031	.013
Within	15	.0381	.000842	.000425	.0369	.107
Adj.Means	3	.3917**	.006264**	.005098**	.0051	.379**

Table XXVIII. Means and Variances for Weight of Hair.

(grms.)	Mean	Variance
HMTL	.097	.000084
LCA	.088	.000326
LCB	.085	.001052

Table XXIX. Analysis of Variance for Weight of Hair.

Source	df	MS
Between	2	.000302 NS
Within	12	.000487

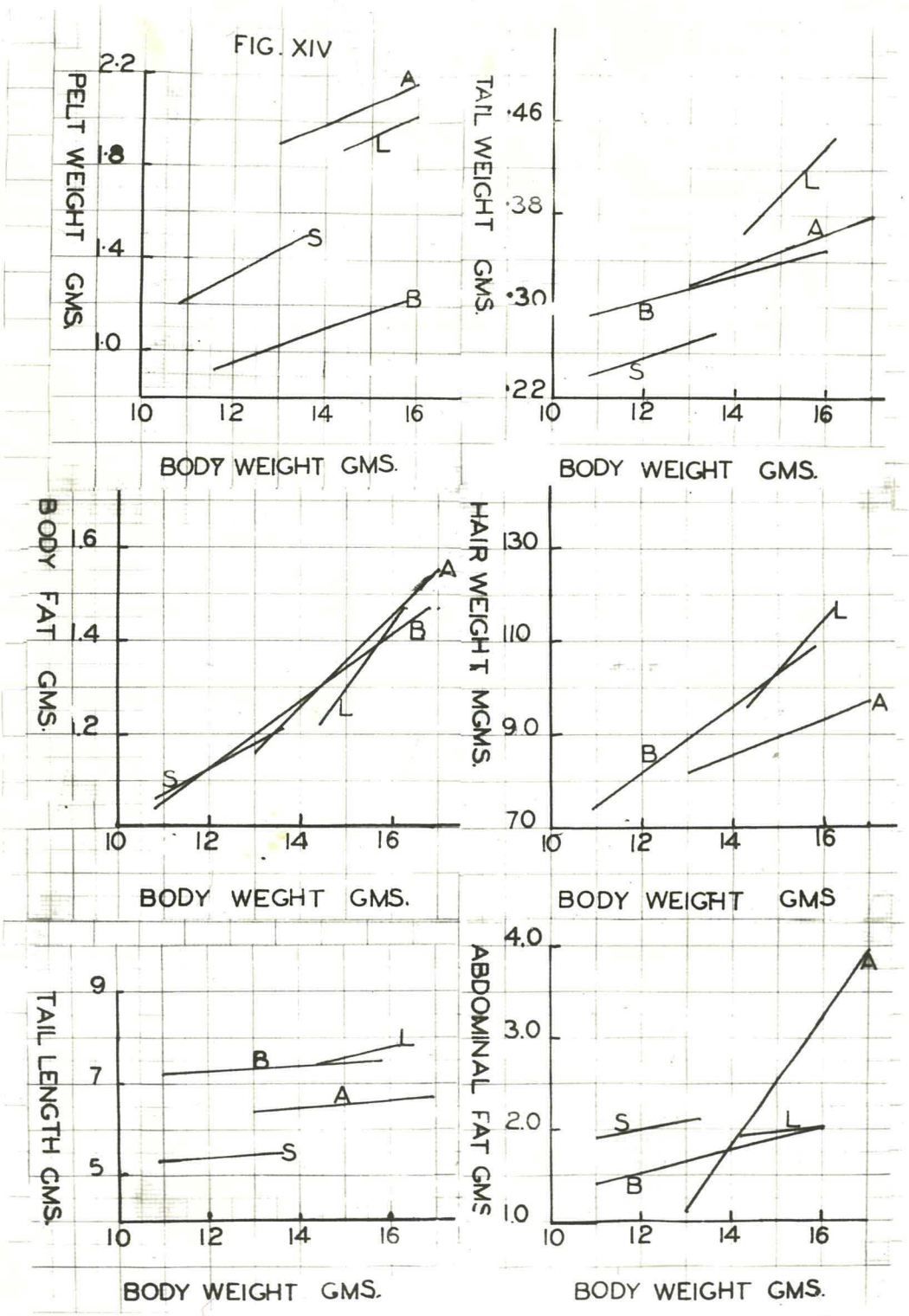


Figure XIV: The relationship between tail length, tail weight, pelt weight, weight of abdominal fat and weight of total body fat with bodyweight for the control mice. A, LCA; B, LCB; L, HMTL; S, HMTS.

In the cold and medium treatment groups, however, the regression lines have positive slope in all four strains which would suggest that mice with a high body temperature had a higher body weight at the end of the experiment than did mice of lower body temperature. In the HMTL, HMTS and LCA strains the slope of the cold treatment group regressions were steeper than the slopes of the medium treatment regressions which would suggest that body temperatures has had a greater influence on bodyweight in the cold treatment groups than it has in the medium treatment groups.

D) Carcass Analysis Trial.

(i) Control Mice (623 days of Age.)

The means and variances of body weight, tail length, tail weight, pelt weight, weight of abdominal fat and weight of total body fat are shown in Table XXV. The analysis of variance (Table XXVI) shows that significant differences exist between the four strains for all of these characteristics except for total body fat where no significant differences were present. Because of the body weight differences between the four strains, covariance analyses were carried out for all characteristics adjusted for body weight as the independent variable. These results are shown in Table XXVII and show that for tail length, tail weight, pelt weight, and weight of abdominal fat, the slopes of the regression lines for the four strains showed not heterogeneity but the adjusted means were significantly different. The regression lines for each of these characteristics are shown in Figure XIV. The slopes of the regression lines for total body fat showed no heterogeneity and the adjusted means also were not significantly different. Therefore for all characters except total body fat strain differences are present when the weight of the character is adjusted for body weight. For total body fat however, no strain differences were present when weight of total body fat was adjusted for body weight between the four strains.

Table XXVIII shows the means and variances for the weight of hair for the three strains, HMTL, LCA and LCB. The HMTS strain was excluded from this analysis because the strain was infested with lice. The analysis of variance for hair weight (Table XXIX) showed no significant differences between the weight of hair in the three strains. The covariance analysis of hair weight adjusted for body weight shown in Table XXX shows that the slopes of the strain regression lines showed no heterogeneity and the adjusted means were not significantly different. The regression lines are shown in Figure XIV.

Table XXXI shows the means for all characteristics adjusted to a body weight of 13.0grms.

II. Treatment Mice.

A. Body Weight, Tail Length and Tail Weights.

i. Analysis of Variance.

Table XXXII shows the means and variances for body weight and

Table XXX. Covariance Analysis for Weight of Hair Adjusted for Body Weight.

Source	df	R.MS
Error	9	.000284
Reg. Coef.	2	.000269
Within	11	.000281
Adj. Means	2	.000345

Table XXXI. Means for Tail Length, Tail Weight, Abdominal Fat, Total Body Fat and Pelt Weight and Hairweight Adjusted to 13.0gms. Body Weight Respectively.

Strain	Pelt Weight	tail Weight	Abdominal Fat	Total Body Fat	Tail Length	Hair
HMTL	1.811	.305	.191	1.079	7.1	.097
HMTS	1.401	.265	.207	1.175	5.5	
LCA	1.914	.322	.110	1.158	6.4	.082
LCB	1.047	.319	.175	1.235	7.3	.089

Treatment Mice (Carcass Trial).

Table XXXII. Means and Variances for Body Weights, Tail Lengths at the Start of the Experiment and Body Weights, Tail Lengths and Tail Weight at the End of the Experiments.

Start.	HMTL		HMTS		LCA		LCB	
Body Weight (gms.)	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
Hot	14.0	2.228	12.5	5.093	14.2	1.628	12.6	0.508
Med.	13.7	5.753	12.2	3.920	14.6	2.328	12.2	2.288
Cold	13.4	4.620	12.4	1.888	14.9	4.973	12.8	1.440
Tail Length (cms.)								
Hot	7.4	.193	5.5	.233	6.5	.178	7.9	.775
Med.	7.3	.388	5.4	.128	6.6	.113	7.8	.273
Cold	7.2	.590	5.4	.115	6.5	.235	7.8	.128
Finish.								
Body Weight (gms.)								
Hot	21.7	3.493	21.4	1.568	22.2	.0163	19.3	4.268
Med.	24.0	1.228	20.3	1.553	24.8	6.248	19.5	3.112
Cold	20.4	1.808	17.4	4.033	21.6	2.860	19.0	2.025
Tail Length (cms.)								
Hot	9.6	.095	7.4	.088	8.2	.053	9.5	.023
Med.	8.5	.090	6.2	.115	7.6	.138	8.4	.260
Cold	7.8	.335	5.8	.143	7.2	.064	8.2	.43
Tail Weight (grms.)								
Hot	.615	.002866	.480	.000995	.583	.000406	.640	.000072
Med.	.522	.002378	.331	.000401	.488	.004295	.487	.004473
Cold	.433	.008137	.292	.000769	.447	.003369	.438	.000695

Table XXXIII. Analysis of Variance of Body Weight and Tail Length at the Start of the Treatments. (Mean Squares).

Source	df	Body Weight ms.	Tail Length ms.
Strains	3	16.020 **	16.380 **
Treatments	2	.185	.045
S x T	6	.477	.013
Individuals	48	3.037	.220

Table XXXIV. Analysis of Variance for Body Weight, Tail Length and Tail Weight at the Finish of the Treatments.

Source	df.	Body Weight ms.	Tail Length ms.	Tail Weight ms.
Strain	3	46.713	16.020 **	.0835 **
Treatment	2	32.680	10.580 **	.1635 **
S x T	6	6.583 *	.195	.0020
Individuals	47	2.758	.123	.0024

Table XXXV. Covariance Analysis for Final Body Weight Adjusted for Initial Body Weight.

Source	df	RSS	RMS
Strains with Treatments Pooled.	d		
Strains	3	60.6168	20.2056 **
Within Strains	54	210.6900	3.9017
Treatments with Strains Pooled.			
Treatments	2	67.5056	33.7528 **
Within Treatments	55	203,8013	3.7055
Strains & Treatments.			
Strains and Treatments.	11	165.5538	15.0503 **
Within S & T.	46	105.7531	2.2990
Interaction	6	37.4314	6.2386 *

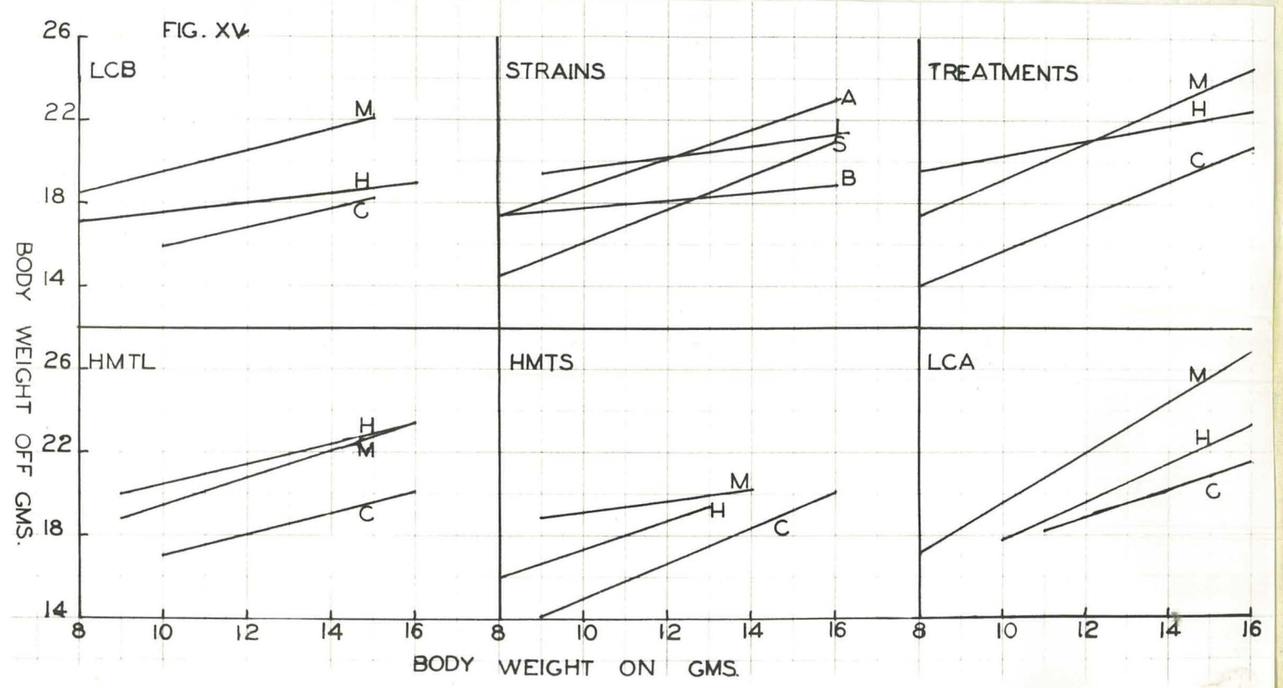


Figure XV: The relationship between final bodyweight and initial bodyweight for all subgroups. H, Hot; M, Med; C, Cold; A, LCA; B, LCB; L, HMTL; S, HMTS.

