

**Developmental Studies on Lines of Mice Selected
for Body Weight**

by

N. J. Mills

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PREFACE

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CHAPTER I

INTRODUCTION

The growth of an organism may be measured by recording its increase in size during convenient intervals of time. Usually the rate of increase changes during the growing period, proceeding fast during the early stages and tapering off towards maturity. Accompanying this increase in size in the young organism is an increasing degree of differentiation of tissues, changing proportions of body parts and the gradual emergence into function of various anatomical structures and physiological systems. As with growth, the speed at which development proceeds changes with time, being faster at young stages and gradually ceasing when the adult stage is reached.

To this extent, then, the processes of growth and development are coincident in time: they both commence at the moment the zygote is fertilised, their tempo of change is generally in harmony and they are both completed when the mature state is reached.

This thesis investigates first of all whether, and if so to what degree, the processes of growth and development follow an identical path in time between the initiation and the termination of the formative phase.

One extreme position is that the processes are independent of each other, so that a measurement of size at a particular time would yield no information about the state of development at that time. The other extreme position is that development is itself an expression

of growth, or vice versa. In this case measurement of size gives a direct indication of development, and developmental events occur at predictable sizes.

The relationship between these two aspects of the unfolding genome may be investigated by causing one variable to change and observing the consequent alteration in the other.

Size may be influenced in the laboratory either by interfering with the environment or by altering the genotype through selective breeding for large or small size. Environmental or genetic variation may also be used to alter the state of development independently of size.

Outside the laboratory, the extent of variation in these processes may be assessed by examining the differences between existing populations, between species, between sub-species and between breeds.

The review which follows examines the available evidence on the relationship between the size and development of organisms from field and laboratory investigations.

A second aspect of this study is concerned not with the growth and development of the entire organism, but with the degree to which its component parts are independent of the body as a whole in growth and development. This aspect, also, can be investigated by causing changes to occur in the total body size and then observing whether the components of the organism have all changed to the same degree.

The relevance of this study for human biology is in the light it may throw on the variation that exists in growth and development between different communities and on the problem of evaluating the growth and development of children. In the first case it is important

to be able to discern when the observed variation in growth and development may be deliberately influenced by environmental, e.g. dietary, means. In the second case it is important to be able to distinguish individuals whose growth is not proceeding normally for nutritional reasons from those who are underweight solely through their being small but normally mature.

BODY SIZE AND DEVELOPMENT TIME

All other things being equal, an animal which is capable of reproducing relatively quickly compared with the rest of a competing population has a greater likelihood of producing offspring. Since the speed with which sexual maturity is reached is an important component of fitness it seems likely that genetic variation in this character must be low due to the effect of natural selection eliminating genes prolonging development time. On the other hand, since the conditions which any species is exposed to over several generations fluctuate from periods of plenty to times of acute food shortage, the possession of a short development time may not always result in the most efficient conversion of available food into biomass. What kind of variation, then, exists in the growth and development of organisms to maturity?

There are three theoretical possibilities for varying the relationships of growth and development between organisms. Their final sizes may be varied by:

- (1) altering the growth rate of similar sized zygotes so that in the same time different sized adults are produced. This implies the possibility of varying the speed with which food is ingested and turned into tissue.
- (2) varying the size of the initial mass of tissue while keeping the growth rate and duration of growth constant.

(3) finally, both initial size and growth rate may be constant but the duration of growth may be lengthened or shortened, producing a larger or smaller adult size.

In the first case the correlation between development time and final size is zero, but final size is positively correlated with growth rate. In the second case the correlation between development time and final size is also zero, but final size is positively correlated with the initial mass. In the third case there is a positive correlation between development time and final size.

These are the three fundamental possibilities; what, empirically, is the nature of the variation in the growth and development of organisms to maturity?

1. Environmental Variation

A. Post-natal Environmental Variation in Development Time

(i) Experimental. In mice small variation in the timing of puberty may be brought about by the proximity of the opposite sex, the presence of males (Ropartz, 1969) or merely the odour of males, accelerating puberty by a day or two. Similarly, constant darkness or light have small effect on the timing of puberty in mice (Relkin, 1967) or in human females (Zacharius and Wurtman, 1969, Magee et al., 1970).

More extreme variation in development time may be produced by dietary control. Age at puberty in cattle may thus be altered by providing different diets to different groups (Sorenson et al., 1959, Hanson, 1956, Crichton et al., 1959). Although the chronological time taken to reach puberty is thus considerably influenced by diet there is little accompanying variation in the size of the animal when it reaches puberty (Penzhorn and Meintjes, 1968). Underfeeding heifers produced a 9.2% difference in weight at puberty and a 62.9% difference

in age at puberty compared with adequately fed heifers (Joubert, 1952). This susceptibility of age of puberty to diet is also present in rats (Widdowson, 1950), and fowls (Pym and Sledge, 1969), while a similar responsiveness of time taken to reach pupation exists in *Drosophila* (Robertson, 1959), with little variation in adult size.

Not only is the event of first oestrus influenced by diet, but, in sheep, also the capacity to mate and the fertility of females (McGuire et al., 1968). Particular metabolic effects have been detected at a hormonal level, reduced feeding in cattle causing a reduction in the ability to generate testosterone and secrete fructose in the seminal plasma (Baronos et al., 1969). Careful control of diet, then, has measurable effects in the chronological time taken to reach puberty, and the effects on the physiology of the animal can be detected at several levels from mating behaviour to hormone production.

(ii) Naturally Occurring. There is evidence that similar effects, found under field or uncontrolled conditions, may be attributed to random differences in nutrition during growth. The availability of food for offspring depends upon the number of young sharing the same source, the ability of the mother to provide a continuous flow of food and, in domestic animals, on the efficiency of the farmer in making food available. Variation in the quality or quantity of food available will influence growth rate of offspring and thus the time it takes them to reach the weight appropriate for sexual maturity. In rats, age at puberty is closely associated with growth rate (Asdell and Crowell, 1935, Bogart et al., 1940) and litter size plays an important part in determining the mean growth rate and thus age at puberty (McCance, 1960).

As animals reach puberty the variation in growth rate found earlier, and therefore in body size, declines and is considerably lower among adults. So long as the conditions causing growth rate variation

continue to operate, differences in time to reach a certain size are detectable. Variation in size in mice is high before weaning and falls off once the restrictions of the suckling environment are lifted (Montiero and Falconer, 1966). Among buffaloes differences between farms give rise to the same increasing, environmentally caused, variation, which falls off in the adult state (Desai, 1969).

Variation in early post-natal nutrition, then, whether experimentally induced or occurring randomly, causes variation in time taken to reach the same end-point in size and physiological state. The physiological time scale is prolonged in accommodating to a lower level of food supply, without necessarily altering final size. That is the correlation between development time and final size is zero.

B. Pre-natal Environmental Variation in Development Time.

To what extent is the degree of variation evident in post-natal growth a consequence of the development state at birth? There is a range of variation in weight and state of development among newborns which might influence their growth rate thereafter. When a reciprocal cross is made between a large and a small breed of cattle there is little difference in the sizes of the adult hybrids born from either parents (Dickinson, 1960). The trajectory of growth taken differs, however, depending on the mother. Small mothers produced small offspring at birth and vice versa so that significant differences in birth weights exist. These differences become insignificant at two years of age. The pre-natal environment, then, gives rise to a similar fluctuation in size variation as can be detected during post-natal growth. Evidence of a similar reduction over time in post-natal body-weight variance comes from comparisons of co-twin cattle (Taylor, 1962) or of cattle twins with singletons (Donald, 1958).

Where dietary control over the pregnant female's food intake is exercised, the variation in body size of offspring may be altered. During the pre-natal period environmental variation in maternal diet may so depress the progress of the fetuses that a permanent deficit in weight is brought about. Thus, the offspring of cows reared on a low, or high, nutritional diet during pregnancy differed in birth weight by twelve pounds (Hight, 1966). At the time of weaning this difference had increased to thirty-six pounds. Even if the dietary manipulation is confined to only the early part of pregnancy of ewes, the effects in body weight are measurable in later life (Shinkel and Short, 1968). The degree to which the effects on growth are permanent depends upon the degree of nutritional deprivation, the length of time it is maintained and the number of fetuses in the same uterus. Shinkel and Short point out that nutritional deprivation produces more severe effects during the period when the mass of foetal tissue is increasing fast. The same generalisations apply to pre-weaning growth in rats, very large litters causing a degree of growth depression which resulted in permanently reduced adult size. Nutritional deprivation after weaning, however, from nine till twelve weeks, while it temporarily depressed body weight did not result in measurable differences among adults (Widdowson, 1950).

Environmentally induced variation in diet thus has effects on subsequent growth which are more serious the earlier the variation is brought to bear. A difficulty in assessing the developmental consequences in the foetus or offspring arises from the fact that not only may weight-gain in the offspring or litter be depressed, and as a consequence development be delayed, but the uterus itself may be involved in delayed parturition, thus altering the age of the offspring when they are born. Gestation time is inversely related to

litter size (Biggers et al., 1963) and the total mass of foetal and placental tissue is the major determinant of gestation length (McLaren, 1963). Which of these, gestation length or development of offspring, responds most readily to dietary control of the mother is not known.

C. Effect of Removal of Environmental Stress.

It is clear, then, that once a restriction is removed, whether it be the uterine environment, the suckling environment, or experimental dietary control, those individuals suffering from depressed growth are prevented from reaching a normal weight only by the extreme severity of the nutritional restriction they have experienced or by the restriction having been experienced early in development. They usually take longer to reach normal weight for sexual maturity and immediately the restriction is lifted put on weight faster than those of the same age which are closer in weight to the appropriate weight for puberty. This negative correlation between pre-weaning and post-weaning gains has been demonstrated among mice (Young and Legates, 1965) and attributed to differences between mothers in producing milk. After weaning individual mice have unlimited access to food in the cage and the weight deficit, with its associated developmental delay, among poorly-fed mice is diminished by increased food consumption and/or food utilisation. A similar acceleration in rate of growth upon the lifting of an environmentally-imposed restriction occurs when foetuses emerge from the confines of the uterus. Dickinson's offspring from reciprocal crosses between large and small cattle showed this "rebound" phenomenon, the hybrid offspring from small mothers exceeding in growth rate the hybrid from the large mother. An experiment transplanting ova between genetically large and small mice also showed that where the growth of an individual from a large line was depressed by transplantation into a smaller mother, a similar acceleration in growth

rate occurred as soon as the foetus became free of the small mother's environment (Brumby, 1960).

The events of birth and weaning are, therefore, similar in that at this time one mode of nutrition is replaced by another and individuals relatively deprived under the earlier circumstances compensate in the new situation by growing faster. There exists, thus, a capacity for animals to accommodate to environmentally induced stress by slowing down their rate of physiological development in harmony with their rate of weight gain.

D. Conclusions.

1. There is evidence from a wide variety of organisms that environmental variation during the growing period causes the physiological-time scale for body weight development to be prolonged. This may be observed as increase in chronological time taken to reach the mature state, there being little variation in the weight at this stage when it is reached. Under these conditions the correlation between development time and size is zero.
2. Extreme nutritional deprivation causes not only delay in development but also prevents appropriate weight for development stage being reached. Final size is diminished especially if the stress is imposed early in development without the opportunity for later compensating growth to occur. Here there is, therefore, a negative correlation between development time and final size.
3. Following removal of environmental stress an increase in growth rate occurs tending to diminish the deficit caused by environmentally-induced slower growth. Whether the delay in physiological time is removed at the same rate that the deficit in weight is made up or whether a residual time deficit remains after weight reaches normal levels is not clear.

2. Genetic Variation

A. Between species.

The variation in development time described thus far relates to environmental influences. What evidence is there for variation in development time which is independent of environmental effects? Genetic differences of large degree, such as those existing between species are associated not only with differences in size but also in time taken to mature. Among animal species of greatly different adult size the chronological time to develop is proportional to the mature weight raised to the 0.27th power (Taylor, 1965). The growth curve by means of which mature size is reached is also very similar between species, merely being larger in the time and weight scale for larger species (Laird et al., 1965). The variation in the shape of the growth curve, that is to say the position along the curve relative to the starting and end points of changes in growth rate, is similar among species including birds and mammals (Laird, 1966). The similarities include the pre-natal period, species differing only in the chronological time scale and weight scale. Not only the changes in growth rate, but also the sequence of stages of development is invariant between species (Adolph, 1970) and the relative timing of cessation of growth of different bones (Ford and Horn, 1959).

Different species, then, differ widely in the time they take to mature, but there is abundant evidence that this difference is related to difference in size of the adult. There appears to be little difference in the rate at which growth or development occurs which is independent of the size of the organism. The differences mainly reside in duration of growth rather than in the growth rate with which the adult form is created.

B. Within Species.

Within species and between varieties there exists a similar correlated relationship between adult size and development time, as was described between species. Comparing, for example, Shetland ponies and draught horses, the larger variety takes longer to mature.

Variation in development time which is independent of mature body size implies that the growth curve is susceptible to variation in shape. Within a random breeding population, however, not much genetic variation exists in the shape of individuals' growth curve (Taylor, 1965). Little variation was detected in the shape of the mean growth curves of populations of mice (Timon and Eiscu, 1969) after many generations of selection.

(i) Effect of directional selection for altered development time.

When selection pressure is put on development time, however, moderate response is detected. Selection for long and short time till the day when the vagina opened in rats produced a heritability estimate of seventeen per cent (Warren and Bogart, 1952). This accords well with the heritability estimate among unselected mice of sixteen per cent (Montiéro and Falconer, 1966). In *Drosophila* it corresponds closely with a heritability estimate for time to pupation of twenty to twenty-five per cent (Sand and Clayton, 1957) and eighteen per cent (Tigerstedt, 1970). An asymmetry exists for development time causing the response to be more readily obtainable for slower than for faster development. Putting selection pressure on swine yields a response for slower but less so for faster development (Knider et al., 1946) and reduction in development time in *Drosophila* (Sand and Clayton, 1957) and other species (Hollingsworth and Smith, 1955; Hunter, 1959) is difficult. That this asymmetry is in some degree related to asymmetry in phenotypic variance for

development time is illustrated by similar selection experiments on *Tribolium castanetum* (Englert and Bell, 1969). Here the skewed distribution in development times till pupation allowed a higher selection differential in one direction than the other.

(ii) Effect of inbreeding on development time.

While directional selection yields information regarding the small amount of additive genetic variation to be found in development time, use of inbred strains provides an insight into other aspects of the genetic variation. Inbred lines of mice differ in the age at which they reach puberty (Yoon, 1955), confirming that genetic variation for this character exists. Inbreeding itself also affects the responsiveness of the development of mice to environmental influences. The greater the degree of inbreeding, the greater the magnitude of non-heritable variation in age at puberty (Yoon, 1955). Similarly, increased susceptibility of pure-bred cattle to a low-nutrient feeding regime caused increased development time (Wiltbank et al., 1969). That this increased susceptibility to environmental influence is the same general phenomenon as that described above following weaning in rats or mice is illustrated by the body weights of inbred cattle measured during growth. Inbreeding depressed all weights from birth till forty-eight months with an associated delay in puberty. The weight of adults was not below the outbred level, however (Young et al., 1969). Genetic variation thus influences the mean value of the character "body weight" and reduction of genetic variation increases the variation in development time independently of mean size.

(iii) Correlated effect on size of selection for development time.

Since variation in development time and size between species shows a positive correlation, it is instructive to know whether

directional selection for development time also produces a positive correlation in size. In rats, the production of a seventeen per cent change in development time was accompanied by only a three per cent change in body size (Warren and Bogart, 1952). In *Tribolium Castaneum* selection pressure on development time produced significant changes in the primary character but very little alteration of adult size (Englert and Bell, 1970). Similarly, two-way selection for development time in *Drosophila* does not significantly affect moulting weight or egg size (Bakker, 1969). Additive genetic variation is independent of body size.

(iv) Correlated effect on development time of selection on body size.

If selection for development time has little effect on final body size, does the converse situation obtain, i.e. is development time unaffected by selection for body size? The evidence on this point is not consistent. A comparison of two different lines of mice selected for large size with an unrelated random-bred strain showed that the age at vaginal opening of the unselected line was intermediate between the two large lines (Gall and Kyle, 1968). An inbred line was older at puberty than the other three, as expected. Comparisons of development time have also been made by fitting growth curves to either individual data or the mean sizes for a series of ages for a population. Comparison is then made between either the computed point of inflection in the fitted curve or the asymptote of the respective curves. Using two different fitted curves, logistic and Richards', the point of inflection occurred about a day later than control mice in a population selected for fast post-weaning gain to six weeks (Timon and Eisen, 1969). Comparison of the asymptotic growth of mice selected for large or small mice revealed no consistent alteration in the rate at which different mature body sizes were attained (Clarke, 1969), although there is evidence that some larger strains take chronologically

longer to reach asymptote (also Roberts, 1961).

The inconsistency of response of different replicates derived from the same common stock by selection for high or low six week weight was illustrated by comparing the correlation between time to mature and asymptotic mature size in six sets of such replicates (Clarke, 1969). Some of the replicates showed a positive correlation between the variables, as predicted (Taylor, 1968), while others had reached larger size by means of a shorter development time. The average regression for the entire group, however, was close to Taylor's value. Those replicates showing shorter time to asymptote differed in the shape of the mean curve between thirty and seventy days, their curves before and after this period being similar. These differences in the time taken to reach asymptote were found among large lines, five out of the six of the small lines showing a predictable decline in development time with smaller size. Noteworthy in these experiments was the fact that in no case did weight selection prolong development time beyond the range of development times found among the controls, although five out of six large replicates exceeded the range of mature size of the controls. There was little difficulty producing a correlated reduction in development time below the range of the unselected lines, but only two of the small replicates were able to reduce body size below the unselected range. There appears to be some evidence, then, that in these replicates difficulty was encountered in selecting body weight below the unselected range and in increasing correlated asymptotic development time beyond the unselected range. Increasing body weight or reducing correlated time taken to mature were not so difficult. The fitting of asymptotic curves to inbred and hybrid data on mice reveals the predictable delay in reaching maximum growth among inbred mice (Laird and Howard, 1967).

Thus, in the growth curve from birth till maturity, i.e. over a hundred days of age, not much genetic variation in shape, i.e. in growth rate, is detected by selection; where variation is found it occurs mainly between thirty and seventy days and in the large lines.

Since selection response depends in the first instance upon the phenotypic variation found in the character being selected, it is important to know how the variation in time and weight changes at different periods during growth. In unselected populations of mice, weight variation increases until weaning and then begins to diminish till maturity (Montiero and Falconer, 1966). Evidence for a similar oscillation in variability is presented for other animals above.

An indication of the variation in timing may be obtained by examining the heritability for this character under selection. An asymmetry for heritability of development time exists in *Triboleum Castaneum*. Selection for shorter development time decreases variance and progress is soon exhausted. Heritability is high, however. Selection for longer development time, on the other hand, increases phenotypic variance. This occurs through increasing the environmental component of variation causing the heritability to decline. Whether a similar difference in heritability of development time also obtains for mammals is not known. However, the differences in response for selection for early and late puberty are similar to those found in other animals. Reduction of development time occurs (or increase in growth rate) but soon reaches a limit, while selection for late development makes more progress before ceasing.

C. Conclusions.

The association between body size and development time depends upon the method used to measure the association. Natural selection, in the creation of different species, has brought about a positive

correlation between time to mature and mature size. Existing subspecies reflect this association.

