A STUDY OF SOME ASPECTS OF GROWTH AND REPRODUCTION IN TWO INBRED LINES OF MICE AND THEIR CROSSES

A thesis presented in partial fulfilment
of the requirements for the
Degree of Master of Agricultural Science in
Massey University of Manawatu

BY

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November, 1964

PREFACE

The mating of closely related individuals is generally believed to result in inferior offspring. Recognition of this danger has been suggested as the reason for evolution of human social customs. However, historical researches suggest that inbreeding unavoidably occurred in small, isolated, ancient communities, and marriage of close relatives was encouraged in the Egyptian, Greek and Hebrew civilisations (Zirkle, 1952).

Inbreeding is likely to have occurred in the isolated flocks of nomads, but it is probable that inbreeding depression, as such, was not recognised until the eighteenth century when the early livestock improvers are reputed to have found that the fertility of their animals deteriorated with continued inbreeding.

Hybrid vigour from cross breeding plants was described by Koelreuter (1766) and confirmed by later botanists. After much detailed work, Darwin (1876) concluded:

> " ... cross fertilisation is generally beneficial and self fertilisation injurious." and that

(a) Mechanisms exist widely for the avoidance of inbreeding.

(b) Inbreeding has effects likely to incur selective disadvantages.

No suitable hypothesis could account for the usually deleterious effects of inbreeding, or the reverse phenomenon of hybrid vigour until the rediscovery of Mendel's work in 1900. The effects of inbreeding can now be explained in terms of the dominance and recessiveness of genes. (Falconer, 1960).

The aim of experiments reported in this thesis was to study growth, reproduction and mortality in two inbred lines of mice, derived from common ancestors, to find if differences between tham had arisen during inbreeding. Reciprocal crosses and matings of the F_1 progeny of these crosses were made to find if hybridisation of the parent lines led to improvements of these characteristics.

This study was suggested by Drs. D.S. Flux, M.F. McDonald and R.E. Munford to whom the author is indebted for advice and guidance.

The author also wishes to acknowledge the assistance of Mr. E. Ormsby in histological preparations, of Mr. P.S. Dale in photomicrography, and of Mr. C. Muir who drew some of the figures reproduced in this thesis. Thanks are also due to the staff of the University Library for their efforts in obtaining literature.

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CHAPTER I

REVIEW OF LITERATURE

A. THE IMPORTANCE OF INBREEDING AND INBRED ANIMALS:

I. Laboratory Animals

As well as being used in genetic studies, highly inbred lines of laboratory animals are used for assays of pharmacological substances, physiological experiments, and nutritional trials. These animals, which theoretically closely approach a homozygous state, are considered to provide genetically uniform biological material which increases the efficiency of such experiments.

Recent work has indicated that many inbred sub-lines derived from originally highly inbred lines, have diverged from one another with respect to some morphological characteristics (Grünneberg, 1954; McLaren and Michie, 1954; Deol, Grünneberg, Searle and Truslove, 1957; Carpenter, Grünneberg and Russel, 1957; Bailey, 1957).

This divergence is probably a result of the occurrence and rapid fixation of mutations in inbred sublines rather than residual segregation (McLaren and Michie, 1954).

Comparisons of inbred and outbred animals have indicated that the heterozygote seems more stable to environmental influences (Lerner, 1954). Robertson and Reeve (1952) suggest that this is because of greater "biochemical versatility" in the heterozygote which enables it to buffer itself against environmental changes.

Because the uniformity of inbred lines is suspect, and because of lowered variability in heterozygous animals it has been suggested that F_1 animals, resulting from the crossing of highly inbred lines, may be more suited to many types of experiment (Grünneberg, 1954; Biggers and Claringbold, 1954; Biggers, McLaren and Michie, 1958). Such animals combine lowered variability with genetic uniformity. There is the possibility that further experimentation with crosses of various inbred lines may produce F_1 animals peculiarly suited to different types of experiment.

Regardless of whether inbred or hybrid animals prove to be more suitable, the practice of inbreeding is necessary to provide genetically uniform experimental animals of both types.

II. Domestic Animals

Because it increases homozygosity and allows the elimination of unwanted genetic material while allowing desired characteristics to be fixed inbreeding, allied with careful selection, is used to develop breeds and strains of livestock with particular inherited characteristics. Such methods were reputedly used in the eighteenth and nineteenth centuries to establish the breed types which are the ancestors of modern sheep and cattle breeds.

The development of the Santa Gertrudis beef breed is a recent example of the use of inbreeding to establish a new breed with particular inherited characteristics (Rhoad, 1949, 1955).

When the numbers of livestock in a country, such as New Zealand, are considered in relation to the numbers originally imported, and the grading up processes involved in changing from a predominantly multipurpose national herd to specialised beef and dairy breeds it is apparent that inbreeding has occurred (Stewart, 1952, 1954).

Likewise pure-breeding and line-breeding systems, as employed by breeders to protect a family thought to have particular merit from contamination by grade stock, or to preserve the genetic material of an admired ancestor, lead to mild, but unwanted inbreeding (Lush, 1948; Stewart, 1954).

Inbreeding can also be used to uncover undesirable recessive characteristics (King, 1955). It is doubtful if losses of this type are of importance relative to the cost and time involved in testing. Such testing may be desirable where animals are to be widely used, as in artificial breeding, since wide dispersal of undesirable recessive genes could occur. The effects of these genes could become apparent when semen from the same, or a related sire, is used on progeny of the original carrier.

In view of the wide occurrence of inbreeding in animal husbandry, as well as the probability of quicker rises in inbreeding levels with intensive artificial breeding, knowledge of the effects of inbreeding on growth conformation, production and reproduction is important (Dickerson, 1940).

Inbred lines of domestic animals have been established in attempts to produce "hybrid vigour" in animals by crossing selected inbred lines. Advances in this field are slow and costly because of the long generation intervals and low reproductive rates of livestock.

In addition characteristics associated with reproductive performance, as well as being most susceptable to hybrid vigour, have low heritabilities and are severely affected by inbreeding depression (Rae, 1953; Sang, 1956).

Since many inbred lines become extinct during inbreeding, and in order to increase the probability of superior lines occurring, it is necessary to set up numerous inbreeding lines. Surviving lines can them be tested for general and specific combining ability and used to establish hybrid lines (Fisher, 1949; Falconer, 1960).

Systematic studies of the effects of inbreeding and testing of inbred lines for crossing ability have been carried out by the Regional Swine Breeding Laboratory in the United States, and the Agricultural Research Council of Great Britain. The results of these experiments are reviewed by Craft (1943, 1953), Donald (1955), and Fredeen, (1956).

The intensive conditions under which pigs can be managed allows records to be kept with relative ease while their prolificacy and short generation interval makes pigs suitable for this type of work. In other livestock inbreeding, progress is slower, and there is less probability of having progeny of each sex to continue an inbred line. It is possible that the application of superovulation of immature animals (Marden, 1953) and ovum transfers techniques (Averill and Rowson, 1958) could be used to aid the establishment of inbred lines of livestock.

The theoretical effects of inbreeding and genetic mechanisms involved are discussed by Lush (1948), and Fredeen (1956). Mathematical considerations of inbreeding are discussed by Fisher (1949), and Lush (1948) reviews methods used to measure inbreeding.

B. OBSERVED EFFECTS OF INBREEDING:

I. Domestic Animals

(a) Occurrence of Abnormalities:

Inbreeding of swine was accompanied by an increase in the occurrence of abnormalities from 4.4 to 9.4 percent (Donald, 1955). Similar increases were observed in the United States work reviewed by Craft (1953).

The incidence of deformed calves in an inbred Guernsey line indicated the presence of a recessive gene (Woodward and Graves, 1935). No such defects occurred in Friesians (Woodward and Graves, 1946), but Rollins, Mead, Regan and Gregory (1949) reported dwarfs in an inbred Jersey line and Thompson, Cranek and Ralston (1957) suspected that lethal recessives were the cause of stillbirths in American Red Danish cattle.

In sheep blindness inherited as a partial recessive has been reported (Ragab and Askar, 1954).

(b) Mortality, Growth Rate and Feed Economy:

Growth rate and feed economy were variously affected in different inbred lines of pigs (Craft 1953; Fredeen, 1956). A decline, with increased inbreeding, in litter and individual weights between birth and weaning has been shown by Dickerson, Blunn, Chapman, Knottman, Krider, Warwick and Whatley (1955), and Bradford, Chapman and Grummer (1958). Hodgson (1935) found birth weight to be unaffected by eight generations of brother-sister mating. Winters, Cummings and Stewart (1947) and Fine and Winters (1952) suggest that less rapid inbreeding combined with a flexible system of selection can minimise the deleterious effects of inbreeding so neither inbreeding of the dam, or of her litter, affects growth and survival between birth and weaning.

The birth weight of inbred Holstein cattle (Woodward and Graves, 1946; Dickerson, 1940) and Black Pied cattle (Nowicki, 1963) declined with inbreeding.

Bartlett, Reece and Lepard (1942) suggest that selection can be used to prevent depression of birth weight in cattle if inbreeding coefficients do not exceed .20 and Martin, Jacobson, McGilliard and Homeyer, (1962) find inbreeding has little effect on birth weight or growth rate at coefficients below .15.

The growth rate of Holstein cows was depressed by inbreeding (Hawke, Tyler and Casida, 1954; Menge, Mares, Tyler and Casida, (1960). Inbreeding of animals of beef breeds led to lighter weights for age and poorer growth rates and feed economies (Stonaker, 1954; Burgess, Landblom and Stonaker, 1954; Swiger, Gregory, Koch and Arthaud, 1961; Alexander and Bogart, 1961; Brinks, Clark and Kieffer, 1963).

At maturity inbred Friesians were lighter than outbreds, but differences were less marked than at birth, (Woodward and Graves, 1946). The inbreds had a higher mortality and were conspicuously less active as mature cows.

Body weight, growth rate, withers height, and heart girth of mature animals were depressed in inbred Holstein and Jersey cows (Baker, Mead and Regan, 1945; Rollins, et al., 1949). As inbreeding progressed Jerseys were more affected than Holsteins.

Swett, Matthews and Fohrman, (1949) found an increase of inbreeding coefficient to .646 decreased body weight, but only slightly affected skeletal size of Holstein cows. Organ weights were variously affected. There was a tendancy for endocrine glands to be reduced in size with inbreeding although thyroid weight increased. No general effect was noted on the variability of the factors studied.

After a series of trials it was concluded that, if genetically superior foundation stock are used, and intensive selection practiced, weight and size of Holstein cows could be maintained provided inbreeding coefficients did not exceed .20 (Bartlett, et al., 1942; Bartlett and Margolin, 1944; Margolin and Bartlett, 1945).

Levels of inbreeding ranging from 0 to .4 (average 0.1) in weaning, yearling and mature ewes and rams of the Rambouillet, Colombia, Corriedale, and Targhee breeds indicated that inbreeding in sheep had its most detrimental effects on mutton characteristics - body weight, type and condition (Hazel and Terrill, 1945a, b; 1946; Terrill, Sidwell and Hazel, 1947, 1948a, b.)

A similar depression of body weight, growth rate and survival, was noted in inbred Merino sheep (Morley, 1954; Doney, 1957, 1958), and birth and weaning weights of singly born lambs were lower in inbred Ossimi sheep (Ragab and Askar, 1954). Greater mortality occurred in inbred lambs than in outbreds between birth and weaning (Glembockii, 1957; USDA, 1952).

Doney (1959) found that administration of pituitary extract to inbred, but not to outbred lambs, improved growth rate. He concluded that inbreeding may partly exercise its detrimental effects by depression of pituitary function.

(c) Reproduction:

Inbred pigs have been shown to be slower in maturing than outbred pigs. Thus at puberty, as shown by age at first oestrus, inbred gilts were older than outbred gilts (Foote, Waldorf, Chapman and Self, 1956; Squiers, Hauser, Dickerson and Mayer, 1949; Warnick, Wiggins, Casida, Grummer, 1951), and inbred gilts were older at first farrowing (Donald, 1955).

Over two years five inbred strains of gilts maintained their characteristic weights, and ages at puberty. Although within lines weight at ages between birth and puberty was significantly negatively correlated with age at puberty, lines which tended to be heaviest at 154 days were slower to reach puberty.

(Warnick et al., 1951). They suggest that inbreeding with selection for growth rate has led to lines with characteristic ages and weights at puberty, and that lines genetically superior for growth are retarded in rate of sexual maturation.

A similar negative correlation between growth rate and age at puberty has been noted by Comstock and Winters (1944).

Such a relationship of growth to rate of maturation was not found in boars (Wiggins, Warnick, Grummer, Casida and Chapman, 1951). They found that lines of inbred boars differed in age and weight at puberty as shown by willingness to mate. Age at puberty was negatively correlated with weight at earlier ages and positively correlated with growth rate. Sperm was present in the testes of animals of these lines before they showed willingness to mate.

The growth of testes, development of the seminiferous tubules and the onset of stages of spermatogenesis were slower in inbred lines of boars (Green and Winters, 1945a; Andrews and Warwick, 1949; Hauser, Dickerson and Mayer, 1954). Differences in the age at onset of sexual behaviour and the extent to which it was displayed could be related to differences between lines in 17-ketosteroid excretion. (Green, Winters, Rash and Dailey, 1942; Green and Winters, 1945b). These differences between lines in 17-ketosteroid excretion probably reflect differences in the levels of male steroid production.

Hodgson (1935) reported a reluctance of inbred animals to mate.

A delay in the attainment of puberty associated with slower growth has been found in Holstein cows. (Hawke, et al., 1954; Menge, et al., 1960).

Longer oestrous cycles were found in inbred sheep (Hulet, Blackwell, Ercanbrack, Price and Wilson, 1962). The daughters of inbred cows had longer oestrous cycles than the daughters of outbred cows, while inbreeding of the daughters themselves, only slightly affected oestrous cycle length. This change in mean cycle length was due to the absence of short cycles. No reason is apparent for this maternal effect (Mares, Menge, Tyler and Casida, 1961a).

A decline in reproductive success with inbreeding in livestock has been generally observed.

From an analysis of Berkshire pedigrees Hughes (1933) concluded that an inbreeding coefficient of .74 had been reached. Inbred pedigree pigs had larger litter sizes than random bred pigs. No indication as to the extent of selection in these two herds is given. McPhee, Russel and Zeller (1931) reported a decline in pig fertility and vigour with inbreeding, while Hodgson (1935) found no effect on litter size.

More recent work, reviewed by Craft (1953) and Donald (1955), has revealed a decline in litter size, vigour at birth, and a poorer survival rate in inbred pigs. This decline in litter size averaged one third of a pig at farrowing and half a pig at weaning per ten percent increase in inbreeding. Similar results are shown by other workers (Dickerson, et al., 1955; Bradford, et al., 1958; Rognoni and Brambilla, 1958).

The observed reduction in litter size was attributable to both inbreeding of the sow and of her litter (Dickerson, et al., 1955; Bradford, et al., 1958).

The number of services per conception was more in inbred dairy cattle (Woodward and Graves, 1946). Inbred beef cattle raised fewer calves than outbreds (Stonaker, 1954), and barren Hereford and Aberdeen Angus cows and their presumed conceptuses had higher inbreeding coefficients than did pregnant cows (Bovard and Priode, 1963).

Inbred sheep suffered more abortions, had lower lambing percentages and were older when they reached maximum reproductive performance than were random bred sheep (Morley, 1954; Glembockii, 1957; Doney, 1957, 1958).

Heritable recessive defects in sperm morphology leading to complete infertility in bulls have been reported (Donald and Hancock, 1953; Gregory, Mead, Regan and Rollins, 1951). It has been suggested that an increase in sperm abnormalities and a general decline in semen quality observed in inbred bulls could be partly due to a deterioration in the environment for sperm maturation with inbreeding. (Harris, Faulkner and Stonaker, 1960; Sostak, 1961).

Except in cases of complete sterility, which occurred more often in inbred pigs (Donald, 1955), once they reached puberty inbred boars were no less fertile than outbred boars (Wiggins, et al., 1951; Hauser, et al., 1954). There was no difference between inbred and outbred lines in the proportion of ova fertilised (Squiers, Dickerson, and Mayer, 1954).

Evidence for differences in ovulation rate between inbred and oubred animals is inconclusive. Inbred lines of gilts had characteristic ovulation rates, but inbreds did not have poorer ovulation rates than outbreds, (Squiers, Hauser, Dickerson and Mayer, 1949). Inbred sows in this trial tended to have poorer ovulation rates, but few were examined. In another study (Squiers, et al., 1954) ovulation rate in gilts was depressed by .55 ova per ten percent of inbreeding.

Since evidence for poorer fertilisation rates and, or lower ovulation rates in inbred animals is not conclusive post fertilisation losses could be a cause of lower reproductive performance. Such losses could be due to the segregation of lethal genes and to poorer viability of inbred embryos associated with a deteriorated uterine environment in inbred dams.

TABLE I:

The effect of a one percent increase in inbreeding coefficient on production by dairy cows

Breed	Level of Inbreeding	Milk Yield lbs	Fat Yield lbs	Fat %	Source
Holstein-Friesian	.37	- 74	-2.3	-	Tyler, et.al., 1949.
Holstein-Friesian	.50+	- 30 (Approx)	-1.9	-0.007	Woodward and Graves, 1946.
Holstein	.44	-209.8	-4.9	+0.008	Laben, et.al., 1955.
Holstein-Friesian	Mild	- 66	-2.0	+0.003	Laben and Herman, 1950.
Friesian	-	-	-4.5	-	Nelson and Lush, 1950.
Friesian	-	-30.0	-	0.0	Robertson, 1954.
Holstein	.34	- 54	-1.74	+0.003	Krosigk and Lush, 1959.
American Red Danish Cattle	-	- 23.1	-0.3	-	Thompson, et.al., 1957.

Pomeroy (1952) concluded that early embryonic death and resorption was the most likely cause of lowered fertility in inbred pigs.

A decline of .80 embryos per ten percent inbreeding in pigs examined at 25 days was found by Squiers, et al., (1954). Vernon, et al., (1952) found still births increased by 1.6 percent per ten percent increase in inbreeding of the dam.

In cows, inbreeding of a potential calf resulted in a poorer fertilisation rate or more early embryonic losses; inbreeding of the dam promoted more later embryonic losses, particularly in mature cows. (Mares, et al., 1961b). Losses up to 150 days of gestation were greater in inbred cows (Hawke, et al., 1955).

Some evidence for an increase in gestation length in inbred pigs is cited by Craft (1953). An association between inbreeding of a cow, but not of her calf and gestation length was reported by Rollins, Laben and Mead (1956). Other factors may have been confounded with the effect of inbreeding on gestation length reported here. Foote, Tyler and Casida (1959) could show no effect of inbreeding or gestation length in Jersey cows.

(d) Lactation:

Early workers, relying on analysis of pedigrees, were unable to demonstrate any clear effect of inbreeding on production by dairy cows. Joyce (1931) concluded that inbreeding, as calculated from pedigrees, did not affect Ayrshire production ; and Asdell (1945) concluded that the top producing 100 cows in the Guernsey and Friesian breeds were no more or less inbred than the breed average. British Jerseys producing over 10,000 pounds of milk per lactation were less inbred than the national average (Smith and Buchanan, 1928). Clear effects of inbreeding on production are shown when experiments to deliberately inbreed dairy cattle are conducted.

It can be seen in Table 1 that inbreeding is associated with a fall in milk and butterfat yields. The effect on butterfat per centage is variable. A similar effect was reported in a Polish experiment (Nowicki, 1963) but Davis et al., (1953) report that levels of inbreeding with selection of .41 were reached over 30 years and milk and fat yields were improved.

The increases in fat percentage reported are not surprising since it is widely known that environmental factors which depress milk yield, often have a compensating effect to increase butterfat percentage. Negative genetic correlations between milk yield and fat percentages have also been reported (Robertson, Waite, and White, 1956).

Laben, Cupps, Mead and Regan, (1955) suggest that the effect of inbreeding on production may be curvilinear and serious declines in production may not occur until inbreeding coefficients exceed .20.

Inbreeding of Sardinian sheep led to a decline in milk yield and fat percentage (Dassat, 1950).

II. Laboratory Animals

(a) Growth and Mortality:

It is reported by Lerner (1954) that inbreeding led to an increase from 3.9 to 45.5 percent in the preweaning mortality in rats as the inbreeding coefficient was raised from 0 to .41 in the experiments of Ritzema Bos (1894). Feldman (1926) observed that inbred rats were more susceptible to disease, but other workers have found no loss of vigour in inbred rats (King, 1918-19; Craig and Chapman, 1953). In these experiments selection for vigour was applied.

Inbreeding increased post natal mortality in guinea pigs (Eaton, 1932), and in mice (Chai, 1959).

Feldman (1926) found inbred rats grew less well than outbreds and Craig and Chapman (1953) found that inbred lines had lower body weight at 13 weeks than did outbred rats from the same source. However, King (1918-19) was able to carry out twenty two generations of brother-sister mating, with selection, and produce inbred rats superior in mature size to random bred rats.

The growth rate of Dutch rabbits declined with inbreeding (Mierowsky and Königs, 1927), but Pease (1928) found inbreeding had no effect on mature weight in Polish rabbits. Ragab, Asker and Madkour (1961) inbred rabbits to an inbreeding coefficient of .33; although birth weights declined weaning weights were improved.