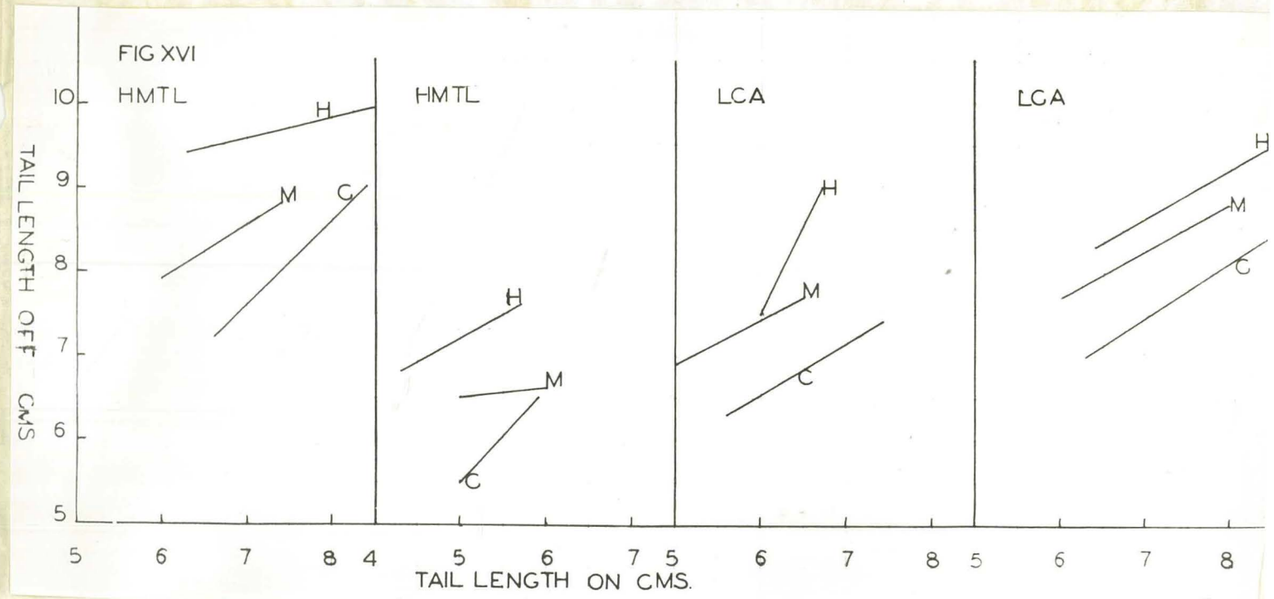


Figure XVI: The relationship between final tail length and initial tail length for all subgroups. H, Hot; M, Med; C, Cold.

tail length at the start of the treatment and body weight, tail length and tail weight at the finish of the treatments for all possible subgroups. The analysis of variance for body weight and tail length respectively at the start of the treatment shown in Table XXXIII shows that only strain differences were present. By the end of the treatments however, the analyses of variance for body weight and tail length presented in Table XXXIV showed significant strain and treatment effects for tail length but a significant strains X treatment interaction was present for body weight.

The presence of the interaction for body weight would suggest that the treatments have affected the four strains differently. For tail length however, the lack of an interaction would suggest that the treatment effect is additive; that is the treatments have affected the four strains similarly.

The analysis of variance for weight of the tail, shown in Table XXXIV, also shows strains and treatment differences to be present, and the treatment effects appear to be additive because of the lack of a strains X treatment interaction.

ii. Covariance analyses of Final Body Weight & Tail Length adjusted for Initial Body Weight & Tail Length Respectively.

The covariance analysis of final body weight adjusted for initial body weight is shown in Table XXXV and shows that a significant (P. 5%) strain plus treatment interaction was present between the adjusted means. A significant interaction was also present in the covariance analysis of final tail length adjusted for initial tail length shown in Table XXXVI. These covariance results would suggest that the treatments have not had an additive effect in the four strains; that is the treatments have not affected the four strains similarly. The regression lines are shown in Figure XV and XVI respectively.

iii. Growth Rates for Body Weight and Tail Length.

Table XXXVII shows the mean body weight and tail length for all possible subgroups on days 1, 2, 3, 6, 9, 15 and 21. The means are shown in Figures XVII and XVIII. The body growth data shows differences in the way that body weight is reached at the end of the treatments. In the HM strains the hot treatments cause an acceleration in growth which in the HMTS strain is maintained for the whole 21 days. In the HMTL strain however, the medium treatment groups has overtaken the hot groups by day 15. In the LCA strain the hot treatment has retarded the rate of body weight gain for the first few days on the treatments and it is not till day 15 that the hot treatment group overtakes the cold treatment group. In the LCB strain the hot and medium groups have similar growth rates for the whole of the 21 days.

The cold treatment groups showed a slower body weight gain in the HM strains but in the LCA strain body growth is actually accelerated in the first 9 days. In the LCB strain body growth in the cold group continuous at a similar rate to the control and

Table XXXVI. Covariance Analysis for Final Tail Length Adjusted for Initial Tail Length.

Source	df	RSS	RMS
Strains with Treatments Pooled.			
Strains	3	2.0550	.6850
Within Strain	54	22.3587	.4122
Treatments with Strains Pooled.			
Treatments	2	17.6526	8.8263 **
Within Treatment	55	6.6611	.1211
Strains and Treatments.			
Strain and Treatment	11	22.3217	2.0292 **
Within S & T	46	1.9920	.0433
Interaction	6	2.6141	.4356 **

Table XXXVIIa. Mean Body Weights on Day 1, 2, 3, 6, 9, 15 and 21. For all Possible Subgroups.

Day	1	2	3	6	9	15	21
HMTL (gms.)							
Hot	14.0	14.6	15.0	17.6	18.5	20.5	21.7
Med.	13.7	14.1	14.6	16.5	17.3	20.9	24.0
Cold	13.4	13.8	14.3	14.8	16.0	18.8	20.4
HMTS (gms.)							
Hot	12.5	13.0	13.7	15.6	16.8	19.3	21.4
Med.	12.2	12.4	13.1	14.5	15.5	17.8	20.3
Cold	12.4	12.3	12.6	14.1	14.7	16.0	17.4
LCA (Gms)							
Hot	14.2	13.7	15.2	16.5	17.9	20.0	22.2
Med.	14.6	15.0	15.6	16.7	18.7	22.0	24.8
Cold	14.9	16.3	16.0	17.8	18.8	19.8	21.6
LCB (gms)							
Hot	12.6	12.8	13.1	14.2	15.9	17.9	19.3
Med.	12.2	12.6	13.1	13.9	15.4	18.2	19.5
Cold	12.8	12.3	14.1	14.6	15.0	17.4	19.0

Table XXXVIIb.

Means Tail Lengths on Day 1, 2, 3, 6, 9, 15 and 21 for all possible Subgroups.

Day	1	2	3	6	9	15	21
HMTL							
Hot	7.4	7.5	7.6	8.1	8.6	9.2	9.6
Med.	7.3	7.3	7.4	7.6	7.8	8.2	8.5
Cold	7.2	7.2	7.3	7.4	7.5	7.7	7.8
HMTS							
Hot	5.5	5.6	5.7	6.1	6.4	7.1	7.4
Med.	5.4	5.5	5.6	5.8	5.9	6.1	6.2
Cold	5.4	5.4	5.4	5.5	5.6	5.7	5.8
LCA							
Hot	6.5	6.6	6.7	7.0	7.3	7.8	8.2
Med.	6.6	6.6	6.6	6.8	7.1	7.2	7.6
Cold	6.5	6.5	6.5	6.6	6.7	6.9	7.2
LCB							
Hot	7.9	7.9	8.0	8.2	8.4	8.9	9.5
Med.	7.8	7.8	7.9	8.0	8.1	8.2	8.4
Cold	7.8	7.9	7.9	8.0	8.0	8.1	8.2

Table XXXVIII.

Covariance Analysis of Final Tail Length Adjusted for Final Body Weight.

Source	df.	RSS	RMS
Strains	3	47.8383	15.9461**
Adj. Means Within Strains.	54	21.9228	.4060
Treatments	2	18.4382	9.2191**
Adj. Means Within Treatments.	55	51.3229	.9331
Strains & Treatments.	11	66.8028	6.0730**
Adj. Means Within S. & T.	46	2.9583	.0643
Interaction.	6	.5261	.0877

Table XXXIX.

Covariance Analysis of Tail Weight Adjusted for final Body Weight.

A. Strains with Treatments Pooled.

Source	df	RSS	RMS
Error	51	.35880	.00704
Reg. Coef.	3	.00534	.00178
Within	54	.36414	.00674
Adj. Means	3	.20375	.06792**

B. Treatments with Strains Pooled.

Source	df	RSS	RMS
Error	53	.24078	.00454
Reg. Coef.	2	.05743	.02871**
Within	55	.29821	.00542
Adj. Means	2	.26987	.13494

C. Strains and Treatments.

Source	df	RSS	RMS
Error	35	.04189	.00119
Reg. Coef.	11	.01285	.00116
Within	46	.05675	.00119
Adj. Means	11	.51731	.04703**