Genetic selection on a population to alter the time variable reveals a moderate amount of genetic variation in this character which is independent of body size. Over many generations, however, a positive correlation between the response in development time and body size is brought about. An asymmetry of heritability exists, however, response occurring more readily for shorter than longer development time, the latter being increasingly confounded by environmental sources of variation so that progress is halted. For a moderate amount of selection, as for a moderate degree of nutritional deprivation, the association between development time and size is zero.

When selection pressure is put on body weight considerable genetic variation is revealed. The pooled results from upward and downwards selection reveal a positive association between development time and size. This association is also reflected in lines selected downwards, whereas those selected upwards show either a positive or zero association.

The nature of the genetic variation found in these characters through inbreeding experiments reveals a close association between degree of heterozygosity and susceptibility to the environment. The less genetic variation there is in a population the more its development will be delayed by environmental influences. This alteration of development time may occur whether or not there is any change in final size.

In selecting for altered development time a limit was more readily reached for earlier than later development, with little change in size in either case. In selecting for large and small size only limited progress in size reduction was achieved, while prolongation

of development time was also not very great. Reduction of development time occurred in both lines and increase in body weight was considerable.

3. Body Size, Development Time and Body Conformation.

When different animals are caused to differ in their age at puberty as a consequence of variation in diet it was pointed out that on the day when sexual maturity becomes evident the animals are generally of a uniform weight. Does this mean that an early and a later-developing animal differ only in the chronological time taken to reach the same stage? Or can a clue to their different histories be discovered in the construction of the adult body itself? Where the differences in time taken to reach a particular stage are induced by genetic means, the question to be asked is whether fast or slow maturing, or large and small selected, animals are identical in their physical make-up, or whether certain parts differ as a consequence of their differing environmental or genetic histories.

In studying variation among body proportions in populations it is important that comparisons be made between animals at comparable stages of development. Since body proportions change during ontogeny and, as described above, the chronological time taken for the same physiological state to be reached may vary as a consequence of purely environmental influence, comparison at a similar age may be misleading. Moreover, since a severe degree of environmental stress may cause permanent stunting in the adult, especially if it is applied early in development, it is important to ensure that the groups to be compared do not differ in an extreme degree in the environmental component of variation in size.

Differences in body proportions may be interpreted, as is the case for differences in gross size, as a consequence of variation in the growth rate, duration of growth and final size of the different

parts of an organism. The origin of variation in shape among subspecies, or species, may be examined by comparing these components of growth in different populations. The possibilities here are, either that the growth of the constituent parts of an organism are all uniformly subservient to the growth of the entire body, or that the growth and development of different parts may be differentially altered either by genetic or environmental influences. Hammond (1940, 1961) was an advocate of the second position, claiming that domestic animals had been altered through man's extending and steepening the different growth gradients within the body, i.e. by altering the body proportions. It was also his view that environmental influences exerted their effects differentially on different body parts depending on the "priorities of growth" of different components, some being more and some less resistant to environmental stress.

Alternatively, the immutability of body form has been described thus: "genetic interference or rigorous nutritional stress has little effect on the allometric relations of different parts or body components" (Everitt, 1965, also Wallace, 1948), and "The form of an animal depends almost solely on its absolute size, and restricted nutrition causes more or less uniform retardation of development except in so far as fatty tissue is concerned" (Stephenson, 1964). It is thought that the impact of stress is absorbed by directing growth and development along a slower time path without differentially affecting different parts. What, then, are the theoretical limitations on the possibility of transforming body shape?

1. Limitations on Dimensional Change

A. Ontological Sequence.

It has already been pointed out that the sequence of ontological

stages is highly consistent among widely different species. Presumably this consistency extends to the order of maturation of body parts. Within the overall invariability of sequence it is theoretically possible to introduce extensive final proportional differences in size by varying the timing of one stage relative to that preceding and that following it. But, since ontological stages, or maturation of parts, are not separate physical processes but expressions of the unfolding of an entire genome, any modification of a particular part's development must occur in conjunction with appropriate modification of those parts in close physical or functional relation to it. Since, during ontogeny, the possibility of change at any stage in development is considerably determined by the amount of development that has already occurred, it seems likely that alteration of body form at the terminal end of growth and development would be rather limited. Continuation of the duration of growth of all parts uniformly would increase gross size, but not form. Increasing the duration of growth of a particular part would in a short while be obstructed by the disharmony of function caused by the limited capacity of other parts to alter radically in conformity with it. The possibility of finding variation either in the growth rate of individual parts or their duration of growth appears unlikely. In both cases the growth characteristics of adjacent tissues mediate against the production of substantial alteration in growth rate of a particular part. Variation in the initial quantities of tissue made available for particular structures seems the most likely means of producing large-scale; alteration of form.

For example, while a large range of organisms share the same arrangement of genetic material which gives rise to the pentadactyl limb, the modification of its form to suit a particular adaptive

Regarding the physical dimensions of the parts of an organism, the limits imposed by the nature of the organic material itself provides a restriction on increase in size due to the influence of gravity. Increasing size without altering shape requires a differential increase in width of the supporting elements of a terrestrial organism. Thus

the growth rate should also show little variation. given the stability of metabolic activity in different organisms, that stroke volume (Forbes 1955, Stahl, 1963). It does not seem surprising, another, e.g. blood volume, vital capacity, cardiac output, cardiac Other functional indices are similarly invariable from one species to organs, metabolism, oxygen intake, haemoglobin mass (Brody, 1945). for basic physiological parameters are close to unity, e.g. major species as diverse as mouse and elephant, the allometric coefficients the physio-chemical limits of organic material and processes. Across tions on transformation of shape. The extreme limit is provided by adult individuals provides information regarding two further limita- The investigation of proportional differences between groups of

B. Physiological Constants.

posed here operates. ally in order to determine at what points the genetic variation ex- similar proportional changes must, therefore, be studied ontogenetic- The genetic alteration of tail length/body weight ratio or other ferentially.

an unvarying ontological sequence than variation of growth rates dif- keeping growth rate constant, would be more readily incorporated into seems likely, also, that alteration of the initial quantities, while have totally suppressed the development of a number of digits. It instance, the family Caviidae have incorporated a set of genes which requirement may be traced to very early stages in development. For

among fossil Pelycosaurs of different sizes, bone widths sort with body weight in a factor analysis rather than with their respective lengths (Gould, 1967). The influence of gravity upon bone shape can also be detected during the growth of the individuals within a population from small size to maturity. In fowl, the growth in shank length relative to body weight conforms closely to simple allometry, while there is no correlation between shank length and shank width at any body weight (Cock, 1963). So, across species generally a positive allometric relation may be found between linear measures and body weight and, once weight is controlled, a persistent association between body weight and the width of bones; this reflects mechanical gravitational constraints.

Some measurements show considerable species variability, tail length and abdominal circumference especially showing little allometric uniformity across species (Stahl and Gummerson, 1967). These measures possibly reflect the local, fluctuating environmental conditions, tail length showing some relationship with temperature and abdominal circumference clearly undergoing considerable diurnal and seasonal change.

In comparing body proportions among different populations, therefore, the relevant comparison must not only be made at the same stage of growth but, also, the dimensions to be measured must neither be of such a general nature that they reflect the universality of physiological constants among homeotherms or the size-determining effect of gravity, nor, on the other hand, so particular that they relate only to a single individual at the moment of its death, or to a temporary response to fluctuating environmental conditions.

C. Gradients.

A third area of regulation comprises the close inter-relationship

of metamerically or segmentally derived parts. Here gradients of growth have been described which integrate the relative speeds of growth and final size of parts into a cephalo-candally or proximo-distally organised sequence. A "growth-gradient" in the pentadactyl limb or axial skeleton may be readily distinguished in many species. Interspecific differences in final form have been brought about without rearranging the general direction or stability of the gradient.

The second area of local influence is possibly subsumed as a special case of that described above. This is the growth gradient associated with the development of the head. Whether the body form is altered by nutritional means or genetic selection, structures close to the brain appear to be more resistant to transformation than other parts (Cock, 1959).

2. Environmental Variation.

A. Naturally Occurring.

Evidence was provided above concerning the stability of pubertal weight in many animals in which it was found that response to variation in diet was in the time parameter of growth. This ability of slower growing individuals to achieve a normal conformation at a stable weight is disturbed only by extreme nutritional deprivation.

Offspring from reciprocal crosses between mothers of widely different size differed greatly in birth weight (Dickinson, 1960). As adults the differences in size were insignificant. Here the pre-natal environment exerted an effect on body size which disappeared by maturity. The effect on the component parts of the body was by no means uniform, however, local variation among body parts being present in the degree of susceptibility to early environmental influences. Early maturing characters were least affected by pre-natal environment, while a larger influence was found in late maturing characters which retained a

measurable effect for many months after the restriction. A similar effect may be detected in monozygous twin cattle whose different experience of the same pre-natal environment causes not only a small difference in birth weight but also differences in the timing of development of parts. There is an initial divergence in early-maturing parts which later converge in size, while later-maturing body parts continue to diverge for a number of years (Taylor, 1962). Generalising this situation, it can be shown experimentally that if two groups of animals take different times to reach the same weight, the faster growing are likely to have shorter long bones at that weight than those which grew more slowly. This was found to be the case among white American girls; those reaching puberty later did so at the same weight as early maturers, but they were taller (Frisch and Revelle, 1970). Similar data is available from domestic animals (Pursér, 1963).

The origin of the variation in growth rate is environmental in the work mentioned above. The order of stress is not so great, however, that it prevented the appropriate weight for puberty from being reached, yet, under these conditions, proportional differences in body shape were induced.

B. Experimental.

It is hardly surprising, therefore, that severe sub-nutrition accentuates proportional change. When growing pigs are given a calorifically inadequate diet their weight may be reduced by 96.7% at the end of a year (McCance et al., 1968). The proportional reduction in weight of teeth at this age was 71%. Following the restoration of an adequate diet, as might be expected, animals grow far more quickly and approach normal values in weight, size, etc. The final deficit in body weight was reduced to 20% and in teeth weight to 30%. Since

the laying down of new enamel had ceased before a normal diet was provided, the opportunity for the teeth to partake in a compensatory growth acceleration was not available. Therefore tooth weight suffered a permanent deficit. As was pointed out in Wilson and Osbourn's (1960) review of the subject, the probability of permanent alteration of body form depends on the degree, intensity and duration of stress and, most importantly, upon the time when the stress is experienced in relation to the growth curve of the parts concerned. Crucial to the accomplishment of a normal size following environmental stress is that normal dietary conditions should be available before the growth curve for the particular structure reaches its final plateau.

C. Conclusions.

Environmental influences operating during growth cause the entire growth curve of the individual to be prolonged. The effect is more pronounced the earlier the environmental stress has been brought to bear. Different parts of the body grow at different rates, but all converge upon the adult form in an orderly ontological sequence varying little among species. Differences in body form may be produced through the influence of the environment. Generally, the effect is to prolong development so that late maturing measurements in particular are longer when they reach final size.

3. Genetic Variation.

A. Experimental.

(i) Direct Selection. It is a familiar finding that most dimensions of the individuals comprising a population are distributed normally. In general they are closely associated with body size, but selection experiments have shown that they can be genetically altered independently of body size. Thus, although wing size in *Drosophila* is highly correlated with body size and highly stable within a population, response

to selection for increasing or decreasing wing/thorax ratio may be obtained (Robertson, 1962). Similarly, the proportion of tail length to body size in mice may be altered by selection in either direction (Falconer, 1949, Cockrem, 1959). These results indicate the presence of modifying genes with purely local effects which can influence development so as to differentially alter final size. Whether the much larger proportional differences which can be found between different species are also the accumulated consequence of the action of the same modifying genes with local effects is not known and is difficult to explore experimentally.

(ii) Correlated Response. The extent to which body components respond in concert to selection upon one of them has been studied extensively in those experiments directed at measuring correlated responses. Much of the work on laboratory animals has sought to relate selection response for weight-gain to the amount of fat in the carcass (Roberts, 1966, Clark, 1969). The findings here are by no means clearcut, the age at selection and method of fat collection having a considerable influence on results aside from considerable strain variability.

B. Naturally Occurring.

Body shape may show variation within the limitations outlined above under the continuous transforming influence of natural selection. Demonstration of the short-term effects of such slow alteration may be made by comparing the allometric plots of local races of existing species; over a longer period the phyletic changes occurring in a known sequence of fossil forms may be exposed in the same way. In these comparisons the frequent finding (White and Gould, 1965) is that the main difference between groups is not so much in duration of growth or the slope of the allometric plot, but in the position of the plot. That is to say, transformation of shape was achieved by altering the

relative proportions at the beginning of allometric growth. Similar findings have been reported in Cock's extensive review of the subject (Cock, 1966).

c. Conclusions.

The existence of independent additive genetic variation for a number of linear measurements has been demonstrated from selection experiments. The presence or absence of such genetic variation depends upon the resultant from a number of influences operating upon body form. Thus, limits to the amount of change possible in a structure are imposed by the physio-chemical nature of the tissue itself and the metabolic system which serves it, by the pervasive influence of gravity, by the presence of adjoining tissues with less ability to alter their shape or size and, overall, by the integrity of the epigenetic regulatory system. The concurrence of these several influences upon the growth and development of different body parts maintains the size and shape of individual members of a population at a stable value over many generations.

While direct selection for a particular linear variable reveals additive genetic variation, if selection is put on body weight as a whole it is likely that such genetic variance in particular characters will not be detected except through differences in correlated response between different variables. Theoretical and field studies lead one to expect that where such differences are detected they are brought about through the activity of genes which operate early in development.

When body weight as a whole is altered through environmental means, provided the stress is not extreme or prolonged, differences in body form may be brought about. In this case the transformation is produced by the lowering of growth rate which causes the limit in prolongation of development time to be reached at different times in

different tissues, later maturing tissues being less susceptible to stunting.

RELEVANCE TO HUMAN BIOLOGY

Studies of the growth and development of human populations have been pressed forward over the last several decades in the Western world. The information collected is diverse but concentrates upon those variables which emerge as having some social significance. Thus military and insurance agencies have collected material on height and weight gain, while medical institutions have paid close attention to the events of birth and puberty. Many of the conclusions drawn from these studies parallel those drawn from animal studies, but the necessity to observe individuals as much as populations has provided more data than has been satisfactorily analysed. Neonatology has developed as a special study due to the fact that the new-born child encounters problems which are unique to this stage in life and which can be understood only by studying this period. It is here that a complete understanding of the relationship between growth and development is most important from the practical point of view. To an animal breeder it matters little what stage of development is reached at a certain weight; for the paediatrician it may be crucial, since decisions about future patient-management turn upon a correct evaluation of developmental status.

Many findings in human growth studies constitute corroboration of conclusions arrived at elsewhere in experiments on laboratory or farm animals.

Thus the event of birth, and therefore birth weight, is determined primarily by the environmental circumstances of the foetus rather than the genotype or developmental state of the foetus itself. On the other hand, puberty reflects in larger degree the genotype of the

individual and here both genetic and environmental influences are detectable (Vandenberg and Falkner, 1965). When the environmental variation is diminished by making comparisons within families, the genetic differences between sibs are the primary determinants of difference in age at puberty (Plomley, 1970).

The stability of body weight at puberty among white American girls (Frisch and Revelle, 1970) in contrast to the variation in their age at this stage probably demonstrates that the human species responds to environmental influence by altering the rate of development while keeping the weight at maturity constant just as was shown to be the case for many other species. Independent demonstration of this comes from data relating degree of maturity to growth rate: individuals with a higher growth rate are more mature at any age (Garn et al., 1965).

In laboratory populations the effects of genetic or environmental influences may be measured during the lifetimes of individuals and also from one generation to the next. Conditions may be controlled so that the sources of variation in the observed parameters derive from particular manipulations. In contrast to this situation it is difficult to control the environment of the human species during the entire growth period. Consequently the number of individuals who have been studied for the duration of their childhood and adolescence is small. Moreover, the conditions of ordinary civilised life make it impossible to follow a lineage of persons over a number of generations. Samples of individuals taken from the same community taken at intervals over a long period of time indicate how stable the "base population" was; against this must be measured variation found in different contemporary groups.

A consistent finding among Western European and North American

populations is a gradual change in the mean values of measured samples. This secular change is most extensively reported in stature and shows that contemporary adolescents are taller at all ages than their predecessors. It is clear that the "base" population has not remained stable in size over the past several decades.

Explanations for this change invoke genetic or environmental causes. Over the period of time that the measurements have been recorded the general level of health of the population has been greatly improved. The nutritional status of all age groups has been enhanced and the mean number of children in a family has diminished. For these reasons it is likely that stunting of growth due to sub- or mal-nutrition has gradually been lessened with a corresponding gradual increase in stature. Thus the secular change reflects changing environmental influences.

More recently an alternative explanation has been put forward. This postulates that until several decades ago the communities comprising Western Europe consisted mainly of local neighbourhoods or villages in which marriage occurred far more frequently between individuals within rather than between localities. Consequently it is thought that a certain degree of homozygosity will have been built up in these inbreeding populations. One of the important changes to affect these communities during the last hundred years was great individual mobility which came with the advent of public transport facilities. The result of this was an increase in marriages between communities and an increase in the relative heterozygosity among the offspring. This heterosis is put forward as a partial explanation of the increase in stature.

The evidence from animal studies indicates that an increasing degree of homozygosity is associated with an increase in development

time rather than diminution in final size exclusively. Increasing heterozygosity conversely would produce individuals larger and more mature at immature ages.

The effect of improving the environmental circumstances of populations during their period of growth would be expected, from the evidence reviewed above, progressively to eliminate those factors which previously had caused the growth curve to be diverted to a lower, more prolonged trajectory. Thus the effect would also be to create a situation where successive generations were larger and more mature at immature ages. Where nutritional circumstances had caused stunting of adult size, the gradual alleviation of this stress would be associated with an increase in final stature.

Although the genetic explanation may account for a portion of the difference in immature size when comparing a relatively inbred generation with its relatively more hybrid offspring, it cannot alone provide an explanation for the continuation of the trend over several generations. It seems likely that the overwhelming influence has been environmental.

Examination of the records of the status of British men over the last hundred years lends credibility to the explanation that dimensions of adults have changed little through time but they have reached their adult height at progressively younger ages (Morant, 1950).

Information on the age of puberty over the last century or so comes from many different sources. Invariably the findings indicate that puberty has been reached at earlier and earlier ages in Europe and North America; about three years earlier today than in 1870 (Tanner, 1968). Whether there was an accompanying increase in weight at puberty is not recorded. Likewise, during those decades when development occurred slowly, adult stature was not reached until the

mid-twenties. Since recordings of height and weight were normally recorded at military ages, between eighteen and twenty-one, the presence or absence of a change in adult stature cannot readily be detected from this date, though from a review of world literature Tanner (1968) shows an increase of 3' over the last 100 years.

General support for the environmental factors being most important comes from a consideration of the social categories involved in the change. It is the underprivileged classes which have more conspicuously benefitted from Public Health measures, etc., over the last few decades and it is in these groups that the changes are most readily observed.

Evidence that the progressive increase in growth rate has reached a limit comes from both sides of the Atlantic, where differences in growth between classes are disappearing (Malina, 1969).

If the growth characteristics of other world populations are evaluated in terms of this growth model it is clear that environmental factors cannot account for all of the differences found. Mean menarcheal age of different populations are marked below:

POPULATION	MEAN MENARCHEAL AGE (TANNER, 1963)
<u>Earliest-</u>	
Cuban Negroes	12.3 years
Cuban Whites	12.4
Hongkong Chinese	:
Italians & East Europeans	:
Upper & Middle-class W. Europeans	12.9
Well-off Africans)	:
Poor Africans)	15
	(18.8 "
New Guinea Highlanders.....	(
<u>Latest</u>	(20.0 "

It is likely that the differences between these groups reflect the presence of genetic differences in this character. It is not known, however, whether correlated differences exist in weight at menarche between these populations or in final size.