In guinea pigs (Wright, 1922; Eaton, 1932) changes were observed in the ranking of five surviving inbred lines over 25 years of inbreeding.

In these experiments five lines of the original 25 inbred lines survived inbreeding, and these were originally above average in their reproductive performance. These experiments seem to show a general decline in litter size, growth rate, and an increase in mortality with inbreeding. The origins of the random bred control, which was begun some years after inbreeding began, are not stated.

Inbreeding affected the lactational performance of mice as measured by litter weight at twelve days (Falconer, 1947). This was partly due to impaired lactation in the inbred dam and partly due to impaired growth rate in the inbred litter. Inbreeding with selection enabled inbred dams to equal outbred dams in lactational performance when suckled by outbred litters.

Pease (1928) found that maternal ability was impaired by inbreeding in rabbits.

(b) Reproduction:

Many inbred lines became extinct through failure to produce enough young to carry on inbreeding (Guinea pigs : Wright, 1922; Rabbits : Mierowsky and Königs, 1927; Mice : Bowman and Falconer, 1960).

A decline in litter size with inbreeding has been shown in rats (Ritzema Bos, 1894; Feldman, 1926), rabbits (Pease, 1928), guinea pigs (Eaton, 1932), and in mice (Bowman and Falconer, 1960). King (1918) found that rats, inbred with selection, were superior in fertility to random bred rats after twenty two generations of inbreeding with selection.

Highly inbred rats of the King albino strain grew as well, reached maturity earlier, and had a superior ovulation rythm to hybrids of the Long Evans strain (Evans, 1928). Inbreeding was accompanied by greater variability in time of vaginal opening in mice (Yoon, 1955) and increased the age at which first litters were born (Strong and Fuller, 1958; Strong, 1960).

Loziak(1959) found that oestrous cycles were often prolonged in inbred mice and some stages of the cycle were often missing.

Congenital sterility as well as difficult parturition was considered to be the cause of lowered fertility in rats (Feldman, 1926).

Male sterility characterised by cryptorchidy and abnormal fatness was found in Polish and Flemish rabbits (Pease, 1928). This condition was possibly due to a recessive gene as it was eliminated in Polish rabbits after seven generations of inbreeding.

Krzanowska, (1960a) found inbred male mice had lower fertilisation rates than outbred males.

Less viable sperm was found in ejaculates from inbred rabbits than in cross bred rabbits (Sokolovskaja, 1950), and inherent line and breed differences in morphological and physiological characteristics of sperm from inbred lines of mice and rabbits have been reported (Beatty and Napier, 1960; Beatty and Sharma, 1960; Sharma, 1960). These differences could lead to variations in fertility between lines, although Falconer and Roberts (1960) found inbreeding of the male parent had no effect on fertility.

Impaired sex behaviour in inbred rats resulted in no copulation occurring in 70 per cent of inbred matings compared with 10 per cent in non-inbred matings (Evans, 1928).

No difference in ovulation rate was seen in inbred and outbred mice by Falconer and Roberts (1960) but Krzanowska (1960b) found inbreds produced slightly fewer ova.

Failure of fertilisation and, or greater pre-implantation losses were found to be the cause of smaller litters in inbred mice (Falconer and Roberts, 1960). Post-implantation losses were similar in both inbred and outbred mice.

Greater pre- and post- implantation losses in inbred mice than in outbred mice were observed by Krzanowska (1955, 1960b) and Leziak (1959). The extent to which losses occurred at different stages of gestation was characteristic of different inbred lines (Krzanowska, 1960b).

C. CONCLUSIONS FROM REVIEW OF LITERATURE

Since inbreeding occurs in both laboratory and domestic animals, knowledge of its effects on variability, growth, reproduction and production of milk and meat is important. Research into the possibilities of crossing selected inbred lines could lead to the development of improved or specialised hybrid strains of animals.

Experiments in which animals have been inbred have indicated that :-

1. Inbreeding is often accompanied by increased mortality and reduced reproductive performance, so that many inbred lines become extinct. The extent to which inbreeding depression has its effects varies between inbred lines; lines surviving to high levels of inbreeding often do not have their preformance severely depressed.

2. Slow rates of inbreeding with constant selection have sometimes enabled high levels of inbreeding to be reached with accompanied improvements in the performance of the inbred animals. Inbreeding is not incompatible with animal improvement, but selection cannot generally combat the depressive effects of inbreeding if inbreeding coefficients exceed .20.

3. It seems generally assumed that the amount by which a character is depressed by inbreeding increases linearly as inbreeding coefficients rise. Some workers suggest this relationship may be curvilinear so that inbreeding effects are not marked at low levels of inbreeding.

4. There is some evidence to suggest that the variability of some characteristics is greater within inbred lines than in the F_1 hybrids of inbred lines.

5. Probably as a result of the segregation of deleterious recessive genes, an increase in the incidence of abnormalities occurs with inbreeding.

6. Inbred animals are often slower to reach puberty than non inbred lines.

7. Complete infertility, probably as a result of segregation of recessive genes, has been reported in some inbred male animals. Differing sperm, and semen characteristics have been found in inbred lines and poorer quality semen from inbred animals has been reported.

8. A decline in reproductive performance has generally been found to accompany inbreeding. Evidence of lower ovulation rates is inconclusive; apparently depressed performance results from increases in embryonic deaths caused by lethal recessives and, or poorer viability of inbred embryos associated with a deteriorated uterine environment in inbred females.

9. Post-natal mortality is generally greater in inbred than in outbred animals.

10. Inbred animals at birth are often lighter than outbred animals and grow less rapidly.

11. Owing to slower growth, and lighter mature weights meat production by inbred livestock is poorer than in outbred animals. Milk production declines with inbreeding in cows and sheep. Poorer milk production by inbred laboratory animals may partly account for the slower growth of their litters.

12. The poorer performance of inbred animals is possibly associated with an imbalance of the endocrine system.

CHAPTER II

MATERIALS AND METHODS

Animals

New Zealand Cinnamon (NCN) mice were used in this study. This strain, bred and maintained by Dr. R.E. Munford at Massey University of Manawatu, was developed from the progeny of a cinnamon coloured male which resulted from crossing a New Zealand Chocolate Male (NZC) with an albino female.

This male was found to have the coat colour genotype A'a Cc bb. (Gene symbols from Mouse News Letter). Progeny of this male when mated to his full sister, a chocolate female of genotype aa C-bb, were backcrossed to albino and NZC strains to detect heterozgotes for cinnamon coat colour. Presumed homozygotes for cinnamon colour were used to establish a number of inbreeding sublines. The pedigrees of the surviving sublines NCN77 and NCN44 used in this experiment are shown in Appendix I. These mice have the genotype AA CC bb for coat colour.

These sublines were inbred by brother sister mating from July 1960 to December 1962 when random breeding colonies of each line were established. At this time the lines NCN44 and NCN77 had undergone ten, and thirteen generation of inbreeding resulting in inbreeding coefficients (Sewall Wright) of .942, and .966 respectively.

From their common ancestry (see Appendix I) these two lines theoretically had originally 39.6 percent of common genetic material. Mutation and segregation during inbreeding following the establishment of these separate lines, would have led to further divergence of the lines, and a reduction of this estimate.

Random bred albino females used in a preliminary trial of the technique of ova recovery were obtained from the Veterinary Department, Massey University of Manawatu.

Housing

Animals were kept in a naturally south lighted room at a temperature of 70 to 75 degrees Fahrenheit.

They were housed in metal covered, plastic cages measuring 30 x 15 x 11 centimetres. Feed hoppers and water bottles were let into the metal covers of cages.

Cage floors were covered in sawdust and wood-wool was provided for nesting. Clean cages were provided weekly.

Diet

Drinking water, and a pelletted mixture of wheatmeal, barley meal, ground oats, butter milk powder, wheat germ meal, lime and salt was fed *ad libitum*. This basic diet was supplemented by a weekly ration of a powdered mixture of maize meal, barley meal, butter milk powder, sodium chloride, and potassium iodide.

At weaning litters were given a powdered mixture of about 30 grams of a 2:1 mixture of wholemeal flour and skim milk powder in a jar in their cage. This supplement was found to assist their survival until they were better able to thrive on the normal diet.

Auriomycin was added to the drinking water immediately any sign of scouring was observed. Mice with persistant scouring were killed.

In March 1964 many mice were scouring and in poor health. For a week all mice were given a daily ration of one tablespoon per cage per day of a 2:1 mixture of barley meal and skim milk powder, plus penicillin as supplied in "Vespen" by Glaxo Laboratories.

Subsequently some mice in the colony were found to be infected with tapeworms (*Hymenolepsis nana*). The contents of the ileum of all mice killed was examined thereafter for the presence of tapeworms. Precautions were taken to prevent young mice coming into excessive contact with older, and possibly infected animals. At weaning all young were dusted with flea powder to kill lice, a known intermediate host of this tapeworm species.

Breeding Procedures

Experimental animals were mated in groups of one male and two females. Space did not allow a 1:1 ratio to be used, although this would have been preferable where records of litter growth were being kept. In practice there was no difficulty in distinguishing litters in the same cage. Unavoidably some cross fostering probably occurred but the incidence of two litters in the same cage together was not high.

Progeny of the original mice were used to make up 30 to 40 matings each of NCN77 x NCN77 and NCN44 x NCN44. Later progeny were used for matings of NCN77 x NCN44 and NCN44 x NCN77. The F_1 progeny of these reciprocal crosses were used in matings $\overline{\text{NCN77 x NCN44}} \times \overline{\text{NCN77 x NCN44}}$ and $\overline{\text{NCN44 x NCN77}} \times \overline{\text{NCN44 x NCN77}}$

These mating systems provided data on inbred dams bearing inbred litters, inbred dams bearing outbred litters, and outbred dams bearing outbred litters.

Pups were weaned at twentyone days and individually identified by earmarks.

A fortnight after weaning the sexes were separated. Sufficient males for later matings were kept and the remainder killed. Females were caged in groups of six and examined daily for vaginal opening.

On the day of vaginal opening females were paired with a ten week old male to ensure that mating was not delayed due to male immaturity. No male was mated with more than two females. If, following a number of copulations, neither female became pregnant a new male was placed with the females. Females who consistantly failed to conceive were paired with males known to be fertile in order to test if sterility was attributable to the female.

Replacement of males was done at least a fortnight after the last observed vaginal plug to avoid complicating an established pregnancy by the presence of a strange male (Bruce, 1960).

Observation and Recording

Weighings and examinations were made each morning. Since most feeding, matings and births occur at night (Snell, 1941) handling of mice at this time should least affect their behaviour, and variation due to differences in weight of ingesta should be minimized by weighing at the same time each day.

On the day of puberty, as judged by opening of the vagina, the age and weight of females were recorded. Thereafter females were examined daily for the presence of a vaginal plug. The day of oestrus could be estimated with some success by examination of the external genitalia. Where a vaginal plug was not obvious in an oestrus female, a metal probe was used to assist in examination of the vagina. The probe was used as little as possible to avoid accidental initiation of pseudo-pregnancy. In these lines vaginal plugs were regularly formed; very few females became pregnant without its being observed.

From puberty females were weighed every three days until mating occurred (day 0) and thereafter on days 3, 6, 9, 12, 15, 18, 19, 20 and 21 of gestation, unless a further vaginal plug indicated failure of the pregnancy.

At parturition the dam was weighed. Numbers of live pups and stillborn and, or partly eaten, but identifiable remains of pups were recorded. Living pups were weighed. Further weights of dam and litter were taken on days 3, 6, 9, 12, 16 and 21 days of lactation.

Six pups was considered to be the most satisfactory number a female could suckle. Litters larger than six were reduced to six by random culling the day after birth.

After the first litter was weaned females who had become pregnant at post partum matings were weighed daily until the second litter was born. If females were not pregnant when the first litter was weaned daily examinations for vaginal plugs were resumed. Records of weights over the second gestation and litter size and dam and litter weights over the second lactation were kept as for the first litter.

The occurrence of post partum matings after first and second parturitions meant that in some females second pregnancy was concurrent with lactation. This could have been avoided by removal of males after first mating, but such an arrangement was not practicable because:

(a) with two females per cage, it was necessary to have the male present to serve the other female;

(b) a large number of pregnancies resulting from post partum matings were required to provide sufficient mice for completion of the experiment in the time available.

Assessment of Prenatal Losses

Examinations of the reproductive tracts of females were made at three stages during gestation.

Insufficient puberal females were available to fill all replicates of the three treatments at one time. Pairs of females reaching puberty were mated with a male, and each female allotted at random to one of the three treatments till all replicates of each treatment in the six mating systems had been filled.

Attempts were made to recover ova from the ovarian bursa and upper Fallopian tube on the morning after mating (Runner and Palm, 1953) and by flushing the Fallopian tubes at various stages after mating. The easiest method of recovering ova, and the most consistent recovery rates were achieved by flushing the uterus in the afternoon of the third day after a vaginal plug was observed (McLaren and Michie, 1956; Michie and McLaren, 1959). To do this, females were killed with ether and the complete reproductive tract removed. Ovaries and Fallopian tubes were separated from the uterus above the utero-tubal junction. Traces of blood, which were found to interfere with the observation of recovered ova, were removed by drying the uterus on blotting paper. The uterine horns were separated above the cervix and each uterotubal junction teased open with watchmakers forceps. A 1 ml. tuberculin syringe fitted with a blunted, 26 gauge, $\frac{1}{2}$ inch hypodermic needle was inserted in the cervical end of the uterine horn and held in place with forceps. Each uterine horn was flushed with 0.25 to 0.5 ml of Ringer Locke solution at room temperature.

Recovered ova were located at a magnification of 17.5 diameters, and examined at magnification x70 with light from below the stage transmitted through the watch glass containing the ova.

Assuming ovulation occurred between 12.00 and 4.00 am on the morning vaginal plugs were observed, ova would be 82 to 86 hours old at recovery. Accordingly (Snell, 1941; Michie and McLaren, 1959; Austin, 1961) ova at late morula or blastocyst stage were classified as normal and viable. The appearance of ova at stages other than these was recorded.

On days 3, 12 and 17 after mating ovaries were dissected free from the ovarian bursa and the corpora lutea counted under the microscope (x10.5).

At twelve days implantation sites and live embryos in each uterine horn were counted and at seventeen days, the number of live foetuses and the appearance of any resorbing sites was noted.

A number of ovaries from females of each line were collected for histological study.

The contents of the vas deferens of mature males of each line was diluted on a glass slide with Ringer Locke solution and examined for the presence of sperm. Testes from mature males were collected for histological examination.

Histological Procedure

Ovaries and testes taken for histological examination were placed in Bouins fixative, dehydrated in alcohol, and embedded under vacuum, using Caltex White Spirit, in paraffin wax. Blocks were serially sectioned at six microns and stained with PAS - haematoxylin - orange G. (Lostroh, Johnston and Jordan, 1963).

Statistical Methods and Presentation of Results

The data has been analysed using where appropriate, the methods of analysis of variance, analysis of covariance, and t - Test (Snedecor, 1956). Data which was not normally distributed, has in some cases, been transformed by taking logarithms (Kempthorne, 1952, p. 153).

The adjusted error mean square used in calculating t where analysis of covariance has been used to adjust data was calculated by Finney's Approximate Method (Cochran and Cox, 1957, p. 86).

Enumeration data have been compared using Fishers Exact Test and Chi Square (Goulden, 1952, p. 368).

Where possible results are presented in tabular form with means, standard deviation and variances quoted for each line. Reported means have, for convenience, been adjusted to one significant decimal, whereas, the differences between means which were tested for significance retain the second significant decimal. Where comparisons between lines or pooled means of lines are made, the probabilities of the observed difference arising by chance are quoted. The type of analysis used is indicated in individual tables.

Hereafter inbred lines NCN77 and NCN44 are referred to as lines 77 and line 44 and the term "inbred matings" refers to matings NCN77 x NCN77 and NCN44 x NCN44. "Crossbred matings" refers to the reciprocal matings NCN77 x NCN44 and NCN44 x NCN77 hereafter written as 77 x 44 and 44 x 77. The F_1 progeny of these reciprocal matings are denoted as $\overline{77 \times 44}$ and $\overline{44 \times 77}$ respectively. Matings of these F_1 progeny are written as $\overline{77 \times 44}^2$ and $\overline{44 \times 77}^2$ and referred to collectively as outbred, or F_1 matings.

TABLE II:

Weight (gms) of females at puberty as shown by vaginal opening in inbred (77;44) and outbred ($\overline{44x77}$; $\overline{77x44}$) strains of mice.

Strain	Number of Mice	Weight	Variance
77	57	14.6 ± 0.16	1.43
44	38	17.5 ± 0.14	0.74
$\overline{44 \times 77}$	28	16.5 ± 0.16	0.71
$\overline{77 \times 44}$	31	16.3 ± 0.18	1.06
All Inbred	95	15.8 ± 0.18	3.23
All Outbred	59	16.4 ± 0.12	0.89

Comparison of Strains	Difference in Weights (gms)	*Significance of Difference
44 vs 77	2.93	$P < 0.001$
$\overline{44 \times 77}$ vs $\overline{77 \times 44}$	0.23	$0.3 < P < 0.4$
Outbred vs Inbred	0.63	$P < 0.001$

* Probabilities from t - Test following analysis of variance

TABLE III:

Age (days) of females at puberty as shown by vaginal opening in inbred (77;44) and outbred ($\overline{44x77}$; $\overline{77x44}$) strains of mice.

Strain	Number of Mice	Age	Variance
77	53	50.5 ± 1.21	77.67
44	38	50.2 ± 1.29	63.41
$\overline{44 \times 77}$	28	42.3 ± 1.31	47.78
$\overline{77 \times 44}$	31	41.8 ± 1.14	40.33
All Inbred	91	50.4 ± 0.89	71.08
All Outbred	59	42.1 ± 0.86	43.17

Comparison of Strains	Difference in Ages. (days)	*Significance of Difference
77 vs 44	0.3	$0.8 < P < 0.9$
$\overline{44 \times 77}$ vs $\overline{77 \times 44}$	0.5	$0.8 < P < 0.9$
Inbred vs Outbred	8.3	$P < 0.001$

* Probabilities from t - Test following analysis of variance

CHAPTER III

RESULTS

Age and Weight at Puberty

The ages and weights of female mice in each line at puberty as shown by vaginal opening are presented in Table II and III.

Females of the two inbred lines were the same age at puberty, but line 77 females were significantly lighter than line 44 females. Outbred mice from the reciprocal crosses of lines 77 and 44 were younger at puberty than inbred females of both lines. At puberty outbred females weighed significantly more than the mean weight at puberty for inbred females; i.e. when two inbred lines which were characterised by a low and a high weight at puberty were crossed, the resultant F_1 animals reached puberty at weight greater than the midpoint between the weights at puberty for the parent lines.

In comparing variances each inbred line (77 and 44) was individually compared with the pooled variance for outbred mice. The pooled variances for inbred animals shown in Tables II and III includes the effect of differences between inbred lines and this cannot, with validity, be regarded as the variance of a homogeneous group as was done with the pooled variance for outbred animals.

Comparison of the variances of lines for these pubertal characteristics (Snedecor, 1956, p. 96) indicated that line 77 females were more variable ($P < 0.05$) for weight at puberty than 44 or the pooled outbred females. The variability associated with age at puberty was similar in both inbred lines, but each line was more variable than outbred females. ($P < 0.05$)

Since it was apparent that variances and means were related (Table III) the original data for age at puberty has been transformed by taking logarithms (Kempthorne, 1951 p. 154). The results of analysis on this transformed scale, shown in Table IV, indicated that non-normality of the original data was not sufficient to invalidate conclusions based on an analysis of variance of this data.

TABLE IV:

Age (days) of females at puberty as shown by vaginal opening in inbreed (77;44) and outbred ($\overline{44x77}$; $\overline{77x44}$) strains of mice. Original data transformed by taking logarithms.†

Strain	Number of Mice	Age	
		Log.	Days
77	53	1.697	49.7
44	38	1.696	49.7
$\overline{44 \times 77}$	28	1.621	41.8
$\overline{77 \times 44}$	31	1.617	41.3

Comparison of Strains	Difference in Ages (Log.)	* Significance Of Difference
77 vs 44	0.001	$P > 0.9$
$\overline{44 \times 77}$ vs $\overline{77 \times 44}$	0.004	$0.7 < P < 0.8$
Inbred vs Outbred	0.077	$P < 0.001$

† Kempthorne, 1951 p. 154.

* Probabilities from t - Test following analysis of Variance.

TABLE V:

Mean interval (days) between puberty and observation of a vaginal plug indicating first copulation in each mating system. Females were paired with a mature male on the day of puberty.

Mating System	Number of females mated	Days	Variance
77 x 77	27	10.1 ± 1.56	63.7
44 x 44	38	15.3 ± 2.02	155.6
44 x 77	27	21.7 ± 2.93	231.6
77 x 44	17	3.2 ± 0.59	5.9
$\overline{44 \times 77}^2$	19	2.7 ± 0.46	4.0
$\overline{77 \times 44}^2$	20	2.5 ± 0.69	9.5
77 Male	44	7.5 ± 1.09	52.2
44 Male	65	18.0 ± 1.73	193.9
77 Female	54	15.9 ± 1.82	178.9
44 Female	55	11.6 ± 1.60	140.2
Inbred Mating	109	13.7 ± 1.22	162.6
Outbred Mating	39	2.6 ± 0.41	6.7

Comparison of Mating Systems	Difference in interval (days)	*Significance of difference
77 x 77 vs 77 x 44	6.9	$0.02 < P < 0.05$
44 x 77 vs 44 x 44	6.4	$0.001 < P < 0.01$
44 Male vs 77 Male	10.5	$P < 0.001$
77 Female vs 44 Female	4.3	$0.02 < P < 0.05$
Inbred vs Outbred	11.1	$P < 0.001$

* Probabilities from t - Test following analysis of variance.