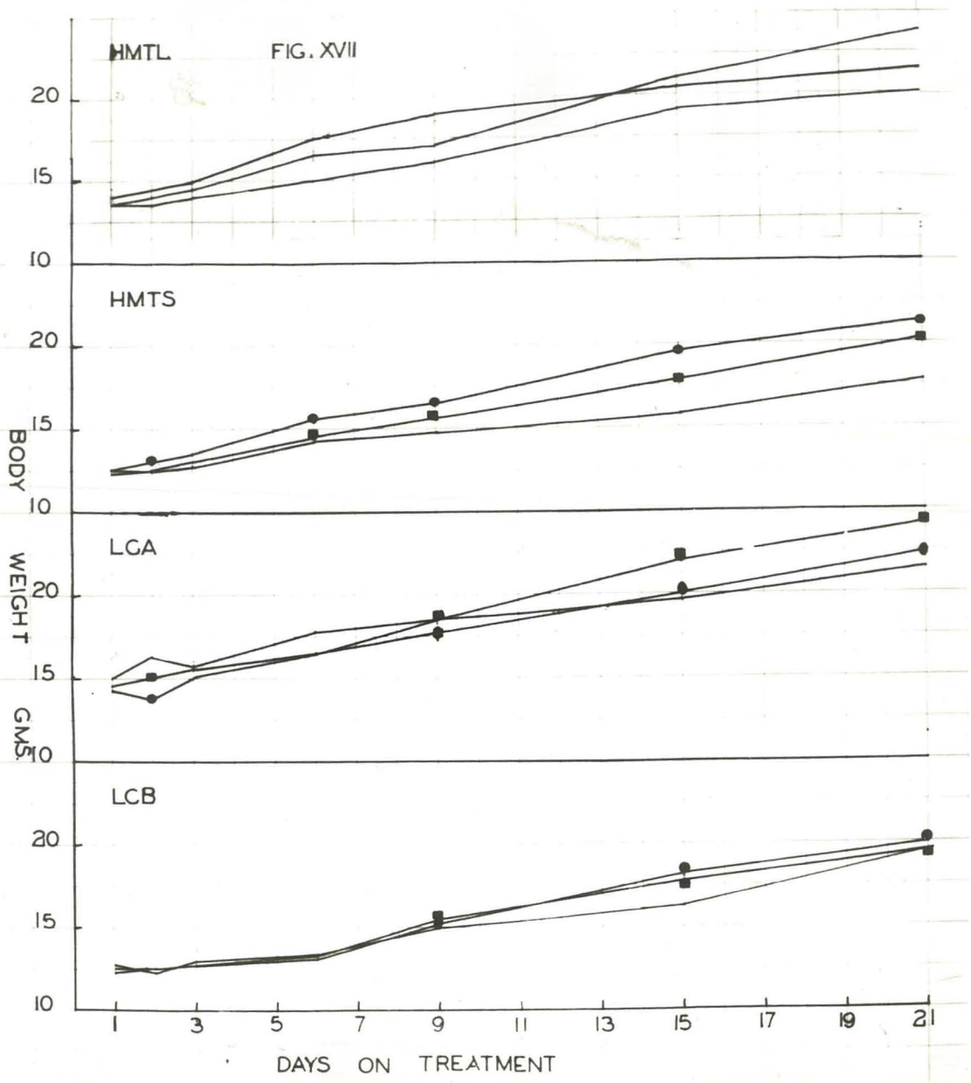


Figure XVII: Mean body weight for all subgroups on days 1, 2, 3, 6, 9, 15 & 21.
H, Hot; M, Med; C, Cold.
——— Hot, ——— Med, ——— Cold.

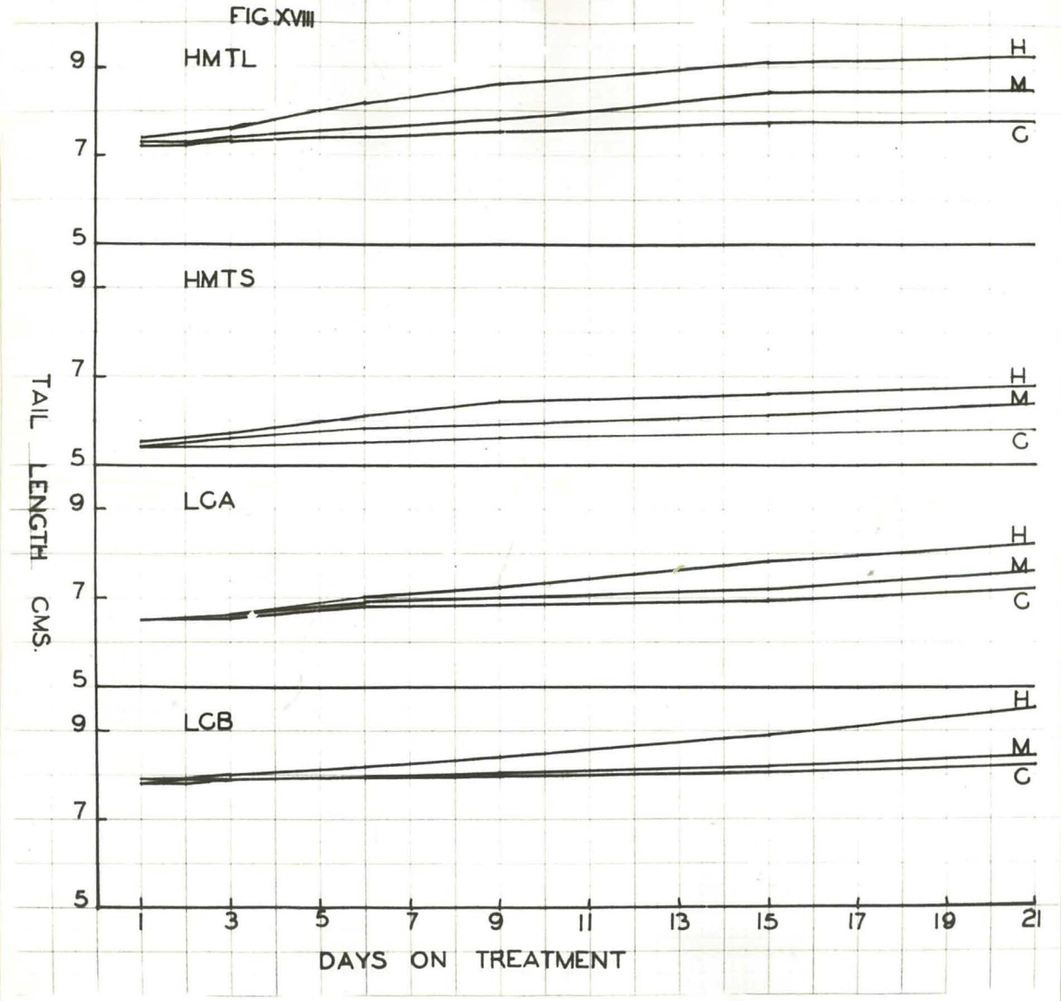


Figure XVIII: Mean tail length for all subgroups on days 1, 2, 3, 6, 9, 15 & 21. H, Hot; M, Med; C, Cold.

FIG. XIX

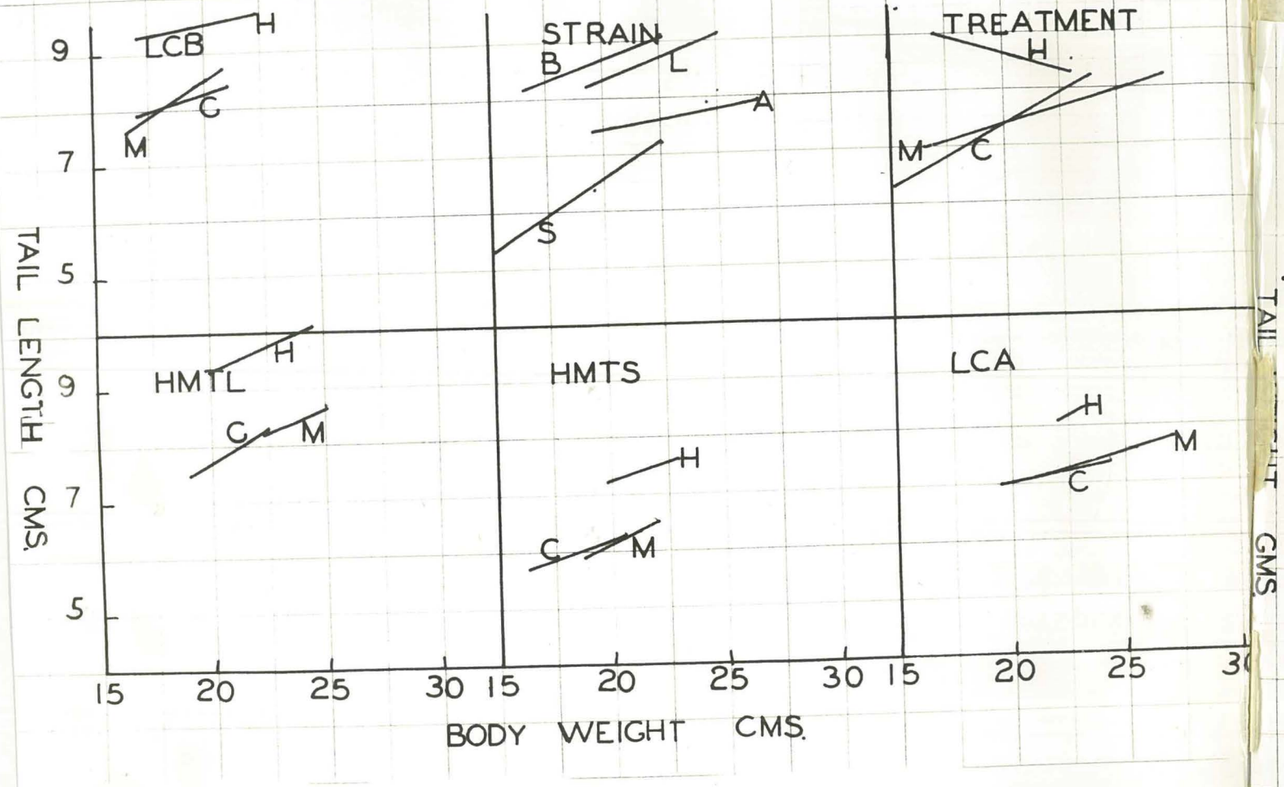


Figure XIX: The relationship between final tail length and final bodyweight. H, Hot; M, Med; C, Cold; A, LCA; B, LCB; L, HMTL; S, HMTS.

FIG. XX

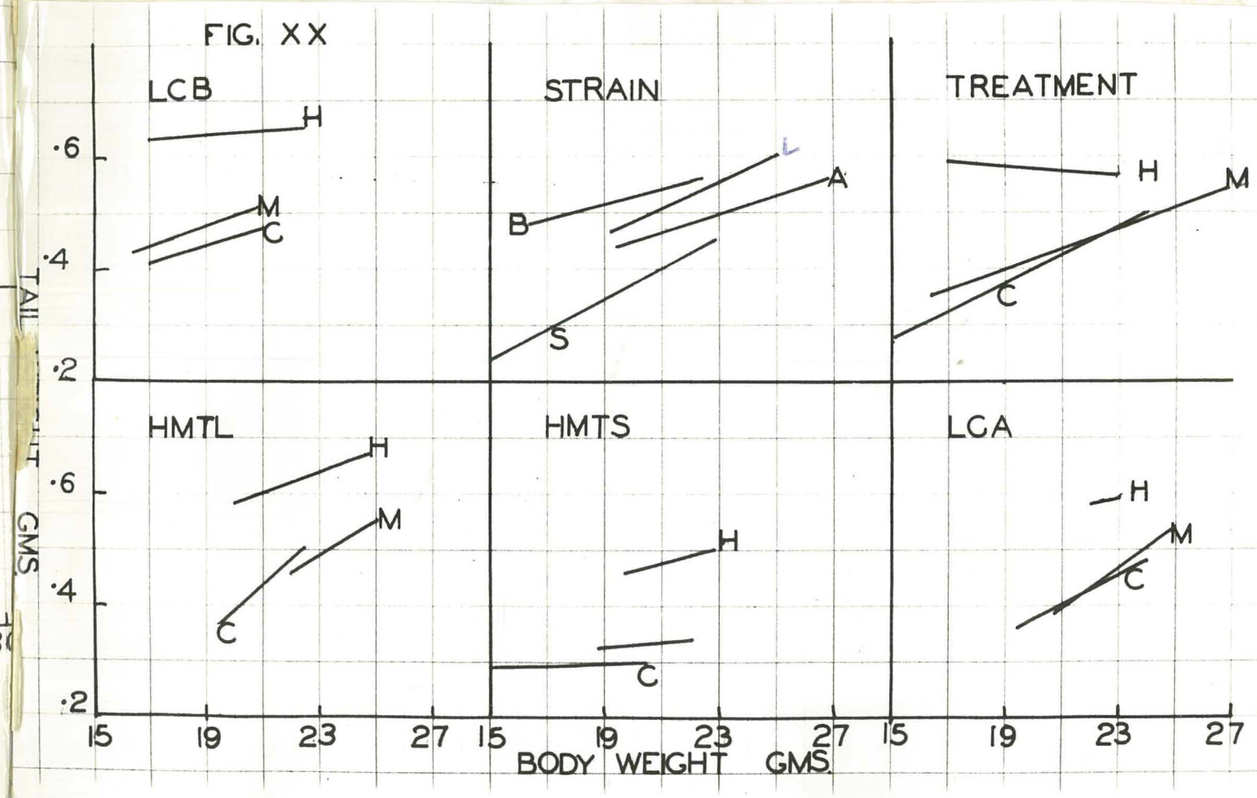


Figure XX: The relationship between tail weight and final bodyweight for all subgroups. H, Hot; M, Med; C, Cold; A, LCA; B, LCB; L, HMTL; S, HMTS.

hot groups up to day 9 when it falls off.