The variables reviewed thus far as an indication of human growth and development were age at puberty and size. Dental development, too, reflects the processes of growth and to some extent conforms with evidence on body size and development time which emerged from other studies. Thus not only is age at puberty correlated with growth rate but also age at eruption of teeth (Garn, 1965). Conversely, heavier children have more teeth erupted than lighter ones at any immature age.

Some data which does not hang well with these reports comes from New Guinea. Variation in age at puberty there is said to reflect the quality of nutrition (Malcolm, 1970), those receiving less protein suffering a greater delay in development time than those better fed. Animal studies and data from East U.S.A. support this association. Malcolm was able to demonstrate a correlation between growth rate and final height and also between age at menarche and final height. Experimental studies indicate that the condition where a positive correlation between growth rate and final size is discovered is also associated with prolonged development time, consistent with Malcolm's findings. In all cases this association is brought about by environmental causes rather than genetic. Generally these are nutritional in nature, operate for a long period of time, especially affect the early stages of growth and are not followed by a period of adequate nutrition before the growth period terminates. Malcolm's data, then, seems to indicate the presence of chronic malnutrition among those of small stature.

Since dental development also indicates the condition of growth of

an individual it might be expected that, like puberty, tooth eruption would be delayed in nutritionally disadvantaged groups. This also appears to be the case in New Guinea populations (Malcolm, 1970), populations growing slowly to a small adult height showing delayed dental eruption.

Difficult to reconcile, however, is the data comparing puberty among different racial groups with that comparing dental development. Since New Guinea populations are, compared with other recorded populations, nutritionally worse off it seems unsurprising that their mean pubetal age should be delayed. Yet, while dental development is universally shown to reflect growth rate, the mean ages of tooth eruption for New Guinea populations are "earlier than European figures despite the more rapid growth and earlier maturation of the European child." (Malcolm, 1970).

Evidence from theoretical and laboratory studies reviewed above indicates that there is very little variation present in the growth curve of mammals, even under direct selection for body size. Yet, Malcolm's data appears to indicate that, compared with European populations, New Guineans possess genes bringing about both faster development at early ages and slower development later. In any population the effect of the environment appears to act uniformly throughout development and its consistent effect is to cause slower development with lower levels of nutrition.

A possible explanation for Malcolm's findings might be that the populations sampled at earlier and later ages were not the same in that the older groups lacked individuals carrying genes which favoured early development, thus shifting the mean value for development at later ages. Malcolm's suggested explanation for the association between growth rate and final size is a similar one, being genetic

in nature.

Since the importance of discovering whether the explanation for this discrepancy is genetic or environmental is an important Public Health issue, it is worth considering the evidence put forward.

Malcolm suggests that the relationship between growth rate and final size might be brought about by natural selection operating in those nutritionally deprived populations to favour slow growth and shorter adults. The selective mechanism might operate during early childhood when individuals carrying genes for rapid growth may be especially at risk when food is short. Other evidence shows that the toddler-stage is especially susceptible to environmental influences (Desai et al., 1970). The long-term consequences of severe infantile malnutrition indicates that individuals treated in hospital for several weeks as infants for this condition were taller, heavier, broader in chest and thicker in bone and leg muscle than their sibs who had not suffered from severe malnutrition (Garrow and Pike, 1967). It was suggested that those who were most seriously affected by malnutrition were those carrying genes for larger size.

From experimental work on animals it is clear that this is not a necessary conclusion from the evidence. An alternative explanation might be that the hospital conditions enabled a compensatory growth mechanism to operate such that a "rebound" effect was created, the surge in growth carrying the hospital inmates' physical dimensions beyond those of their sibs who were not so seriously afflicted.

Whether or not the explanation for these findings lies in gene-environment interaction or in the fact that the two samples had been subjected to different environments, cannot be answered from a study of data from human populations. Populations of animals subjected to suitable genetic and environmental operations might, at least, provide

evidence on what are the limits to which genetic and environmental factors may exert an influence on the timing of development or final size.

GENERAL CONCLUSIONS

1. Body Size and Development Time.

- (a) Both genetic and environmental variation are associated with zero correlation between body size and development time under moderate conditions of selection or stress.
- (b) Continued selection for body weight produces a positive correlation between final size and asymptotic development time which is demonstrable for increasing and decreasing size. Increase in size, however, is also likely to produce zero correlation with development time.
- (c) Extreme environmental stress brings about a negative correlation between size and development time.

2. Body Size, Development Time and Body Conformation.

- (a) Genetic variation may be detected in the body proportions of adults altered by direct selection.
- (b) Theoretical and field studies indicate that when selection is on overall size little independent genetic variation between different parts will be revealed.
- (c) When differences are produced it is likely that they are generated by genetic variation acting early in development, rather than on rate or duration of growth during the later period of development.
- (d) Environmental stress causes prolongation of development time in all tissues but affects the early and late maturing parts differently. Late maturing parts may be larger in the adult.

3. Human Biology.

Although the secular change in human size and development time is susceptible to a genetic explanation, the negative correlation

between increasing size and decreasing development time indicates the presence of an important environmental component. Evidence from immature and mature stages of human development suggest that the processes of growth and development may be different at early and late stages of development.

INTRODUCTION TO EXPERIMENTAL WORK

Sexual maturity is reached at a stable weight in an unselected population. This stage of development becomes evident when the genes responsible for producing the enzymes which stimulate sex-hormones production become active. An equivalent state in *Drosophila* is the commencement of pupation. It appears from work reviewed above that both of these terminal developmental events appear at constant body mass. Moderate interference with diet or genotype advances or retards the event but does not much affect the weight at which it occurs.

Four questions are generated by these results:

1. The stability of weight described above relates to a terminal developmental event, i.e. puberty; is the appearance of earlier developmental events controlled by a similar mechanism or, as suggested by Malcolm's work, are different processes controlling early development?
2. At a certain level of genetic or environmental interference the weight stability breaks down, producing either positive or negative correlation between development time and size. What is the nature of the limit to the weight-regulation of development and what are the consequences of exceeding it?
3. What are the limits to alteration of development time?
4. When growth rate is selected for many generations considerable changes in adult mean size are produced. How does the alteration in gross size affect the skeleton? Are all parts affected equally by

the transformation or are there differences in response between different parts as theoretical and field studies predict? If changes are produced, at what point in the growth do they become measurable?

Two separate experiments were set up to examine these questions

Experiment 1.

A population of random-bred mice was followed through from birth to sexual maturity. Birth weight and the weight on the day of the appearance of a number of developmental events or states were recorded. This population was used to examine the regulatory processes controlling time and size during development.

Populations of mice derived from the same source as the above population had been selected for high and low growth rate. The effect of the consequent alteration in growth rate upon rate of development and associated body weight was examined.

Experiment 2.

Samples of mice were collected at a series of ages from birth to one hundred and twenty days to provide data for comparisons of body weight and skeletal proportions. The samples were representative of unselected and selected lines of mice.

CHAPTER II

MATERIALS AND METHODS

The mice used in these experiments were the offspring from the 18th generation of the Q strain (Falconer, 1968) and their progeny. This strain consisted of six replicates designated A to F. Each of the six replicates had been separated into three lines - a large line selected for high six-week body weight by intra-litter selection, a small line similarly selected for low six-week body weight, and a control line maintained without selection. Mating was designed to minimise in-breeding. There were, thus, eighteen separate lines.

The first experiment was a longitudinal study whereby randomly selected mice were followed through from birth to maturity and the age and weight recorded when certain developmental stages were reached. The second experiment was a cross-sectional study where a cohort of mice was sacrificed at a series of different ages from birth to one hundred and twenty days in order to represent the growth of the different lines throughout life.

In the first experiment matings were set up among the offspring of the 18th generation. The offspring of these matings yielded the individuals for the longitudinal study. It was aimed to select two males and two females from each of three matings within each replicate. Owing to failed matings and varying numbers of small litters this balanced situation could not be achieved and the basic data is shown below:

<u>Line</u>	<u>Large</u>	<u>Control</u>	<u>Small</u>
Replicates	6	6	6
Families	14	21	20
Individuals (Male)	26	41	32
(Female)	26	39	32

Following weaning, the female mice were further observed to record the attainment of sexual maturity. The male mice were sacrificed at six weeks of age and their carcasses prepared for examination. The numbers involved in these aspects of the experiment were fewer than in the first part owing to the death of various individuals. The remaining samples were as follows:

<u>Line</u>	<u>Large</u>	<u>Control</u>	<u>Small</u>
Replicates	6	6	6
Families	14	21	20
Individuals (Male)	25	40	28
(Female)	24	39	29

In the longitudinal study the method followed was observation of selected individuals over a period of time. When a litter was born two male and two female mice were randomly selected from the litter and identified by means of toe clipping. These mice were observed daily from the sixth day and their body weight recorded on the day that the upper right central incisor tooth was seen to have broken through the gum. They were examined daily thereafter until the globe of the right eye was seen to reflect light between the eyelids. Body weights were again recorded. Further daily observation was carried

out until the cusps of the lower right molar tooth were observed to have broken through the gum, when once more body weights were recorded. Body weights were again recorded on the day of weaning, twenty-one days. Mice were then removed from their mothers and placed in cages containing up to six mice of single sex per cage.

From the twenty-second day the chosen females were examined by gentle probing of the vaginal area with a blunt probe. On the day that the vagina was observed to have opened the body weight was recorded. On the same day the female was placed in a separate cage with an unselected male whose fertility had been proven in a previous mating. Observation was continued until the vagina was plugged. Body weight was again recorded. Ages of the female mice were recorded when the litters were born.

The male mice were treated in a similar manner to the females during suckling and weighed on the day of weaning. They were removed from their mothers and placed in cages containing no more than six mice. They were sacrificed at six weeks of age and weighed. The carcasses were skinned and boiled with papein until the bones could be easily picked out. Selected parts of the skeleton were washed, dehydrated and de-fatted and measurements recorded. These were as follows:

1. Occipital length.
2. Humerus length.
3. Tibia length.
4. Mid-humerus diameter.
5. Mid-femur diameter.
6. Scapula length.
7. Body weight.

Animals were killed with ether, weighed and skinned. Their carcasses were digested with papein and selected skeletal parts were removed for measurement. At birth and at five days a fore and hind limb were removed before papein digestion and stained, using the Alizarin method (Isaacson)¹⁹⁶³, for signs of calcification in the carpels, tarsels and epiphyses.

Statistical Methods

In the longitudinal study, differences between mean values were tested by t tests and by an analysis of variance. In both longitudinal and cross-sectional studies differences between replicates, in the first case, and families in the second case, were ignored and values pooled within lines of selection.

In both experiments seasonal variation in maturation rate was accounted for by running all lines concurrently so that the seasonal effects would have influenced all lines simultaneously. It was thought unlikely that seasonality would differentially effect the lines.

In the longitudinal experiment the problem of litter size was considered since growth rate is considerably influenced by the number in the litter and this, in turn, has an effect on maturation rate. The general effect is that large litters take longer to mature than small litters. Since the large mice produced larger litters than small mice the effect would be to prolong development time of large mice compared with smalls. Since other evidence shows that large mice were faster maturing in controlled size litters than small mice it was decided to leave litter sizes unaltered so that the combined effect of litter size and body size could be estimated. The effect of litter size would be to diminish the effect of selection on maturation rate and so differences discovered in this experiment would

moderate the actual effect of selection on maturation rate.

Since the largest litters produced slowest maturing mice, in the second experiment litter size was reduced in all cases to six without supplementation of smaller litters. The comparison between lines in this experiment, therefore, represents differences in growth without the additional influence of litter size.

In the longitudinal experiment, differences between means were tested for statistical significance by t tests and analysis of variance. Both of these tests rely on the variance being the same among the samples tested and this was not the case in this material. This did not seriously influence the conclusions, however. In the cross-sectional comparison there were ten samples of offspring in each of six lines and measurements were made as mentioned above.

The total number of comparisons was, therefore, twenty-one at each of ten ages, totalling 210 comparisons for each of two replicates. Of these, at the 5% level of statistical significance, twenty comparisons which may not have been statistically different would so have been judged. In order, therefore, to simplify the comparison the percentage difference from the control value was calculated for each age and, where possible, the result plotted on a graph. For the main character, that is the character selected, body weight, statistical tests were unnecessary since it was evident that eighteen generations of selection had produced great differences in body weight. What was of interest was at what ages these differences became established and how bone sizes followed the change in the main character.

Comparisons were therefore made between percentage difference between selected lines and controls at different ages and the percentage difference which existed between the two control values at that age. The percentage difference between the two control lines was ascribable

to differences between the two populations which had founded the two replicates and, in addition, random effects over eighteen generations. Since all lines were housed in the same room and treated as a single population from the management point of view it was judged that the differences between controls were random genetic and environmental effects.

The selected lines differed from their own controls by a similar quantity ascribable to random effects, with the addition of a large component due to the effect of selection for high and low body weight. Here, then, it was expected that the difference between the inter control difference and the control-selected comparison would indicate mainly the effect of genetic selection.

In certain comparisons interpolation was necessary to estimate values from the mean growth curves of the lines at ages or weights which were not directly measured. In this case the graphs were drawn twice and the interpolation made twice in order to obtain an error value for the comparisons. The direct measurements made on the skeletal material were re-measured on a ten per cent sample to obtain an error value for the measurement operation.

Throughout, statistically significant differences of $p = 0.05$ is indicated by one asterisk and $p = 0.01$ by two asterisks.

CHAPTER III

FIRST EXPERIMENT

1. Preliminary

The first part of the experiment attempted to discover whether development at early ages was associated with body weight thresholds as had been shown by Montiero and Falconer for puberty. Following this, the effect of body weight selection at forty-two days on the relationship between body weight and development was examined.

It was necessary, first of all, to establish the fact that the circumstances determining puberty in Montiero and Falconer's unselected mice were also at work in this experimental population.

Montiero and Falconer showed that the phenotypic variance of body weight, V , could be broken down into the following components:

- V_A - additive genetic variance,
- V_D - dominance variance,
- V_{EW} - environmental variance within litters,
- V_{EC} - environmental variance between litters
of different mothers.

They showed that the component V_{EC} accounted for the largest proportion of phenotypic variation during the first six weeks of life and that this represented the variation between mice of different litters attributable to maternal effects.

In the present analysis regressions of age at puberty, on weight at puberty and on weaning weight were calculated using family mean age

TABLE 1 Mean Body Weight and Age at Puberty

	Mean $\pm$ s.e.	Variance	V_p (Montiero and Falconer)
Age at puberty (days)	35.2 $\pm$ 0.70	19.17	15.60
Weight at puberty (grams)	18.3 $\pm$ 0.37	5.27	3.43

TABLE 2 Regression of Body Weight on Age at Puberty (g/day)

	b $\pm$ s.e.	P	b $\pm$ s.e. (Montiero & Falconer)	P
Family Means	0.077 $\pm$ 0.092	n.s.	0.007 $\pm$ 0.032	n.s.
Within-Family Deviations	0.48 $\pm$ 0.077	**	0.33 $\pm$ 0.022	**

TABLE 3 Regression of Age and Body Weight at Puberty on Weaning Weight (g/day)

	b $\pm$ s.e.	P	b $\pm$ s.e. (Montiero & Falconer)	P
Age at Puberty on Weaning Weight	- 1.23 $\pm$ 0.59	*	- 1.28 $\pm$ 0.05	*
Weight at Puberty on Weaning Weight	0.34 $\pm$ 0.25	n.s.	0.16 $\pm$ 0.04	n.s.

at puberty, weight at puberty and at weaning. Here the regression coefficients indicated mainly the effects of maternal environment. Deviations of sibs from their own family means were also calculated and the regressions assumed to represent the remaining components of variance in which within-litter environmental and additive genetic components were most important. The regressions calculated were found to be comparable to those computed by Montiero and Falconer from the various components of their analysis of variance.

The variances for body weight and age at puberty are very similar to Montiero and Falconer's results as shown on Table 1, and the absence of a significant regression of age at puberty on body weight is confirmed from an inspection of the regression of weight on age at puberty, Table 2. When the regression is calculated using deviations of sibs from the family mean the same positive association between time taken to reach puberty and body weight at puberty is revealed as was shown by Montiero and Falconer.

In both experiments the variation in age at puberty is largely determined by the variation in weight at weaning, which is itself an indication of maternal environment. Weight at weaning has a much smaller effect on weight than on age at puberty, as shown in Table 3.

In these unselected populations, then, early environmental variation attributable mainly to differences between families produces an effect on development time taken to reach puberty, but less so on the weight at which puberty occurs. The body weight, or threshold, at which puberty occurs is in the region of 18.3g. and the age range in which it becomes evident lies between thirty and forty-eight days.