Readiness to Mate Following Puberty

Females were considered to have reached puberty the first day the vagina was observed fully open. The swollen, externally cornified appearance of the vagina at this time was characteristic of oestrus.

The interval between puberty and pairing with a mature male, and mating, as evidenced by the observation of a vaginal plug, gives some indication of the readiness of mice of each line to mate after puberty.

The mean interval between puberty and copulation for each line is shown in Table V. Since pairing at puberty may have been too late for mating to occur and females could not therefore mate until the next oestrus, a mean interval of less than five days indicates willingness to mate at or very soon after puberty. Mean intervals in other groups were approximately multiples of five which was consistant with normal five day oestrous cycles.

The extreme differences in variance in Table V made comparisons of mean values for lines and mating systems of doubtful validity. Transformation of these data appeared unlikely to stabilise the variance. The differences between means are large and highly significant except in two cases. The non-normality of data meant conclusion from these slightly significant comparisons should be drawn with caution.

Where 77 females or 44 males were involved in matings there was more variability in the length of the period between puberty and copulation.

Regardless of whether they were mated to 77 or 44 males, 77 females were slower to mate than 44 females. This difference is not highly significant. These results suggest that F_1 and 44 females are willing to mate at, or soon after, puberty, while 77 females are slower in becoming receptive to the male.

Line 44 males were later to mate than 77 males. Observation suggested that 44 males were less enthusiastic in seeking out oestrus females than F_1 and 77 males. The libido of 44 males would appear to be poorer than that of line 77 and F_1 males.

TABLE VI

Conception rate as shown by the number of full term pregnancies resulting from the first observed mating of females in each mating system.

Mating System	Number of Matings	Matings resulting in Successful Pregnancy	Barren Matings	% Conception
77 x 77	34	29	5	85.3
44 x 44	38	14	24	36.8
44 x 77	28	12	16	42.8
77 x 44	19	17	2	89.5
$\overline{44 \times 77}^2$	28	26	2	92.8
$\overline{77 \times 44}^2$	20	16	4	80.0

Comparison of ratio of successful to barren matings	Probability of observed or a more extreme ratio occurring by chance
77 Male - 77 Female vs 44 Female	0.51 †
44 Male - 77 Female vs 44 Female	0.41 *
77 Male vs 44 Male	<0.001 *
77 Male vs Outbred Male	0.42 *
44 Male vs Outbred Male	<0.001 *

† Probability by Fisher's Exact Test (Goulden, 1952 p. 373-374)

* Probability from Chi^2 (Goulden, 1952 p. 372-374)

Conception Rates

The number of females of each line in which parturition occurred within twenty two days of a vaginal plug being observed, are shown in Table VI. Infertile matings were reckoned as those where a second mating occurred after a first with no intervening pregnancy or parturition.

Comparison of conceptions to first mating in Table VI indicates that 77 and F_1 males had equal successes, but 44 males had significantly fewer conceptions. The conception rates of females were dependant on the line of the male to which they were mated.

All five females barren to first mating in 77 x 77 matings conceived at a second mating. Three of these suffered diarrhoea, and this disease may have been the cause of termination of first pregnancy. The remaining two conceived to matings six days after the first.

All females, which had infertile first matings in lines 77 x 44, $\overline{44\text{ x }77}^2$ and $\overline{77\text{ x }44}^2$ conceived at second mating, except for two in $\overline{77\text{ x }44}^2$. Both of these were mated to the same male; after three pseudo-pregnancies each conceived when remated to a new male.

After two to three matings nine of the barren females in line 44 x 44 became pregnant. Thirteen remained barren after an average of three to four matings, but conceived immediately to a new male. Two failed to have litters even when mated to known fertile males.

No changes of male were made in 44 x 77 matings. All females, barren after first matings, eventually became pregnant except for two which failed to have litters after seven and nine matings respectively.

Examinations for vaginal plugs were not made after parturition, so there was no record of the occurrance of post partum matings in each line. In 77 x 77, 77 x 44, $\overline{44\text{ x }77}^2$, and $\overline{77\text{ x }44}^2$ matings all females became pregnant, either at post partum mating, or at the first mating after weaning their first litter. In line 44 x 44, with presumed sterile males removed, seven out of thirty six females failed to conceive at post partum, or post lactational matings. Four of these eventually conceived, two aborted regularly at twelve to fourteen days intervals, and one failed to have a litter after six matings. Ten females in 44 x 77 matings conceived at post partum, or post lactational matings. Of the remainder eight conceived, and seven did not, after an average of five matings following first parturition.

The results for conception rates in the lines and crosses studied suggested that a high proportion of 44 males were sterile. This sterility was not always complete as some became sterile after a period of fertility,

TABLE VII:

Mean number of pups suckled[*] in the first litter by females which became pregnant *post partum* and by females which failed to become pregnant *post partum*.

Mating System	Pregnant. Number of litters	Pregnant. Number Suckled	Not Pregnant. Number of litters	Not Pregnant. Number Suckled	[**] Significance of difference between numbers suckled in each group
77 x 77	22	2.0 ± 0.45	12	3.0 ± 0.59	$0.3 < P < 0.4$
44 x 44	19	2.9 ± 0.49	15	3.1 ± 0.68	$0.8 < P < 0.9$
44 x 77	7	3.6 ± 0.95	19	2.5 ± 0.51	$P > 0.9$
77 x 44	5	2.4 ± 1.12	12	2.3 ± 0.70	$0.5 < P < 0.6$
$\overline{44 \text{ x } 77}^2$ $\overline{77 \text{ x } 44}^2$	29	4.0 ± 0.35	15	4.9 ± 0.37	$0.3 < P < 0.4$

[**] Probabilities from t - Test following an analysis of variance.

[*] Including females whose whole litter died at birth as suckling no pups.

and others became fertile after a period of sterility.

The numbers of young suckled in the first lactation by females of each line classified according to whether or not they conceived at a post partum mating are reported in Table VII. Line 77 females mated to 77 males and females in F_1 matings becoming pregnant at post partum matings suckled fewer pups than those who failed to conceive at this mating. The data in Table VII also suggests that fewer inbred females became pregnant at post partum matings when the litter they were suckling was outbred. None of these effects reached significance.

Since the occurrance of post partum matings was not confirmed by inspection for vaginal plugs, and because of the small numbers in some groups, as well as the complications with sterile line 44 males, no definite conclusions can be drawn from these results.

TABLE VIII:

Lengths of first gestations (days) uncorrected for differences between lines in litter size. The day of observation of a vaginal plug was counted as day 0 of gestation, and the day of parturition the last day of gestation.

Mating System	Number of Pregnancies	Length of Gestation
77 x 77	28	20.0 ± 0.23
44 x 44	28	19.4 ± 0.15
77 x 44	16	19.1 ± 0.12
44 x 77	20	19.6 ± 0.20
$\overline{77 \times 44}^2$ $\overline{44 \times 77}^2$	27	19.4 ± 0.17

TABLE IX:

Lengths of second gestations (days) where full twentyone day lactations were concurrent. The day of first parturition was counted as day 0 of gestation, and the day of second parturition was counted as the last day of the second gestation.

Mating System	Number of Pregnancies	Length of Gestation
77 x 77	13	25.7 ± 0.87
44 x 44	10	24.7 ± 0.75
$\overline{44 \times 77}^2$ $\overline{77 \times 44}^2$	24	27.2 ± 0.61

Length of Gestation

Table VIII shows the mean lengths of gestation in each line uncorrected for litter size. An analysis of covariance between length of gestation and number of pups born showed no significant differences in gestation lengths between lines when allowance was made for differences in litter size. There was a significant regression of gestation length on litter size amounting to a reduction of 0.13 days in gestation length per pup increase in litter size at birth.

Gestation times for second litters where conception occurred post partum, and a lactation of twenty one days was concurrent with gestation are shown in Table IX. The small number of post partum pregnancies in matings 77 x 44 and 44 x 77 are not included in Table IX. No significant differences were shown between lines in the length of second gestation and since few mice were involved no further analysis of gestation length relative to numbers suckled during gestation, or numbers born was attempted. It was apparent that second gestations, where lactation was concurrent, were longer than first gestations in the lines investigated.

TABLE X:

Mean weight (gms) at the end of first gestation of females in each mating system which conceived at first mating, and the mean of these weights transformed by taking logarithms.

Mating System	Number of Mice	Weight (gms)	Variance	Log. Weight
77 x 77	27	26.4 ± 0.53	7.45	1.419
44 x 44	13	27.9 ± 0.58	4.29	1.444
44 x 77	12	25.9 ± 0.62	4.55	1.413
77 x 44	14	28.5 ± 0.70	6.94	1.454
$\overline{44 \times 77}^2$ $\overline{77 \times 44}^2$ } F_1	30	31.4 ± 0.66	13.23	1.493

Comparison of Mating Systems	Difference Between Mean Weights (i) gms	Difference Between Mean Weights (ii) Logs	Significance of Differences (i)*	Significance of Differences (ii)**
77 x 77 vs 44 x 77	0.45	0.006	$0.6 < P < 0.7$	$0.6 < P < 0.7$
77 x 44 vs 44 x 44	0.67	0.010	$0.5 < P < 0.6$	$0.5 < P < 0.6$
44 Female vs 77 Female	1.96	0.033	$0.001 < P < 0.01$	$0.001 < P < 0.01$
F_1 vs 44 x 44	3.59	0.049	$P < 0.001$	$P < 0.001$
F_1 vs 77 x 77	5.05	0.074	$P < 0.001$	$P < 0.001$

* Probabilities from t - Test following analysis of variance

** Probabilities from t - Test following analysis of variance of data transformed by taking logarithms.

TABLE XI:

Mean weights (gms) at conception of females which conceived at first mating in each mating system.

Mating System	Number of Mice	Weight	Variance
77 x 77	27	16.6 ± 0.34	3.04
44 x 44	13	18.5 ± 0.37	1.75
44 x 77	9	17.5 ± 0.46	1.92
77 x 44	14	18.4 ± 0.47	3.11
$\overline{44 \times 77}^{2}$ $\overline{77 \times 44}^{2}$ } F_1	30	17.9 ± 0.31	2.87

Comparison of Mating Systems	Difference in Mean Weights	*Significance of differences
44 x 77 vs 77 x 77	0.89	$0.1 < P < 0.2$
44 x 44 vs 77 x 44	0.15	$0.8 < P < 0.9$
44 x 44 vs 77 x 77	1.90	$P < 0.001$
F_1 vs 77 x 77	1.26	$0.001 < P < 0.01$
44 x 44 vs F_1	0.64	$0.2 < P < 0.3$

* Probabilities from t - Test following analysis of variance.

TABLE XII:

Mean weight gain or loss (gms) from conception to completion of first parturition in females of each line which conceived at first mating.

Mating System	Number of Mice	Weight	Variance
77 x 77	27	2.7 ± 0.22	1.32
44 x 44	13	1.8 ± 0.29	1.08
44 x 77	9	1.2 ± 0.33	0.96
77 x 44	14	1.2 ± 0.47	3.03
$\overline{44 \times 77}^{2}$ $\overline{77 \times 44}^{2}$ } F_1	29	3.3 ± 0.32	2.87

Comparison of Mating Systems	Differences in Mean Weight Gains	*Significance of Differences
77 x 77 vs 44 x 77	1.56	$0.001 < P < 0.01$
44 x 44 vs 77 x 44	0.52	$0.3 < P < 0.4$
77 x 77 vs 44 x 44	0.98	$0.02 < P < 0.05$
F_1 vs 77 x 77	0.56	$0.1 < P < 0.2$
F_1 vs 44 x 44	1.54	$0.001 < P < 0.01$

* Probabilities from t - Test following analysis of variance.

TABLE XIII:

Mean weights *post partum* (gms) of females which conceived at first mating in each mating system.

Mating System	Number of Mice	Weight	Variance
77 x 77	29	19.5 ± 0.32	2.90
44 x 44	13	20.1 ± 0.42	2.33
77 x 44	16	19.2 ± 0.68	7.28
44 x 77	12	18.9 ± 0.35	1.51
$\overline{77 \times 44}^{2}$ $\overline{44 \times 77}^{2}$ } F1	43	20.7 ± 0.44	8.27
77 Female	41	19.3 ± 0.25	2.53
44 Female	29	19.6 ± 0.42	5.07
77 x 77 44 x 44	42	19.7 ± 0.25	2.72
44 x 77 77 x 44	28	19.1 ± 0.41	4.69

Comparison of Mating Systems			Difference in Mean Weights	*Significance of Difference
44 Female	vs	77 Female	0.27	$0.6 < P < 0.7$
77 x 77 44 x 44	vs	44 x 77 77 x 44	0.60	$0.2 < P < 0.3$
F_1 Female	vs	77 Female	1.40	$0.001 < P < 0.01$
F_1 Female	vs	44 Female	1.13	$0.02 < P < 0.05$

* Probabilities from t - Test following analysis of Variance.

Weight Changes of Dams During First Gestation

The weight increases of inbred and F_1 females over their first gestation are illustrated in Fig. 1. The weights of pregnant females in each mating system at the end of first gestation, and the component parts of this weight - weight at conception, weight gain or loss of the dam over gestation, and the weight of the conceptus, are presented in Tables X, XI, XIII and XIV, and is illustrated in Fig. 2.

At first conception (Table XI) females of each line maintained the same ranking of weights as at puberty (see Table II), but 44 females were no longer significantly heavier than F_1 females. Probably because of the longer interval between puberty and mating 77 and 44 females mated to 44 males were heavier, though not significantly heavier at first conception than the same females mated to 77 males.

By the end of first gestation inbred (77 and 44) females were significantly lighter than outbred (F_1) females (Table X). There were no significant difference, at this time, between inbred females bearing inbred, or outbred litters. Line 77 females were significantly lighter than line 44 females. Because of the relationship between means and variances in Table X this data was transformed by taking logarithms (see page 20). The results of analysis of this transformed data are shown in Table X. It is clear that non-normality of this data has not altered the validity of conclusions drawn from the analysis of variance.

After completion of first parturition females of the two inbred lines did not differ significantly in weight (Table XIII). Outbred females were significantly heavier than both lines of inbred females after parturition. There is a relationship between means and variances in Table XIII, but it is unlikely that transformation of the data would change differences revealed by the analysis of variance.

The reason for the changes in ranking of weights over first gestation can be seen in Table XII and Fig. 2. When the weight increase of conceptuses over first gestation was eliminated it was apparent that line 77 and outbred females gained more weight over gestation than line 44 females so that at parturition 77 females were not significantly lighter than 44 females, and outbred females had overtaken 44 females. In the analysis shown in Table XII weight losses over first gestation were regarded as negative weight gains. Only four females lost weight over first gestation; one in 77 x 77 matings and three in 77 x 44 matings.

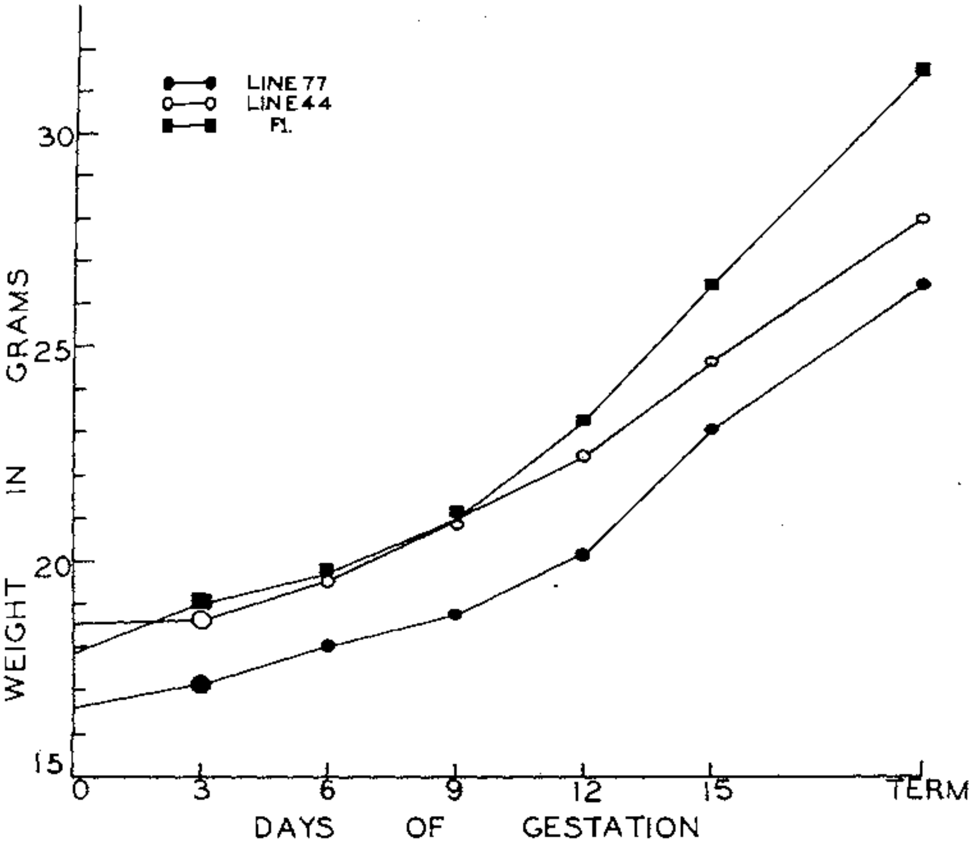


FIG. 1 Weight increases during first gestation of females in lines 77, 44, and F_1 matings.

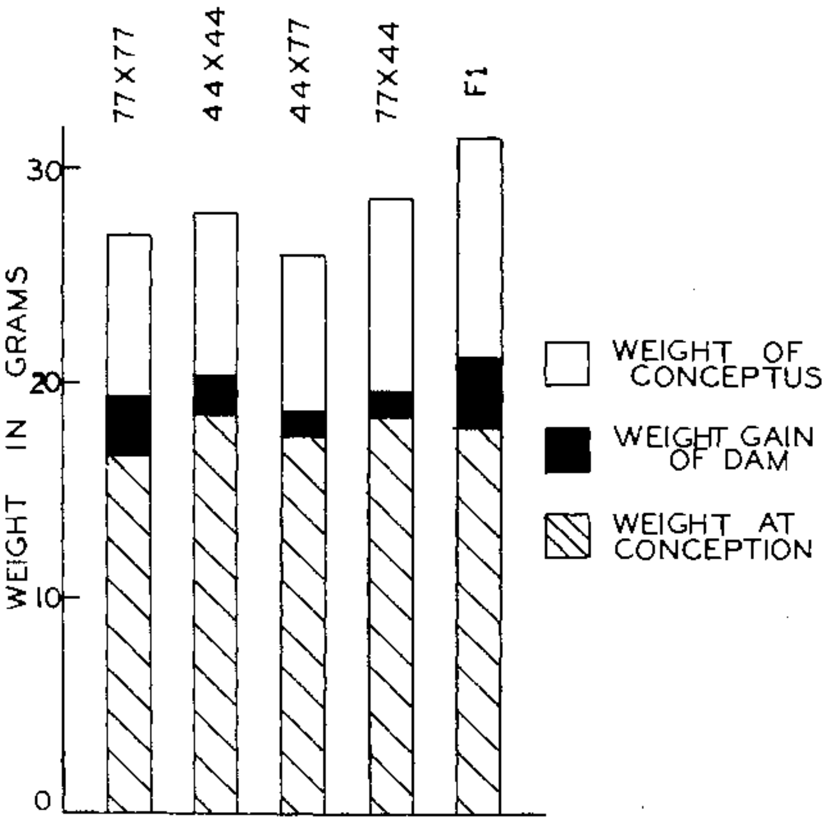


FIG. 2 The components of the mean body weight of pregnant females in each mating system on the last day of first gestation.

TABLE XIV:

Mean weights (gms) of first and second* conceptuses, as shown by the weight lost by females at parturition, in each mating system; and the pooled mean conceptus weights, over two litters, for each combination of inbred or outbred dam with an inbred or outbred litter.

†Inbreeding Status Dam:Litter	Mating System	First Conceptus		Second Conceptus		Difference in Conceptus Weights	**Significance of Differences	Pooled Conceptus Weights		
		Number	Weight	Number	Weight			Number	Weight	Variance
I:I	77 x 77	34	7.4 ± 0.37	34	8.7 ± 0.35	1.31	$0.02 < P < 0.05$	132	8.2 ± 0.23	7.14
	44 x 44	32	8.5 ± 0.49	32	8.3 ± 0.62	0.21	$0.7 < P < 0.8$			
I:0	44 x 77	18	7.4 ± 0.50	18	9.4 ± 0.76	2.03	$0.02 < P < 0.05$	70	8.8 ± 0.35	8.37
	77 x 44	17	8.3 ± 0.60	17	10.0 ± 0.74	1.66	$0.05 < P < 0.1$			
0:0	$\overline{44 \times 77}^2$	27	9.4 ± 0.37	27	10.2 ± 0.62	0.86	$0.6 < P < 0.7$	84	10.0 ± 0.30	7.36
	$\overline{77 \times 44}^2$	15	10.1 ± 0.89	15	10.6 ± 0.57	0.48	$0.2 < P < 0.3$			

Comparison of Inbreeding Combinations	Differences in Pooled Conceptus Weights	**Significance of Differences
I:0 vs I:I	0.54	$0.1 < P < 0.2$
0:0 vs I:0	1.24	$0.001 < P < 0.01$
0:0 vs I:I	1.78	$P < 0.001$

* No distinction has been drawn between second conceptuses conceived at a post partum mating, and those conceived after first lactation was completed.