For tail length the means shown in Table VIb and Figure show that the hot treatment causes an immediate acceleration in growth in tail length and this is clear after day 3. However, the time that the divergence in tail length between the treatment groups continues appears to vary between the four strains. After day 15 the three treatment groups in each of the HM strains appear to increase tail length at the same rate. This is not so in the LC strains, especially the LCB strain, where the growth in tail length between the treatment groups appears to be still diverging at day 21.

Therefore differences in rate of body weight increase under the three treatments are present between the four strains and treatment effects on tail length are also present.

iv. Covariance Analysis of Final Tail Length adjusted for Final Body Weight.

The covariance analysis of final tail length adjusted for final body weight is shown in Table XXXVII. The regression lines are shown in Figure XIX. The results show that in the strains analysis with treatments pooled, significant differences were present between the adjusted means but not between the slopes of the regression lines. This would suggest that once body weights are adjusted for, differences in tail length are present between the strains. The LCB strain having the longest tail while the HMTS strain has the shortest tail.

The treatments analysis with strains pooled showed that the adjusted means were significantly different but not heterogeneity was present between the slopes of the regression lines. This shows that differences are present for tail length between the treatment groups. Much of the difference, however, is between the hot and medium groups while the medium and cold groups have similar regression lines.

v. Covariance Analysis of Tail Weight adjusted for Final Body Weight.

The covariance analysis of tail weight adjusted for final body weight is shown in Table XXXIX. The regression lines are shown in Figure XX. The strains analysis with treatments pooled has shown that significant differences were present between the adjusted means but not between the slopes of the regression lines. This would suggest that differences are present between the strains for the weight of the tail at a constant body weight. The LCB strain had the heaviest tail while the HMTS strain had the lightest tail.

The treatment analysis with strains pooled showed that the slopes of the regression lines showed heterogeneity. The medium and cold groups have similar regression lines which have positive slopes while the hot treatment group has a regression line of negative slope.

Table XXXX.

Means and Variances for Weight of Total Body Fat and Weight of Abdominal Fat.

		HMTL	HMTS	LCA	LCB
Total Body Fat (grms.)					
Hot	Mean	3.103	2.484	3.396	2.017
	Variance	.0858	.1789	.2153	.1100
Med.	Mean	2.062	2.334	3.301	1.875
	Variance	.3158	.2897	1.4333	.0665
Cold	Mean	1.696	1.279	1.915	1.830
	Variance	.1733	.0304	.4558	.0728
Abdominal Fat (Grms.)					
Hot	Mean	.417	.403	.438	.384
	Variance	.00309	.00118	.00279	.00599
Med.	Mean	.397	.383	.535	.368
	Variance	.00123	.00203	.01455	.01161
Cold	Mean	.396	.344	.534	.390
	Variance	.00226	.00357	.00985	.00313

Table XXXXI.

Analyses of Variance for Weight of Total Body Fat and Weight of Abdominal Fat for the Control Mice (Mean Squares).

Source	df	Body Fat	Abdominal Fat
Strains	3	2.746**	.0513**
Treatments	2	6.440**	.0005
S x T.	6	.620	.0068
Individuals	47	.292	.0052

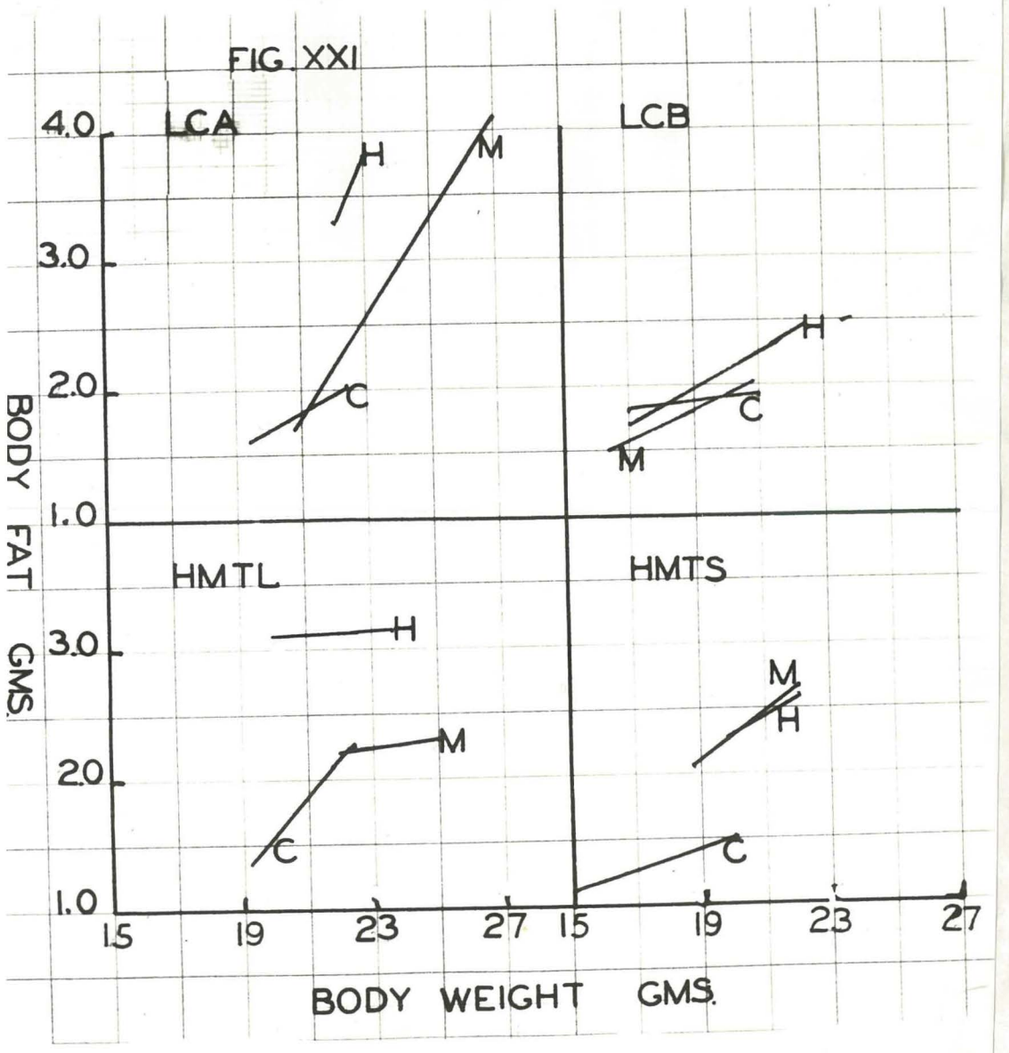


Figure XXI: The relationship between total bodyfat and final bodyweight for all subgroups. H, Hot; M, Med; C, Cold.

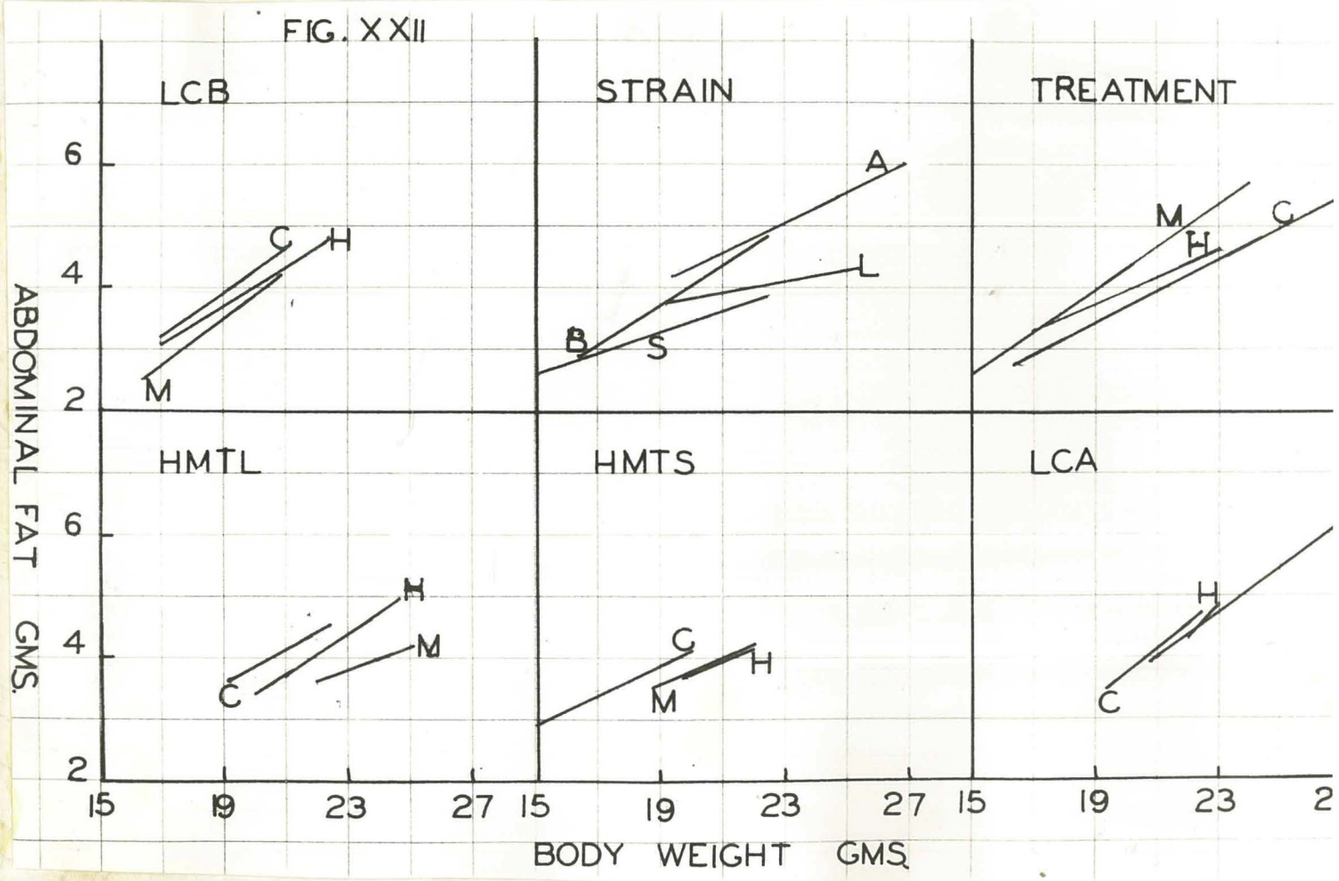


Figure XXII: The relationship between weight of abdominal fat and final bodyweight for all subgroups. H, Hot; M, Med; C, Cold; A, LCA; B, LCB; L, HMTL; S, HMTS.

These results would suggest that in the hot treatment group, mice of lower final body weight had a tail of higher weight than have mice of higher final body weight. This is due to the low-body weight LCB strain having a heavier tail once body weight was adjusted for than did the high-body weight LCA strain. Mice in the medium and cold treatment groups had tails of similar weights in all strains once body weight differences were adjusted for.

B. Abdominal Fat & Total Body Fat.

Table XXXX shows the means and variances for the weight of total body fat and the weight of abdominal fat for all possible subgroups at the finish of the treatments. The analysis of variance shown in Table XXXXI shows that for total body fat both strain and treatment differences were present, while only strain differences were present for abdominal fat.