Having established that the weight determination of the stage of puberty operated in this population, earlier stages of development were examined to ascertain if similar weight thresholds could be discovered

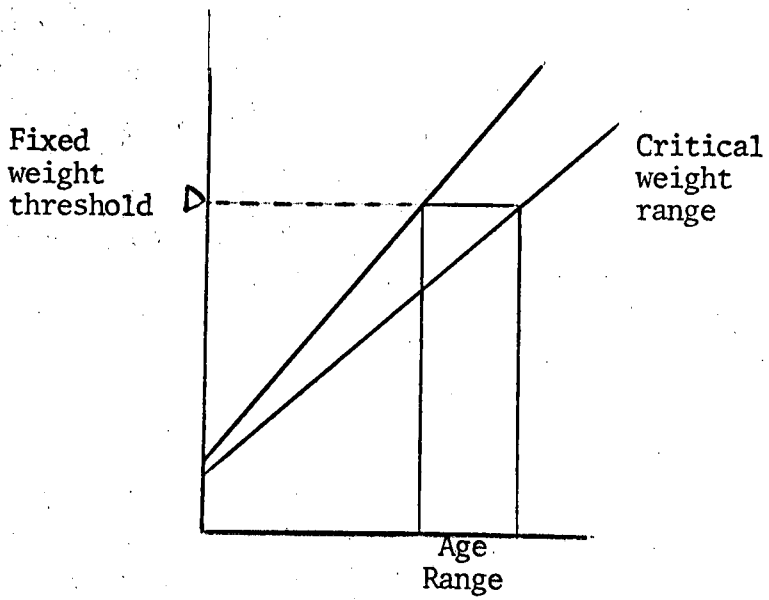


Fig. 1A

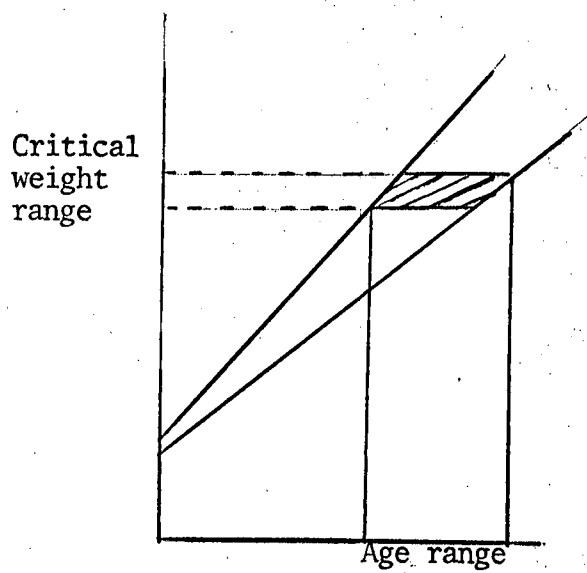


Fig. 1B

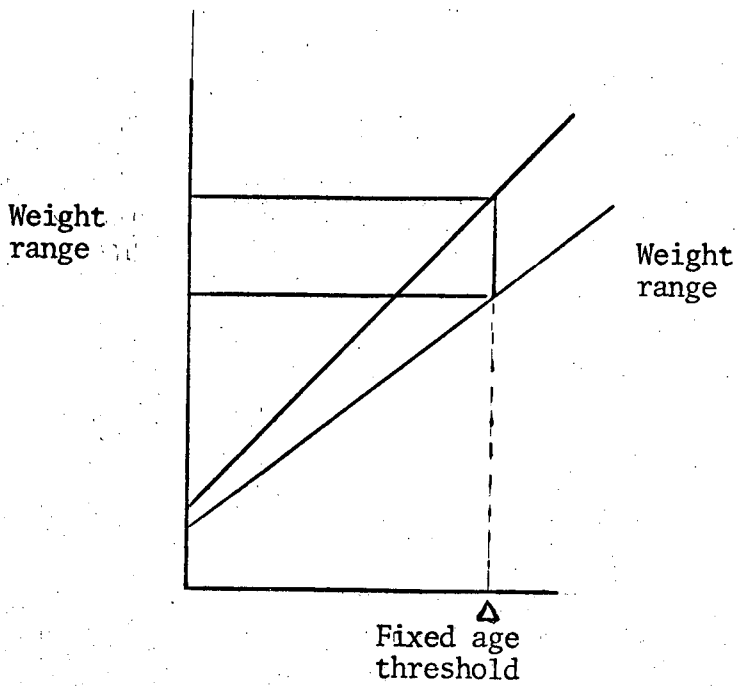


Fig. 2A

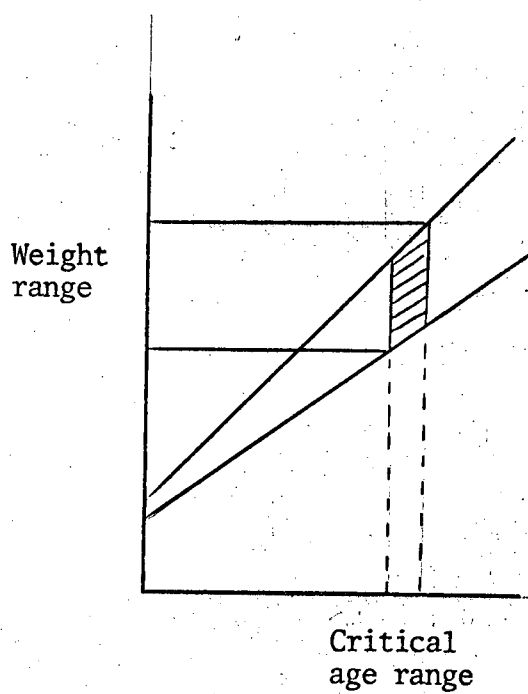


Fig. 2B

for these stages of development. Two events of a similar physiological nature, incisor eruption and molar eruption, and one different event, eye opening, were chosen for this study and observations were made daily, as was the case for vagina opening. The effect of selection for high and low body weight at forty-two days on these events and puberty was also examined.

2. Growth and Development during Suckling and at Puberty in Unselected Mice

There are two extreme possibilities for the control of development: developmental events may occur at a particular weight irrespective of age, or at a particular age irrespective of weight. The consequences are illustrated in Figs. 1A and 2A. Weight is plotted against age, and the two sloping lines represent the growth of the fastest and slowest growing individuals (growth is made linear for simplicity). The experimental observations consist of the weight and age of all individuals at the occurrence of the developmental event. Fig. 1A depicts what we would expect to find if weight were the sole determinant, i.e. the first of the two possibilities. Among the individual observations there is no variation in weight, but there is variation in age: the occurrence of the event appears to be dependent on a 'weight threshold.' Fig. 2A depicts the second extreme possibility, age being the sole determinant. Here there is variation in weight, but more in age; there appears to be an 'age threshold.'

Biological phenomena, however, seldom show complete fixity of regulation and we would therefore expect to find individuals showing a range of values in their thresholds, with consequences as illustrated in Fig. 1B and Fig. 2B. The problem then arises: how do we decide whether the more important determinant is age, as in 1B, or weight, as in 2B? The following procedure seems to provide a solution. Fig. 3

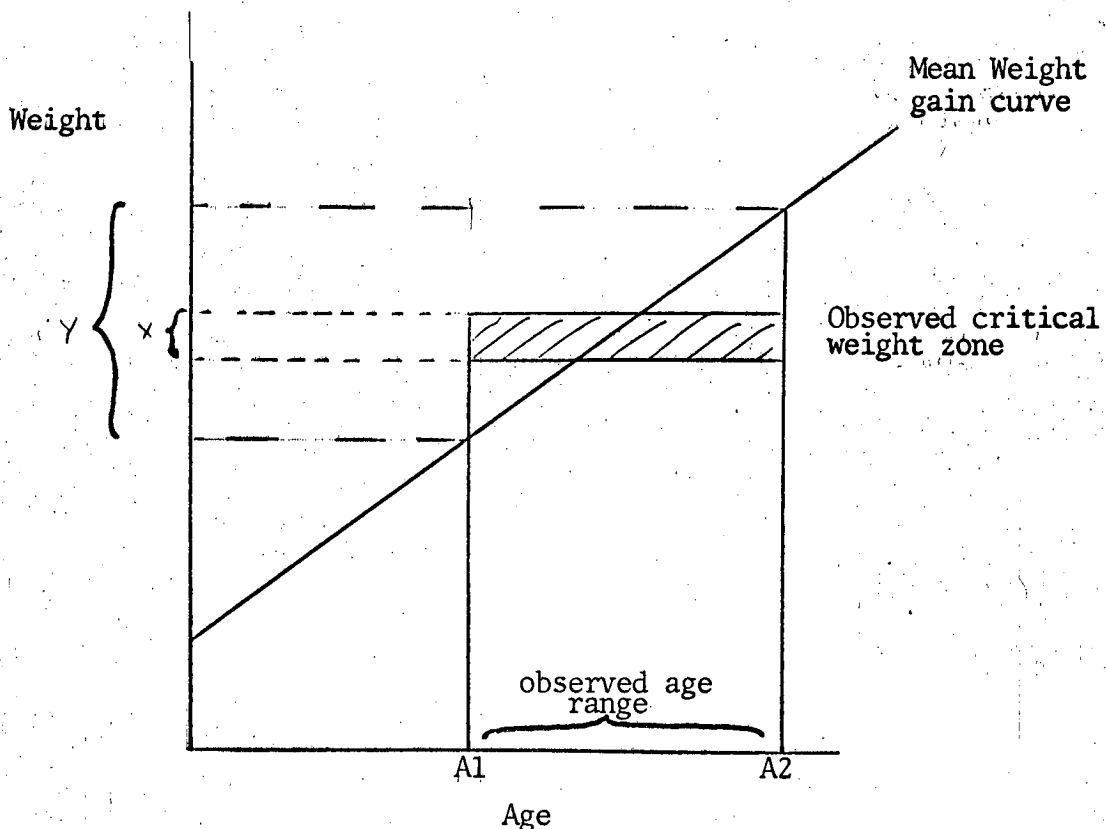


Fig. 3. Comparison of Ranges in Weight Assuming Development is, or is not, Weight Dependent.

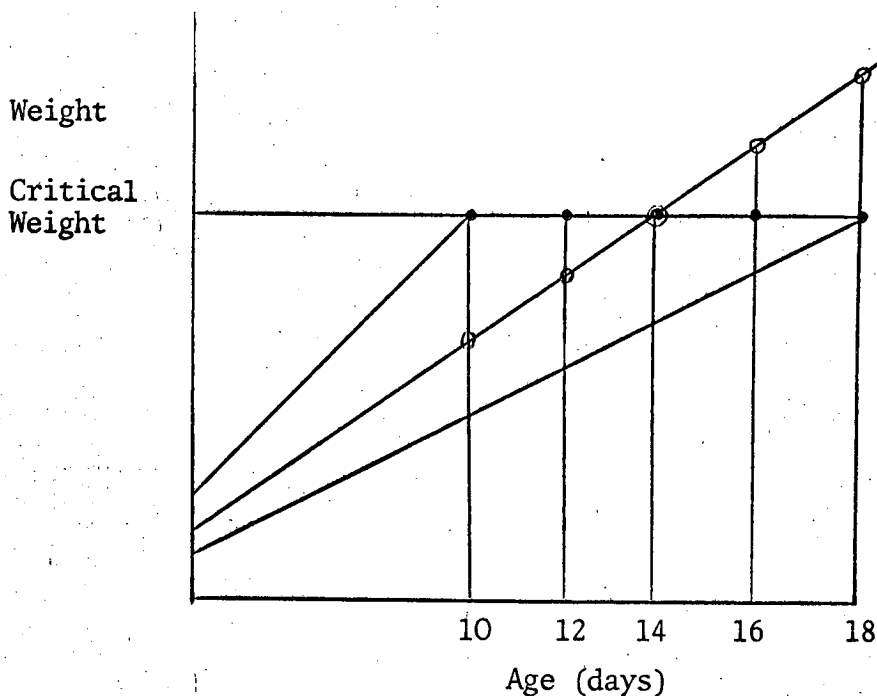


Fig. 4. Comparison of Weights of Same Age Groups on Successive Days Under the Two Conditions.

shows the mean growth curve of the population with weight and age ranges at the occurrence of the event. Let the weight range be X , and let the lower and upper limits of the age range be A_1 and A_2 . Now consider all the individuals in the population whether the event has occurred or not, and find the mean weight at A_1 and at A_2 . Let the range between the mean weights be Y . If the range X is less than the range Y we can conclude that weight has some influence in determining the occurrence of the event.

In Fig. 4 the expectation in deviation of observed weight for age on different days is plotted over a mean weight-gain curve. Here, the assumption is that development is entirely weight-determined. In this situation on day ten, for instance, from the general population there will be some heavier individuals which have reached the critical weight for the event. Thus, initially, the mean weights of individuals reaching the stage of development will be higher than the mean for the population. At the other end of the range of ages, slower growing individuals will be approaching the critical weight and their mean weight will fall below the mean for the population.

Where weight is an important factor in determining development, then, the range in age at which an event occurs may be extended with no great change in the range of weights. Also the distribution of mean weights of individuals passing through the phase falls with time, first lying above the mean weight gain line and later below it.

The objective, therefore was to demonstrate that the mean weight of individuals passing through the stage of development on successive days would first lie above and then below the mean weight-gain line, and secondly that they would reveal a smaller range in weight than that predicted by the mean weight-gain data, calculated as the average weight-gain between birth and weaning. Other evidence supports

TABLE 4 Comparison of predicted and observed range in weights
during development of control mice

<u>Event</u>	<u>Weight range predicted from</u> <u>curve(g)</u>	<u>Observed weight range (g)</u>
Incisor eruption	6.26 - 4.80 = 1.46	6.7 - 5.9 = 0.80
Eye opening	7.40 - 5.60 = 1.80	8.20 - 7.0 = 1.20
Molar eruption	8.90 - 6.26 = 2.64	8.30 - 6.9 = 1.70

TABLE 5 Unselected mice: regression coefficients of mean family
weight (g) on age (days) at suckling events

<u>Event</u>	<u>Regression Coefficient (b) $\pm$ s.e.</u>
Incisor eruption	- 0.08 $\pm$ 0.10 g/day
Eye opening	- 0.13 $\pm$ 0.10 g/day
Molar eruption	0.05 $\pm$ 0.14 g/day

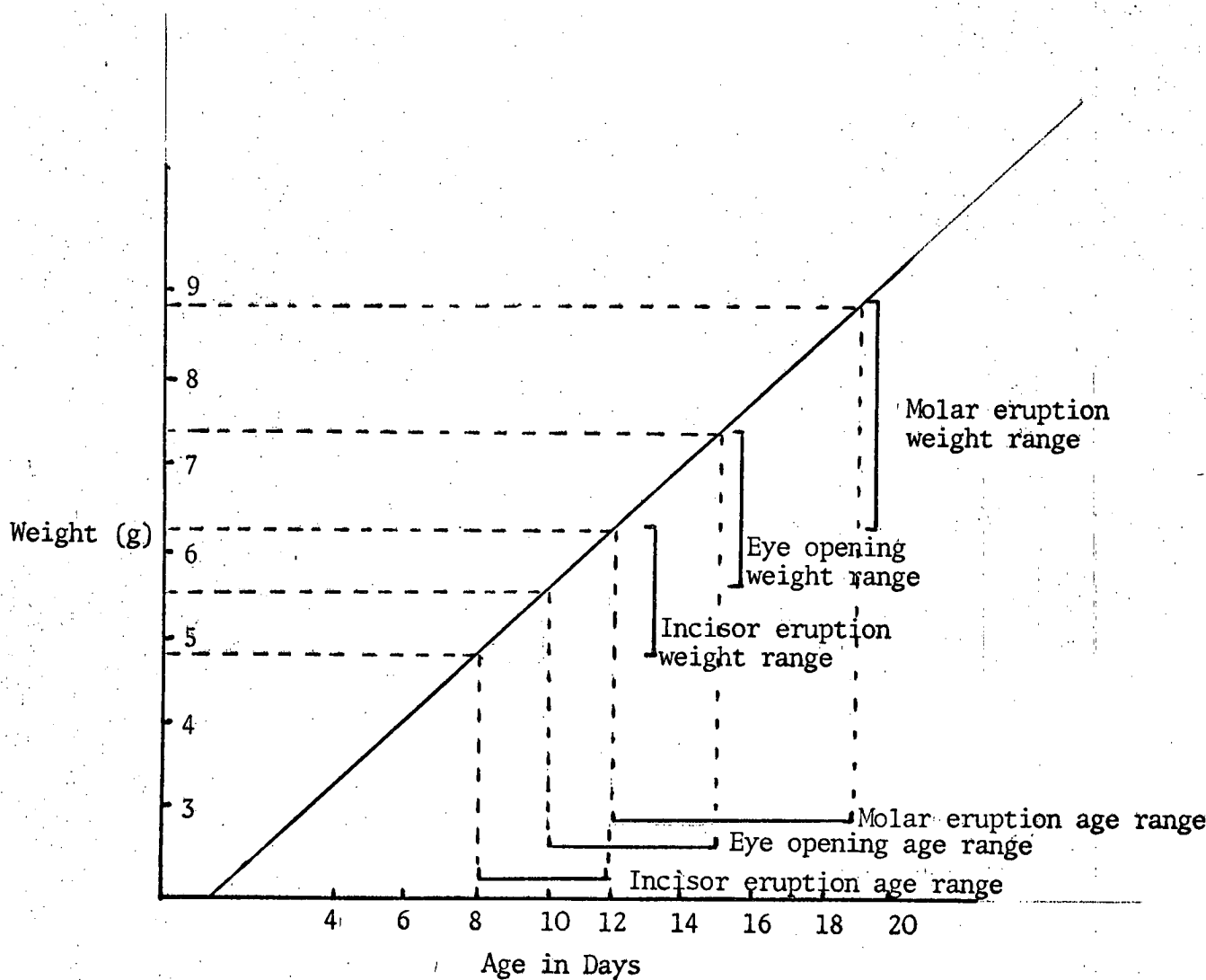
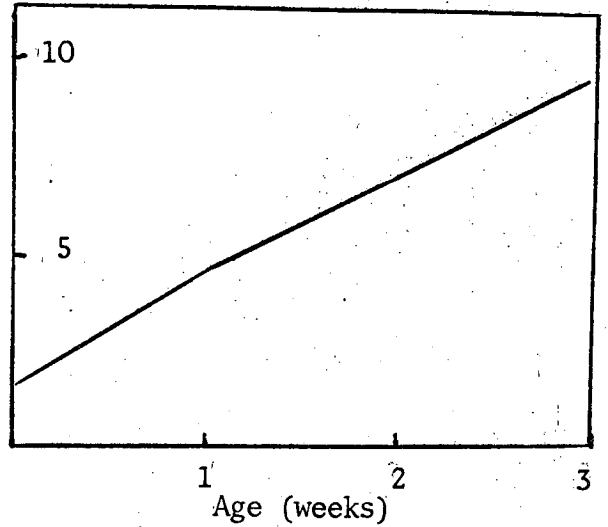
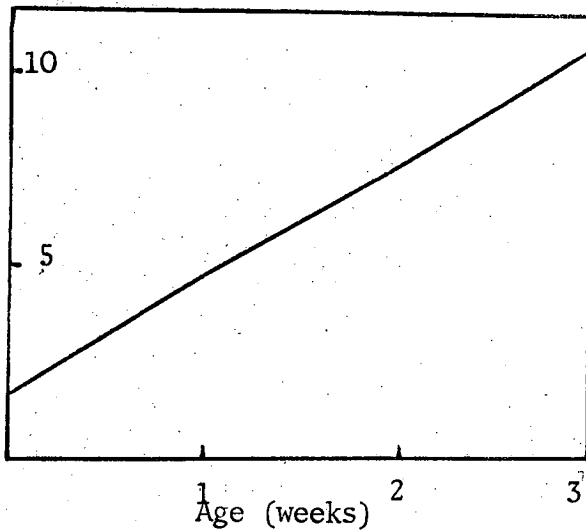
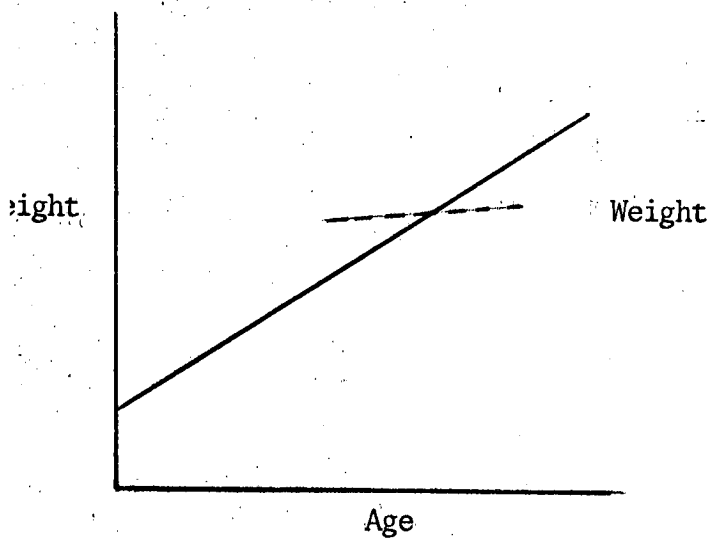


Fig. 7. Unselected Mice, Predicted Weight Range for Developmental Events Assuming Weight Gain is Linear and Weight has no Influence on Development.

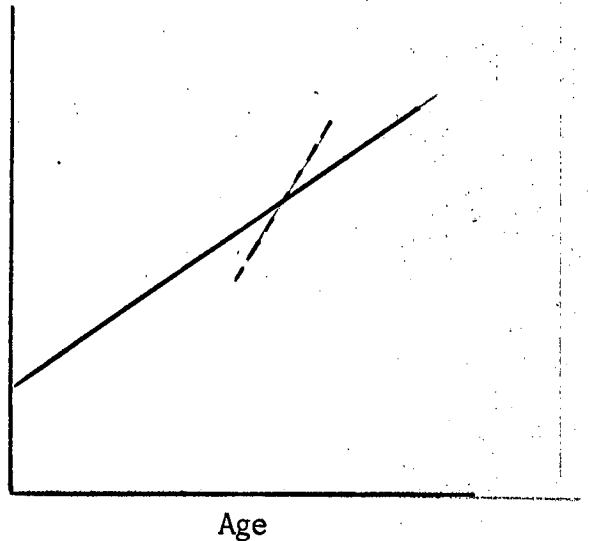


A. Brumby's unselected longitudinal data. (Brumby, 1960). B. Montiero and Falconer's unselected data. (Montiero and Falconer, 1966).