** Probabilities from t - Tests following analysis of Variance.

† I:I inbred dam bearing an inbred conceptus.

I:0 inbred dam bearing an outbred conceptus.

0:0 outbred dam bearing an outbred conceptus.

TABLE XIV:

Mean weights (gms) of first and second* conceptuses, as shown by the weight lost by females at parturition, in each mating system; and the pooled mean conceptus weights, over two litters, for each combination of inbred or outbred dam with an inbred or outbred litter.

†Inbreeding Status Dam:Litter	Mating System	First Conceptus		Second Conceptus		Difference in Conceptus Weights	**Significance of Differences	Pooled Conceptus Weights		
		Number	Weight	Number	Weight			Number	Weight	Variance
I:I	77 x 77	34	7.4 ± 0.37	34	8.7 ± 0.35	1.31	$0.02 < P < 0.05$	132	8.2 ± 0.23	7.14
	44 x 44	32	8.5 ± 0.49	32	8.3 ± 0.62	0.21	$0.7 < P < 0.8$			
I:0	44 x 77	18	7.4 ± 0.50	18	9.4 ± 0.76	2.03	$0.02 < P < 0.05$	70	8.8 ± 0.35	8.37
	77 x 44	17	8.3 ± 0.60	17	10.0 ± 0.74	1.66	$0.05 < P < 0.1$			
0:0	$\overline{44 \times 77}^2$	27	9.4 ± 0.37	27	10.2 ± 0.62	0.86	$0.6 < P < 0.7$	84	10.0 ± 0.30	7.36
	$\overline{77 \times 44}^2$	15	10.1 ± 0.89	15	10.6 ± 0.57	0.48	$0.2 < P < 0.3$			

Comparison of Inbreeding Combinations	Differences in Pooled Conceptus Weights	**Significance of Differences
I:0 vs I:I	0.54	$0.1 < P < 0.2$
0:0 vs I:0	1.24	$0.001 < P < 0.01$
0:0 vs I:I	1.78	$P < 0.001$

* No distinction has been drawn between second conceptuses conceived at a post partum mating, and those conceived after first lactation was completed.

** Probabilities from t - Tests following analysis of Variance.

† I:I inbred dam bearing an inbred conceptus.

I:0 inbred dam bearing an outbred conceptus.

0:0 outbred dam bearing an outbred conceptus.

WEIGHT OF CONCEPTUS

The weight of conceptus was measured by the loss in weight of females at parturition. Only in line 77 females was the conceptus of second pregnancy significantly heavier than that of first pregnancy (Table XIV).

No significant differences could be shown between the mean weight of conceptus over two litters within inbred (77 x 77 vs 44 x 44), crossbred (77 x 77 vs 44 x 77), or outbred ($\overline{77 \times 44}^2$ vs $\overline{44 \times 77}^2$ matings. These groups were therefore pooled to calculate a mean conceptus weight over two litters for each of the three types of mating. These means are compared in Table XIV.

Outbred females had significantly heavier conceptuses than inbred females with inbred, or outbred conceptuses. The difference in weight between inbred and outbred conceptuses borne by inbred dams did not reach significance.

TABLE XV:

Mean numbers of pups (including still-births) at birth in first and second litters in each mating system; and the pooled mean litter sizes over two litters for each combination of inbred or outbred dam with inbred or outbred litters

Inbreeding Status Dam:Litter	Mating System	First Litter		Second Litter		*Significance of Difference in Size of 1st and 2nd Litters	Pooled Litter Size
		Number of Litters	Pups	Number of Litters	Pups		
I:I	77 x 77	33	4.2 ± 0.28	34	4.7 ± 0.31	$0.2 < P < 0.3$	4.5 ± 0.17
	44 x 44	36	4.5 ± 0.37	32	4.3 ± 0.42	$0.6 < P < 0.7$	
I:0	44 x 77	26	4.4 ± 0.34	18	5.6 ± 0.37	$0.02 < P < 0.05$	5.0 ± 0.22
	77 x 44	18	4.4 ± 0.51	17	5.8 ± 0.52	$0.02 < P < 0.05$	
0:0	$\overline{44 \times 77}^2$	28	4.9 ± 0.34	28	5.8 ± 0.36	$0.05 < P < 0.1$	5.5 ± 0.22
	$\overline{77 \times 44}^2$	18	5.9 ± 0.54	16	5.6 ± 0.59	$0.6 < P < 0.7$	

Comparison of Inbreeding Combinations	Difference in Pooled Mean Litter Sizes	*Significance of Difference
I:0 vs I:I	0.52	$0.05 < P < 0.1$
0:0 vs I:0	0.53	$0.05 < P < 0.1$
0:0 vs I:I	1.05	$P < 0.001$

* Probabilities from t - Tests following analysis of variance.

** I:I - Inbred dam with inbred litter; I:0 - Inbred dam with outbred litter; 0:0 - Outbred dam with outbred litter.

LITTER SIZE AND MORTALITY

The mean litter size at birth of first, and second litters, and a pooled mean for each combination of inbred and outbred litter and dam are presented in Table XV. The means were calculated for all pups observed at birth including the remains of stillborn and partly eaten pups. This was the most accurate estimate possible of the number of foetuses completing gestation in each line. Since no difference could be shown between the weights of second conceptuses on a basis of whether they were conceived post partum, or after first lactation no distinction has been made in Table XV between second litters born under these categories. Only inbred dams with outbred litters had significantly more pups born in second than in first litters.

Since there were no significant differences between subgroups within each combination of inbred or outbred dam with inbred or outbred litters, the pooled mean litter sizes over two litters for each of the three combinations were calculated and are compared in Table XV.

Inbred dams with inbred litters had one mouse per litter less than outbred dams with outbred litters. This difference is apparently made up of non-significant increase in litter size of .5 pups when inbreeding of the litter is dispersed (Inbred dam, inbred litter vs Inbred dam, outbred litter), and a further non-significant gain of .5 of a pup when inbreeding in the dam is dispersed (inbred dam, outbred litter vs outbred dam, outbred litter).

The total number of pups born in two litters in each mating system and the losses from parturition to weaning at twentyone days of age are shown in Table XVI. Litters with more than six pups at birth were culled to six to ensure that all pups were properly fed, and that growth and the prospects of survival of pups in large litters was not penalised because of excessive competition within litters. Neonatal losses comprised losses occurring after birth and before three days of age.

Comparisons in Table XVII show that line 44 females had significantly more pups born dead than line 77 females. Inbred dams lost fewer pups if their litters were outbred rather than inbred. Fewer outbred pups were born dead to outbred dams than to inbred dams.

The extent of neonatal losses analysed in Table XVIII, was similar in inbred dams regardless of the inbreeding status of the litter. There were significantly fewer neonatal losses when dams were outbred. Further losses from three to twentyone days were similar in all lines (Table XVIII).

TABLE XVI:

Mortality of pups from birth to weaning.

(Data from first and second litters in each mating system)

	77x77	44x44	44x77	77x44	$\overline{44x77}^2$ $\overline{77x44}^2$
Total Pups Observed at Birth	299	302	215	178	495
Born Dead	24	37	10	16	18
Born Alive	275	265	205	162	477
Culled	5	14	10	14	45
Neonatal Deaths	39	52	34	19	35
Losses 3 to 21 Days	24	14	13	8	24
Weaned at 21 Days	207	185	148	121	373

TABLE XVII:

The ratio of stillbirths : total births (live births plus stillbirths) as affected by dams of different inbred lines, inbreeding status of litters born to inbred dams, and the inbreeding status of dams bearing outbred litters.

Comparison	Matings	% of Total Pups Born Dead	Chi^2	*Significance of Difference in Ratio Births : Stillbirths
77 Female	77 x 77	6.6	4.612	P = 0.016
	44 x 77			
vs	44 x 44	11.0		
44 Female	77 x 44			
Inbred Dam	77 x 77	10.1	2.746	P = 0.045
Inbred Litter	44 x 44			
vs				
Inbred Dam	44 x 77	6.6		
Outbred Litter	77 x 44			
Inbred Dam	44 x 77	6.6	3.152	P = 0.037
Outbred Litter	77 x 44			
vs				
Outbred Dam	$\overline{44 \times 77}^2$	3.6		
Outbred Litter	$\overline{77 \times 44}^2$			

* Probabilities from Chi^2.

TABLE XVIII:

The effect of dams from different inbred lines, inbreeding status of litters born to inbred dams and the inbreeding status of dams on the survival of pups from birth to weaning.

Comparison	Mating Systems	Neonatal Losses*			Losses 3-21 Days**			Overall Survival (1)+			Overall Survival (2)++		
		%	Chi^2	P‡	%	Chi^2	P	%	Chi^2	P	%	Chi^2	P
77 Female	77 x 77 44 x 77	15.2			9.4			76.3			71.1		
vs			0.1660	0.341		1.191	0.138		<0.001	0.500		0.1918	0.330
44 Female	44 x 44 77 x 44	16.6			6.7			76.7			67.7		
Inbred Dam Inbred Litter	77 x 77 44 x 44	16.9			8.8			75.2			67.4		
vs			0.5488	0.230		0.3227	0.284		0.1162	0.456		0.5088	0.239
Inbred Dam Outbred Litter	77 x 44 44 x 77	14.4			7.2			78.4			72.9		
Inbred Dam	77 x 77 44 x 44 77 x 44 44 x 77	15.9			8.2			76.5			69.5		
vs			15.33	0.001		1.213	0.136		1.790	0.090		3.981	0.023
Outbred Dam	$\overline{44 \times 77}^2$ $\overline{77 \times 44}^2$	7.3			6.0			86.3			82.9		

‡ P - Probability of the ratios being compared being the same. Probabilities are calculated from Chi^2.

* Neonatal losses (i.e. losses occurring between birth and three days of age) relative to the numbers of pups alive at birth.

** Losses between three and twentyone days of age relative to the numbers of pups alive at three days.

\+ The number of pups weaned relative to the number of pups alive at birth, less those pups culled.

\++ The number of pups weaned relative to the total number of pups born (i.e. Live births plus stillbirths less those culled).

Of the total number of pups alive at birth outbred dams weaned more, but not significantly more, than inbred dams (Table XVIII). Consideration of the number of pups weaned in relation to the total number of pups born, - alive and dead, less those culled in large litters, indicated that overall outbred dams lost fewer pups than inbred dams. Outbreeding of litters did not significantly affect the extent of overall losses to weaning sustained by inbred dams.

TABLE XIX

Mean of average pup weights (gms) per dam and litter weights (gms) corrected for litter size in each mating system at birth, 12 days and at weaning for first and second litters.

Inbreeding Status	Mating System	Parity	BIRTH			12 DAYS			WEANING		
			Number of Litters	Mean Pup Weight	Corrected Litter Weight	Number of Litters	Mean Pup Weight	Corrected Litter Weight	Number of Litters	Mean Pup Weight	Corrected Litter Weight
Inbred Dam	77 x 77	1st	26	1.4 ± 0.04	6.27	21	4.7 ± .16	18.54	20	6.7 ± .35	26.03
		2nd	32	1.5 ± 0.03	7.85	32	5.4 ± .13	24.35	32	7.2 ± .23	32.22
Inbred Litter	44 x 44	1st	28	1.4 ± 0.04	5.98	25	4.5 ± .14	17.70	25	6.1 ± .24	24.47
		2nd	28	1.4 ± 0.04	7.27	22	4.8 ± .15	22.37	20	6.8 ± .23	30.32
Inbred Dam	44 x 77	1st	26	1.4 ± 0.04	6.10	18	4.8 ± .25	18.81	18	6.8 ± .38	26.23
		2nd	18	1.4 ± 0.05	7.44	17	5.0 ± .24	23.55	16	8.0 ± .35	35.74
Outbred Litter	77 x 44	1st	14	1.2 ± 0.05	5.48	11	3.9 ± .17	15.30	9	5.2 ± .23	21.28
		2nd	16	1.4 ± 0.06	7.30	14	4.6 ± .28	21.70	15	6.4 ± .24	28.55
Outbred Dam	$\overline{44 \times 77}^2$	1st	27	1.3 ± 0.04	5.62	17	5.3 ± .18	20.39	25	7.5 ± .25	29.34
		2nd	28	1.4 ± 0.03	7.54	27	5.5 ± .15	26.20	26	7.8 ± .22	35.77
Outbred Litter	$\overline{77 \times 44}^2$	1st	17	1.3 ± 0.05	5.82	12	5.0 ± .24	20.63	12	6.8 ± .37	28.00
		2nd	15	1.4 ± 0.03	7.65	15	5.4 ± .20	25.96	15	7.6 ± .60	36.87

Comparison of Corrected Litter Weights	Difference in Means gms		† Significance of Difference		Difference in Means gms		† Significance of Difference		Difference in Means gms		† Significance of Difference	
	1st Litter	2nd Litter	1st Litter	2nd Litter	1st Litter	2nd Litter	1st Litter	2nd Litter	1st Litter	2nd Litter	1st Litter	1st Litter
77 Female vs 44 Female	0.38	0.42	0.01 < P < 0.02	P < 0.001	1.70	1.97	0.02 < P < 0.05	0.02 < P < 0.05	2.50	3.83	0.05 < P < 0.1	0.01 < P < 0.02
Inbred Dam Inbred Litter vs Inbred Dam Outbred Litter	0.24	0.21	0.1 < P < 0.2	0.02 < P < 0.05	0.61	0.82	0.4 < P < 0.5	0.3 < P < 0.4	0.58	-0.77	0.6 < P < 0.7	P > 0.9
Outbred Dam Outbred Litter vs Inbred Dam Inbred Litter	-0.42	-0.01	0.001 < P < 0.01	P > 0.9	2.41	2.57	0.001 < P < 0.01	0.001 < P < 0.01	3.74	4.68	0.001 < P < 0.01	0.001 < P < 0.01
Outbred Dam Outbred Litter vs Inbred Dam Outbred Litter	-0.18	0.20	0.2 < P < 0.3	0.02 < P < 0.05	3.02	3.39	P < 0.001	P < 0.001	4.32	3.91	0.001 < P < 0.01	0.01 < P < 0.05

† Probabilities from t - Test following analysis of covariance.

Growth of Litters from Birth to Weaning

The mean litter weights of first and second litters in each mating system at birth, twelve, and twentyone days, are shown in Table XIX. These mean weights have been corrected by analysis of covariance for differences in litter size. Since litter sizes differed, and dead and partly eaten pups could not be weighed at birth, the actual weights of litters at birth do not enable comparisons to be made between lines. Consideration of the corrected litter weights in relation to the numbers of pups born in litters enables conclusions to be drawn regarding the weights of pups in each line at birth. The mean weights of individual pups in each mating system, at each stage, are also shown in Table XIX. These mean weights were calculated from the average weights of pups in each dams first and second litter. The growth curves of first and second litters in each of the two inbred lines, reciprocal crosses and F_1 matings, and the mean growth curves over two litters for each mating system are illustrated in Figs. 3 to 6.

Litters born to line 77 females weighed significantly more than those born to line 44 females. Since there was no significant difference between the litter sizes at birth in these lines, it seems that pups born to line 77 females were heavier at birth than the pups of 44 females.

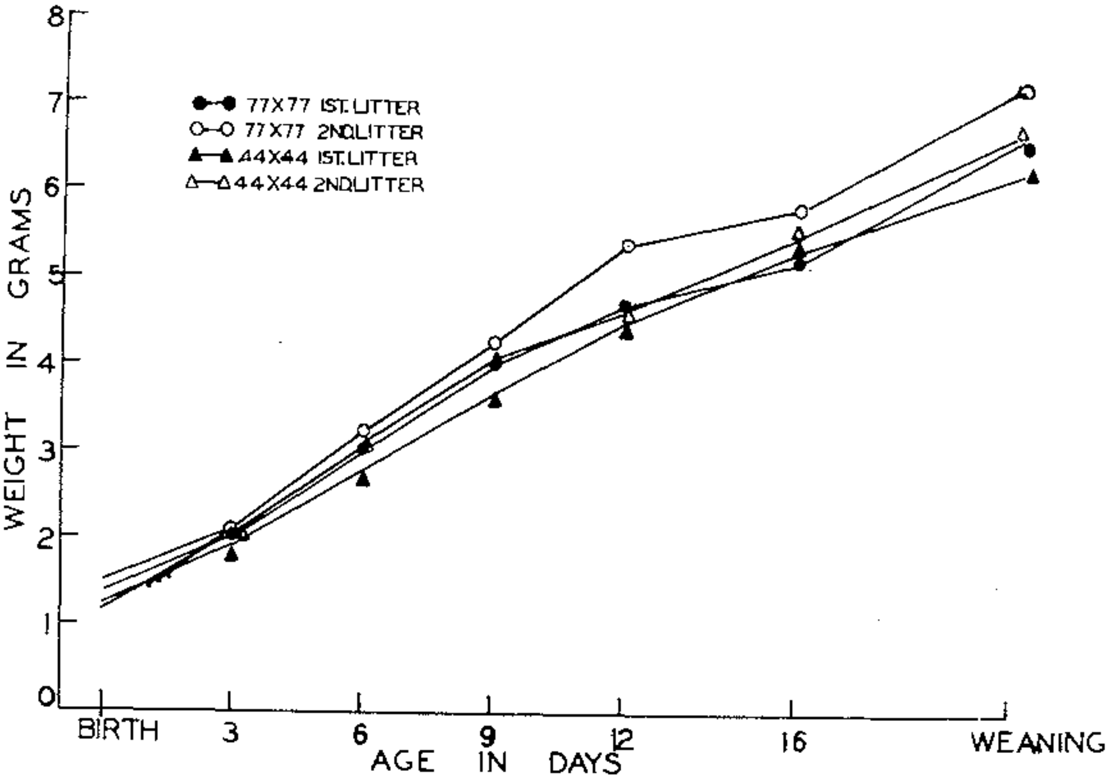
Inbred dams had heavier corrected litter weights at birth when their litters were inbred rather than outbred. This difference was significant at second parturition. This difference probably results because outbred litters were larger, especially at second parturition, than inbred litters. If individual pups in larger litters were lighter, corrected litter weights would also be lighter. For the same reason lighter pups in the larger litters of outbred females would cause the lighter corrected litter weights seen in outbred females at first parturition when they were compared with the corrected litter weights of inbred females with smaller inbred or outbred litters. At second parturition, although they had more pups in their litters, outbred females had corrected litter weights equal to those of inbred dams with inbred litters, and which were significantly heavier than the corrected litter weights of inbred dams with outbred litters.

The corrected litter weights at twelve and twentyone days revealed that at both these ages the litters of 77 females were heavier than the litters of 44 females. There was no significant difference in the weights of inbred or outbred litters nursed by inbred dams. The slight difference in favour of the inbred litter was due to the poorer performance of outbred litters suckled by line 44 females (see Fig. 4).

Outbred females reared their outbred litters to significantly heavier weights at twelve and twentyone days than did inbred females with inbred or outbred litters.

FIG. 3 The growth curves from birth to weaning of inbred pups in the first and second litters in line 77 and 44.

FIG. 4 The growth curves from birth to weaning of outbred pups in first and second litters from the reciprocal crosses of lines 77 and 44.