The covariance analysis of total body fat adjusted for body weight is presented in Table XXXXII. This shows a significant strain X treatment interaction to be present. An examination of the individual strain covariance results shown in Table XXXXIII show that in the two low-body weight strains (HMTS and LCB) no differences were present in the slopes of the treatment regression lines or the adjusted means. The regression lines are shown in Figure XXI. In the two high-body weight strains, however, (HMTL and LCA) differences were present between the adjusted mean and the slopes of the treatment regression line showed no heterogeneity. These results would suggest that the treatment has not affected total body fat in the low-body weight strain but has in the high-body weight strain. The weight of total body fat in the hot treatment groups corrected for body weight for the high body weight strains is higher than in the hot and medium groups which have similar amounts of fat.

The results of the covariance analysis for weight of abdominal fat adjusted for body weight are shown in Table XXXXIV. The individual strain regression lines are shown in Figure XXII. The individual strain analysis showed that within each of the four strains the slopes of the treatment regression lines showed no heterogeneity. For all strains except the HMTL strain no differences were present in the adjusted means but in the HMTL strain differences ($P < 0.5$) were present.

These results would suggest that no differences were present between the treatment groups within each strain. However, an analysis of strains with treatment effects pooled showed that strain differences were present.

C. Pelt Weight and Hair Weight.

Table XXXXV shows the means and variances for the weight of the pelt and weight of hair for all possible subgroups at the finish of the treatments. The analysis of variance for pelt weight and hair weight are shown in Table XXXXVI. These results show that both strain and treatment differences were present for pelt weight, but a significant strain and treatment interaction was present for hair weight. The lack of an interaction in the

Table XXXXII.

Covariance Analysis for Weight of Total Body Fat Adjusted for Body Weight.

Strains	3	1.32190	.44063
Within Strains	54	21.19461	.39249
Treatments	2	6.23461	3.11821**
Within Treatments	55	16.28010	.29600
Strains and Treatments	11	12.99426	1.18130
Interaction.	6	5.43595	.90599**

Table XXXXIII.

Covariance Analysis of Total Body Fat Adjusted for Body Weight. (individual Strain Analysis).

		HMTL	HMTS	LCA	LCB
Source	df	R.M.S.	R.M.S.	R.M.S.	R.M.S.
Error	9	.11235	.19670+	.43972	.05370
Reg. Coef.	2	.33815	.01563	.30566	.04217
Within	11	.15341	.16048+	.41534	.05160
Adj. Means	2	2.75003**	.40894	2.15122*	.04212

+ One df. deducted because of one death.

Table XXXXIV.

Covariance Analysis for Weight of Abdominal Fat Adjusted for Body Weight.

		HMTL	HMTS	LCA	LCB
Source	df	R.M.S.	R.M.S.	R.M.S.	R.M.S.
Error	9	.001118	.001826+	.006470	.004382
Reg. Coef.	2	.000092	.000007	.000289	.000199
Within	11	.000932	.001329+	.005347	.003621
Adj. Means	2	.005484*	.000367	.019208	.001912

+ One df. deducted because of one death.
* P70.5

Table XXXXV.

Means and Variances for the Weight of the Pelt and Hair at the Finish of the Treatments.

		HMTS	HMTL	LCA	LCB
Pelt Weight (grms.)					
Hot	Mean	2.585	2.728	3.079	2.403
	Variance	.0713	.0133	.1669	.3103
Med.	Mean	2.937	2.534	3.134	2.367
	Variance	.0515	.1773	.4950	.1648
Cold	Mean	2.563	1.860	2.465	2.281
	Variance	.1407	.0947	.2380	.0791
Hair Weight (grms.)					
Hot	Mean	.254		.136	.152
	Variance	.000399		.000583	.001501
Med.	Mean	.251		.141	.196
	Variance	.001688		.001453	.001392
Cold	Mean	.221		.203	.226
	Variance	.002441		.001187	.000984

Table XXXXVI.

Analysis of Variance for Weight of Pelt and Weight of Hair at the End of the Experiment.

		Hair Weight		Pelt Weight
Source	df	Mean Square	df	Mean Square
Strains	2	.025751	3	1.0308 **
Treatments	2	.004820	2	1.2345 **
S + T.	4	.005352 **	6	0.2439
Individuals.	36	.001285	47	0.1705

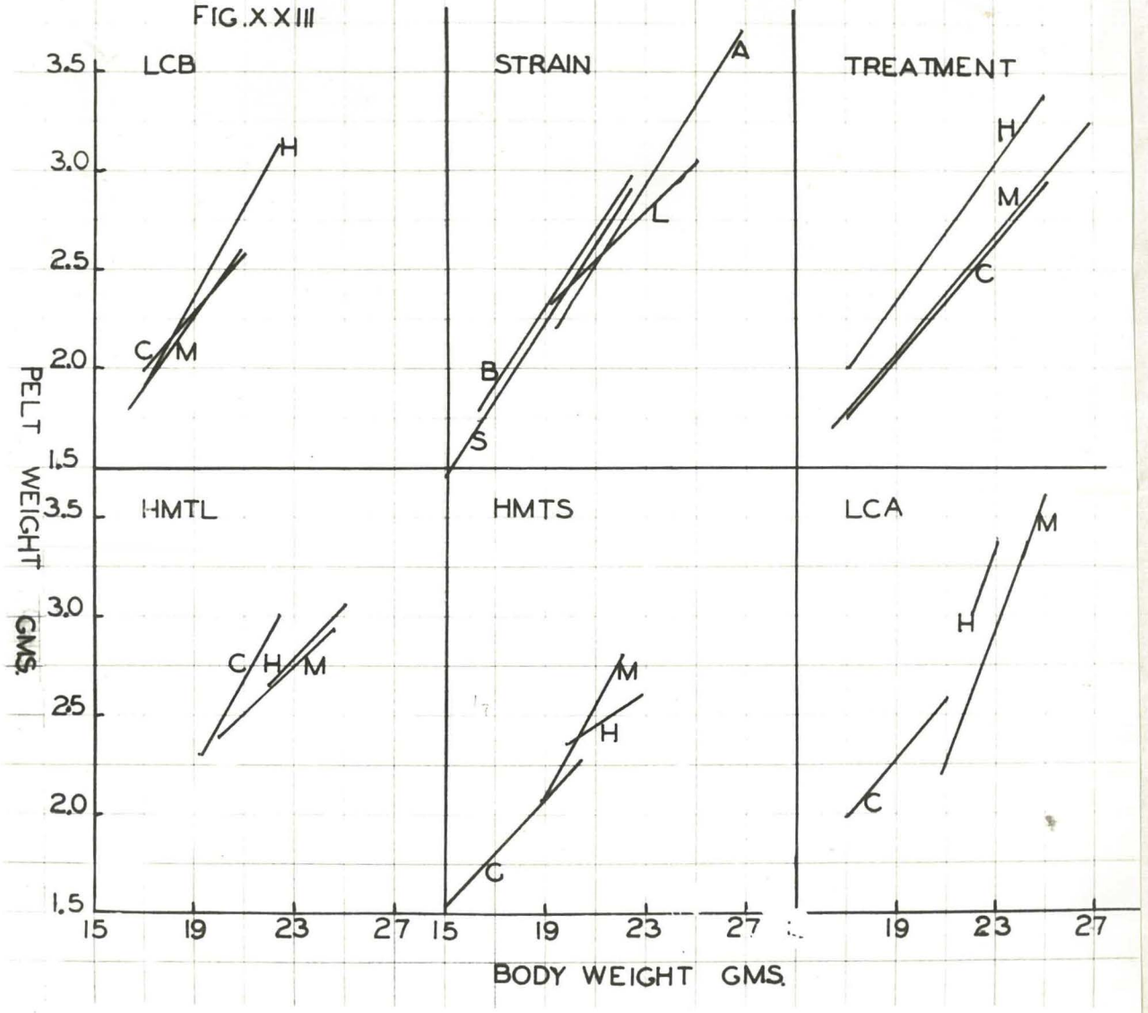


Figure XXIII: The relationship between pelt weight and final bodyweight for all subgroups. H, Hot; M, Med; C, Cold; A, LCA; B, LCB; L, HMTL; S, HMTS.

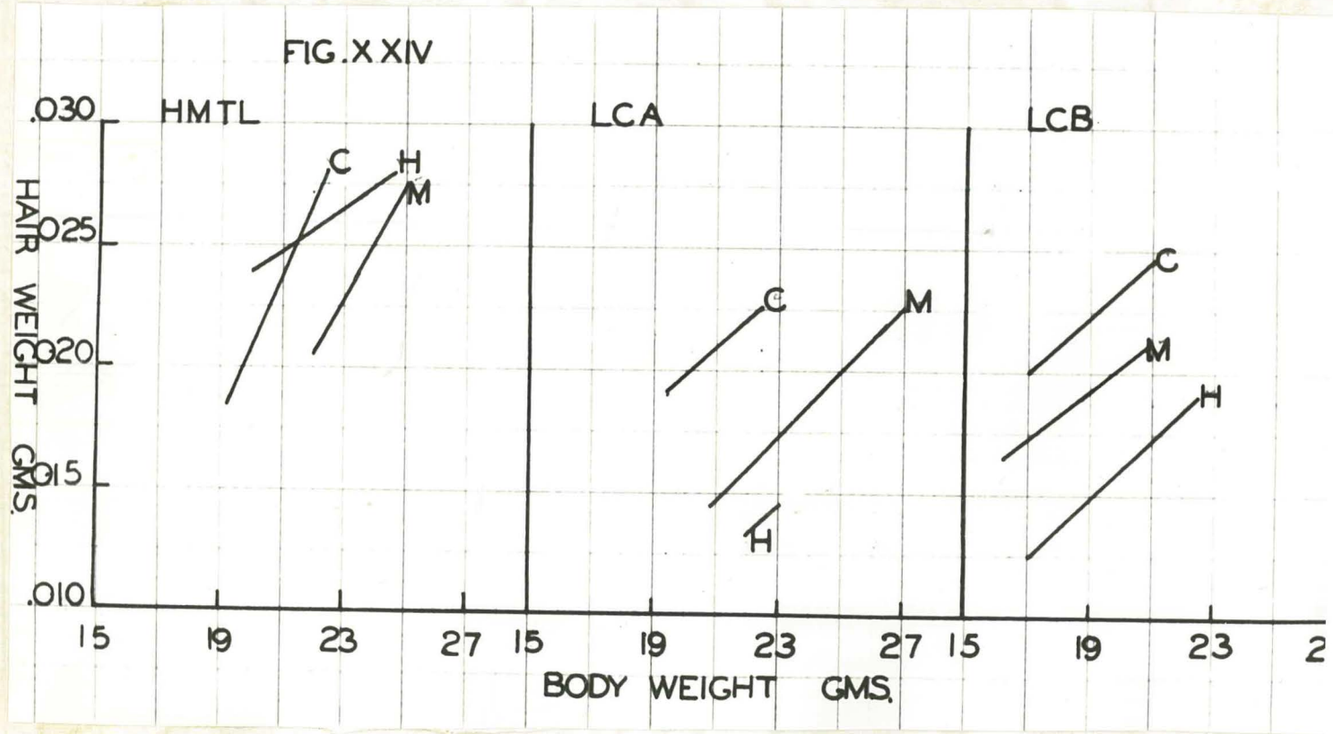


Figure XXIV: The relationship between hair weight and final bodyweight for all subgroups. H, Hot; M, Med; C, Cold.

pelt weight analysis would suggest that the treatments have affected the four strains similarly. However, the presence of the interaction in the hair weight analysis would suggest that the strain and treatment affects are not additive; that is the treatments have affected the three strains differently (the HMTS strain was excluded from the analysis because of the infestation of lice in the early stages of the treatments had caused some shedding of hair).