Fig. 5. Mean Weight Gain of Unselected Mice.



A.



B.

Fig. 6. Mean Weight Gain Lines Showing Regression Line of Body Weight on Day of Event. A. Weight More important than time. B. Time More Important Than Weight.

the contention that the rate of weight gain over this period is practically linear. Fig. 5 illustrates data on weight gain from two longitudinal studies during the first twenty one days after birth.

In order to demonstrate that weight is not only important, but more important than time in controlling development, the regressions of body weight on age on the day the event occurred is plotted over the mean weight-gain regression line. The two theoretical possibilities are shown in Figs. 6A and 6B. If weight were more important than age, the regression would lie as shown in Fig. 6A. If age were more important, the regression could slope the other way, Fig. 6B.

Results. In Fig. 7 the observed range in ages is marked off on the mean weight gain curve for the control population. From the Y axis we obtain the predicted ranges in weight if development were to occur entirely under the control of age. These ranges are compared with the observed ranges in weight obtained from the mean weights of individuals demonstrating the event on successive days. Table 4 shows the difference between predicted and observed results in weight range at the three events. In the third event, molar eruption, the slowest two individuals were ignored since their weights were widely divergent from the rest of the population.

The figure shows that the observed range in weight is smaller than that predicted by the mean weight-gain curve, supporting the hypothesis that body weight is important in determining development.

Regression of weight on age of individuals in the population at the occurrence of the event are plotted in Fig. 8 over the regression curve of weight on age in the whole population. Once more, the slope of the regression supports the hypothesis that weight is more important than age in determining development. Regression coefficients are shown in Table 5.

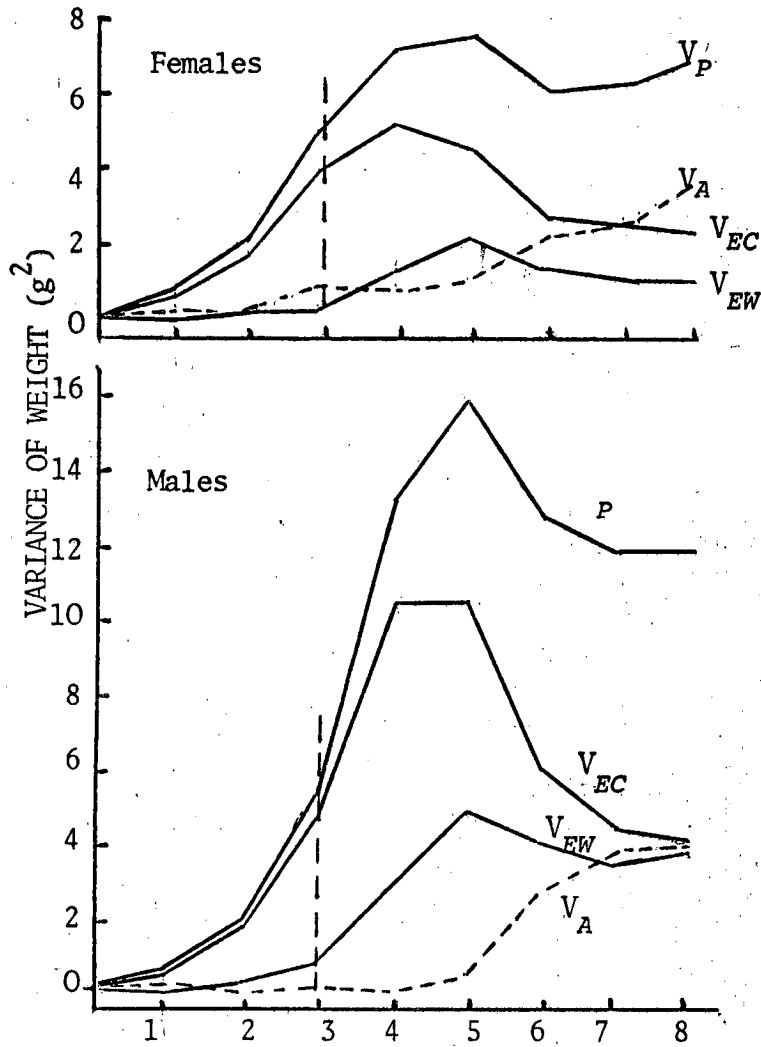


Fig. 9. Partitioning of Phenotypic Variance of Body Weight at Weekly Intervals (Montiero and Falconer, 1966).

This data was comprised of the values of individuals neglecting family and replicate differences. It was shown earlier that by distinguishing among families a major source of variation in growth rate during suckling could be identified. Fig. 9 is reproduced from Montiero and Falconer's paper indicating that between-family variation greatly exceeds within-family variation. For females, 77.5% of the variance of weaning weight is due to maternal environment and 84.3% of the variance of male weaning weight. They showed that at puberty also the maternal environmental factor influenced age more than weight.

The association between weight and age at stage of development was calculated, as has been done above, for puberty, using family means. Next, the association between weight and age was calculated using individual deviations from family means. The results are shown in Tables 6 and 7.

TABLES 6 & 7 Comparison of Regressions (b) Between- and Within Families
(Control Mice)

Regression (b) of age on Wt at:	Between Families			Within Families		
	Reg:	Coeff:	s.e. P	Reg:	Coeff:	s.e. P
Incisor Eruption	-0.21	0.20	n.s.	0.29	0.10	*
Eye Opening	-0.09	0.16	n.s.	0.02	0.14	n.s.
Molar Eruption	0.04	0.16	n.s.	-0.20	0.13	n.s.
Vagina Opening	0.19	0.58	n.s.	0.72	0.17	**

Between-family associations are all close to zero and indicate that mean litter weight at any stage of development is relatively independent of age at that stage, as was the case for puberty. The within-family deviations show a similar positive association between development time and body size as was found for puberty, i.e. late developers are heavier than early developers. This relationship does not hold for molar eruption, which is a similar physiological event to incisor eruption and therefore

TABLE 8 Association of Speed of Development with Birth Weight

Regression (b) of age on birth wt. (family means)	Regression Coefficient + s.e.	P
Incisor eruption	0.08 $\pm$ 0.02	* *
Eye Opening	0.07 $\pm$ 0.02	* *
Molar eruption	0.04 $\pm$ 0.02	*
Regression (b) of age on weaning wt. (family means)		
Vagina opening	-1.23 $\pm$ 0.59	*

TABLE 9 Regression(b) of Age at Event (days) on Absolute Growth Rate/(g/day) at Time of Event

Event	b $\pm$ s.e.	P
Incisor eruption	-4.74 $\pm$ 0.99	* *
Eye opening	0.55 $\pm$ 0.34	*
Molar eruption	1.21 $\pm$ 0.66	*

TABLE 10 - Please see page 50B.

TABLE 10. Mean Weights(g) Of Mice Grouped By Age When Event Occurred. (Numbers at each age are shown beneath weight).

Line	Age in Days													Event	Mean Weight (g)
	8	9	10	11	12	13	14	15	16	17	18	19	20		
Large		6.0 5	7.6 14	7.4 24	6.7 7	4.9 2								Incisor eruption	6.5
Control		6.1 6	5.9 3	6.7 22	6.1 45	6.4 3									
Small	5.0 2	5.0 2	6.0 8	5.4 23	5.4 27	4.8 3									
Large						9.5 10	9.3 27	7.9 14	4.4 1					Eye opening	7.8
Control				7.2 2	8.2 3	8.2 7	7.1 32	7.4 28	7.0 7						
Small						7.7 12	6.4 16	6.0 20	5.9 13	3.8 3	4.9 1				
Large								10.6 10	11.8 12	8.4 21	6.5 4	9.4 1		Molar eruption	9.3
Control						7.7 2	6.9 2	8.3 2	7.5 19	7.8 36	8.2 17	5.5 2			
Small								7.6 10	7.1 11	6.7 20	5.8 18	6.6 4	3.0 1		
	8	9	10	11	12	13	14	15	16	17	18	19	20		
Age in Days															

would be expected to show a similar association. Other inter-litter environmental factors probably influence this result.

A further similarity with pubertal data was the association of development time with body weight measured at a particular point in time before the event. For puberty litters putting on weight fast, that is, litters which were heavier at weaning (21 days), were closer to the puberty weight threshold and therefore reached it sooner. Consequently there was a strong negative association between weaning weight and age at puberty. A similar association was calculated for age at the three developmental stages during suckling and the mean litter weight at an earlier fixed age - that at birth. As for puberty, a strong negative association was revealed - Table 8.

Alternatively, the age at which the event occurred could be regressed upon the absolute growth rate from birth till the event occurred. A similar strong negative association was shown at all three stages, Table 9. Under the alternative hypothesis of time-dependence rather than weight-dependence for these events, the strong positive associations would not be possible.

It was concluded, therefore, that the developmental events during suckling showed weight-dependence just as had been shown for the event of vagina-opening in this and Montiero and Falconer's material.

Next, it was attempted to estimate the weight thresholds, the age limits for each developmental event and the effects of six week weight selection on all four events.

The basic data is shown in Table 10, arranged by age groups at each successive developmental event. The table shows mean body weights at each age and the numbers which contribute to that mean. The overall mean body weight of successive daily weights are shown in the right hand column. These means represent the weight threshold associated with each event in the control population.

For unselected mice they are as follows:



TABLE 11 Weight thresholds and age ranges for developmental events of unselected mice

	<u>Weight thresholds (g)</u>	<u>Age range in days</u>
Puberty	18.4	30 - 48 (18)
Molar eruption	8.7	13 - 19 (7)
Eye opening	7.5	11 - 16 (6)
Incisor eruption	6.2	9 - 13 (5)

The data on selected mice was used to examine the stability of the weight thresholds and the presence of age limits enclosing the range of ages shown above.

3. Effect of selection on growth and development

What can we predict the effect would be on sexual maturity and suckling characteristics, of increasing or decreasing six week body weight by artificial selection? Such selection had already been carried out for eighteen generations by within-litter selection.

Regarding the association between mature size and time taken to mature among populations which are genetically different from one another, there is evidence for a positive association. Taylor, (1965, 1967) and Laird, (1966) and Howard, (1967) showed that by examining the relationship between the scaling factors necessary to transform the growth curve parameters of different species, a systematic relationship between them could be estimated. Taylor showed that there was a proportionality between the time a species takes to mature in live weight, and its mature weight raised to the power of 0.27, which is close to Brody's regression coefficient of 0.273 and Laird's coefficient of 0.28.

Between strains within species and between sexes within strains Taylor found that the coefficient was higher, 0.35, and still positive.

TABLE 12 Large mice. Regression coefficients of mean family
weight (g) on age (days) at suckling events

<u>Event</u>	<u>Regression Coefficient (b) $\pm$ s.e.</u>
Incisor eruption	0.05 $\pm$ 0.26 g/day
Eye opening	1.15 $\pm$ 0.44 g/day
Molar eruption	1.12 $\pm$ 0.41 g/day

TABLE 13 Small mice. Regression coefficients of mean family
weight (g) on age (days) at suckling events

<u>Event</u>	<u>Regression Coefficient (b) $\pm$ s.e.</u>
Incisor eruption	0.11 $\pm$ 0.10 g/day
Eye opening	0.61 $\pm$ 0.00 g/day
Molar eruption	0.65 $\pm$ 0.10 g/day

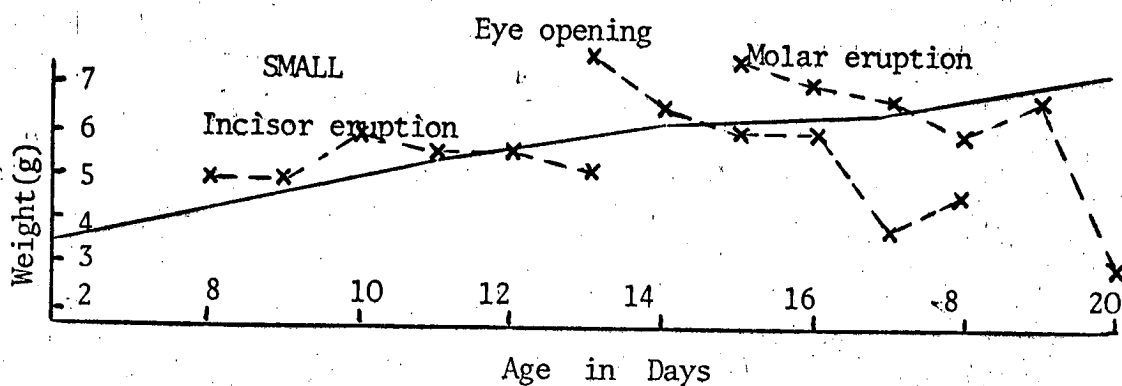
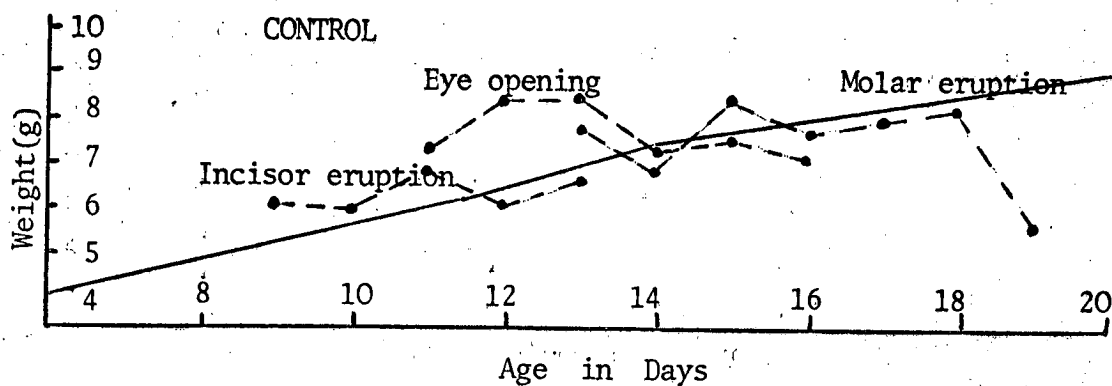
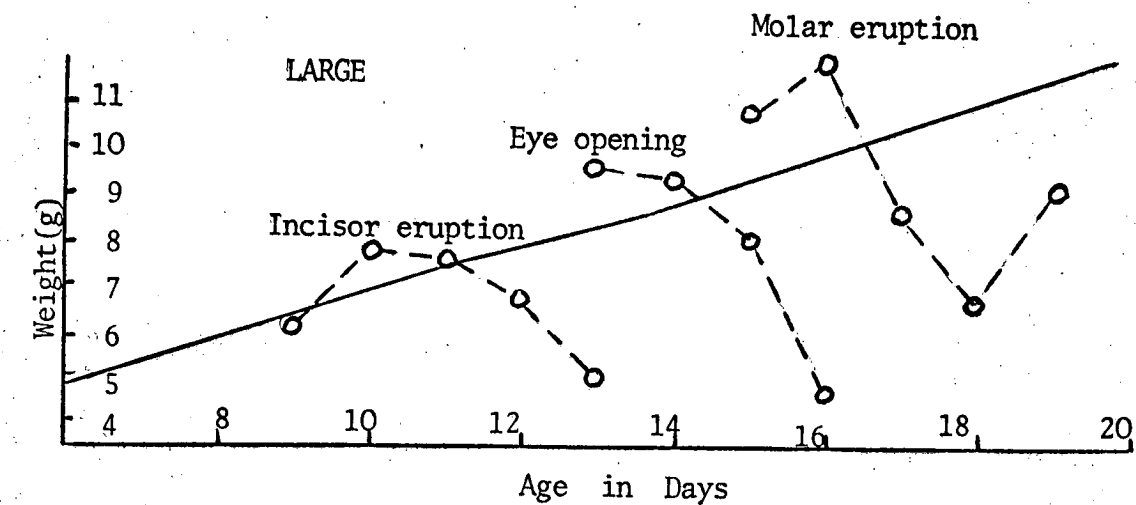


Fig. 10. Mean Weights of Animals Grouped by Age Passing Through Stage.

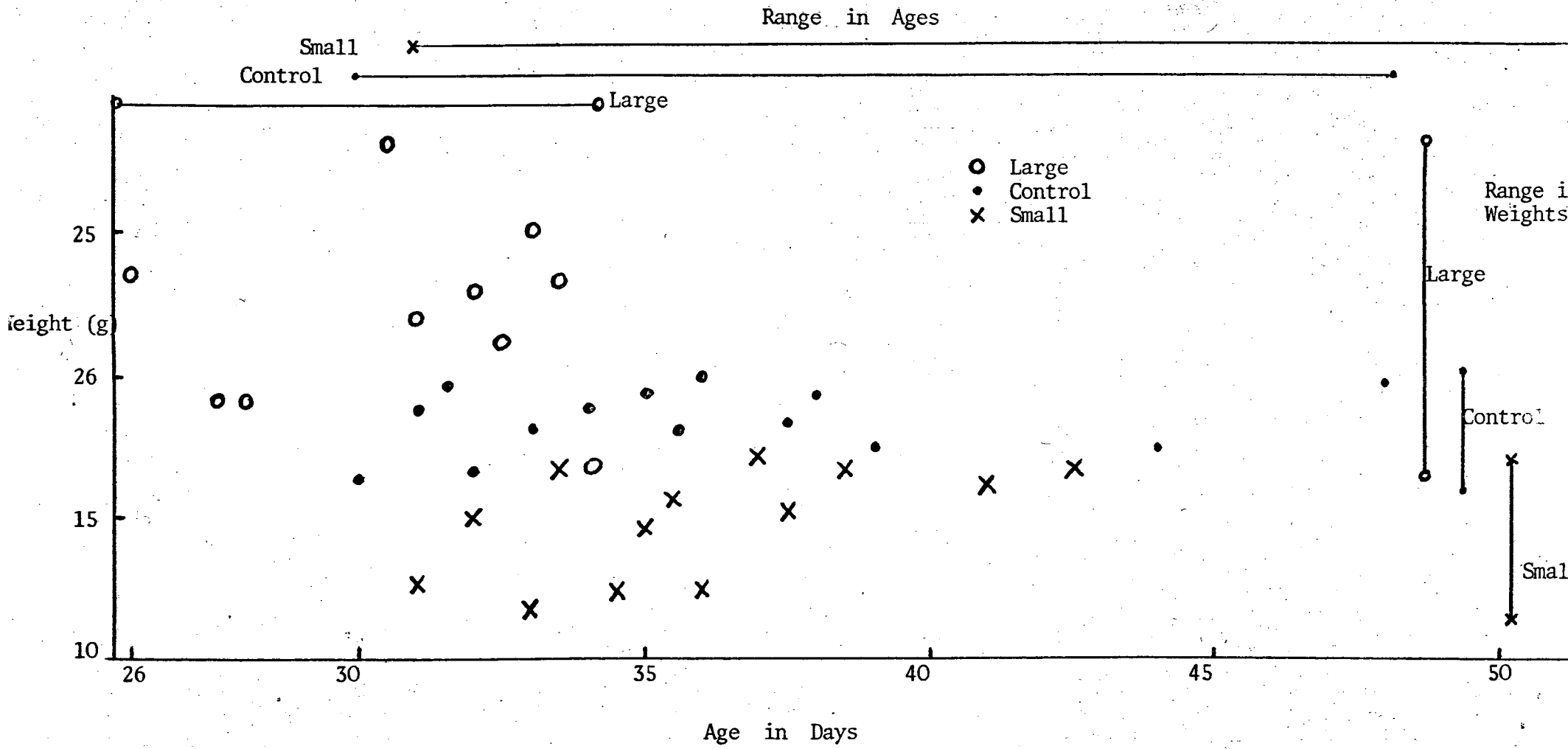


Fig. 11. Mean Weights and Ages of Female Sibs at Opening Vagina.