77X77 IST.LITTER
77X77 2ND.LITTER
44X44 IST.LITTER
44X44 2ND.LITTER
WEIGHT IN GRAMS
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1
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8
BIRTH
3
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9
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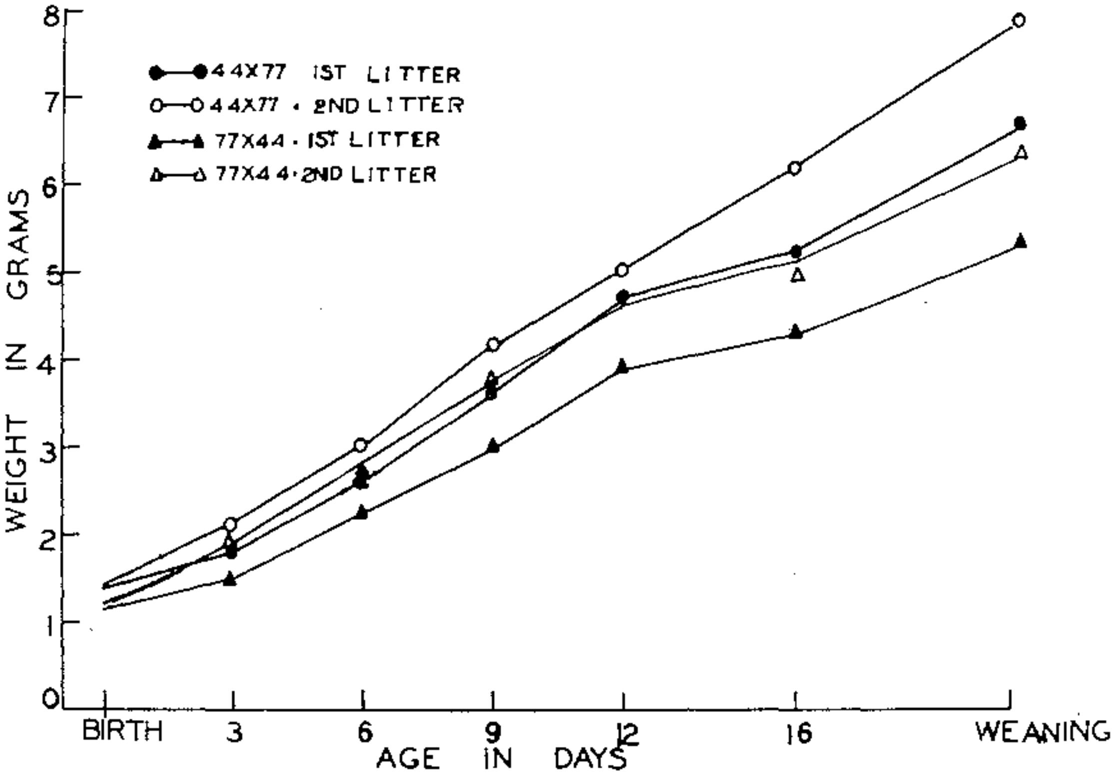
16
WEANING
AGE IN DAYS

FIG. 3


44X77 IST LITTER
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77X44 · IST LITTER
77X44·2ND LITTER
WEIGHT IN GRAMS
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3
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5
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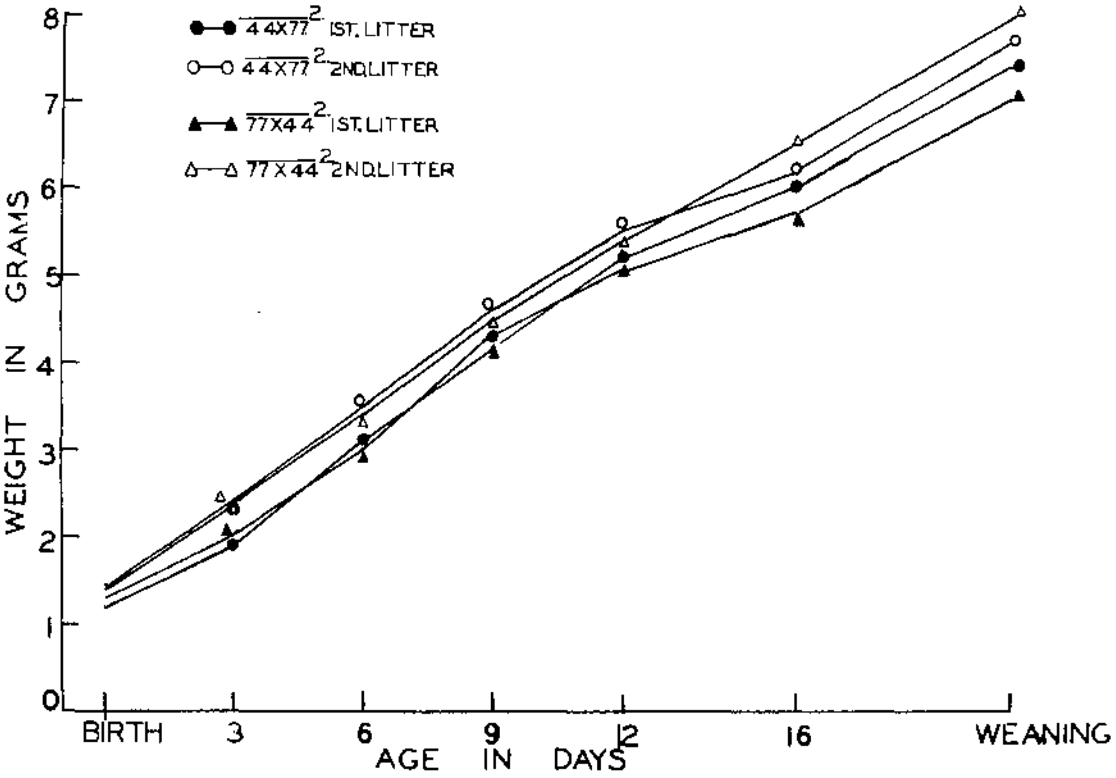
8
BIRTH
3
6
9
12
16
WEANING
AGE IN DAYS

FIG. 4

FIG. 5 The growth curves from birth to weaning of outbred pups in first and second litters from F_1 matings.

FIG. 6 The mean growth curves (two litters) from birth to weaning of inbred pups nursed by 77 and 44 dams, outbred pups nursed by 77 and 44 dams (44x77; 77x44) and outbred pups nursed by outbred dams (F_1).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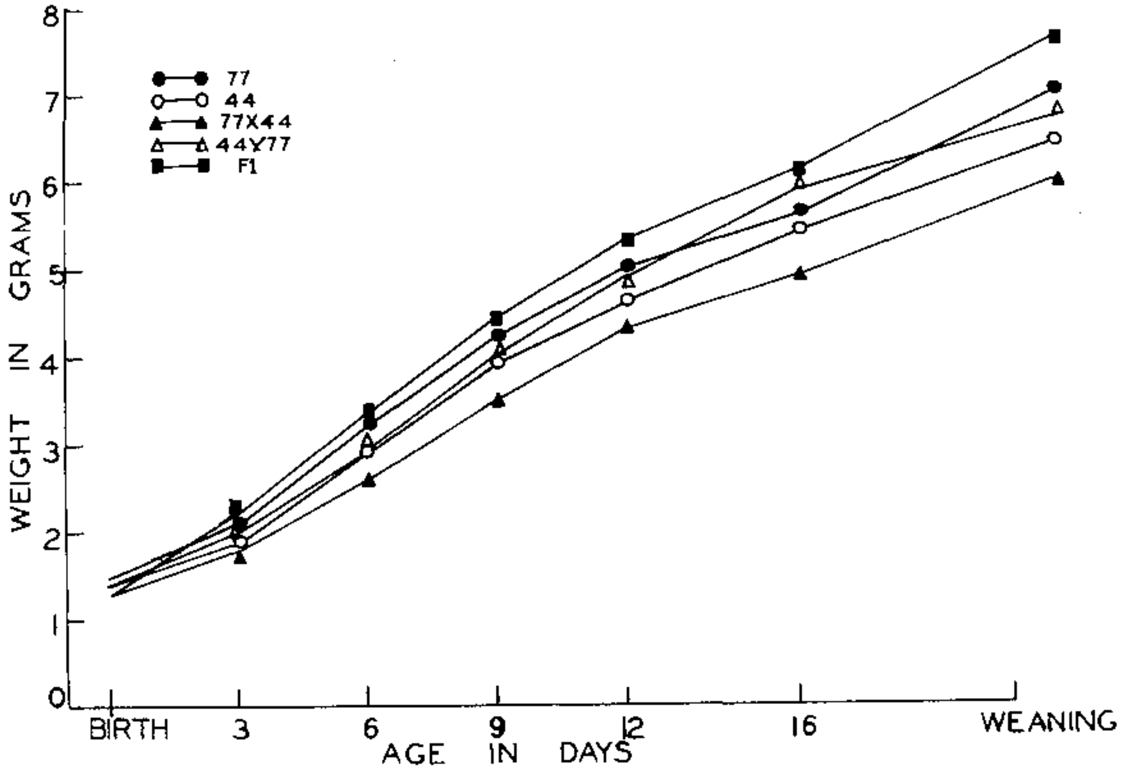
WEANING
AGE IN DAYS

FIG. 5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WEIGHT IN GRAMS
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BIRTH
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16
WEANING
AGE IN DAYS

FIG. 6

TABLE XX:

Mean mature weights (gms) of females in each mating system at second parturition

Mating System	Number of Mice	Weight
77 x 77	33	22.9 ± 0.31
44 x 44	33	25.7 ± 0.36
44 x 77	18	24.3 ± 0.85
77 x 44	17	23.9 ± 0.62
$F_1\ \overline{44 \text{ x } 77}^2$	16	25.4 ± 0.52
$F_1\ \overline{77 \text{ x } 44}^2$	28	25.0 ± 0.48
77 Female	51	23.4 ± 0.37
44 Female	50	25.1 ± 0.34
F_1 Female	44	25.1 ± 0.35

Comparison of Mating Systems	Difference in Weights	† Significance of Difference
44 x 77 vs 77 x 77	1.40	$0.02 < P < 0.05$
44 x 44 vs 77 x 44	1.74	$0.01 < P < 0.02$
44 Female vs 77 Female	1.65	$P < 0.001$
F_1 Female vs 44 Female	0.03	$P > 0.9$

† Probabilities from t - Test following analysis of variance.

Mature Weight of Females

The mean weights of mature females of each line following second parturition are shown in Table XX. Although the females were not of equivalent age at this time, they should have been approaching mature weight, and were all in the same reproductive state.

Line 77 and 44 females mated to 44 males were significantly heavier at this time than when the same females were mated to 77 males. This was probably a result of their greater age, since conception occurred sooner with 77 than with 44 males.

Line 44 females were significantly heavier than line 77 females, but were similar in weight to F_1 females at this time.

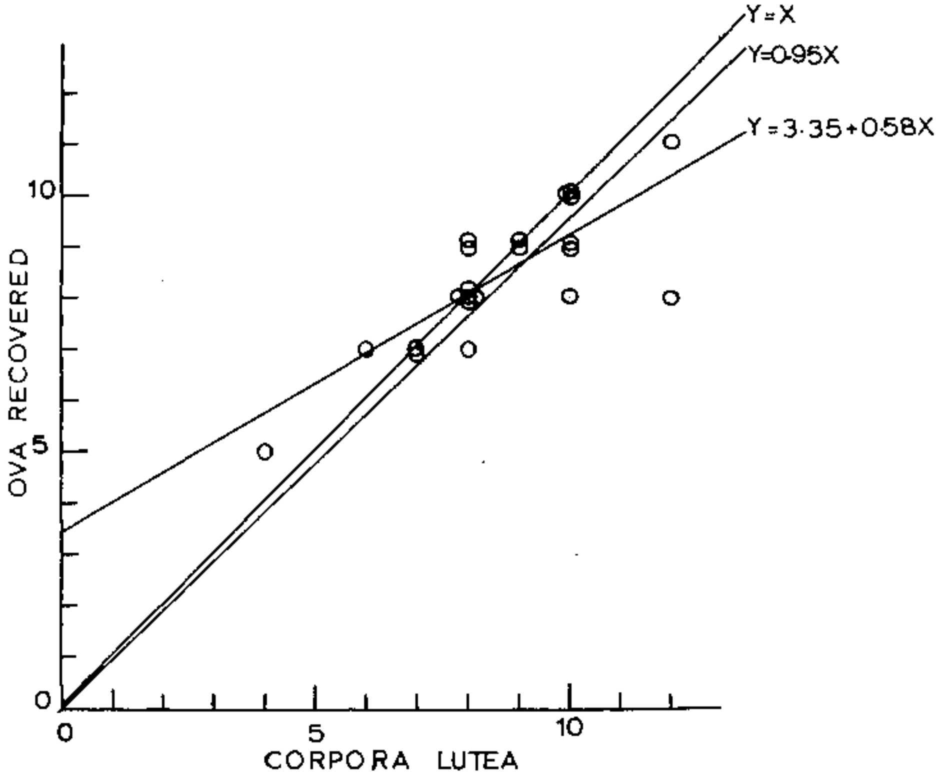

FIG. 7 Regression of the number of ova recovered after flushing the uterus 3½ days *post coitum* on the number of ova shed, as shown by number of corpora lutea, in albino mice.

Ovum Recovery

In a preliminary experiment the technique of ovum recovery, described in Chapter II, was used to recover ova from random bred albino females. A total of 190 corpora lutea were counted in twenty two females, and 184 ova were recovered, giving a recovery rate of 97 percent of the ova ovulated.

The regression of ova recovered on ova shed, as shown by the numbers of corpora lutea on the ovaries, indicated that proportionately fewer ova were recovered at higher ovulation rates. (Fig. 7).

It can be seen from Fig. 7 that in four animals, more ova were recovered than corpora lutea. It was difficult where corpora were close together to decide whether one or more corpora lutea were involved. These discrepancies are therefore more likely to be due to erroneous counts of corpora lutea than to poly-ovular follicles.

Corpora lutea counts were made before ova were recovered and it was considered unwise to recount corpora again after ova had been recovered.

The recovery rates from this trial were considered satisfactory enough to allow the technique to be used to recover ova in order to assess fertility in the experimental lines.

TABLE XXI:

Mean number of ova shed at ovulation by inbred and outbred females. Data from counts of corpora lutea at three, twelve and seventeen days of gestation.

Mating System	Number of Mice Examined	Mean Number of Corpora lutea	Variance
77 x 77	40	6.3 ± 0.14	0.73
44 x 44	32	6.8 ± 0.23	1.64
77 x 44	34	6.4 ± 0.16	0.91
44 x 77	21	6.2 ± 0.25	1.29
F_1 $\overline{77 \times 44}^2$ F_1 $\overline{44 \times 77}^2$	65	6.9 ± 0.13	1.08

Comparison of Mating Systems	Difference Between Means	*Significance of Difference
44 Female vs 77 Female (44x44) (77x77) (77x44) (44x77)	0.31	$0.05 < P < 0.1$
F_1 Female vs 77 Female	0.63	$P < 0.001$
F_1 Female vs 44 Female	0.32	$0.05 < P < 0.1$
F_1 Female vs Inbred Female	0.47	$0.001 < P < 0.01$

* Probabilities from t - Tests following analysis of variance.

Ovulation Rates and Losses During Gestation

Female mice paired with mature males at puberty were killed at three, twelve and seventeen days after mating. The data from these experiments is shown in Figs. 8 to 12.

No differences between corpora luteal counts made at different stages of gestation within lines could be shown. The results presented in Table XXI are the pooled means from corpora luteal counts made at all three stages of gestation in each mating system.

Assuming each corpus luteum represented one ovulation, line 77 females had significantly fewer ovulations than F_1 females. Differences between line 44 and 77, or F_1 females did not quite reach significance, but the mean overall ovulation rate for inbred females was significantly less than for outbred (F_1) females.

Ova flushed from the uterus three days after mating were examined microscopically. Ova which had reached blastocyst or morula stage were classified as viable. Classification on this basis meant that the number of ova able to begin implanting, as shown by implantation sites counted at twelve days, was less, in some mating systems, than the number of ova classified as viable. This difference was most marked where line 44 females were involved. A second classification of viable ova, according to whether they had cleaved at three days, brought the numbers of viable ova into better agreement with the number of implantation sites recorded. No conclusions can be drawn from this secondary classification as it was based on notes and sketches made at the time of first classification of recovered ova.

On the twelfth day of gestation corpora lutea, implantation sites, and numbers of live embryos were recorded; at seventeen days corpora lutea, live foetuses and resorbing sites were recorded.

From Figs. 8 to 12 it can be seen that recovery rates averaged 0.3 - 0.4 ova less than the number of ova shed, except in 44 x 44 mating, where the recovery rate was poorer.

The number of implantation sites visible at twelve days was significantly less than the number of ovulations in inbred females, regardless of the inbreeding of potential embryos. Losses between ovulation and implantation were not significant in outbred females (Table XXII).

TABLE XXII:

Mean numbers of ova shed and numbers of implantation sites observed on the twelfth day of gestation in each mating system.

Mating System	Number of Ova Ovulated		Number of Implantation Sites		*Significance of Difference Between Ovulations and Implantations
	Number of Mice	Ovulations	Number of Mice	Implants	
77 x 77	40	6.3 ± 0.14	16	4.9 ± 0.57	$0.001 < P < 0.01$
44 x 44	32	6.8 ± 0.23	15	4.8 ± 0.86	$0.01 < P < 0.02$
77 x 44	34	6.4 ± 0.16	13	4.9 ± 0.81	$0.02 < P < 0.05$
44 x 77	21	6.2 ± 0.25	10	3.9 ± 1.13	$0.01 < P < 0.02$
F_1 $\overline{44 \text{ x } 77}^2$ $\overline{77 \text{ x } 44}^2$	65	6.9 ± 0.13	27	6.2 ± 0.46	$0.05 < P < 0.1$

* From t - Test following an analysis of variance.

In all lines there were fewer live embryos at twelve days than implantation sites. The numbers in each mating system were too few to allow conclusions to be drawn regarding losses occurring after implantation had begun.

The apparent marked losses from twelve to seventeen days in 77 and 44 females mated to 44 males has arisen because of sterility in 44 males. In 44 x 44 matings three out of fifteen females killed at twelve days, and three out of twelve killed at seventeen days, no sign of pregnancy was evident. Similarly in 44 x 77 matings three out of ten and five out of ten females were barren when examined at twelve and seventeen days, respectively. With the small numbers involved this high incidence of sterility, which was probably a result of male infertility, made accurate comparisons of the extent of losses over pregnancy in the different lines impossible.

The results of the slaughter experiments indicated that ovulation rate was higher in outbred females, and preimplantation losses were greater in inbred females. Even with matings involving 44 males excluded, overall losses were greater in inbred than in outbred females.

FIG. 8 Prenatal mortality during first gestation in 77 x 77 matings.

FIG. 9 Prenatal mortality during first gestation in 44 x 44 matings.

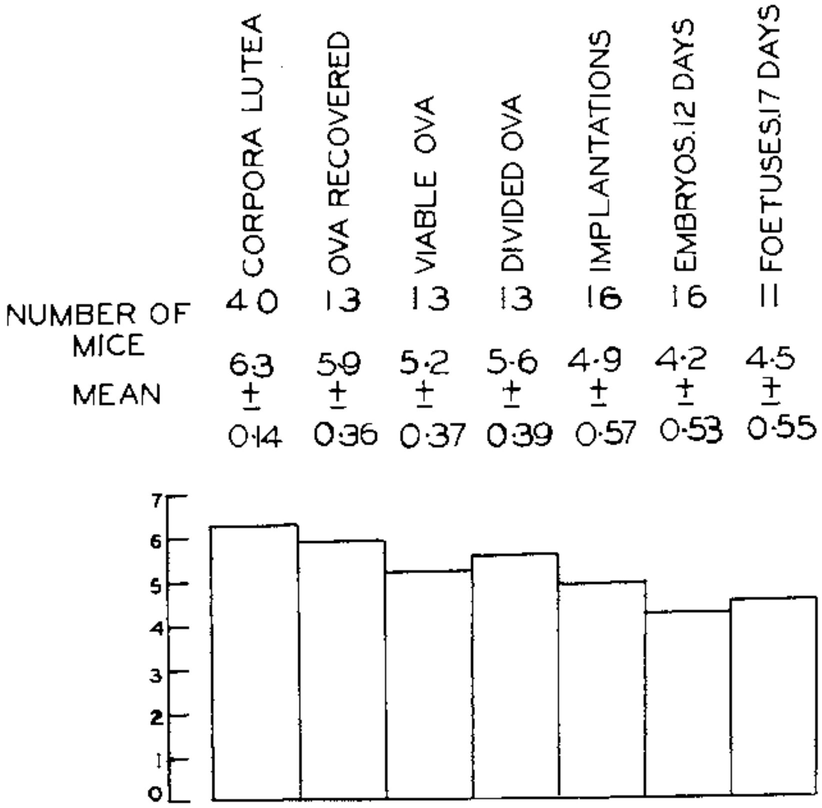
CORPORA LUTEA
OVA RECOVERED
VIABLE OVA
DIVIDED OVA
IMPLANTATIONS
EMBRYOS.12 DAYS
FOETUSES.17 DAYS
NUMBER OF MICE
40
13
13
13
16
16
11
MEAN
6·3 ± 0·14
5·9 ± 0·36
5·2 ± 0·37
5·6 ± 0·39
4·9 ± 0·57
4·2 ± 0·53
4·5 ± 0·55
7
6
5
4
3
2
1
0

FIG. 8

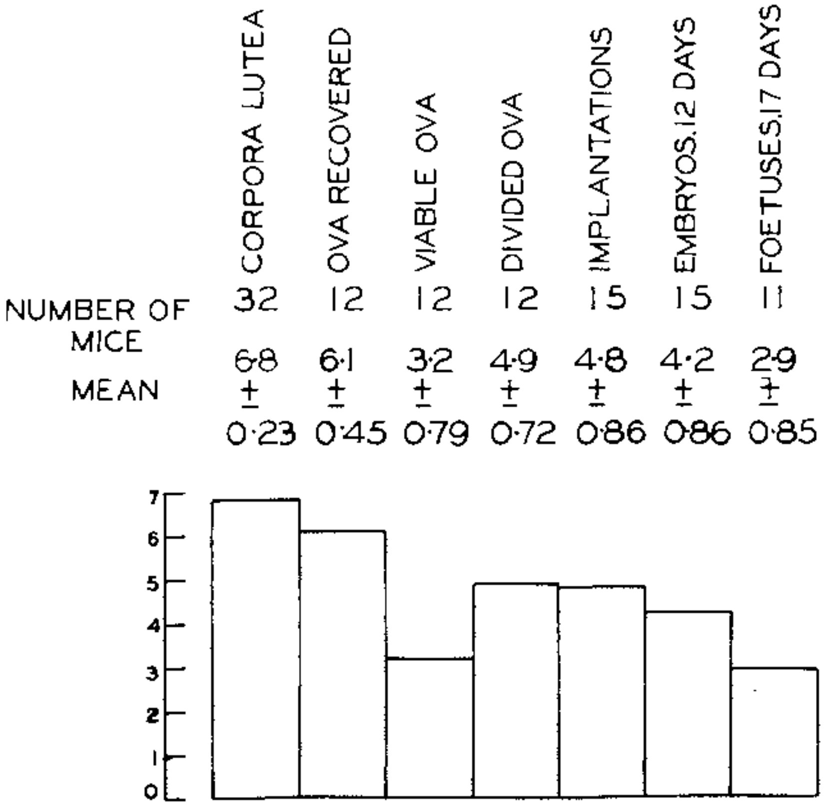
CORPORA LUTEA
OVA RECOVERED
VIABLE OVA
DIVIDED OVA
IMPLANTATIONS
EMBRYOS.12 DAYS
FOETUSES.17 DAYS
NUMBER OF MICE
32
12
12
12
15
15
11
MEAN
6·8 ± 0·23
6·1 ± 0·45
3·2 ± 0·79
4·9 ± 0·72
4·8 ± 0·86
4·2 ± 0·86
2·9 ± 0·85
7
6
5
4
3
2
1
0

FIG. 9

FIG. 10 Prenatal mortality during first gestion in 44 x 77 matings.