The results of the covariance analysis of pelt weight adjusted for body weight shown in Table XXXXVII show no differences between the strains or between the treatments. The regression lines are shown in Figure XXIII. Table XXXXVIII shows the results of the covariance analysis for hair weight adjusted for body weight and show a significant strain X treatment interaction was present. The regression lines are shown in Figure XXIV. These results would suggest that the treatments have had no effect on the weight of pelt adjusted for body weight, but they have effected the weight of hair adjusted for body weight. The individual covariance analysis for each of the three strains for hair weight adjusted for body weight showed that the HMTL strain had no significant differences between the slopes of the regression lines for the three treatments and the adjusted means were not significantly different. For the two LC strains however, although no differences were present in the slopes of the treatment regression lines, the adjusted means were significantly different. The hair weight for the cold treatment adjusted for body weight was significantly greater than the hair weight for the hot treatment group for the LC strains.

Therefore it would appear that the treatments have not affected the weight of the pelt adjusted for body weight in the four strains nor the weight of hair adjusted for weight in the HMTL strain. They have however, effected the weight of hair adjusted for body weight in the LC strains.

D. Food Intake.

Table XXXXIX shows the total amount of food consumed by five mice in each of the possible subgroups. The analysis of variances presented in Table L shows that a significant strain X and treatment interaction was present. The within days effect was included in the error sums of square, and only strain, treatment and the strain X treatment interaction sums of square were taken out in the analysis. The presence of the significant interaction would suggest that the four strains had reacted differently to the treatments in the amount of food consumed. Figure XXV shows the amount of food consumed each day by the 5 mice in each subgroup.

Table LI gives the total amount of food consumed by 5 mice in each subgroup, total body weight gain of 5 mice in each subgroup and the amount of food consumed per gram body weight gain for each subgroup. In each of the four strains the hot treatment subgroups consumed less food per gram body weight gain, while the cold treatment subgroups consumed the most food per gram body weight gain. However, within each of the treatments, differences in the efficiency of food utilization were present

Table XXXXVII.

Covariance Analysis of Pelt Weight Adjusted for Body Weight.

Covariance Analysis (Adjusted Means).

Source	df	Residual SS	Residual MS
Pelt Weight			
Strains	3	.14312	.04777 NS
Within Strains	54	4.43928	.08221
Treatments	2	.39476	.19738 NS
Within Treatments	55	4.34213	.07895
Strains & Treatments	11	1.14103	.10373 NS
Within S & T.	46	3.44137	.07481
Interaction.	6	.60315	.10053 NS

Table XXXXVIII.

Covariance Analysis of Hair Weight Adjusted for Body Weigt.

Source	df	R.SS	R.MS
Strains	2	.055893	.027947
Within Strains	41	.063992	.001555
Treatments	2	.006723	.003462
Within Treatments	41	.113162	.002760
Strains & Treatments	8	.089858	.011232
Within S & T.	35	.030027	.000858
Interaction	4	.027242	.006811 **

Table XXXXIX.

Total Amount of Food Consumed by Each Subgroup (grms.)

	HOT	MED.	COLD
HMTL	325.4	482.4	506.6
HMTS	297.8	465.0	548.0
LCA	307.0	509.8	631.2
LCB	433.4	470.8	561.0

Table L.

Analysis of Variance for the Amount of Food Consumed Consumed Each Day.

Source	df	Mean Square.
Strains	3	200.453
Treatments	2	2610.690
S x T	6	153.688 **
Error	228	33.654

Table LI.

Total Amount of Food Consumed, Total Body Weight Gain and Food Consumed per grm. Body Weight Gain for the Five Mice in Each Subgroup.

		Total Food	Total Body Wt. Gain	Food/Gm. Body Wt.
HMTL	Hot	325.4 grms	38.6 grms	8.43
	Med.	482.4 "	51.6 "	9.35
	Cold	506.6 "	35.2 "	14.39
HMTS	Hot	297.8 "	44.8 "	6.65
	Med.	465.0 "	40.4 "	11.51
	Cold	548.0 "	25.3 "	21.66
LCA	Hot	307.0 "	40.4 "	7.60
	Med.	590.8 "	51.0 "	11.58
	Cold	631.2 "	33.4 "	18.90
LCB	Hot	433.4 "	33.6 "	12.90
	Med.	470.8 "	36.2 "	13.01
	Cold	561.0 "	30.8 "	18.21

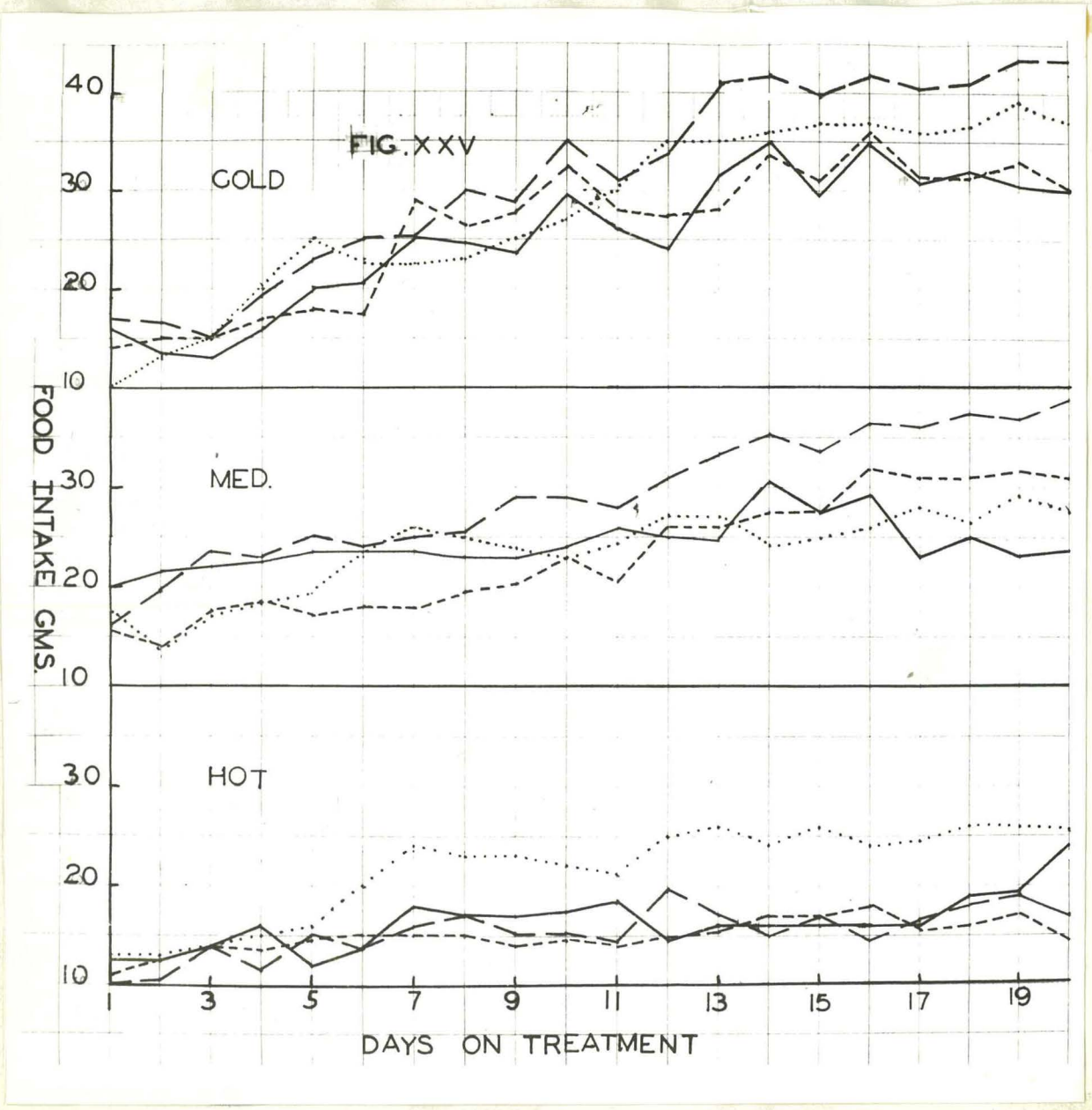


Figure XXV: Total daily food intake for the 5 mice in each subgroup during the treatments. —— ——LCA, ... LCB, ——— HMTL, - - - - HMTS.

between the four strains. The HHDS strain was the most efficient strain in the hot environment, but was the least efficient in the cold environment. The HHTL strain was the most efficient strain in the medium and cold treatments. The high-body weight LCH strain was more efficient than was the low-body weight LCL strain in the hot and medium treatments but both strains showed similar efficiencies in the cold treatment. The high-body weight HHTL strain was more efficient in all treatments except the hot treatment where the low-body weight HHTL strain was the more efficient strain.

In the three treatments the strain which showed the highest body weight gain during the treatment was also the most efficient strain.

Chapter VI.

GENERAL DISCUSSION.

A. Body Weight and Tail Length.

The results obtained for tail length confirm those of other observers (e.g. Harrison etal. 1959) that rearing mice at a higher temperature results in a longer tail. This was true even though the treatments were for a period of only three weeks. These results differ from those of Harrison etal. (1959) in that the short-tail strains did not show a greater response to the hot treatments than did the long-tail strains. Within the two long tail strains however, mice of shorter initial tail length have shown a greater response to the hot treatments than have mice of longer initial tail length. Within the two short-tail strains however, all mice in the hot treatment groups have shown similar responses. Therefore the results for the long-tail strains agree with the observation of Harrison etal. (1959) but this was not so for the short-tail strain.

In the cold treatment groups, all mice had responded similarly to the treatment, and the longer absolute tail-length of the long-tail strains, was the result of differences present at the start of the treatments.

Body weights were reduced in all strains in the cold environment even after body weight differences at the start of the treatments were adjusted for. The hot treatment also reduced body weights in all strains except the HMTL strain where the medium and hot groups had similar body weights once initial body weight differences were adjusted for.

The analysis of strain with treatments pooled showed that the short and long-tail strains made different response over the treatment period. Mice of lower initial body weight in the short-tail strains had made a greater gain in body weight than have mice of lower initial body weight in the long-tail strains.

B. Body Temperature.

The results obtained are similar to those obtained by Fleischner and Sargent (1959) for rats. The mice in the cold treatment groups showed hypothermia for the first few days of exposure after which body temperature rose to a level above that of the medium groups. In the hot and medium groups there was a gradual decline in body temperatures over the treatment period. However, the hot treatment caused body temperatures to be raised above the medium group in the early stages of exposure after which body temperatures gradually declined and by the end of the treatment period the body temperatures were above the medium treatment groups but below the cold groups. These results would suggest that in the early stage of exposure to the treatments the mice had difficulty in regulating body temperatures. Mice in the cold environment had difficulty in controlling heat loss which

caused a decline in body temperature. This probably caused an increase in the metabolic rate of the mice which caused food intake to be increased above the level of the medium groups and body temperatures started to rise again. In the hot treatment groups however, body temperatures would be raised because of the difficulty to dissipate the excess heat as the metabolic rate was lowered, and possibly voluntary muscle activity, so food intake was lowered and body temperature declined. The decline in body temperatures in the medium groups would be the result of the normal decline in body temperatures with increased age.

The carvariance analysis of mean body temperature over the last 10 days of the treatments adjusted for final body weight supports these conclusions. The results suggest that the control of heat loss has had some effect on body weight. In the hot environment mice with the higher final body weight were the mice with the lower body temperatures. This would suggest that the mice in the heat that are able to control heat loss and therefore keep body temperatures down are the mice that showed the greater final body weight. In the cold treatment groups however, the mice that were able to reduce heat loss and therefore maintain body temperature at a higher level showed the greater final body weight.

C. Growth Rates.

a. Body Weight.

The results obtained for the HM strains confirm the observations of Harrison etal (1959) that rearing mice at a higher temperature promotes early growth. However, this was not so for the LC strain where early growth was not evident. The LCA strain actually showed a reduced rate of growth in the hot environment for the first few days of exposure and it was not till day 15 that the hot treatment groups had a higher mean bodyweight than did the cold treatment group. The LCB strain had a rate of body weight increase in the hot environment that was similar to the medium group. For the HM strain growth was faster during the early stage of exposure in the HMTL strain but growth rate was reduced in the latter stages. The HMTS strain had a higher body weight at all stages.