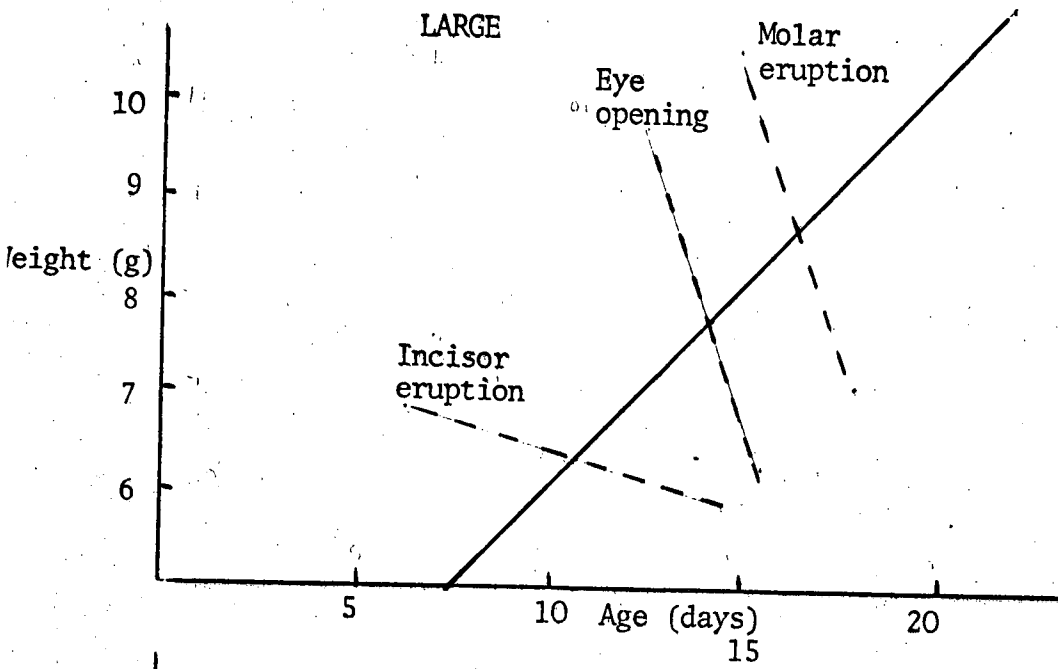


Fig. 12.

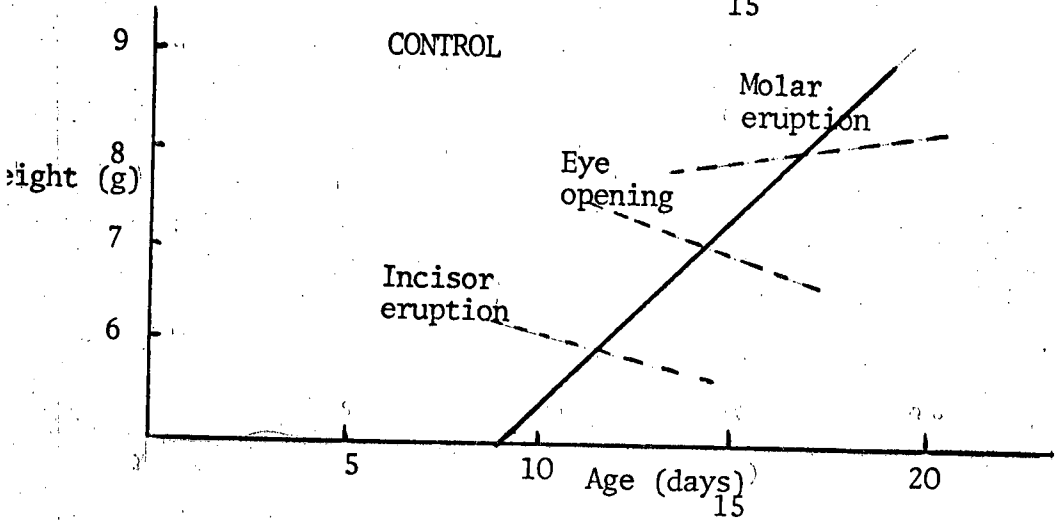


Fig. 8

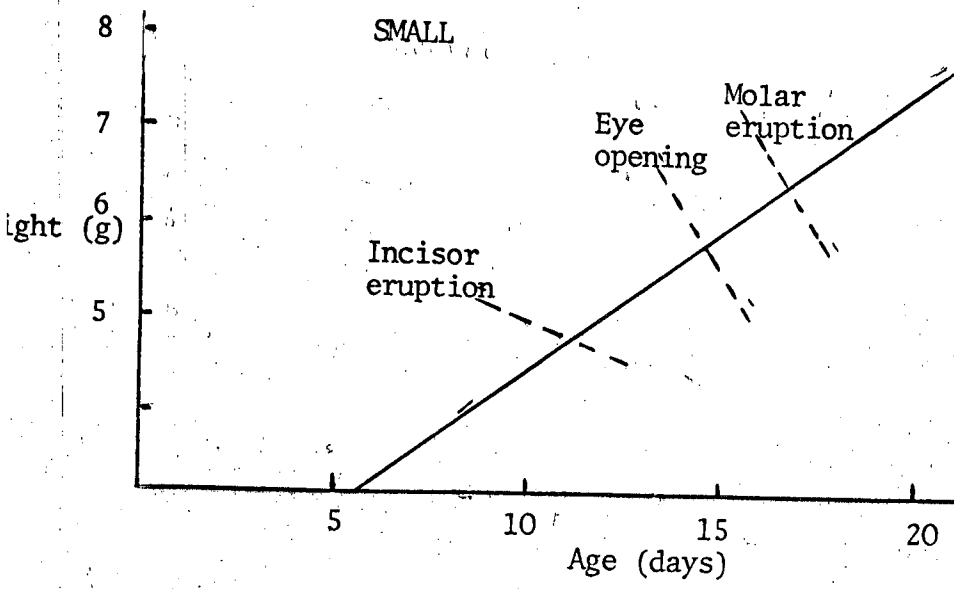


Fig. 13.

Regression Lines of Body Weight on Age at Time of Event

TABLE 14 Variance of Developmental Age and Weight Among Lines

	<u>Large</u>	<u>Control</u>	<u>Small</u>
Incisor Eruption			
Age (days)	0.89	0.86	1.08
Weight (g)	3.21	0.92	0.95
Eye Opening			
Age (days)	0.53	1.10	1.45
Weight (g)	5.62	1.61	1.25
Molar Eruption			
Age (days)	1.09	1.25	1.49
Weight (g)	9.70	2.06	1.26
Vagina Opening			
Age (days)	7.78	19.17	43.46
Weight (g)	18.66	5.22	6.66

TABLE 15 Coefficient of Variation of Developmental Ages and Weights

<u>Coefficient of Variation of Ages and Weights Among the Three Lines</u>			
	<u>Large</u>	<u>Control</u>	<u>Small</u>
Incisor Eruption			
Age (days)	8.8	8.1	9.3
Weight (g)	25.9	15.2	18.0
Eye Opening			
Age (days)	5.2	7.3	8.1
Weight (g)	27.2	17.1	17.7
Molar Eruption			
Age (days)	6.6	6.7	7.1
Weight (g)	33.1	18.4	17.3
Vagina Opening			
Age (days)	9.1	12.2	17.8
Weight (g)	19.6	12.4	17.4

differentially at puberty. The range in age is greatly decreased in the large line and slightly increased in the small line. Body weight range is increased in both selected lines compared with controls. These differences are more dramatically shown by comparison of the variances for sexual maturity, Table 14. In absolute measures, the large line has undergone a great reduction in age variance and an increase in weight variance. Conversely, the small line has undergone a great increase in age variance and a small increase in weight variance. When coefficients of variation are compared, however, the differences are less than indicated by the variances, Table 15. Like the control line, variation is approximately equal in age and weight in the small line, but both are 50% larger than the corresponding control value. The large line has also undergone a real increase in weight variation, and a 25% decrease in age variation, which is independent of the scale effect.

The results during the suckling stage are not very different. Variation in time is slightly reduced in the large line and weight variation is substantially increased. The variation at these stages in the small line, however, is very little different from what it is in the control line. In general the variation in weight has been altered more than that in age.

Because of the difference in variance, comparisons between mean results have to be treated with caution. A least squares analysis of variances was carried out for the following observations:

- birth weight and length,
- weight and age at each suckling event,
- weaning weight.

Differences between lines contributed significantly only to

TABLE 16 Least Squares Analysis of Variance of Suckling Character

<u>Character</u>	<u>Source of Variation: P Value</u>		
	<u>Line</u>	<u>Replicate</u>	<u>Family</u>
Birth Weight	**	n.s.	**
Birth Length	**	n.s.	**
Age at Incisor Eruption	n.s.	n.s.	**
Weight at Incisor Eruption	n.s.	n.s.	**
Age at Eye Opening	n.s.	n.s.	**
Weight at Eye Opening	n.s.	n.s.	**
Age at Molar Eruption	n.s.	n.s.	**
Weight at Molar Eruption	n.s.	n.s.	**
Weaning Weight	*	*	**

t Tests Between Mean Values for Body Weight at
Different Developmental Events and Several Fixed Ages

TABLE 17 Mean of Family Means for Each Line Compared at Different
Events in Age and Weight

WEIGHT COMPARISON (in gm)
+ s.e.

<u>Event</u>	<u>Line Large</u>	<u>t test</u>	<u>At Same Stage</u>		
			<u>Line Control</u>	<u>t test</u>	<u>Line Small</u>
Incisor Eruption	6.9 ± 0.5	n.s.	6.3 ± 0.2	*	5.4 ± 0.2
Eye Opening	8.7 ± 0.7	n.s.	7.4 ± 0.3	*	6.3 ± 0.3
Molar Eruption	9.4 ± 0.9	n.s.	7.8 ± 0.3	*	6.5 ± 0.3
Vagina Opening	22.0 ± 1.0	*	18.4 ± 0.3	**	14.8 ± 0.5
<u>At Same Age</u>					
Birth	1.72 ± 0.04	*	1.54 ± 0.0	n.s.	1.46 ± 0.04
Weaning (Males)	12.4 ± 1.2	*	9.4 ± 0.4	*	7.4 ± 0.3
Six Weeks (Males)	34.6 ± 1.8	**	24.6 ± 0.7	**	17.9 ± 0.9

TABLE 18 Mean Age of Family Means for Each Line,
Compared at Different Events

AGE COMPARISON (days)					
+ s.e.					
Event	Line Large	t test P	Line Control	t test P	Line Small
Incisor Eruption	10.7 $\pm$ 0.2	5%	11.5 $\pm$ 0.2	n.s.	11.2 $\pm$ 0.2
Eye Opening	14.1 $\pm$ 0.2	n.s.	14.3 $\pm$ 0.2	n.s.	14.8 $\pm$ 0.3
Molar Eruption	16.4 $\pm$ 0.3	n.s.	16.8 $\pm$ 0.2	n.s.	17.1 $\pm$ 0.3
Vagina Opening	30.8 $\pm$ 0.9	5%	35.8 $\pm$ 1.4	n.s.	37.04 $\pm$ 1.4

variation in birth weight and length and weaning weight, i.e. to weights measured at fixed ages - see Table 16. Only family differences significantly affected weight or age at the three fixed stages investigated. Since the variances among these different characters were different, however, confirmation of the absence of significant line effects was sought by carrying out t tests on the family means. The results are shown in Tables 17 and 18. Once more, no statistically significant difference was found between the ages of control or small lines at all four stages of development, although the small line fell behind in mean value. The large line was in advance of the control at all stages although this was significant only at incisor eruption and vagina opening.

The small line was consistently significantly smaller in body weight than the control line at all stages. The large line was consistently larger, but only statistically significantly at puberty. Comparing weights at fixed ages, however, all comparisons are significant except the small-control comparison at birth.

However, since t tests also assume equal variances, the comparisons between weights in the large and control lines especially are not reliable, nor is the comparison between ages at puberty between the selected and control lines.

It emerges clearly, however, that weights at fixed ages are substantially and significantly altered by selection.

Comparing mean values among the lines, then, it is clear that selection has had the expected result on weights at the different stages: large mice are consistently heavier and small mice consistently smaller, though the statistical significance of the comparisons is variable.

The effect on age at the different stages is not in the expected

direction, however; large mice develop and mature slightly faster where we would expect them to be slow, and vice versa for small mice. It was shown in the unselected mice that speed of development was closely associated with absolute growth rate. Growth rate, it was also shown, was governed mainly by environmental factors in early life. It appears, therefore, that the altered mean development time may be a reflection of, respectively, the enhanced growth rate necessary for increasing six week weight in the large mice, and the depressed growth rate in the small line. This implies that within-litter selection has produced a change in growth rate which is a combination of environmental and genetic effects, and a change in development rate which is an entirely environmental effect. These results are the reverse of what we would expect from Montiero and Falconer's calculations. They found a heritability of 16% for age at sexual maturity, but no additive genetic variance for weight at sexual maturity. It could be, however, that genetic differences in early growth rate, like environmentally induced variation in growth rate, influence development time without altering weight at puberty. Thus, it is not clear whether the differences in time taken to reach sexual maturity among the lines are brought about by genetic differences or a correlated environmental effect.

It is possible that both are present. If the selected line was differentiated from the control line genetically in the expected directions, both for age and weight, then the mean weights for large would be (and are) greater, and the ages would likewise be greater. We might expect the mean age and range in age to be delayed compared with controls, and the mean age for the small line to occur earlier than controls. If, however, the selected lines were influenced by a correlated environmental effect on development time, the alteration

brought about genetically might be eliminated by the effect of environment, since these two factors appear to work in opposite directions in respect of development time. Thus it would not be possible to predict how the age limits might alter.

What might be the expected results of either progressively prolonging or shortening development time through its genetic correlation with increased or decreased body weight or, on the other hand, progressively shortening or prolonging development time through its environmental correlation with increased or decreased body weight? A continued change in development time would be evinced so long as there was a direct response in the character selected (though the limits of genetic correlation might be reached before the main character selected reached a plateau).

A continued change in development time due to environmental correlation with body weight might be expected, also, to continue so long as there was a direct response in the character directly selected. The limits in ages for development for control mice are compared below with those for selected mice:

TABLE 19 Comparison of limits in age for different
developmental events. (Days)

<u>Event</u>	<u>Large</u>	<u>Control</u>	<u>Small</u>
Incisor eruption	9 - 13	9 - 13	8 - 13
Eye opening	13 - 16	11 - 16	13 - 18
Molar eruption	15 - 19	13 - 20	15 - 20
Vagina opening	26 - 34	30 - 48	31 - 52

It is evident that the ranges in age have been scarcely changed by selection at suckling stages, the limits in time found in the control line for the most part being the same as those in the selected lines.

At puberty, however, both the earlier and later time limits have been moved to earlier positions in the large line, while in the small line only the later limit in time has been altered.

The stability of limits in time for developmental events during suckling, regarded in the light of the corresponding stability of mean ages at those ages under the effect of body weight selection, indicates that, at this period, the limits to development time are resistant to either extreme correlated genetic or environmental transformation. However, as was mentioned above, at all three suckling events the mean values have been shifted in a direction suggesting an environmental influence. This, accompanied by the reduction in variance in the large line at all stages, suggests that, in this line, an earlier time limit has been reached due to environmental effects.

In control mice increased growth rate was associated with earlier development and little change in mean body weight at the different developmental stages. Progressive increase in growth rate, as occurred in the large line, appears to have encountered an earlier limit in time, which can be reduced only a little. Increase in growth rate, therefore, might be associated with increase in body weight at each stage among those mice which grew fastest, and an increase in the proportion of these mice; slower growing mice would not differ much from controls.

Reduction in rate of growth might, conversely, cause slower developing mice to encounter a limit in time beyond which development cannot be much delayed. Here, then, reduction in growth rate brought about by low six-week weight-selection would produce a proportion of mice showing a negative association between body weight and speed of development. Briefly, once the limits in time had been encountered, the weight-threshold for development found in unselected mice no longer was operative; the faster a mouse grew, the greater

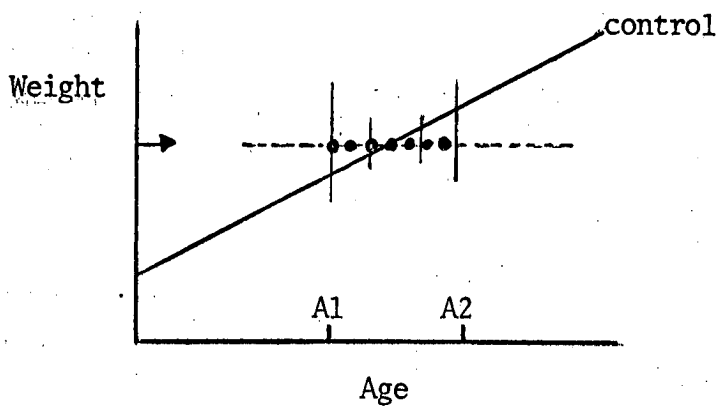


Fig. 14

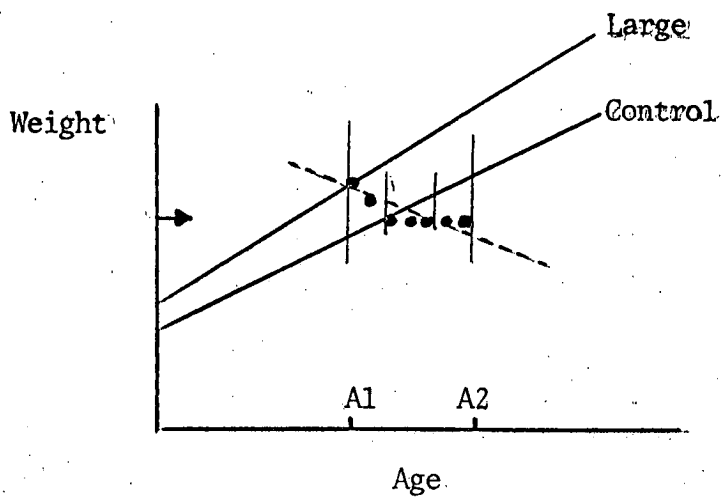


Fig. 15

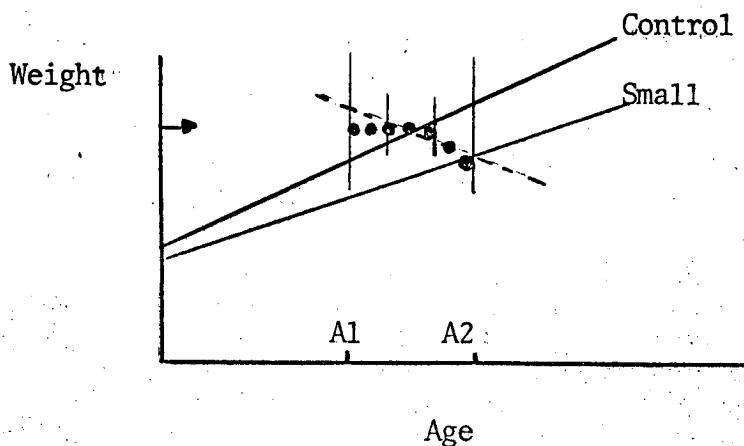


Fig. 16

Mean Weight Gain Curves of Idealised Large, Control and Small Mice Passing Through an Identical Age Range for a Developmental Stage.