FIG. 11 Prenatal mortality during first gestation in 77 x 44 matings.

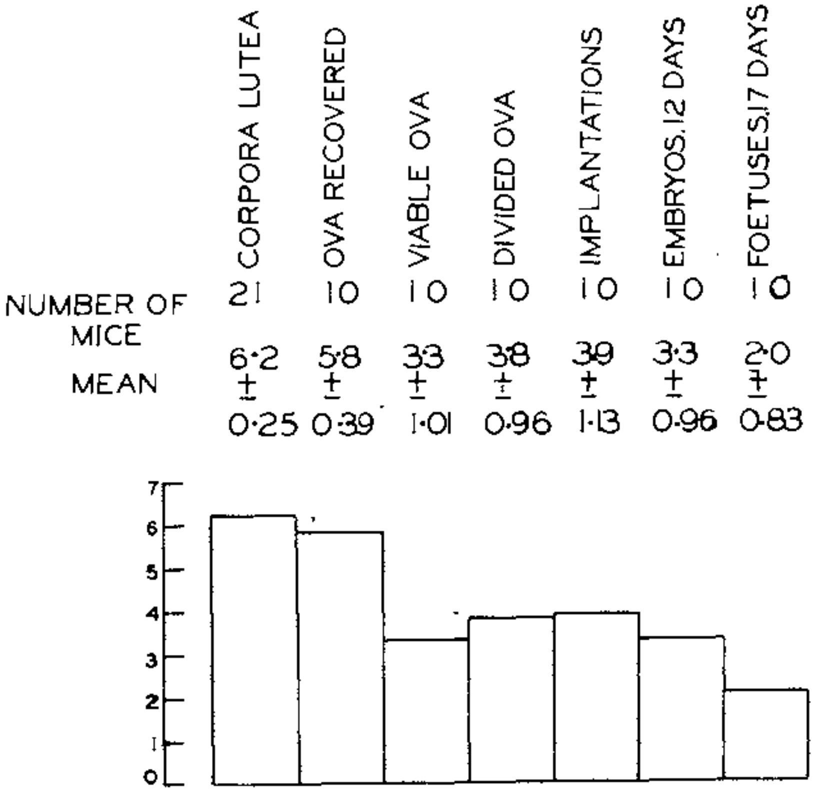
CORPORA LUTEA
OVA RECOVERED
VIABLE OVA
DIVIDED OVA
IMPLANTATIONS
EMBRYOS.12 DAYS
FOETUSES.17 DAYS
NUMBER OF MICE
21
10
10
10
10
10
10
MEAN
6·2 ± 0·25
5·8 ± 0·39
3·3 ± 1·01
3·8 ± 0·96
3·9 ± 1·13
3·3 ± 0·96
2·0 ± 0·83
7
6
5
4
3
2
1
0

FIG. 10

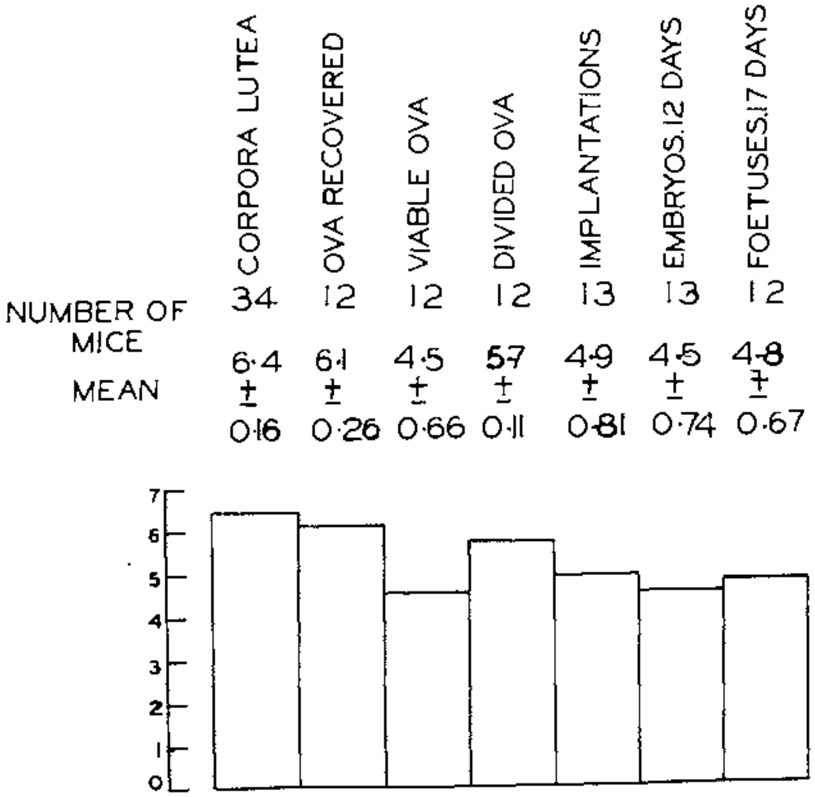
CORPORA LUTEA
OVA RECOVERED
VIABLE OVA
DIVIDED OVA
IMPLANTATIONS
EMBRYOS.12 DAYS
FOETUSES.17 DAYS
NUMBER OF MICE
34
12
12
12
13
13
12
MEAN
6·4 ± 0·16
6·1 ± 0·26
4·5 ± 0·66
5·7 ± 0·11
4·9 ± 0·81
4·5 ± 0·74
4·8 ± 0·67
7
6
5
4
3
2
1
0

FIG. 11

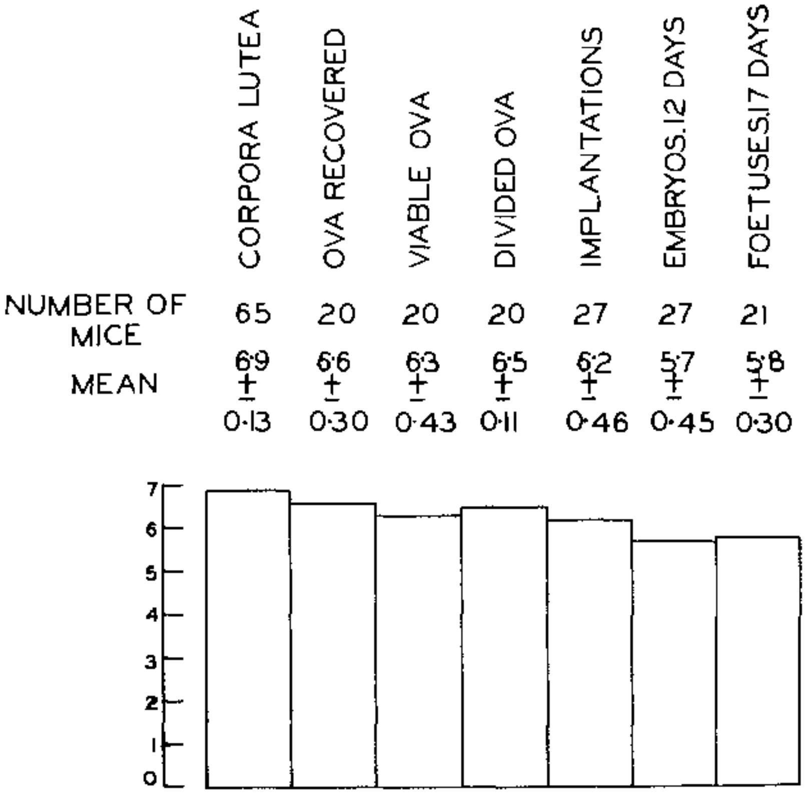

FIG. 12 Prenatal mortality during first gestation in F_1 matings ($\overline{77x44}^2$; $\overline{44x77}^2$).

Histological Examination of Reproductive Organs

During microscopic examination of ovaries it was noted that the corpora lutea in 44 females were often not as well developed, at three days *post coitum* as were corpora lutea in F_1 and 77 females.

Histological examination of ovaries, taken from twelve females in each line, indicated that at three days after mating there were differences between lines in the extent to which luteal tissue had invaded the blood clot formed at ovulation (see Figs. 13a, b and 14a, b). A number of histological sections from each ovary were selected at random, and classified subjectively according to the size of the blood clot remaining in the corpora lutea. It was found that the majority of F_1 and 77 females had a small, or no blood clots remaining. There may have been a tendancy for the corpora lutea of F_1 females to be better developed than those of 77 females. The corpora lutea of 44 females were markedly less well developed than in females of the other lines; most corpora lutea had blood clots classified as large or medium in size. In some line 44 females luteinization of unovulated follicles was evident (Figs. 15a, b). No other differences in ovarian histology were apparent.

The contents of the vas deferens was examined in males of each line. In two groups of line 77 males, aged 2 to 3 and 4 to 5 months respectively, the vas deferens were filled with motile sperm. Eighteen out of twenty F_1 males, aged 2 to 5 months, had sperm in similar concentrations to males in line 77. The remaining two F_1 males had poor concentrations of sperm and many were dead. Motile sperm were found in seven out of ten line 44 males aged 2 to 4 months, but sperm concentration was generally poorer than in 77 and F_1 males. In the remaining three 44 males only a few dead sperm were found in the vas deferens. In line 44 males older than four months only two out of ten males were found to have good concentrations of motile sperm; the remainder had few or no sperm present and sperm were usually dead.

A histological examination was made of the testes from males aged 3 to 4 months. Normal spermatogenesis appeared to be occurring in line 77 and F_1 males and in some 44 males (Figs. 16a, b). Line 44 males which had proved infertile in matings, and which failed to have motile sperm in their vas deferens, were found to have abnormal testes. The testes of these sterile males were smaller and darker in colour than those of fertile males. Microscopic examination revealed that normal spermatogenesis was not occurring in these sterile males. This abnormality seemed characterised by a failure of spermatazoa to develop past the spermatid stage (Figs. 17a, b). Sterile males seemed to have more interstitial cells than fertile males, but no conclusions regarding the functional ability of interstitial tissue could be made from histological preparations.

FIG. 13 Fully developed corpora lutea as found in the ovaries of 77 and F_1 females three days *post coitum*.

(a) x 80; (b) x 180.

FIG. 14 Poor luteal growth with large blood clot evident. This was typical of the appearance of the ovaries of line 44 females three days *post coitum*.

(a) x 80; (b) x 180.

FIG. 13 (a)

FIG. 13 (b)

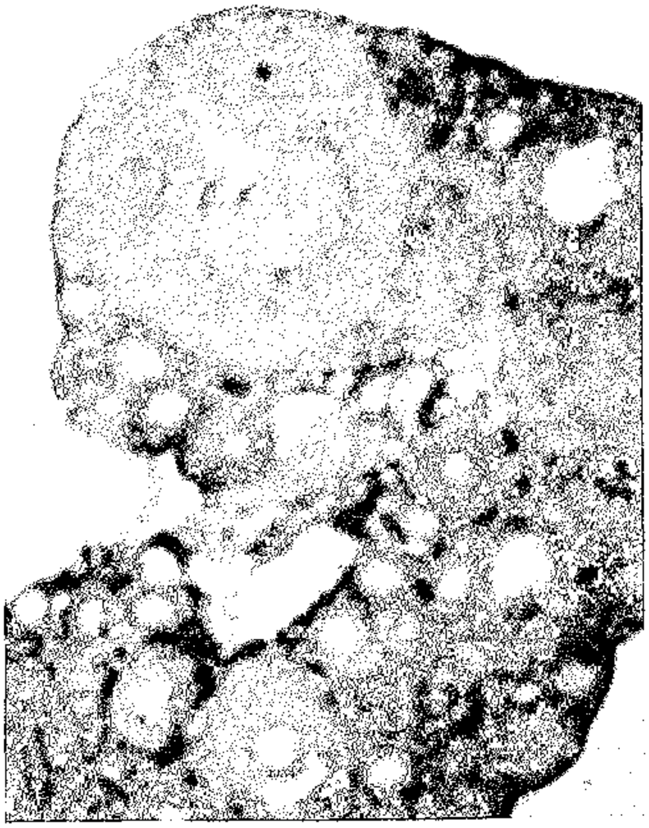

FIG. 14 (a)

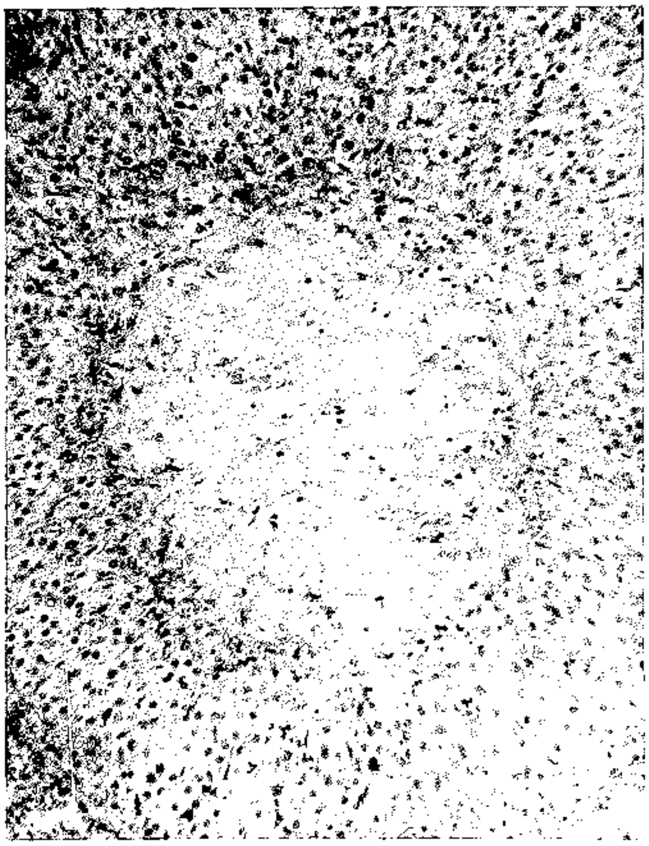

FIG. 14 (b)

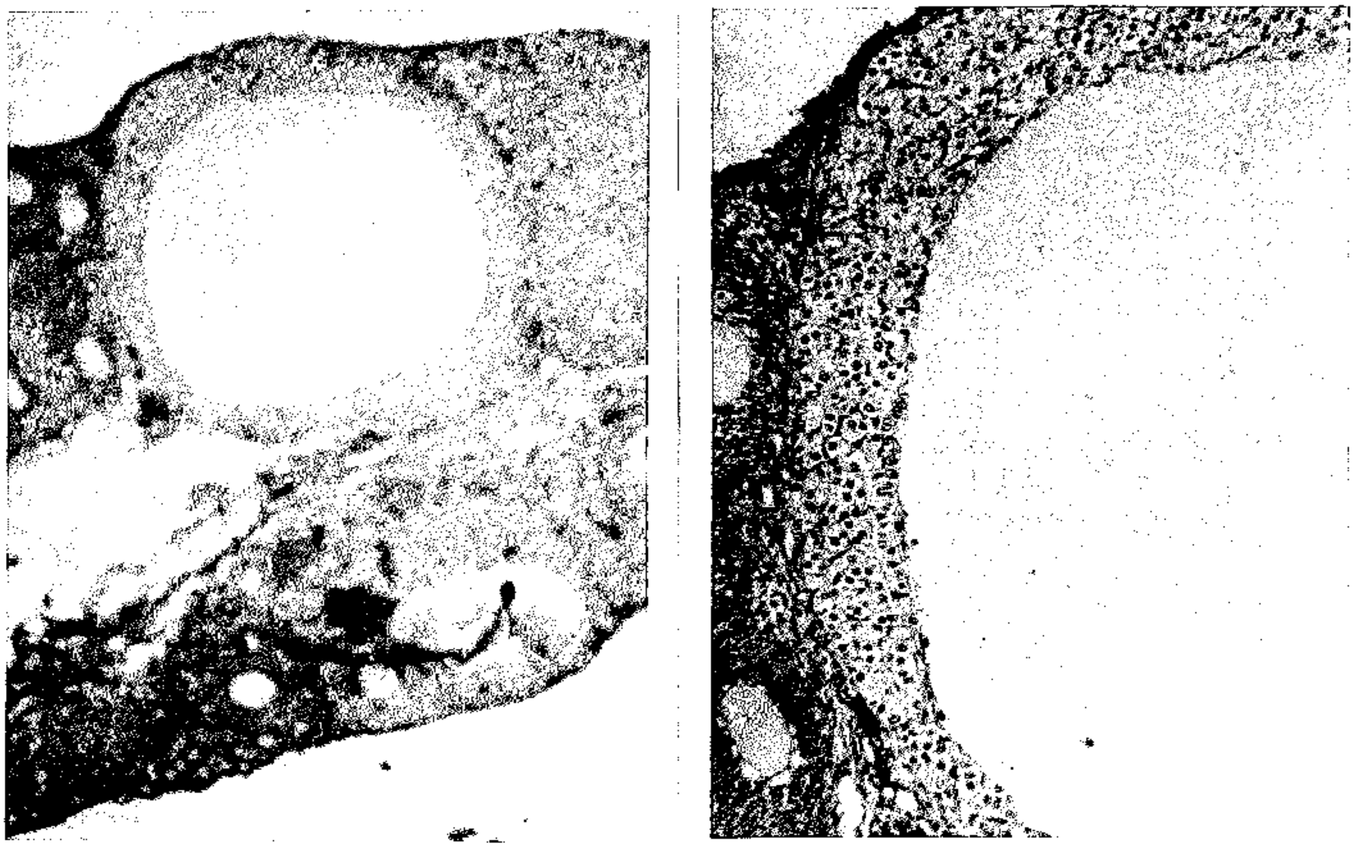

FIG. 15 (a)

FIG. 15 (b)

The ovary of a line 44 female three days *post coitum* showing an unovulated follicle with luteal tissue developing about the periphery. (a) x 80; (b) x 180.

FIG. 16 The typical appearance of testes of fertile 77, 44 and F_1 males showing active spermatogenesis.

(a) x 180; (b) x 800.

FIG. 17 Testes tissue from a sterile line 44 male showing shrunken seminiferous tubules (c.f. Fig. 16) and no evidence of spermatozoa. There appears to be more interstitial tissue in the testes of these sterile animals than in fertile animals.