The results obtained for mice in the cold confirm the observations of other workers (e.g. Barnett and Manly 1956) that growth is retarded in the cold. However, during the early stages of exposure the LCA strain actually showed a higher rate of body weight increase than did the medium group but by day 9 the mean body weight of the cold group was less than the medium group. The LCB strain had a similar growth rates in both the medium and cold groups up to day 9 after which the growth rate was reduced. In the HMTL strain however, growth was retarded during the early stages of exposure but after day 9 growth rateswere similar for the cold and medium groups. A slower rate of growth in the cold was present at all stages for the HMTS strain.

b. Tail Length.

The results obtained for growth in tail length confirmed the observations of Harrison etal. (1959) that exposure to a higher temperature causes an acceleration in the growth of tail length. The increase in tail length in the hot treatment groups was clear by day 3. Growth in tail length was retarded in the cold. The length of time that the divergence in growth in tail length continued between the treatments differed between the four strains. After the fifteenth day of exposuretail length in the three treatment groups increased at a similar rate in the HM strains but in the LC strains the divergence in tail length was still continueing. These results would suggest that the HM strains have responded more quickly to the treatments than have the LC strains. By day 15 the HM strains have shown the full response to the treatments while the LC strains are still showing a response to the treatments.

d. Tail Weight.

At three weeks of age the strain had tails of different weights once body weight differences were adjusted for. The HMTL strain had the heaviest tail while the HMTS had the lightest tail. The LC strains had tails of similar weight even though they differed in tail length.

At the finish of the treatment the LCB strain had the heaviest tail which was significantly heavier than the LCA tail once body weight differences were adjusted for. Therefore the LCB strain has shown a greater increase in tail weight than has the LCA strain during the treatment period.

The hot treatment caused a considerable increase in the tail weight while the cold and medium treatment groups had tails of similar weight even though differences in tail length were present between the treatment groups.

e. Abdominal Fat and Total Body Fat.

The results for body fat confirm the observations of Fowler (1958) that at three weeks of age no differences are present for the weight of body fat in mice selected for high and low body weight even when body weight differences are adjusted for. At 6 weeks of age, the high body weight strain had a higher weight of body fat. Once body weight differences were adjusted for however, mice of the low body weight strain tended to have a higher weight of fat than the high body weight strain, an observation also made by Fowler (1958).

Fowler (1958) showed that after about 35 days of age, mice of the high body weight strain commences to lay down considerable amounts of fat whereas the low body weight strain does not and continues protein disposition and bone growth to an advanced age. Therefore, Fowler suggests that the different rates of growth in mice of high and low-body weight may account for some of the differences in carcass composition.

The results have shown that both body weight and body fat are less in the cold treatment group but that the weight of fat but not body weight were increased in the hot treatment groups. Once body weight differences between the treatment groups were adjusted for the low-body weight strains showed no differences in body fat between the treatments. However, the high body weight treatment groups with the hot treatment group having a greater weight of fat than the medium and cold groups which had similar amounts of fat.

Barnett etal. (1959) showed that both body weight and body fat (expressed as fat per 100gms. of homogenized carcass) were less in mice born and reared in the cold than in mice born and reared in the control environment. In the present experiment if the results were expressed as a percentage of body weight then large differences are also present between the treatment group. Once body weight differences are adjusted for by the use of covariance technique however, differences are present only between the hot and medium groups of the two long tail strains.

Therefore the situation found for rearing mice in the cold and medium environments is similar to that found for selection for high and low body weight at 6 weeks of age. Heroux (1958) has shown that for mice exposed to cold temperatures (6°C) the reduced rate of body growth is due mainly to a reduced rate of protein deposition in the muscles. Other observers (Fleischner and Sargent 1959, and Sealander 1952) have shown that if the cold temperature conditions are not too severe, or if heat loss can be kept to a minimum by huddling, nesting etc., food intake is increased to a level which satisfies the heat requirements and permits further growth but at a reduced rate.

The results for body weight and body fat found in the study support these conclusions. The reduced rate of growth in the cold being due probably to a reduced rate of protein deposition with a corresponding reduction in the rate of fat deposition.

The reasons for the higher amounts of fat in the hot treatment groups of the high body weight strains is not clear. Young and Cook (1955) have suggested that the fat derived from the diet is put to two primary uses.

1. it may be stored
2. it may be oxidized to supply energy.

They have suggested "that for mice in the hot environment there is little if any need for fat storage as an insulating device or as an available energy store. Because of the relatively low energy requirements of these mice however, they have no alternative but to store fat."

The present evidence does not support these conclusions as the two low body weight strains did not lay down a greater amount of fat in the hot environment. The increased weight of fat in the hot environment is more likely to be due to an acceleration of the normal fat deposition processes that normally take place from about 35 days of age onwards and not because the mice were forced to

store fat. Fowler (1958) showed that in mice of the high body weight strain show an increased rate of fat deposition from about 35 days of age onwards and the hot treatment could have caused this process to start earlier or else caused the rate of fat deposition to be accelerated above the normal level from 35 days onwards. Fowler (1958) also showed the mice of the low body weight strain did not show this increased rate of fat deposition at any stage but appear to continue protein deposition and bone growth to advanced ages.

The results obtained for abdominal fat have shown differences to be present between the strain both at the start and finish of the treatments however, no differences were present between the treatment groups within the strains except for the HMTL strain were significant (P 70.5) differences were present between the hot and medium groups.

Barnett etal. (1959) have shown that the weight of abdominal fat (expressed as weight per 100gms. of carcass weight) was less in mice born and reared in the cold than in mice born and reared in the ~~cold~~ control Environment. In this study differences were present between the treatment groups if the weight of abdominal fat is expressed as a percentage of body weight. Once body weight differences were adjusted for by the use of a carvariance technique no differences were present except for the HMTL strain.

f. Hair Weight and Pelt Weight.

The results for weight of hair for the LC strains confirm the reports of other observers (Barnett 1959 and Harrison 1959) that the weight of hair grown depends on the temperature at which the mice are reared. This was so even though the treatments were for a period of only three weeks.

The results for the HMTL strain, however, do not agree with the results of other observers as no differences in the weight of hair adjusted for body weight were present between the treatment groups. The reasons for the differences between the response of the LC strains and the HMTL strain to the treatments is not clear. There are however three possible causes of differences in hair weight between the treatment groups, if it is assumed that all mice starting the treatment had completed the first hair cycle.

Firstly the treatments may have delayed the start of the second hair cycle and so clipping the hair at a set time (i.e. 6 weeks of age) would result in the hair being removed before the second hair cycle is completed. Secondly the treatment may have speeded up the hair cycle and by six weeks of age the third hair cycle may be started. Therefore in this case hair from the third hair cycle would have been included in the weight of hair. Thirdly the treatments may not have influenced the hair cycles but may have influenced the amount of hair grown within the second hair cycle.

If the differences between the LC treatment groups are true differences and not the result of hair cycle differences then it

would appear that differences exist between the LC and HMTL strains, in the response to temperature treatments. Thus differences between the LC treatment groups were expressed after three weeks of exposure, but a longer period of exposure may be needed before the HMTL strain would show treatment differences.

It has been suggested that in the sheep the gradient of wool growth on different parts of the body are related to the amount of blood supplied to these various positions on body (Cockrem 1961). An indirect method of measuring blood supply is by measurement of skin temperatures. Heroux (1959) has shown that cold-acclimated rats have a greater number of capillaries and a higher tissue temperature in the skin than control animals. These results indicate a greater blood circulation in the skin of the cold-acclimated rat. If blood supply to the skin is related to hair growth in rats, then it is likely that the higher peripheral temperatures and greater blood supply to the skin of the cold-acclimated rat would promote the growth of a greater weight of hair.

Cockrem & Wickham (1961) has reviewed the possible mechanisms by which a greater blood supply to the skin would promote the growth of a greater weight of wool. These are:

1. The speed of chemical reaction is influenced by temperature and a higher skin temperature may cause a faster rate of conversion of substrates from the blood to wool.
2. Higher skin temperatures are related to a greater cutaneous blood flow and therefore it is likely that a better supply of nutrients reach the follicle.
3. Diffusion rates in general, tend to increase with temperature and a higher skin temperature may result in a greater supply of fibre substrate reaching the follicle.

The results of Heroux (1959) and the discussion of Cockrem & Wickham (1961) may explain the lack of response of the HMTL strain to the treatments. This strain may not have been able to elevate peripheral blood flow and therefore peripheral temperatures to the same level as the LC strains in the cold treatment groups. Therefore, because of the lower blood flow and peripheral temperatures the HMTL strain was perhaps unable to increase the weight of hair grown in the cold due to one or more of the reasons discussed above.

Chase (per com.) has suggested that differences in the weight of hair between the LC groups could be the result of differences in the density of hair. He has suggested that within each of the treatment groups different numbers of hair follicles are actively producing hairs. A greater number of follicles would be active in the cold treatment groups and the least number active in the hot treatment groups.

This would explain the greater number of hairs found on rats (Heroux 1961) and mice (Sealander 1951) acclimated to cold than in controls.

The results obtained for pelt weight confirm the reports of other observers (Heroux and Gredgema 1958, Barnett 1959, and Sealander 1951). The treatments had no effect on the weight of pelt adjusted for body weight nor were differences found between the four strains once body weight differences were adjusted for.

The interest attached to the pelt in thermoregulation lies in its insulative properties, which, as Heroux (1961) has pointed out, can hardly be deduced from its weight. The hair, according to Heroux (1958), provides most of the insulation. No attempts have been made in this study to measure the insulation of the pelt or hair but Heroux etal. (1959) and Hart (1953) have not been able to show any improvement in the insulative properties of the increased hair weight in cold-acclimated rats and mice respectively. Barnett (1959) has some evidence which suggests that there may be an increase in pelt insulation in mice in the cold.

The results for pelt and hair weight for the strains suggest that differences in the insulating properties of the pelt & hair may be present between the strain. Also it appears likely that the strain have made different adjustments to the insulating properties of the pelt and hair during the treatment period.

Thus if the hair provides most of the insulation, as Heroux (1961) suggests, then it is likely that at 6 weeks of age HMTL and LCB strains have a greater amount of insulation in the medium environment provided by the greater weight of hair. At three weeks of age however, all strains would appear to have similar amounts of insulation provided by the hair because of similar weights of hair.

g. Food Intake.

Many workers have now shown that the amount of food consumed by an animal depends to a large extent upon the temperature at which the animal is living. Generally the lower the environmental temperature the greater will be the amount of food consumed. Efficiency of food utilization has also been shown to be dependant on the environmental temperature and generally the lower the environmental temperature the lower is the efficiency of food utilization.

The results observed in this study confirm the observation of Fowler (1958) that mice of the high body weight strains consume more food and utilize it more efficiently than do mice of the low body weight strains. Fowler (1958) concluded that the efficiency with which food is utilized is thus evidently gene controlled and capable of modification by selecting changes in efficiency and appetite being positively correlated with changes in growth rate.

This correlation is to be expected as body weight changes are used to calculate efficiency. However, this does not alter the conclusion that the efficiency of food utilization is capable of being modified by selection.

Food intake was increased in the cold treatment groups and decreased in the hot treatment groups as compared to the medium groups but strain differences were present in the degree of adjustments to the treatments. Efficiencies of food utilization were lowest in the cold treatment groups and highest in the hot treatment groups. The strain that showed the greatest body weight increase in each treatment was also the most efficient strain. The HMTS strain showed the highest weight gain in the hot treatment group and had the highest efficiency of food utilization. This strain showed the lowest weight gain in the cold and had the lowest efficiency of food utilization.

Chapter VII.

FINAL DISCUSSION.

This study was designed to investigate differences in the responses of four strains of mice to high and low temperatures. The four strains differed in body weight and tail length and the relationship between these factors.