Dotted Line a Regression Line.

its weight at the earlier time limits at the different stages of development, and the slower, the less its weight at those stages.

In Figs. 14, 15 and 16 three possible conditions are illustrated. Where development is controlled by a weight threshold with a certain age range as in Fig. 14, the mean weights for successive days line almost parallel to the X axis.

Where the earlier developing individuals reach the early age limit, increased growth rate causes them to be heavier than control but no longer younger at each stage. Consequently the regression of weight on age becomes negative largely because of the high proportion of fast developers within the same age range - Fig. 15.

The opposite condition occurs where individuals growing slowly encounter the later age limit and develop at weights below the control threshold. The regression of weight on age becomes increasingly negative - Fig. 16.

In both cases individuals growing at intermediate speeds will not be different weights between the lines, but at the extremes in age they will be.

This expectation was tested by regressing age at developmental stage on weight at that stage using family mean data - Table 20.

TABLE 20. Comparison of Regression Coefficients at Fixed Stages of Development. ($b \pm s.e.$) g per day.

<u>Event</u>	<u>Large</u>	<u>Control</u>	<u>Small</u>
Incisor eruption	0.03 $\pm$ 0.64	-0.25 $\pm$ 0.25	-0.23 $\pm$ 0.23
Eye opening	1.58 $\pm$ 1.1	-0.17 $\pm$ 0.30	-0.79 $\pm$ 0.17 *
Molar eruption	1.36 $\pm$ 0.92	0.08 $\pm$ 0.34	-0.85 $\pm$ 0.20 *
Vagina opening	0.18 $\pm$ 0.53	0.08 $\pm$ 0.09	0.09 $\pm$ 0.07

* stat. sig. at 5% level

TABLE 21

Comparison of weights of large, control and small
mice erupting incisor teeth,
(1) early, i.e. on day 9 or 10

	<u>Large</u>	<u>P</u>	<u>Control</u>	<u>P</u>	<u>Small</u>
Mean Weight $\pm$ s.e.(g)	7.3 $\pm$ 0.5	*	6.0 $\pm$ 0.2	n.s.	5.8 $\pm$ 0.3

(2) late, i.e. on day 11

	<u>Large</u>	<u>P</u>	<u>Control</u>	<u>P</u>	<u>Small</u>
Mean Weight $\pm$ s.e.(g)	7.4 $\pm$ 0.5	n.s.	6.7 $\pm$ 0.3	**	5.4 $\pm$ 0.3

Comparison of weights of large, control and small
mice opening eyes,
(1) early, i.e. on day 13

	<u>Large</u>	<u>P</u>	<u>Control</u>	<u>P</u>	<u>Small</u>
Mean weight $\pm$ s.e.(g)	9.5 $\pm$ 0.7	n.s.	8.2 $\pm$ 0.5	n.s.	7.7 $\pm$ 0.2

(2) late, i.e. on day 15

	<u>Large</u>	<u>P</u>	<u>Control</u>	<u>P</u>	<u>Small</u>
Mean weight $\pm$ s.e.(g)	7.9 $\pm$ 0.3	n.s.	7.4 $\pm$ 0.3	**	6.0 $\pm$ 0.3

Comparison of weights of large, control and small
mice erupting molar teeth,
(1) early, i.e. on day 16

	<u>Large</u>	<u>P</u>	<u>Control</u>	<u>P</u>	<u>Small</u>
Mean weight $\pm$ s.e.(g)	11.8 $\pm$ 1.1	**	7.5 $\pm$ 0.3	n.s.	7.1 $\pm$ 0.2

(2) late, i.e. on day 17

	<u>Large</u>	<u>P</u>	<u>Control</u>	<u>P</u>	<u>Small</u>
Mean weight $\pm$ s.e.(g)	8.4 $\pm$ 0.5	n.s.	7.8 $\pm$ 0.3	**	6.7 $\pm$ 0.3

The pattern of the coefficients indicates that in most cases the expectation is borne out, though the regressions are seldom statistically significant. The regressions tend to be more negative in the selected lines and close to zero in the unselected line.

The assumption here was that there existed limits in developmental time, especially during the suckling stage, which environmental influences scarcely moved, merely adjusting the mean slightly at each age and altering the variances. The main effect of selection had been on body weight at the different stages of selection. A comparison of the population means showed that weights of selected mice at fixed ages were substantially different from controls though statistical significance could be tested only on the control - small comparison due to the large increase in weight variance of the large line. Only after weaning was statistical significance established in the large-control comparison.

The change in mean and variance brought about by selection at each of these three stages, then, reflects an alteration in the distribution of individuals within the lines. In the large line there are rather more individuals whose growth rate is high and who develop fast, and fewer slower growing individuals who also develop slowly. Contrariwise, the small line represents fewer individuals gaining weight quickly, but on the other hand contains individuals whose growth rate is slow and who therefore show a delay in reaching any developmental stage.

To illustrate this redistribution of individuals, a comparison was made between the early and late developing individuals across the three lines - Table 21. Significant differences in weight were found between large and control mice reaching a given stage on the same day, if that day was early in range of possible days. Similarly, if the

TABLE 22 Percentage Change in Different Variables Compared
With Controls

<u>VARIABLE</u>	<u>LARGE</u> % deviation from Control	<u>CONTROL</u> Percentage change between 9th & 18th generation	<u>SMALL</u> % deviation from Control
<u>9th Generation</u>			
Wean Weight	+ 11.9%	+ 2.6%	- 18.4%
Pubertal Weight	+ 15.2%	- 1.7%	- 13.2%
Pubertal Age	- 10.6%	+ 5.0%	+ 3.9%
Female 6 wk Weight	+ 26.9%	+ 0.1%	- 16.1%
<u>18th Generation</u>			
Wean Weight	+ 30.5%	+ 2.6%	- 20.8%
Pubertal Weight	+ 20.1%	- 1.7%	- 21.8%
Pubertal Age	- 10.5%	+ 5.0%	+ 3.8%
Male 6 wk weight	+ 41.4%	+ 2.1%	- 28.1%
Female 6 wk weight	+ 43.3%	+ 0.1%	- 30.0%

TABLE 23 Percentage Change in Variables in First and Second
Half of Experiment

<u>VARIABLE</u>	<u>LARGE</u>			<u>SMALL</u>		
% change in gens:	<u>1-9</u>	<u>10-18</u>	<u>Total</u>	<u>1-9</u>	<u>10-18</u>	<u>Total</u>
Wean weight	+ 11.9	+ 18.6	+ 30.5	-18.4	2.4	- 20.8
Pubertal weight	+ 15.2	+ 4.9	+ 20.1	-13.2	- 8.6	- 21.8
Pubertal Age	- 10.6	+ 0.1	- 10.5	+ 3.9	- 0.1	+ 3.8
Female 6 wk weight	+ 26.9	+ 16.4	+ 43.3	-16.1	-13.9	- 30.0%

weights of control mice developing slowly were compared with small mice passing through the stage on the same day a significant difference was found. No significant difference was found, however, between the weight of large mice developing slowly and controls developing slowly, or small mice developing early and controls developing early.

The presence of a limit to the amount development time could be transformed, compared with the ready response in body weight, was further tested by comparing body weights and ages at the eighteenth with the ninth generation of selection, that is, with a half-way point in the selection experiment. There was a large and statistically significant change in the character under direct selection, six week body weight, between these two generations.

In Table 22 the deviation of mean values from controls are compared between the ninth and eighteenth generations. In Table 23 the increments subsequently added to the first nine generations are identified. It is shown that during the first nine generations development time was reduced in the large line by ten per cent, but there was, subsequently, no change at all in development time. Similarly, development time was prolonged in the first nine generations by 3.9% and was, subsequently, virtually unchanged, though this difference is less than that found between the controls of the ninth and eighteenth generation.

Regarding all the other variables, the response in the first half of the experiment exceeds that in the second, with the exception of weaning weight in the large line where the response is greater in the second than the first half. The amount of change brought about in these variables during the second half is not sufficient to make them statistically significantly different from those in the ninth

TABLE 24 t Tests Between Variables of 9th and 18th Generation

	<u>9TH GENERATION</u>			<u>18TH GENERATION</u>			<u>P</u>
<u>SMALL</u>	Mean	$\pm$	s.e.	Mean	$\pm$	s.e.	
Weaning wt.(g) (females)	7.55		0.22	7.61		0.29	n.s.
Pubertal wt.(g)	15.53		0.17	14.96		0.48	n.s.
Pubertal age(days)	37.2		0.7	37.8		1.2	n.s.
 <u>LARGE</u>							
Weaning wt.(g) (females)	10.41		0.28	12.34		0.68	*
Pubertal wt.(g)	20.48		0.45	21.57		0.88	n.s.
Pubertal age(days)	32.0		0.7	31.41		0.6	n.s.

generation, again with the exception of weaning weight in the large line - Table 24.

It appears, then, that a limit in response in development time to puberty had already been reached by the ninth generation. A limit was approached in weight at puberty for both directions of selection and also for weaning weight in the small line. Only in weaning weight in the large line does there appear to be no limit to selection response.

An asymmetry exists in the responses to selection. There is a greater response in development time to puberty in large mice than small, with a reduction of variance, while in the small line there is increased variance.

It is suggested that this asymmetry is associated with the difference in the direct response, which is greater in the large line (43.3%) than in the small (30%). These direct responses are also reflected in weight at weaning which is greater in the large line (30%) than in the small line (20.8%). The comparison of ninth and eighteenth generations suggests that a limit in the response at weaning weight may be present in the small line but not in the large. This lower limit to selection response in the small line may be associated with the existence of a threshold of viability among mice whose development had been delayed greatly by slow weight gain. The presence of these individuals in the line is suggested by the statistically significant negative regression of development time upon body weight for molar eruption which occurs just prior to weaning. Individuals falling below this threshold of viability would not survive to be represented in the next generation selected. Thus, both later body weight and development time would be prevented from being altered by the existence of this threshold. Evidence for the

TABLE 25 Proportion of Total Variation Accounted for
by Litter Size

	<u>Large</u>	<u>Control</u>	<u>Small</u>
<u>Incisor Eruption</u>			
Age	12%	8%	0%
Weight	70%	60%	16%
<u>Eye Opening</u>			
Age	6%	2%	6%
Weight	29%	64%	18%
<u>Molar Eruption</u>			
Age	37%	0%	5%
Weight	64%	59%	14%
<u>Vagina Opening</u>			
Age	24%	0%	1%
Weight	30%	0%	1%

TABLE 26 Mean Litter Sizes of Three Lines

	<u>Large</u>	<u>Control</u>	<u>Small</u>
Mean	8.3	8.6	7.6
Variance	10.3	9.6	4.2

presence of such a threshold is presented in the Mortality and Fertility section.

In unselected mice the major source of variation in body weight during the suckling period was environmental. Litter size alone accounted for much of this. Selected lines differed very little in litter size, although the small line was much less variable - Table 26.

In Table 25 a comparison is made of the contribution of this factor of environmental variation to variation in age and weight at the different developmental stages. Only in the large line does litter size have more than a trivial effect on development time. For pre-weaning development in the large and control lines, however, litter size accounts for the major proportion of variation. In the small, however, although mean litter size is only reduced to a small extent, the reduced variance is reflected in a weaker association with body weight. In the small line genetical and other environmental factors account for most variation.

Conclusion

Unselected mice appear to differ from selected mice in the weight-regulation of development. Control mice demonstrate 'target-seeking' for body weight at a particular stage, the stage appearing within a particular age range. Selected mice possess age ranges for development which are virtually unchanged, although the means and variances are. The correlated effect of selection on body weight, however, is detectable at most stages of development, becoming more pronounced during puberty.

Thus, for unselected mice, weight is more important than time in development, while for selected mice time is more important than weight in the large line. Small mice are not consistent in this

regard, sometimes time, sometimes weight being more important. It is suggested that environmental influences in early growth cause the development time limits to be reached especially in the selected lines.

4. Effect of selection for body weight on the ossification of the skeleton

In the foregoing section the relationship between weight gain and development was examined by making daily observations so that the animal could be weighed on the day that it passed a given developmental stage. In this way the presence of a weight threshold for different development stages could be sought. Increase in body weight is accompanied by progressive ossification of the skeleton and increase in size of bones. It was of interest, therefore, to ascertain whether various stages in the process of enlargement and maturation of the skeleton also revealed the weight-threshold phenomenon. This proved more difficult to ascertain since the female mice had been followed in a longitudinal study so their skeletons were not available for accurate measurements. Material was provided, however, by male mice sacrificed at birth, five days, and several ages thereafter.

The material which was of use to this part of the investigation was collected by examination of ossification in the left foot and limb epiphyses. Since mice had to be killed in order to obtain the material the samples were of uniform age when the comparison was made. The data consisted of the body weights of individual mice and an observation of the number of epiphyses observed in the limbs. For new-borns, the area examined was the left metatarsel region, and individuals fell into one of three ossification categories - two, one or no ossification centres present. The five day old individuals were examined in the epiphysed region of the left fore and hind limbs, and the total number of centres

TABLE 27 Number of Ossification Centres Visible and Weight
in New Borns

<u>Line</u>	<u>Two Centres</u>			<u>One Centre</u>			<u>None</u>		
	<u>Wt(g)</u>	<u>+ s.e.</u>	<u>N</u>	<u>Wt(g)</u>	<u>+ s.e.</u>	<u>N</u>	<u>Wt(g)</u>	<u>s.e.</u>	<u>N</u>
Large C	1.7	0.06	12	1.39		2			0
Control C	1.5	0.05	11	1.2	0.03	3	1.16		2
Small C	1.5	0.04	9	1.3	0.05	5	1.16	0.06	3
Large F	1.97	0.05	25			0			0
Control F	1.69	0.04	17	1.57	0.03	17	1.5		1
Small F	1.7	0.03	7	1.3	0.04	13	1.2	0.08	5

TABLE 28 Body Weight and % Unossified in Three Lines at Five Days

<u>F REPLICATE</u>		<u>Mean Body Weight</u>		<u>% Unossified</u>	
<u>Line</u>	<u>N</u>	<u>Mean (g)</u>	<u>+ s.e..</u>	<u>Fore Limb</u>	<u>Hind Limb</u>
Large	23	5.1	0.02	9.7	62.5
Control	25	4.5	0.02	13.9	66.3
Small	27	3.5	0.01	19.4	71.7

<u>C REPLICATE</u>		<u>Mean Body Weight</u>		<u>% Unossified</u>	
<u>Line</u>	<u>N</u>	<u>Mean(g)</u>	<u>s.e.</u>	<u>Fore Limb</u>	<u>Hind Limb</u>
Large	20	5.4	0.05	10.1	84.1
Control	22	4.04	0.01	29.0	94.5
Small	22	3.7	0.02	34.1	78.6

TABLE 29 Association of Ossification and Body Weight

Regression of Number of Ossification Centres on Body Weight of New-Borns						
<u>Line</u>	<u>LC</u>	<u>CC</u>	<u>SC</u>	<u>SF</u>	<u>CF and LF</u>	
N	14	16	18	25	insufficient	
Regression coefficient	0.36	0.19	0.18	0.27	data	
s.e.	0.16	0.03	0.03	0.04		
P.	0.05	0.005	0.005	0.005		

Regression of Degree of Percentage of Local Unossified on Body Weight at Five Days						
<u>Line</u>	<u>LC</u>	<u>CC</u>	<u>SC</u>	<u>LF</u>	<u>CF</u>	<u>SF</u>
N	18	22	22	21	24	22
Regression coefficient	-0.030	-0.022	-0.015	-0.020	-0.018	-0.010
s.e.	0.01	0.004	0.004	0.005	0.005	0.003
P.	0.005	0.001	0.005	0.005	0.01	0.005

of ossification seen was recorded for each individual. The three-dimensional arrangement of metatarsels prevented accurate enumeration of the number of centres present in five day old mice since most centres were ossified and the absence of a few centres of ossification could not be reliably ascertained.

The samples of new-borns were separated into three groups according to their ossification status and the mean standard error and number in each category are shown in Table 27. The mean body weights of the five day old mice are shown in Table 28.

It was shown earlier that individuals gaining weight fast reached the various developmental stages soonest, that is, there was a positive association between body weight and degree of maturity. All three lines in each replicate were tested for the association between ossification, that is maturity, and body weight, at birth and at five days. The regressions are shown in Table 29. In both cases it is clear that heavier animals are more mature, as was the case in the earlier analysis. These results are compatible with the relationship between development and body weight, such that thresholds for ossification exist at certain weights. At a particular age the heavier animals will have passed through more 'thresholds' for ossification and thus show more centres of ossification than lighter animals of the same weight.

It would be useful to estimate what the weight thresholds are for this material, but the nature of cross-sectional data does not allow this to be calculated as was done previously. If weight thresholds did not exist, say, for the stages of ossification among new-borns, then one might expect that there would be no relationship between ossification and body weight among the lines within a replicate. The mean body weight of the small line sample with one ossification centre, for instance, would not necessarily be lower than the mean body weight

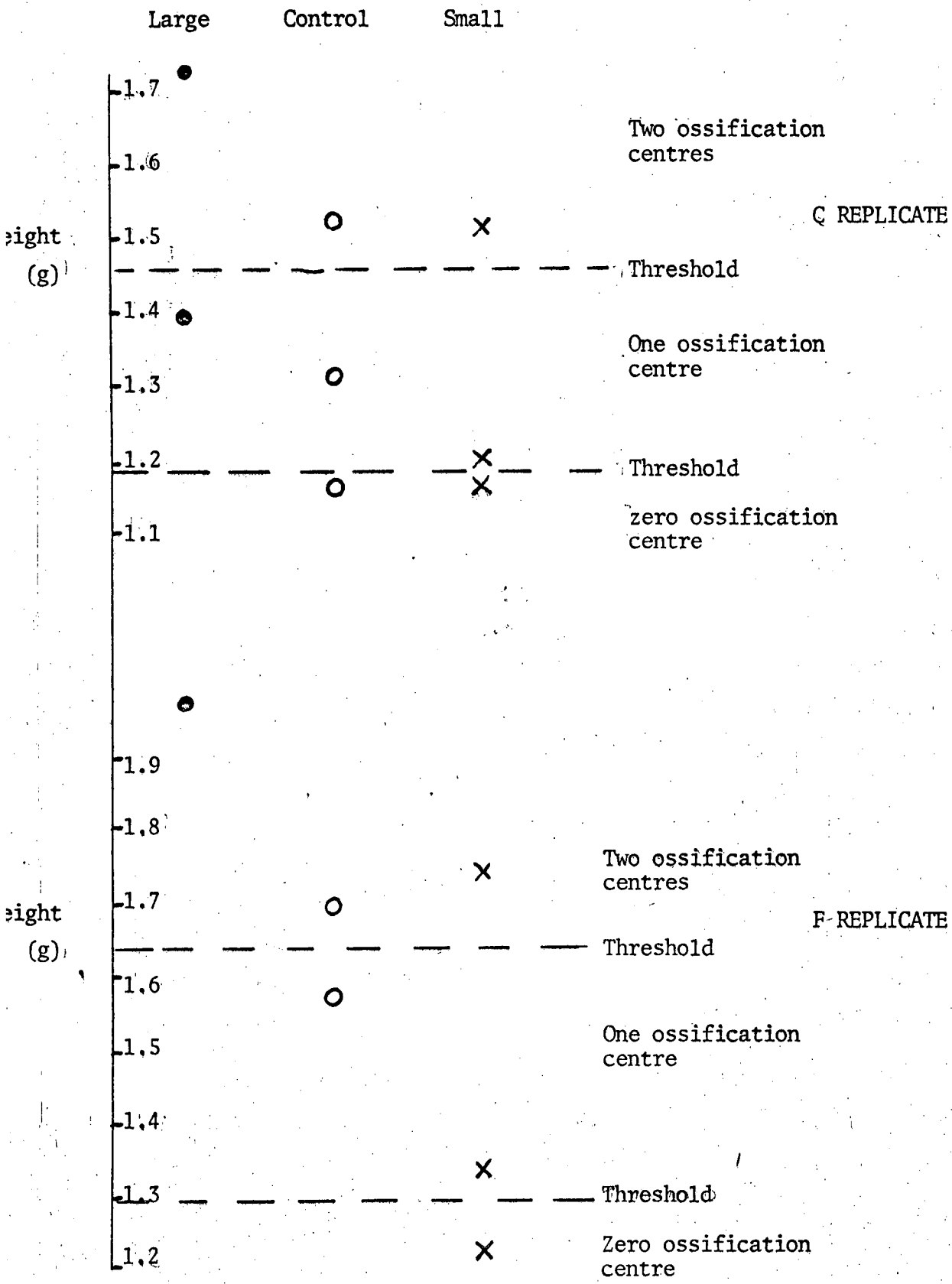


Fig. 17.