(a) x 180; (b) x 800

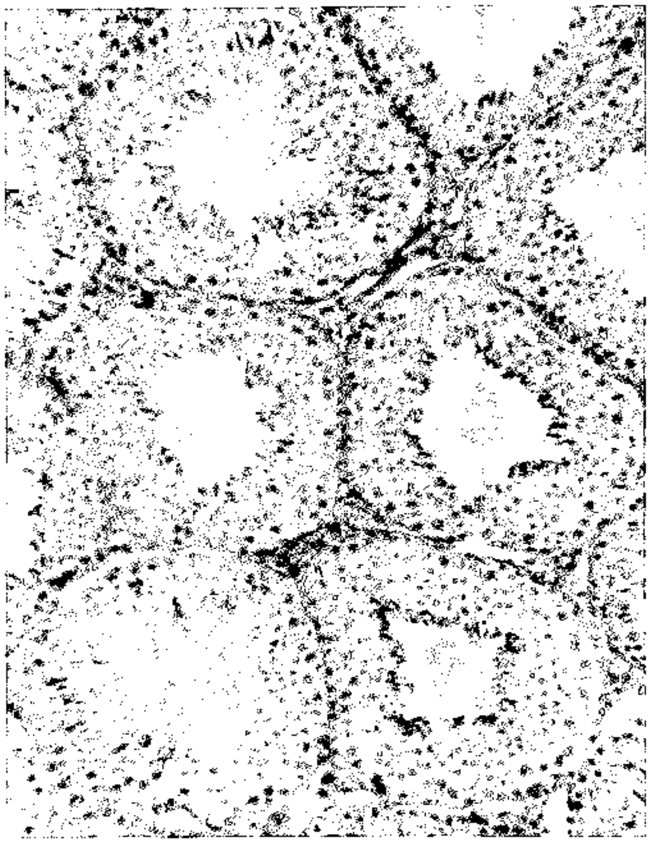

FIG. 16 (a)

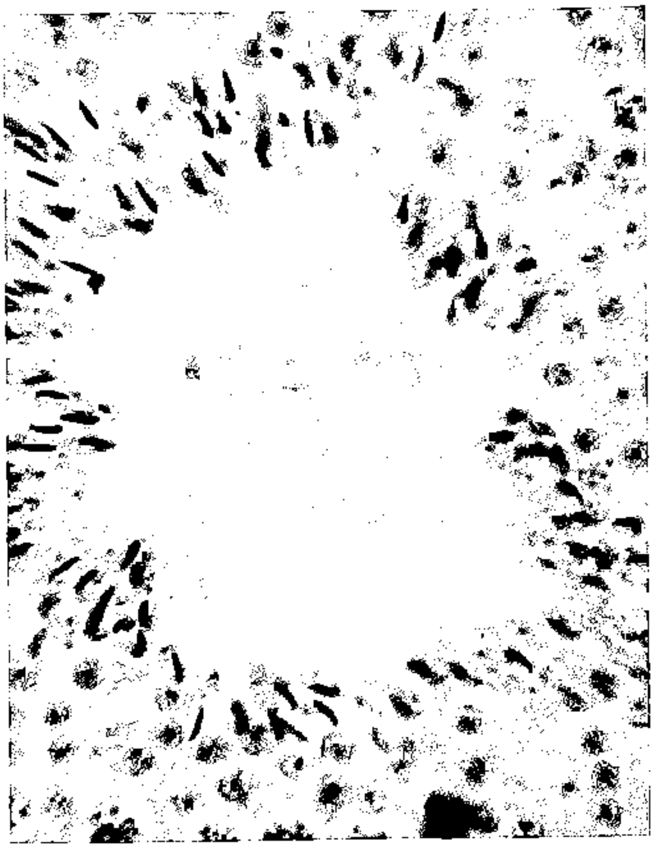

FIG. 16 (b)

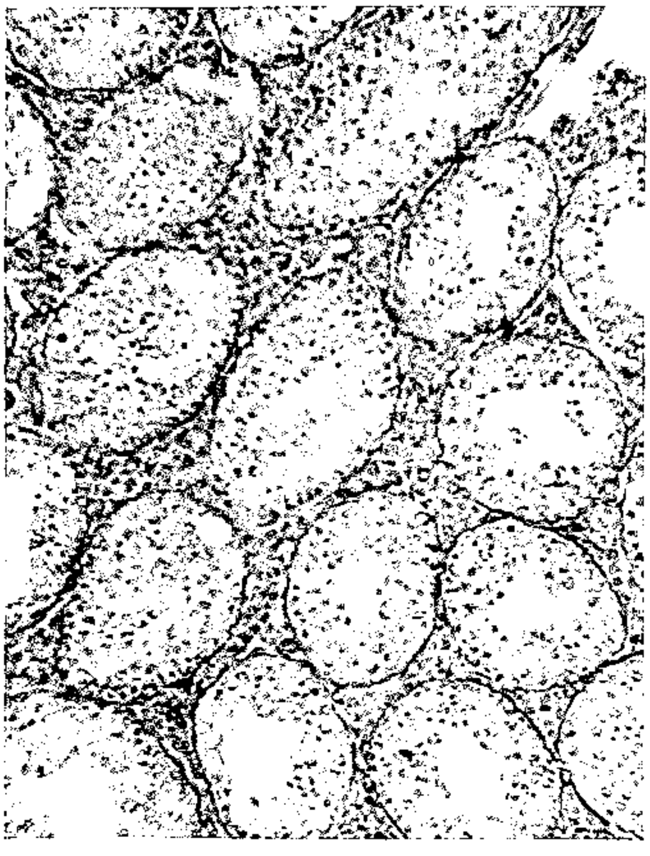

FIG. 17 (a)

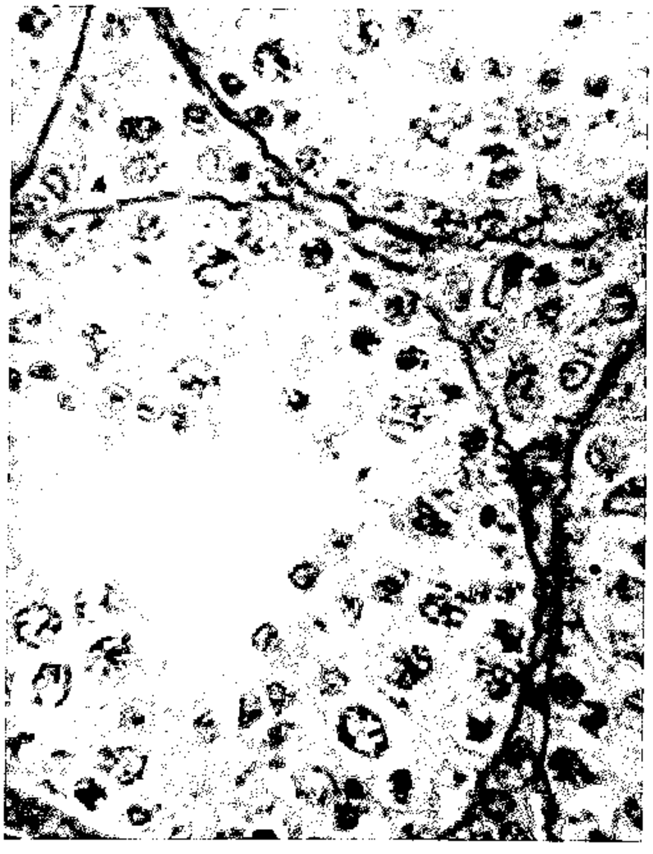

FIG. 17 (b)

Disease Incidence

Scouring in young mice after weaning was often encountered. There seemed to be no difference between lines in the incidence of this disease.

Cystic kidney was noted in mice of all lines and the regular occurrence of stones in the bladders of males older than four months was noted.

The tapeworm *Hymenolepis nana* was found in the ileum of mice in the colony and thereafter all mice killed were examined. In lines 77 and F_1 twenty and thirty percent, respectively, of the mice killed were infected. Eighty percent of line 44 mice were found to be parisitised by tapeworms. Line 77 and F_1 animals rarely had more than one or two tapeworms, but line 44 animals suffered from larger loads of parisites. There would appear to be a difference between lines in their susceptability to tapeworms infection.

CHAPTER IV

DISCUSSION

The aim of these experiments was to compare two inbred lines of mice, of common ancestry, to find if they differed in characteristics associated with growth and reproductive performance. Such differences could arise as a result of segregation of different alleles during inbreeding. Comparison of the inbred parent lines with their reciprocal crosses, and their randomly mated F_1 progeny enabled the effects of dispersal of inbreeding, together with any effects of heterosis from the hybridisation of inbred lines, to be measured. In the absence of a random bred stock, of the same ancestry as the inbred lines, the effects of heterosis and dispersal of inbreeding could not be separated.

Data was collected over the first and second gestations and lactations in each line. Second litters were conceived at both post-partum and post-lactational matings. No significant difference was found in this experiment between the weights of conceptuses born after gestations concurrent with, or which began after, first lactation. In the analysis of results no distinction was made between these two classifications of second gestation. Second gestations concurrent with first lactations could have been avoided if males had been removed after first conception. This procedure was not followed as it was necessary to have the male present to serve the other female in a cage, and it was desirable to have as many females as possible conceiving <u>post-partum</u> in order to provide further experimental animals.

Following first parturition examinations were not made for vaginal plugs as they were not conspicuous, or were quickly lost at this time, and excessive handling of mice at this time may have interfered with their maternal performance.

In the statistical analysis of these experiments it was found that in some cases means and variances were related. Transformation of some of these data did not alter the significance of main differences as revealed by analysis of variance. Where transformations have not been made, but where differences in variance are shown, care should be taken in interpreting results if differences between means barely reach significance.

<u>Litter Size and Pup Weight at Birth</u>:

The reproductive performance of females in each mating system was measured by recording conceptus weights, numbers of young born, and the weights of litters at birth. Conceptus weight was measured by the weight lost by females at parturition. This measure should be closely related to litter size. Litter size at birth included dead and partly eaten pups, and is a measure of the number of foetuses which completed gestation. This assessment of litter size would be erroneous if any stillborn young were completely eaten before litters were found on the morning of birth. Live pups were weighed at birth. Because of differences in litter size these litter weights were corrected, by analysis of covariance, so that litter size was standardized throughout the different lines. Comparisons of these corrected litter weights would reflect differences in individual pup weight at birth. This method was considered more accurate than weighing individual pups because weights could only be recorded to an accuracy of 0.2 of a gram. The mean pup weights reported were calculated from the average weights of pups in each litter born. Since pups had suckled by the time they were found on the morning of birth the measures of litter weight are confounded with the lactational ability of the dam. Differences in the amount of milk available, and greater competition for the available milk in large litters therefore precludes any definite conclusions regarding differences in the birth weight of pups.

Second litters were generally superior to first litters in each of these three characteristics. Such an improvement in reproductive performance, was noted by Murray (1934) and Eaton (1953).

In 77 x 77, but not in 44 x 44, matings there was a significant increase, from first to second litter in conceptus weight. This increase in line 77 x 77 was accompanied by a non-significant increase of 0.5 pups in litter size, and heavier birth weights of their pups. The difference between inbred lines probably occurred because of the greater age of 44 females at first conception. Increased conceptus weights associated with significantly larger litters were found at second parturition in the two reciprocal crosses. This rise in conceptus weight from first to second gestation was of similar magnitude in both crosses, but significant only in line 44 x 77. Outbred females did not show any significant increase in litter size or conceptus weights, from first to second parturition, although $\overline{44 \text{ x } 77}$ females had one more pup in their second litters.

The conceptus weights and litter sizes at birth recorded by line 77 and 44 females were similar when both bore either inbred, or outbred pups. The corrected litter weights at birth for 77 females were significantly heavier than those of 44 females. Since both lines of females had similar numbers of pups in their litters it seems that pups born to 77 females were heavier when weighed. Because no difference could be shown between females in their conceptus weights it is possible that this difference in pup weight at birth was a result of less milk in the stomachs of pups born to 44 females. It was found that milk production, as shown by growth of pups to 12 days, was poorer in 44 females, and observed that some pups born to 44 females died without having suckled successfully. Line 44 females may not have been able to begin lactation as soon, or produce as much milk in early lactation as line 77 females.

Over two litters, outbred litters born to inbred dams had slightly, but not significantly, larger litter sizes and conceptus weights than inbred females with inbred litters. This difference was largely a result of the superior weight and size of outbred second litters. These outbred litters had lighter corrected litter weights at birth than inbred litters which indicated that outbred pups were individually smaller than inbred pups at birth. This is probably a result of the greater number of pups in outbred litters - particularly in second litters. A number of workers have found that individual pup weights were lighter in larger litters (Grüneberg, 1952).

F_1 matings resulted in conceptuses which were significantly heavier than the conceptuses of inbred dams bearing inbred or outbred litters. The litters of F_1 females were significantly (1 pup) larger than inbred litters born to inbred dams but were not significantly larger than outbred litters born to inbred dams.

The cause of mean litter size over two litters differing in reciprocal crosses from both inbred, and F_1 matings was the marked increase in litter size from first to second gestation in the reciprocal crosses. This could indicate that in the primiparous inbred female the uterine environment limited litter size so that any improvement in the viability of heterozygous pups could not be expressed. In her second gestation, when more mature, the inbred dam was able to support a heterozygous litter, larger than the inbred litters of similarly inbred dams, and equal in size to the outbred litters of F_1 females. A simpler reason for this effect may be the fact that proportionately fewer females in the reciprocal crosses conceived at post-partum matings. Since fewer second gestations in these crosses were concurrent with lactation they may have been able to produce larger litters than would otherwise be expected. Reduced litter sizes as a result of concurrent gestation and lactation has been reported by some workers, but Bruce and East (1956) found reproductive performance was not necessarily impaired by post-partum mating and concurrent lactation.

Comparison of corrected litter weights indicated that pups in the smaller first litters of inbred dams were slightly heavier at birth than the outbred pups in the larger first litters of F_1 females. At second parturition the pups in litters of F_1 females were equal in weight to the inbred pups of inbred dams, and significantly heavier than outbred pups born to inbred dams. It can be concluded from these results that outbred F_1 females were able to produce significantly larger litters than inbred females and provide these pups with a superior uterine environment so that they were equal to or heavier at birth than pups born to inbred females. It is doubtful if outbreeding in the litters born to inbred dams gave them any significant advantage over inbred litters.

Such effects of inbreeding resulting in poorer litter sizes and birth weights are in agreement with the results of inbreeding reported for livestock and laboratory animals by other workers (see Chapter I). In mice a decline in litter size of 0.5 young per 10 per cent increase in inbreeding coefficient was shown by Bowman and Falconer (1960) and Roberts (1960). These declines were averaged over a number of lines, and it was found that those lines in which litter size was most affected soon became extinct. Lines which survived to high levels of inbreeding had litter sizes equal to those of random bred control stock. (Bowman and Falconer, 1960). Similar findings were made in inbred guinea pigs (Eaton, 1932). In the absence of a random bred control stock, in the present experiment, it is not known whether there has been any decline in litter size during inbreeding in lines 77 and 44. These two lines are, however, the survivors of a number of inbreeding NCN lines originally established; since their litter size has not declined to the point of extinction of the lines, these lines have been, in effect, selected for performance under inbreeding.

When lines of mice, inbred to coefficients of only 0.50 so that no lines became extinct, were crossed litter size was increased by .51 pups; the litters of the resultant F_1 animals were 2.8 pups larger than the litters of their inbred parent lines (Roberts, 1960). The rise in litter size, when inbreeding of litters was dispersed by crossing inbred lines, was less than the initial decline in litter size when inbreeding began; i.e. when outbred females bore inbred litters. Because of this, it was concluded that inbreeding of the dam imposed a restriction on litter size which could not be overcome by increasing the heterozygosity of the litter. Similarly, Falconer (1960) found that there was no improvement in litter size when the surviving three lines, from twenty inbreeding lines originally set up, were crossed. Matings of the F_1 progeny of these lines resulted in litters two pups larger than the litters of their 0.80 inbred parents. Eaton (1953) showed in one experiment that outbreeding of the litters, born to inbred mice, significantly increased litter size at birth, but in a second experiment inbreeding of the dam was the only factor which significantly affected litter size at birth.

Mortality:

Although equal numbers of foetuses were brought to parturition in 77 and 44 females, significantly more pups were stillborn to 44 females. Line 44 females had more stillborn pups both when their litters were inbred, and when litters were outbred. It seems conclusive that 44 females have poorer maternal abilities than 77 females, but it cannot be said whether these losses at birth were due to difficulties at parturition, as was suggested in rats (Feldman, 1926), or because of improper maternal care after birth. Since litters were often found scattered in the cage, rather than in a nest, and some litters of line 44 females died without having suckled, the latter may be a more likely reason. In females of both inbred lines, outbreeding reduced the proportion of pups which were dead when litters were found. Outbred dams had significantly fewer losses at birth than inbred females with inbred or outbred litters. Vernon et al., (1952) found that there were increased stillbirth in pigs when they were inbred.

Neonatal losses were not significantly different between dams of the inbred lines and heterozygosity of their litters did not significantly reduce the extent of these losses. Outbred dams lost significantly fewer pups, between birth and three days of age, than inbred dams.

Losses between three and twenty one days of post-natal life were similar in all mating systems. There were no significant differences between mating systems in proportion of pups seen alive at birth which were weaned at twentyone days, although outbred dams weaned 10 percent more pups. Overall survival from birth to weaning was similar in litters born to both inbred lines. More (5%) but not significantly more of the pups born to inbred dams survived when litters were outbred. Outbred dams weaned significantly (13.4%) more of the pups born than inbred females.

Eaton (1953) found in one experiment that heterozygosity of litters was most important factor affecting the survival of young mice, but in a later experiment heterozygosity of the mother had a more important effect. Greater mortality associated with inbreeding of dam and offspring has been reported in pigs

(Craft, 1953; Donald, 1955), cattle (Woodward and Graves, 1946), sheep (Glembockii, 1957; USDA, 1952), rats (Ritzema Bos, 1894; Feldman, 1926), guinea pigs (Eaton, 1932), mice (Chai, 1959). Some workers have found no effect of inbreeding on survival or vigour of their animals, but in some of these experiments selection for the most "fit" animals was practiced (see Chapter I).

Growth from Birth to Weaning and Lactational Performance:

The growth of litters of mice is characteristic of the lactational ability of their dam (MacDowell, Gates, MacDowell, 1930). Litter weight at twelve days was found by Falconer (1947) to be a satisfactory measure of the lactational ability of female mice since pups are entirely dependant on their dam for nutrition until this age. Bateman (1954) suggested that this measure was inaccurate because he found pre-natal factors contributed more to the variance of twelve day litter weight than post-natal factors. However, Cox, Legates and Cockerham (1959) showed that post natal influences were responsible for 75 percent of the variability of twelve day litter weight in mice.

In the present experiment large litters were reduced to six pups at birth to ensure that all pups were adequately fed. Because of differences in the numbers of pups suckled litter weights have been corrected by analysis of covariance so that litter weights could be compared at the same litter size.

It is evident from Figs. 3 to 5 that females in all lines were able to support pups to heavier weights at twelve days in their second lactation than in first lactation. This result differs from that of Falconer (1947) who found no difference in lactational performance between first and subsequent lactations.

When both lines of inbred females nursed inbred or outbred litters, line 44 females produced significantly lighter litters at 12 days than 77 females. No significant difference was found, over both lines of inbred females, between the weights of inbred or outbred litters at twelve days. However, outbred litters suckled by line 77 dams were slightly heavier at twelve days than inbred litters while outbred litters suckled by 44 females were considerably lighter at twelve days than inbred litters. Outbred females, despite the fact that they had larger litters, had significantly heavier corrected litter weights at twelve days than inbred dams. Falconer (1947) suggested that poorer lactational performance by inbred dams was in part due to poorer milking ability and in part due to poorer suckling by inbred pups. The slightly greater weights of outbred litters, compared with inbred litters, suckled by 77 mothers may be a result of more vigorous suckling by outbred pups; the poorer performance of outbred litters suckled by 44 mothers may result from greater competition in the slightly larger outbred litters for the limited amount of milk available. The significantly better growth of litters suckled by outbred females suggested that dispersal of inbreeding, as well as any heterosis effects, when inbred lines were crossed has improved lactational ability. Any improvement in the suckling ability, or the efficiency of food conversion in the progeny of F_1 females is confounded with this measure of lactational performance.

A depressing effect of inbreeding on lactational performance has been reported by other workers; (mice: Falconer, 1947; sheep: Dassat, 1958; cattle: Woodward and Graves, 1946; Tyler, et al., 1949; Laben and Hermann, 1950; Nelson and Lush, 1950; Krosigk and Lush, 1959).

The ranking of corrected litter weights of the various lines was the same at weaning as was found at twelve days. Line 77 females weaned heavier litters than line 44 females; this difference was significant only in second litters. Outbred litters had no significant weight advantage at weaning over inbred litters when both were suckled by inbred dams. It can be seen that outbred litters nursed by 77 female gained weight more rapidly from twelve days to weaning than did outbred litters nursed by 44 females. Outbred litters suckled by outbred dams were heavier than litters in any other group at weaning.

Eaton (1953) found in one experiment that heterozygosity of the dam was the most important factor influencing litter weight at fifteen days, but in another experiment heterozygosity of the litter proved more important. In the present experiment heterozygosity of the dam appeared to be the most important factor influencing litter weight at twelve and twentyone days.

The "break" in the growth curves between twelve and fifteen days (see Figs. 3 to 6) has been reported by MacDowell, Gates and MacDowell (1930) and other workers. It appears to be associated with the process of weaning litters from complete dependance on their mothers for nourishment.

Growth and Development of Females from Weaning to Maturity:

Females of both inbred lines reached puberty, as shown by vaginal opening, at the same age. Despite their lighter weights at weaning line 44 females were significantly heavier at puberty than 77 females. Thus from weaning to puberty line 44 females grew faster than line 77 females, even though they were found, when killed during first gestation, to be more heavily infested with tapeworms.

Although they differed in weight at weaning, F_1 females from both reciprocal crosses reached puberty at the same age (42 days); eight days earlier than did inbred females. At puberty F_1 animals weighed significantly more than the mid-weight between the light and heavy parent lines. This meant that they grew faster to puberty and reached it earlier than inbred animals of either parent line.

When F_1 females were reared by 44 dams they were slightly more variable in weight at puberty than when reared by 77 females. This was possibly a result of poorer nutrition in early life. The lighter line 77 females were more variable in weight at puberty than 44 or F_1 females. Females of both inbred lines were more variable in age at puberty than F_1 females. This result is in agreement with that of Yoon (1955) who found that females from each of three inbred lines were more variable in age at puberty than the F_1 progeny of these lines.

Differing ages at puberty have been reported in lines of mice (Mirskaia and Crew, 1930) and pigs (Warnick, et al., 1951). In the present experiment no positive correlation between growth rate and age at puberty was found. Warnick, et al (1951) suggested such a relationship in gilts. Later puberty in inbred lines of pigs has been noted (Donald, 1955; Foote, et al., 1956; Squiers, et al., 1949) and in cows (Hawke, et al., 1954; Menge, et al., 1960). In cows this delay in reaching puberty was associated with slower growth rate.