Harrison (1958) has suggested that the length of the tail of a mouse is related to its ability to loose heat, as a longer tail could be a more efficient 'radiator' than a shorter tail. On this hypothesis therefore, a longer tail should be an advantage in a hot environment and a disadvantage in a cold environment unless compensating physiological changes take place on exposure to hot and cold temperatures.

Harrison (1958) based this hypothesis on two observations:-

1. Mice from which the tails had been removed at 21 days of age were less heat tolerant to very high temperatures (41.7° C.D.B., 29.6°C.W.B.) at 8 weeks of age than were the normal litter mates.
2. Mice acclimatized to hot temperatures (32°C.D.B., 29°C.W.B.) had longer tails and were more heat tolerant to very high temperatures than were control reared litter mates.

The results for tail length differ from those of Harrison *etal*. (1959) in that the short tail strains did not show the greater tail length increase in the hot treatment. However, within the long tail strains, mice of shorter initial tail length showed a greater tail length increase than did mice of longer initial tail length. This was not true for the cold treatment where all strains showed a similar response to the treatments.

The results obtained for body temperatures would suggest that differences are present in the ability of the four strains to adapt to both high and low temperatures. The tail length results, however, suggest that differences are only present in the hot environment.

A. Adaption to Cold.

When homeotherms are exposed to low temperatures physiological adjustments must be made to prevent a fall in body temperatures. The adjustments that take place are:-

i. a reduction in heat loss by improving insulation.
ii. An increase in the rate of heat production.

The results obtained in this study would suggest that these two methods used to maintain heat production are of relatively different importance in the four strains. Within the HMTS strain, which showed the greatest reduction in body temperature in the cold environment, it appears likely that these mice were unable to

raise their metabolic rate to a level high enough to maintain body temperatures. Within the LCA and HMTL strains, the mice appear to have the ability to raise metabolic rate to maintain body temperatures at or near the same level as the control mice. Insulation also appears to differ between the HMTS strain and the LCA and HMTL strains.

The LCB strain had a much higher proportion of deaths in the cold environment than did the other three strains. Mice dying in this strain tended to be the mice of lower initial body weight and all mice dying showed very low body temperatures before death. Therefore, in this strain body weight appears to be related to the ability to maintain body temperature. Body weight in this strain, therefore, is related to either the ability to raise the metabolic rate or to increase the amount of insulation.

Deaths in the HMTS strain in the cold were not related to body weight and therefore body weight in this strain does not appear to have been an important factor in the maintenance of body temperatures.

Body weights were reduced in the cold in all four strains as compared to mice in the medium groups. The results for weight of total body fat showed that the reduced body weight at 6 weeks of age was not due to fat. Once body weight differences were adjusted for, the medium and cold treatment groups had similar regression lines in all four strains. Heroux (1958) has shown that at 6°C. much of the reduction in body weight is due to a reduced rate of catabolism. The results have shown that mice of lower initial body weight in the high body weight strains showed a greater body weight gain than have mice of lower initial body weight in the low body weight strains. These results would suggest that mice of lower initial body weight in the low body weight strains how a greater reduction in catabolism than did mice of lower initial body weight in the high body weight strains.

For the LCB strain it was suggested above that the ability to maintain body temperatures is related to body weight. Therefore, it would appear likely that in this strain mice of lower initial body weight were unable to raise metabolic rate to the same level as did mice of lower initial body weight in the high body weight strains. However, insulation differences between the strain may also have had an influence.

The results for pelt weight at 3 weeks of age showed that differences were present between the strains. The high body weight strains had a significantly heavier pelt than did the low body weight strains, once body weight differences were adjusted for. Hair weights, however, were not significantly different between the strains. Heroux (1961) has pointed out that the insulative ability of the pelt can hardly be deduced from its weight and further points out that most of the insulation is provided by the hair. If the heavier pelt weight of the high body weight strains is the result of a greater weight of subcutaneous fat than it is likely that at three weeks of age the high-body weight strains have a greater amount of insulation than

do the low-body weight strains.

The food intake results support the conclusion that the low body weight strains have less insulation (which includes tail insulation) in the cold. These strains consumed as much food, or more in the case of the HMTS strain, as the high-body weight strain in the early stage of exposure, yet still had difficulty in maintaining body temperatures. This would suggest that the low-body weight strains had a higher heat loss than the high body weight strains. However, Fowler (1958) has some evidence which showed that the high-body weight strain was able to absorb a greater amount of protein from the diet than did the low-body weight strains.

These conclusions are supported by the results of the covariance analysis of mean body temperature over the last ten days of the treatments adjusted for final body weight. In the cold treatments, mice of higher final body weight also had the higher mean body temperatures. This would suggest that mice able to control heat loss or else raise heat production to maintain a higher body temperature were the mice with the higher final body weight.

Changes in pelt and hair weights have occurred in the strains during the exposure period. No differences were present between the strains for pelt weight once body weight differences were adjusted, but the long tail strain (HMTL and LCB) had significantly heavier amounts of hair than did the LCA strain (the HMTS strain was excluded from the hair weight analysis), at 6 weeks of age. As the hair provides most of the insulation (Heroux 1961), it is likely that the LCA strain has less insulation than the two long tail strains. Both the LCA or HMTL strains however, have made similar body weight gains in the cold treatment, even though the LCA strain appears to have less insulation at 6 weeks of age than has the HMTL strain. This may be related to loss of heat through the tail. The short tail LCA strain may loose less heat through the tail than the HMTL strain.

B. Adaption to Heat.

In a hot environment the adjustments that homeotherms can make to regulate body temperatures at a constant level are:-

i. Increase heat loss by reducing the amount of effective insulation. This also includes sweating and panting.

ii. Decreasing the rate of heat production by lowering metabolic rate and reducing the amount of voluntary activity.

The results have shown that the four strains have responded differently to the hot treatment. The LCA strain had the higher body temperature over the early period of exposure andshowed the greatest reduction in food intake and also showed a reduced rate of body growth. Therefore it would appear that this strain is the least adaptable to the hot environment. However, during the middle stages of exposure this strain showed the greatest

reduction in body temperature while the LCB strain showed little or no reduction in body temperature.

It was shown that the high-body weight strains had a greater pelt weight at 3 weeks of age than did the low-body weight strains. This, it was suggested above, would mean that the high-body weight strains had the greater pelt insulation at this age. However, the high-body weight HMTL strain did not show the same body temperature rise that the LCA strain showed. This would suggest that the HMTL strain is able to decrease the amount of effective insulation to a greater degree than has the LCA strain, perhaps as a consequence of the longer tail. The LCA strain also added a smaller weight of hair than did the HMTL and LCB strains which could account for its greater reduction in body temperature during the middle stages of exposure.

It was shown that the high-body weight strains had a greater weight of fat in the hot treatment than in the medium and cold treatment groups. This was probably the result of an acceleration in the normal increased rate of fat deposition that takes place in the high-body weight strains from 35 days of age onwards (Fowler 1958). However, the reduced rate of growth of mice of lower initial body weight in the low-body weight strains as compared to mice of lower initial body weight in the high-body weight strains, cannot be explained in terms of body fat because all mice in the high-body weight strains showed the increased weight of fat in the hot treatment. The explanation is probably the same as for mice in the cold i.e. a reduced rate of catabolism.

In the hot environment, a lower mean body temperature, over the last ten days of the treatment, was related to a higher final body weight. Therefore, the ability to dissipate heat appears to be related to body weight. This would suggest that mice of lower initial body weight in the lower-body weight strains may have had difficulty in dissipating heat. Fowler (1962) showed that mice of the low-body weight strains have a lower energy expenditure per day than do mice of the high-body weight strains. However, at the same body weight both the large and small strains had similar energy expenditures per day. Therefore, it is likely that the LC and HM strains have similar energy expenditures per day in the medium treatment once body weight differences are adjusted for. However, it is likely that differences are present in the hot environment. The LCB strain consumed more food than the LCA strain in the hot which would suggest that this strain had the higher rate of heat production. This strain maintained its body temperature about 0.3°C to 0.5°C above that of the medium group for the whole treatment period. Even with this high body temperature for the duration of the treatment it is likely that the LCB strain has dissipated more heat to the environment than the LCA strain. It was shown however, that the LCB strain showed a greater increase in hair weight during the treatments than did the LCA strain. Therefore, it is likely that the LCB strain had a greater amount of insulation but was still able to dissipate the extra heat. This extra heat may have been dissipated through the longer tail of this strain.

These results would suggest that the tail may be an important means of heat dissipation in the hot treatment. This may however, only be so during the initial stages of exposure to a hot environment.

The results for the HM strains would suggest that body weight is important in determining their heat loss, also. These two strains showed similar rises in body temperature on exposure to heat and had similar food intakes. Therefore, it is likely that they produced similar amounts of heat. Because of body size, however, the HMTS strain would be dissipating more heat per unit area than the HMTL strain, even though it had a shorter tail.

Therefore, the tail as a means of dissipating heat may be of different importance in the long and short tail strains. This would explain the greater response in growth in tail length in mice of shorter initial tail length in the long-tail strains. The short tail strains dissipated heat more efficiently from the body surface than do mice of the long tail strains. On the other hand, the long-tail strains dissipate heat more efficiently through the tail.

These results would suggest that differences are present between the strains in the way in which they adapt themselves to high or low environmental temperatures.

SUMMARY.

Four strains of mice were exposed to 30°C, 21°C and 7°C for the period from weaning (23 days of age) to 6 weeks of age in order to investigate the possible differences that might exist between the strains in their responses to these environments. The four strains differed in body weight and tail length and in the relationship between these two characters. The four strains were:-

LCA High body weight, short tail.

LCB Low body weight, long tail.

HMTL Long tail Himalayan.

HMTS Short tail Himalayan.

The results have shown:-

1. Body weight and tail length at 6 weeks of age was less for mice reared in the cold than for mice reared in the medium environment.
2. Tail length was increased in the hot environment but body weight was not.
3. Mice of lower initial body weight in the low-body weight strains showed a smaller body weight response to all treatments than did mice of lower initial body weight in the high-body weight strains.
4. Mice of shorter initial tail length in the long tail strains showed a greater tail length response to the hot treatment than did mice of longer initial tail length.
5. Rate of body-weight growth differed between the strains within each of the treatments.
6. The medium and cold treatment groups had tails of similar weights within each strain. The hot treatment groups had heavier tails than the medium and cold treatment groups even when body weight differences were adjusted for.
7. The LCB strain showed the greatest increase in tail weight during the treatments while the other strains showed similar increases.
8. Strain differences in pelt weight were present at 3 weeks of age but at 6 weeks of age no differences were present once body-weight differences were adjusted for.
9. No differences were present for the weight of hair at 3 weeks of age but at 6 weeks of age the LCB and HMTL strains had a significantly greater amount of hair than the LCA strain once body weight differences were adjusted for. Treatment differences were present for the LC strains but not for the HMTL strain. The cold treatment groups had a significantly heavier weight of hair than the medium treatment groups, while the hot treatment groups had the least amount of hair in the LC strains.

10. No differences were present between the strain for the weight of total body fat at 3 weeks of age. At 6 weeks of age no differences in the weight of total body fat were present between the treatment groups of the low body weight strains. In the high-body weight strains the hot treatment groups had a significantly greater amount of body fat than did the medium and cold treatment groups.

11. Strain differences were present for the weight of abdominal fat at 3 weeks of age once body weight differences were adjusted for. At 6 weeks of age strain differences were present but treatment differences were present only for the HMTL strain once body weight differences were adjusted for.

12. All strains showed hypothermia during the first few days of exposure to cold, and body temperatures were raised in the hot treatment groups. By the end of the treatments however, the cold treatment groups had the higher body temperatures and the medium groups the lower body temperature.

13. Differences were present in the amount of food consumed between the strains and between the treatments. Efficiencies of food utilization also differed between the strains and between the treatments.

14. Possible differences between the strains in the control of heat loss are discussed in relation to the adaption to the different environments.

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CHAPTER IV.

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