There is generally believed to be an association between growth rate and age at puberty in mammals, but it is not clear from the literature how closely associated these are. The onset of the phenomenon of puberty has been shown to be initiated by the removal of a hypothalamic inhibition of the pituitary, (Donovan and van der Werff ten Bosch, 1959). It is not known what stages of growth and development must occur in the young animal before this hypothalamic block is removed so that pituitary, ovaries and central nervous system can act in concert to begin the oestrous cycle. Kennedy and Mitra (1963) discuss possible connections between hypothalamic inhibition of the pituitary, and growth and metabolic rate in rats.

Because no differences existed in the age or weight at puberty of F_1 females from either reciprocal cross maternal influences are unlikely to be responsible for the differences between parent lines in weight at puberty. Since they are descended from common ancestors it is possible that both inbred lines have retained similar genetic material controlling rate of development and maturation. Thus both lines reached puberty at the same age, but their growth rates were markedly different. It seems unlikely that rate of growth and rate of maturation, in these two inbred lines of mice, are under similar genetic controls. However, had the parent inbred lines been homozygous for the same genetic material regulating age at puberty, the F_1 hybrids would be expected to have the same genotype for this character as the parent lines. The F_1 animals reached puberty earlier than the parent lines. It cannot be said whether this more rapid maturation was related to faster growth rate in F_1 animals, but clearly size per se is not the factor initiating puberty.

Although vaginal changes at puberty are characteristic of oestrus mating does not always occur at this time. Differences between lines of mice in willingness to mate at puberty, has been reported by Mirskaia and Crew (1930). In the present experiment the times between puberty and pairing with a mature male, and copulation, as shown by the appearance of a vaginal plug, were recorded as a measure of the willingness of females to mate. Reluctance of line 44 males to mate made it difficult to compare the readiness of females to mate in the various mating systems. It appeared that the lighter line 77 females was less willing to mate at puberty than F_1 or 44 females. Kennedy and Mitra (1963) found that retarded growth in rats led to separation in time of the usually synchronous events of vaginal opening, first oestrus, and ovulation. There could be some relationship between slower growth of line 77 females and their reluctance to mate after vaginal opening.

During first gestation line 77 and F_1 females continued to grow and gained significantly more weight than line 44 females. The poorer weight increases by inbred dams bearing outbred conceptuses, in their first gestation, may reflect greater demands on the inbred dam from her outbred progeny.

After first parturition line 77 females were lighter than line 44 females but this difference was not significant. F_1 females were significantly heavier than females of both inbred lines at this time.

Butler and Metrakos (1950) found that the growth curves of mice plateaued at 60 days of age. After second parturition, in this experiment, mice were older than sixty days and were all at the same reproductive state. Comparison of the lines at this time should reveal any differences between lines in their mature weights. Line 77 animals were significantly lighter than line 44 females. Line 44 animals were older and had not had as successful first lactation as line 77 females. These factors could contribute to the greater weight of line 44 females, but it seems likely that they were naturally a heavier mouse at maturity. Line 44 and F_1 animals did not differ significantly in mature weight. These results indicate that when a large (line 44) and a smaller (line 77) line of inbred mice were crossed the F_1 progeny were of similar mature weight to the larger parent line. These results differ from those of Butler (1952) and Chai (1956) who found that at 60 days the F_1 progeny of a large and small lines were slightly heavier than the mid-weight between the parent lines. Such a relationship was evident at puberty in the present experiment. Differences between lines in age, reproductive and lactational history may be responsible for the result found in the present experiment.

Gestation Length:

Inbreeding had no effect on gestation length in this experiment. A tendancy for inbreeding to increase gestation length has been suggested in the pig (Craft, 1953) and in cows by Rollins, et al., (1956), but Foote, et al., (1959) could show no effect of inbreeding on gestation length in the cow.

The longer second gestations, where lactation was concurrent, were to be expected, following the results of Mirskaia and Crew (1931) and Bruce and East, (1956). The slightly longer second gestations seen in F_1 mice may be because of larger numbers of pups suckled in first lactation as was found by Enzmann, Saphir, and Pincus (1932).

The Extent of Possible Causes of Infertility and Prenatal Losses:

Records of conception rates indicated that many 44 males were sterile. Line 77 and F_1 males achieved similar conception rates. Histological examination of sterile males indicated that sterility was a result of impaired spermatogenesis. This sterility was not complete as some males were seen to become sterile after a period of fertility and others regained their fertility after a period of sterility. This abnormality was associated with poorer libido in line 44 males. Differences in the libido of inbred lines of boars have been found to be correlated with differences in 17-keto-steroid excretion. (Green, et al., 1942; Green and Winters, 1945b). Hodgson (1935) reported that inbred boars were reluctant to mate, and Evans (1928) found that copulation failed to occur in 70 percent of matings in an inbred line of rats. These differences could be the result of a lack of androgen production which in turn could be caused by failure of the pituitary to produce sufficient gonadotrophins. A second cause could be differences between lines in their thresholds for response to androgens. Chai (1956) found differences between lines in the levels of androgen to which they would respond. No conclusions could be drawn regarding the functional ability of the interstitial tissue in the testes of sterile males.

The recovery of ova achieved in the preliminary investigation indicated that the technique could be used satisfactorily to evaluate fertility in the experimental mice. Although the recovery rate was less than 100% at higher ovulation rates, as indicated by the numbers of corpora lutea, ovulation rates in the experimental mice were located about the centre of the regression line (Fig. 7) where discrepancies between ovulations and ova recovered should be least. In these slaughter experiments corpora lutea were counted before ova or foetuses were counted. This procedure avoided the possibility that prior knowledge of the number of ova recovered, or the number of foetuses present could affect the corpora lutea count.

Outbred females ovulated significantly (0.5) more ova than did inbred females. There was a slight, but not significant, difference in ovulation rate between 77 and 44 females. This difference could be related to the larger body weights of 44 females or to their greater age as a result of the delay when mated to a 44 male. Since histological examination revealed luteinized, unovulated follicles in 44 females it is probable that the presence of these led to a spuriously high corpora lutea count. This would also account for the slightly lower recovery rate of ova in 44 females. Poorer ovulation rates in inbred gilts were reported by Squiers, *et al.*, (1954), although in an earlier experiment (Squiers, *et al.*, 1949) they had been unable to demonstrate this. In mice Falconer and Roberts (1960) could show no difference in the ovulation rates of inbred and outbred animals.

In inbred females significantly fewer ova were implanted than were shed, both when the prospective embryo was inbred and outbred. There was no significant difference between numbers of ova shed and implantation sites in F_1 females. In 77 x 77 matings the number of ova classified as viable was in agreement with the number of implantation sites found and failure of ova to implant appeared to be the greatest source of prenatal losses in this line. The occurrence of sterile 44 males together with the small numbers of mice in each group meant that the extent of pre- and post-implantation losses in 44 x 77 and 44 x 44 matings could not be assessed. In 77 x 44 matings fewer ova were classified as viable than there were implantation sites at twelve days, but the number of ova classified as viable was in agreement with the number of live embryos at twelve days. This may indicate that these less well developed ova were able to elicit a decidual response so that an implantation site was found, but they were not sufficiently developed to survive implantation.

The number of viable ova in F_1 matings agreed with the number of implantation sites seen. Repetition of this experiment with infertile 44 males excluded would be required for an accurate assessment to be made of pre-natal losses in these lines. It seems likely, however, that overall losses were greater in inbred females than in outbred females, that heterozygosity of the potential embryo may not improve the litter sizes of inbred females, and that most losses in inbred females occurred before implantation. The greater extent of pre- and post-implantation losses in inbred than in outbred animals is in agreement with the results of other workers (Krzanowska, 1955, 1960b; Leziak, 1959; Falconer and Roberts, 1960; pigs: Squiers, _et al._, 1954; Pomeroy, 1952; cows: Hawke, _et al._, 1955; Mares, _et al._, 1961b).

Histological examination of the ovaries of line 44 females indicated that the corpora lutea were not as well developed by three days after mating as in other females at this stage. The reason for this slower development could be later ovulation in this line, or slower luteal growth. It was also observed that many ova from line 44 females had not developed to the morula or blastocyst stage as had ova in other lines at three days after mating. The delayed development of these ova may have been simply a result of later ovulation, but may also have arisen because of lack of hormone from the under-developed corpora lutea.

In view of the abnormalities in the ovaries and testes of line 44 animals, it seems likely that these could be caused by an insufficiency of gonadotrophins from the pituitary. It has been shown by Lostroh, Johnson and Jordan (1963) and Lostroh (1963) that both FSH and ICSH were required for spermatogenesis. Both FSH and LH are required for ovulation and corpora luteal development. Interplay of pituitary and ovarian hormones is also necessary for the initiation and maintenance of lactation, and the poorer lactation of 44 females may also be associated with pituitary insufficiency.

It was found by Ladman and Runner (1959) that the number of implantation sites in pregnant mice could be correlated with the weight of the dams pituitary. Baird, Nalbandov and Norton (1952) found that lines of pigs selected for different growth rates differed in their levels of growth hormone per unit weight of pituitary. It is not clear whether their lines selected for high and low growth rate had the same origins, or whether they differed genetically before selection was begun. Edwards (1962) reported that mice selected for large and small body size differed in pituitary weight, but pituitary weight per unit body weight was identical in both lines. It is therefore not clear whether smaller animals are smaller as a result of having smaller pituitaries, or whether the pituitary has merely maintained its size relative to the rest of the body. Large and small lines in Edwards' (1962) experiment had equal gonadotrophin potency per unit weight of the pituitary. Thyroid activity per unit body weight was comparable in both lines and both responded to growth hormone, though the response of the smaller mice was greater. Doney (1959) suggested that poorer growth by inbred lambs was partly a result of pituitary insufficiency.

It seems that inbreeding may have its primary action by depressing pituitary function. In the present experiment it is possible that pituitary function has been depressed in both inbred lines resulting in poorer reproductive abilities than were seen in outbred mice. In line 77 animals pituitary production of growth hormone may have been more severely depressed so that growth was poorer. Line 44 females appeared to suffer from depressed, or improperly balanced gonadotrophin production resulting in some failures to ovulate, slow growth of corpora lutea, and poorer lactation. Crossing of these two lines resulted in improved growth, reproduction and lactation in the F_1 progeny.

It is worth noting sterility in mice selected for large body size by Fowler and Edwards (1960) was caused by lack of libido in males. This effect could be similar to male sterility in the larger line 44 mice in this experiment. However, they found sterility in their small line was characterised by delay, or absence of cyclic oestrus, failure to ovulate and failure of ova to implant by twelve days. These symptoms were shown in the present experiment in the larger line 44 females rather than in the smaller line 77 females.

Disease Incidence:

There were differences in the incidence of tapeworm infection in the two inbred lines and their F_1 progeny. Hunninen (1936) found that mice developed an absolute resistance to this tapeworm soon after first infection. It is possible that during inbreeding line 44 females have lost the ability to develop immunity to this tapeworm.

CHAPTER V

SUMMARY AND CONCLUSIONS

The two inbred lines of mice (NCN77 and NCN44) used in these experiments had inbreeding coefficients of .966 and .942 respectively. These two lines were derived from common ancestors, and, at the time of separation into two inbreeding lines, they had 39.6 percent of their genetic material in common. Data were collected over the first two gestations and lactations in a random breeding population from each inbred line, from reciprocal crosses of the inbred lines, and from random matings of the F_1 progeny from each reciprocal cross. Data from these mating systems were analysed to find whether inbreeding had caused the inbred lines to differ from one another in fertility; pre- and post-natal mortality; birth weight; lactational performance as measured by litter growth to twelve days of age; and growth to weaning, puberty and maturity. Further comparisons of each of the inbred parent lines with reciprocal crosses and F_1 matings enabled measurement of the extent to which dispersal of inbreeding, and heterosis in litters and dams affected the above characteristics.

Sterility, characterised by failure of spermatogenesis and poor libido was found in many line 44 males. This condition was not congenital; some males, initially fertile, became sterile, and some sterile males regained their fertility. Sterility was more common in older males. The functional ability of interstitial tissue in the testes of sterile males was not investigated. However, ovarian abnormalities seen in 44 females suggested that male sterility in this line may be a result of deficient, or imbalanced gonadotrophin production by the pituitary.

Line 77 females were slower to mate at puberty than 44 or F_1 females. When conception occurred litter sizes at birth were similar in females of both inbred lines if both bore inbred, or outbred litters. Outbred litters born to F_1 females were one pup larger than the inbred litters of inbred dams. Heterozygosity of the litters born to inbred dams did not lead to improvement of litter size at first parturition, but second litters in reciprocal crosses were larger than in inbred matings. This difference may have indicated an inability of heterozygosity in litters to express itself in improved first litter size because of limitations imposed by the immature inbred female.

The occurrance of fewer post-partum matings in reciprocal crosses may be the reason for larger second litter sizes in these matings.

Mice were slaughtered during first gestation, and counts of corpora lutea indicated that F_1 females ovulated 0.5 more ova, on average, than inbred females. Losses to implantation, and probably total losses during gestation were less in F_1 than in inbred females. Accurate analysis of losses during gestation were not possible because of sterility in 44 males, and insufficient numbers of animals. It seemed conclusive that F_1 females had larger litters than outbred females as a result of higher ovulation rates and fewer pre-implantation losses. At least in first gestations, following mating at puberty, heterozygosity of the potential litter did not improve the number of implantations or number of pups born to inbred females.

Line 44 females had a slightly greater ovulation rate than 77 females although this difference was not significant ($0.05 < P < 0.1$). If this difference was real it may have been related to the heavier weight, or greater age of 44 females mated to 44 males. Histological examination revealed the presence of luteinized unovulated follicles in some 44 females. The presence of these may have led to an over-estimation of ovulation rate. Corpora lutea in 44 females were less well developed by three days after mating than in 77 and F_1 females. This difference, whether a result of delayed ovulation, or slow luteinization, was probably responsible for the poorer development of the ova of line 44 females recovered three days after mating.

At birth pups born to line 44 females were lighter than pups born to line 77 females. It is concluded that this difference probably arose because of differences in the amounts of milk in the stomachs of pups suckled by these dams. Outbred litters born to inbred females were lighter at second parturition than inbred litters; probably a result of individual pup weights being lighter in large litters. A similar depression of pup weight in large litters was the probable cause of smaller birth weights in pups born in first litter to F_1 females.

Despite their larger litters at second parturition F_1 females gave birth to pups equal in weight to the inbred pups, and heavier than the outbred pups of inbred females. It seemed that when mature F_1 females were able to provide a more favourable uterine environment than inbred females so that they gave birth to larger litters of heavier pups.

Both inbred and outbred litters born to line 44 females suffered a higher incidence of stillbirths than when born to 77 females. The maternal abilities of 44 females seemed poorer than the abilities of 77 females. Heterozygosity of litters and of the dam both led to significant reductions in the extent of losses at birth. Heterozygosity of dams was the only factor which reduced, significantly, the extent of neonatal deaths. The number of pups surviving from birth to weaning was greater in 77 females than in 44 females, and heterozygosity of pups increased their rate of survival over that of inbred pups; neither of these effects was significant. Outbreeding of the dam improved, significantly, the survival rate of pups from birth to weaning.

Lactational performance, as measured by litter weights attwelve days, improved in all lines at second lactation. Line 44 females had a poorer lactational performance than line 77 females. This was observed with both inbred and outbred litters and it appeared that the lactating ability of line 44 females was more severely affected by inbreeding than that of 77 females. Hybridisation of inbred lines restored lactational ability and F_1 females had heavier litters at twelve days that any other line.

At weaning litter and pup weights of the various lines maintained the same ranking as at twelve days so that the litters of F_1 females were heavier than those of 77 females which were heavier than the litters of 44 females.

Females of both lines reached puberty at the same age, but, despite their lighter weight at weaning, line 44 females were heavier at puberty than line 77 females. F_1 animals from the reciprocal crosses reached puberty at the same age and weight in spite of differences in their weights at weaning. At puberty F_1 females were younger than inbreds and heavier than the mid-weight between the weights of the two parent lines at puberty. Inbred animals were more variable in their age at puberty than outbred (F_1) animals. In these experiments neither growth rate or weight per se appeared to control the onset of puberty.

Comparisons of the weights of mature females indicated that inbreeding had led to separation of low (line 77) and high (line 44) body weight lines. Crossing of these lines resulted in F_1 animals with mature weights equal to those of the heavier parent line. Differences between lines in age, and reproductive and lactational history would have contributed to these differences.

Line 44 females were found to suffer from a higher incidence of tapeworm parisitism than 77 or F_1 females.

It seems probable from these experiments that, at least in early reproductive life, limitations imposed by the inbred dam are responsible for restricting litter size, birth weight and growth and survival to weaning. Differences in preweaning nutrition did not affect age, or weight of females at puberty. The observed differences in growth and maternal abilities of inbred lines, and abnormalities found in the reproductive organs of line 44 animals have been discussed in relation to the hypothesis that the pituitary was a primary target of inbreeding depression.

A number of aspects requiring further study are apparent from the present results.

(i) An examination of the histological abnormalities of ovaries and testes in line 44 animals. A useful starting point in the male would be a study of the accessory reproductive organs, e.g. ventral prostate or seminal vesicle weight, to determine if the interstitial tissue of the testes is functional. The levels of 17-keto steroid excretion by male animals could be investigated to ascertain if differences between males are related to total androgen production. The responses of sterile males to exogenous androgen or gonadotrophins could assist in establishing the causes of male abnormality. Likewise the responses of females to exogenous gonadotrophins could give evidence as to whether pituitary deficiences are the causes of ovarian abnormalities. Determination of the times of ovulation in the two inbred lines, and an investigation of the effect of exogenous progesterone on ovum development and implantation may reveal why the ova of 44 females were less developed at three days after mating than the ova of other lines, and indicate the possible cause of preimplantation loss in inbred females.

(ii) Repetition of slaughter experiments with sterile males excluded, and with larger numbers of mice is necessary to accurately assess differences, if any, in gestational losses of inbred and outbred embryos in inbred dams. Transplantation of inbred ova to outbred females could also be used to find if inbreeding of the dam is the main factor limiting intra-uterine survival.

(iii) Further examination of the phases of the oestrous cycle, readiness to mate, and the occurrence of ovulation following puberty would resolve certain of the possible differences between lines and crosses reported here.

(iv) The genetic cause, if any, of the apparently greater susceptability of 44 females to parasitic tapeworms could be investigated. There may well be a significant relationship between susceptibility to parasites, and growth and reproductive performance.

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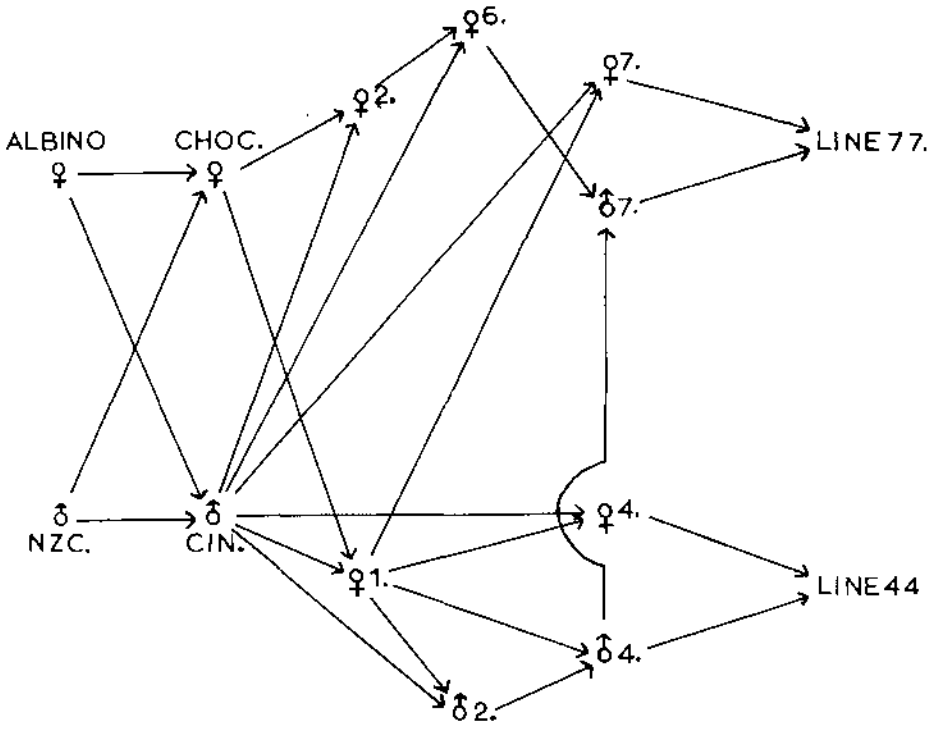
APPENDIX I



The above diagram shows the matings used to establish two inbreeding lines (NCN77; NCN44), which were homozygous for cinnamon coat colour, from the original cinnamon male (CIN o) and his full sib (CHOC ♀).

At the time of separation into two separate lines these two lines had, theoretically, 39.6 percent of their genetic material in common. To the time of the beginning of the present experiment, when each inbred line was expanded into a random breeding population, thirteen and ten generations of brother-sister mating had occurred in lines NCN77 and NCN44 respectively. This inbreeding together with the inbreeding which occurred in establishing the two lines resulted in Sewall Wright coefficients of inbreeding of .966 (line 77) and .942 (line 44).