Mean Body Weights of Different Ossification Status
at Birth, with Theoretical Weight Thresholds.

of the sample with two ossification centres, or greater than the mean body weight of a sample showing no ossification centres. The mean values for body weights of the different ossification classes were plotted to ascertain whether, for each replicate, the presence of a weight threshold could be refuted by the data. The graphs, Fig. 17, show that the data is compatible with the existence of thresholds for each replicate, though it must be conceded that the horizontal lines drawn to indicate the zone where a weight threshold might lie might well have been drawn differently to support other hypotheses.

In the earlier section it was suggested that selection caused age limits in development to be reached which caused selected animals at extreme speeds of development to differ significantly in weight from controls developing at the same speed. Fast developing large mice were significantly heavier than control mice of the same developmental status, while small mice of this status do not much differ from controls. Where numbers were sufficiently large the *t* tests between body weights were made for similar ossification status at birth Table 30. The more advanced stage was two centres ossified.

TABLE 30. Comparison between Body Weights of Mice Showing Two Centres

	C Replicate			F Replicate		
	Mean weight(g)	s.e.	P	Mean weight(g)	s.e.	P
Large	1.72 ± 0.05		* *	1.97 ± 0.04		*
Control	1.52 ± 0.03			1.69 ± 0.03		
Small	1.51 ± 0.04		n.s.	1.74 ± 0.08		n.s.

There were insufficient numbers in the zero centres ossified to enable the comparisons to be made. At least, this data conforms with the situation found for development during suckling. For both replicates it is assumed that a weight threshold exists for the 'two ossification centres' stage, but these are different in the two

replicates. The higher growth rate of large would enable them and more of them, to exceed this threshold on the day of birth. The lower rate of growth of control mice would provide fewer in this category with a lower mean weight. Similarly the few mice from the small line growing fast enough to exceed the weight threshold and become 'two centre' mice do so by only a small amount. Consequently within this category large mice greatly differ from the other two lines, while control and small mice differ little.

Conclusion.

The data on bone ossification does not alone give sufficient evidence that body weight controls ossification by means of 'weight thresholds' as was described in Section I. However, the results conform with expectations and certainly do not refute the hypothesis. It is strongly suggested, then, that body weight is a co-ordinating factor in body development whether external events or skeletal ossification is considered. In order to obtain suitable data to estimate what the weight thresholds are, animals should be examined daily, e.g. by X-ray, and their weights collected at constant ossification status.

5. Comparison of Body Weight/Skeletal Length Proportions at Birth.

So far, the results suggest that at intermediate speeds of development selected lines do not differ in body weight very much: only at extremes of rates of growth is the relationship between body weight and development changed. The data on new-borns was used to find whether, allowing for scale effect by converting body weights to logs, the weight/bone length ratios differed in intermediate range of growth rates.

Data was provided by body weight and length of occipital bone at birth. For each individual occipital length/log body weight ratio was calculated in all three lines of both replicates. The individuals

TABLE 31 Regression of occipital length/log body weight ratio
on body weight among three lines in both replicates

<u>Line</u>	<u>LC</u>	<u>CC</u>	<u>SC</u>	<u>LF</u>	<u>CF</u>	<u>SF</u>
Regression coefficient	-1.53	-2.36	-1.62	-2.59	-1.92	-1.54
s.e.	0.18	0.24	0.33	0.21	0.17	0.13
P	**	**	**	**	**	**
Ratio at 1.56g.	0.45	0.44	0.46	0.45	0.46	0.46

chosen for measurement were all those whose birth weight fell in the same range, i.e. 1.4g to 1.8g. For each line the regression of the ratio on the individual arithmetic body weight was calculated - Table 31. All regressions were significant. Using these regressions, the predicted values of the ratio for a common body weight was found. The ratios are almost identical among the different lines. From this comparison it is evident that the ratio is constant for the same body weight among the different lines. What differs is the mean value for body weight among the lines. This conclusion is consonant with those arrived at earlier - that at intermediate levels of development there are few differences between the lines and body weight appears to be a unifying factor in determining development. Selected lines diverge from this by either greatly increasing or decreasing body weight without much change in development.

6. General Conclusions

Evidence is presented for weight-dependence of development during suckling for observable events related to tooth eruption and eye opening and also for bone ossification. This situation is similar to that at puberty, the main source of variation in age being environmental.

For any event there exists a range in ages between which the event may occur. Between these limits the age at which the event occurs is determined by the attainment of a threshold body weight. These generalisations hold for unselected mice, and are represented by Montiero and Falconer's ideal curves - Fig. 18.

Selection for body weight alters growth rate so that the limits in the age range for the events are encountered by a proportion of the population. For these individuals the events occur within the same age range, but at body weights which do not any longer demonstrate the threshold characteristics of unselected mice. Individuals in the

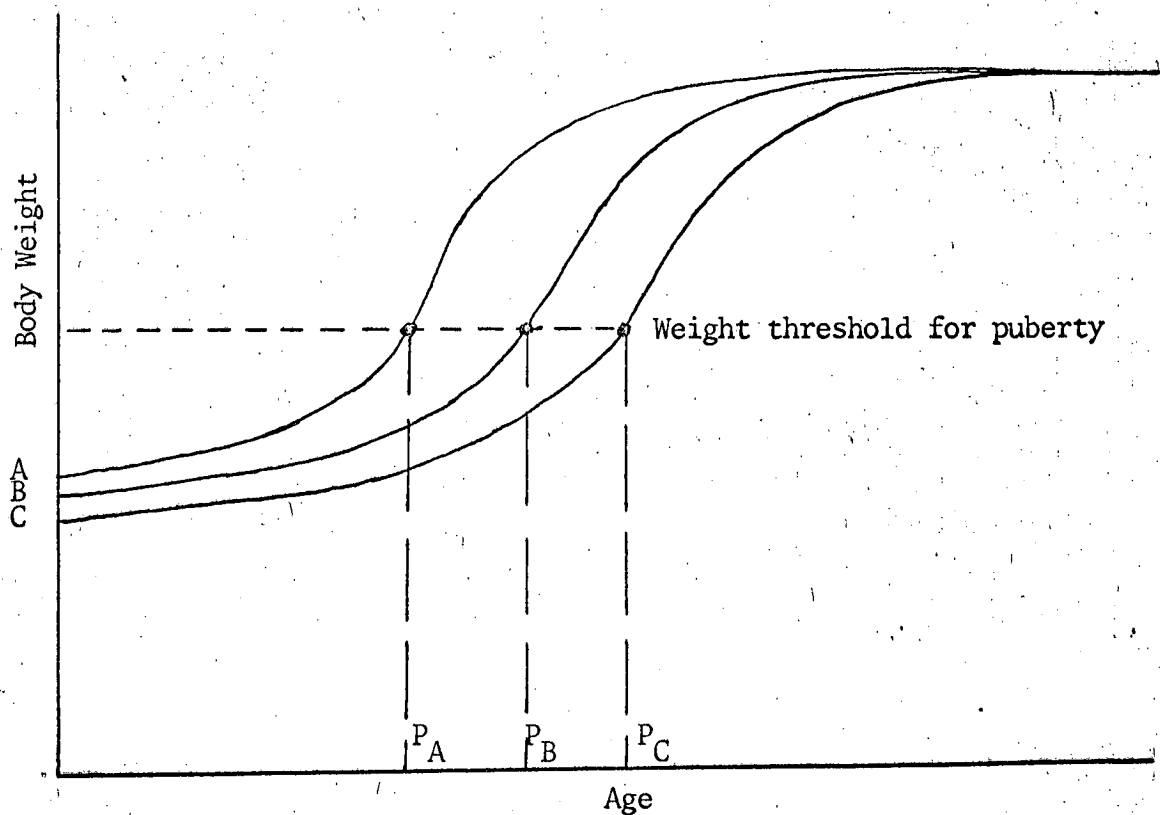
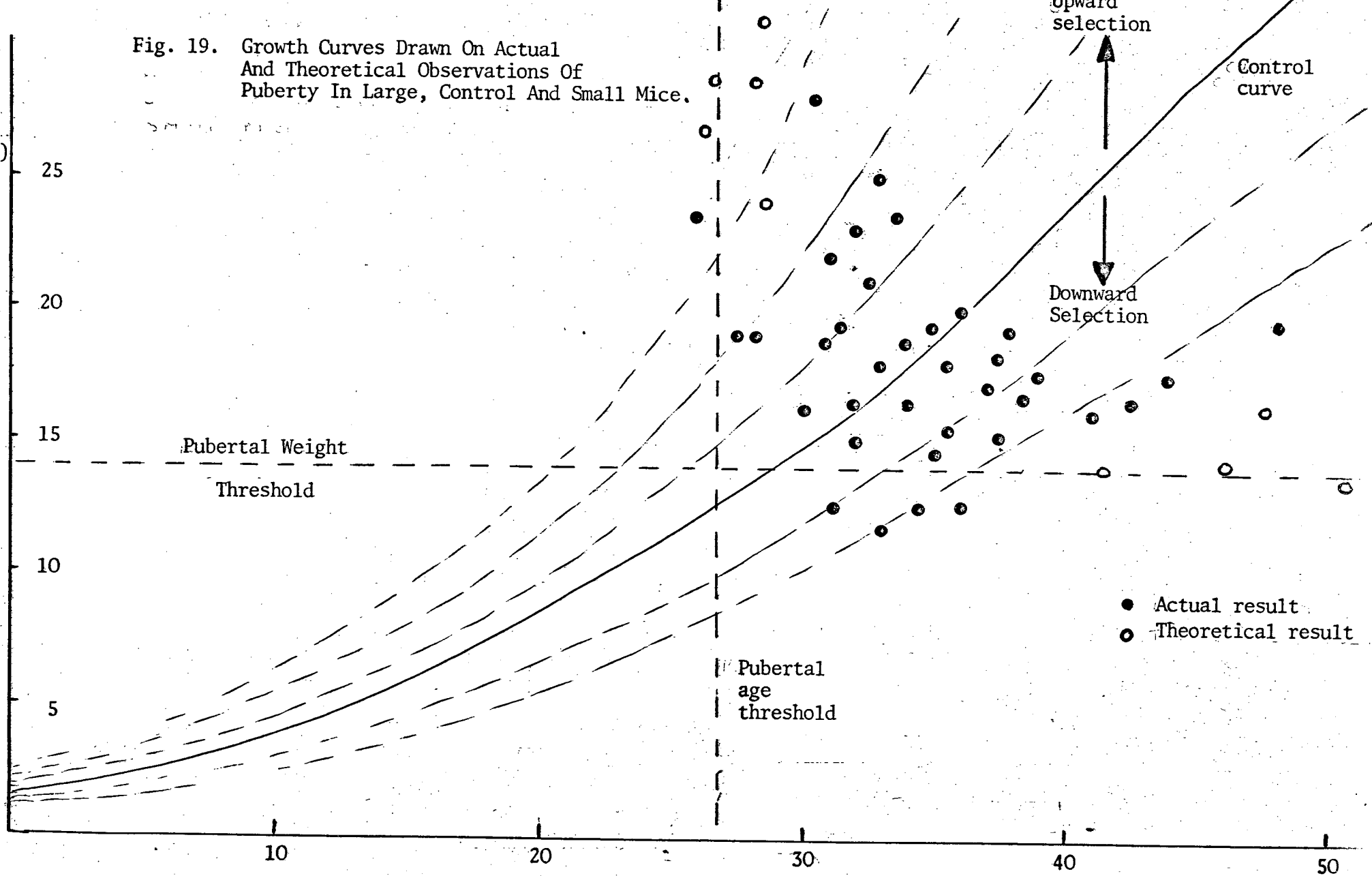


Fig. 18. Idealised Curves for Three Individuals Growing at Different Rates. (After Montiero and Falconer, 1966).

Individual A matures earlier than B or C.

Fig. 19. Growth Curves Drawn On Actual
And Theoretical Observations Of
Puberty In Large, Control And Small Mice.

ht(g)



selected lines growing at intermediate growth rates show the same association of development with a body weight threshold.

Using the real data for puberty, a series of theoretical curves can be drawn demonstrating the growth and development of selected and unselected mice and the effect of a weight threshold and age limit on these processes - Fig. 19.

Similar curves could be drawn for the events observed during suckling. There is an indication that environmental variation found in unselected mice causes a zero correlation between body weight at any stage of development and age at that stage, while any tendency to extremes of growth rate cause the association to be a negative one, as shown by the theoretical graph.

This model may inform us about the discordant observations concerning growth and development in New Guinea and also about the secular trend in Western human growth. It is assumed that adult New Guineans are genetically smaller than Europeans.

In New Guinea it was observed that girls took a much longer time to reach puberty, and were smaller at that stage and as adults, than Europeans. During childhood, however, they erupted teeth earlier than Europeans and also at lower body weights. These contradictory observations may be resolved by comparing genetic and environmental components of variance during growth.

It is probably the case that body weight at various stages of development is positively correlated with adult body weight, between genetically different populations. Thus, populations of large adults show higher body weight at various stages of development than populations of small adults. Correspondingly, the body weights (or thresholds) being lower, they are probably encountered sooner in populations of small than of large adults. Therefore we would expect tooth eruption to occur, on average, sooner and at lower body weight in

populations of small people, e.g. New Guineans, than in Europeans. We would also expect puberty to occur at an earlier age and lower weight.

If we assume, now, that the growth curve of New Guineans has been depressed for environmental reasons, the change in mean age of tooth eruption would not be very great (as predicted from the mouse experiment) although the positive association between growth rate and age at eruption could still be demonstrated. A small delay in the mean age at tooth eruption of New Guineans might not, therefore, cause it to be prolonged until it reached the mean age appropriate to the larger Europeans.

The variance of developmental age at puberty was greater, especially for the population of mice which was selected for lower growth rate, than at earlier stages. Contrariwise, the variance for age at puberty in fast growing mice was greatly reduced at puberty. One could predict, then, that the differences in environment between New Guineans and Europeans would cause the mean age of puberty in the former to be greatly delayed and to occur at a lower weight, and that for Europeans to be greatly reduced and to occur at higher weights. Improvement of New Guinean environmental conditions would be expected, therefore, to cause little change in mean body weight at the different events, a diminution of the difference in age at puberty between them and Europeans, and an increase in differences between them and Europeans for age at, e.g., tooth eruption.

Regarding the secular trend in Western Man, it appears that the increase in growth rate is (predictably) associated with greater size among younger people measured at any immature age, but not necessarily a pronounced change in adult body size. Gradually, environmental improvement causes increased growth rate to be reflected in earlier

occurrence, either of tooth eruption or age at menarche, or in larger measured size of the adult.

The presence of limits in time to the range of ages when these developmental events occur in mice suggests that limits of a similar nature may be found in human growth and development. The secular trend in maturation would be expected to cease when the variance of developmental age for a population was reduced to a low value associated with a mean which lay close to the lower age limit for the event. The trend in body size, on the other hand, might be expected to continue after the trend in maturation has ended.

Contrariwise, populations living under environmentally sub-optimal conditions may be recognised by their delayed maturation and high variance for development time.

CHAPTER IV

SECOND EXPERIMENT

1. Preliminary

The data so far has consisted of body weight and observation of developmental status, these observations of status being readily accessible. Regarding the progressive elongation of the skeleton, which follows the commencement of ossification, it is impractical to collect body weights on the day that different animals reach certain length in various skeletal measurements. The investigation was carried out, therefore, by collecting body sizes at fixed ages and making comparisons between lines at these ages over the entire range of life.

In an earlier section it was shown that litter size exerted an effect on the subsequent growth and development of mice. Litter size was, therefore, controlled to the extent of reducing litter size to six at birth without supplementation. Thus, individuals at the lower extreme of development rate were eliminated from all lines. Two replicates were chosen for investigation, the C and F replicates, being those which showed the greatest difference in their response to selection. Mice were sacrificed at selected ages between birth and one hundred and twenty days in order that comparisons could be made between lines at different points during growth. Following weighing, skeletons were extracted from the carcasses and measurements were made on several bones and teeth. In order to represent as much of the

skeleton as possible the bones selected for measurement derived from widely spread regions. The measurements taken were as follows:-

BONES

Lengths

1. Basi-occiput
2. Scapula
3. Humerus
4. Tibia

Diameters

5. Humerus, mid-shaft
6. Femur, mid-shaft

TEETH

Lengths

1. First molar
2. Second molar
3. Third molar

Comparisons were made between lines to ascertain (1) at what age the differences in weight between lines were established, (2) whether the differences in body weight were accompanied by a proportional change in the size of the skeleton and (3) whether different parts of the skeleton were all equally affected by the alteration in body weight.

TABLE 32. Estimation of Measurement Error

	<u>Occipital</u>	<u>Scapula</u>	<u>Humerus</u>	<u>Humerus dia:</u>	<u>Tibia</u>	<u>Femur dia:</u>
Error as % of mean	1.9	0.89	0.95	5.5	0.18	2.6

Measurement errors were calculated by measuring a sample of all bones twice. The mean difference between the two sets of measurements was then shown as a percentage of the mean value - Table 32. The bones measured twice were the entire sample of individuals in the C replicate sacrificed at eighty days. Errors may be larger in the younger mice since the bones are smaller and less easy to handle.

2. Effect of Selection on Body Size Measured at Fixed Ages

Mice were compared at five different points during growth to assess

what degree of change had been brought about during the foregoing period. The ages for comparison chosen were:-

- (a) birth, in order to indicate differences in pre-natal growth,
- (b) three successive ages between birth and twenty days in order to indicate differences in total maternal environment,
- (c) forty days, to register the differences present at the age when selection was carried out, and
- (d) at eighty and (e) one hundred and twenty days, to show what changes were present when most of the growth had been completed.

Comparisons were made between mean values for sub-lines, the number of animals contributing to each mean being between fifteen and twenty-two. The basis of the comparison was the percentage difference between the control lines in the two replicates. At each age of comparison the percentage difference between the selected and control value was compared with the replicate control's difference. In this way the amount of change brought about by selection and random influences was compared with that caused by random influences alone. The results are shown diagrammatically in Figs. 20 to 29.

Deviations of mean values of selected lines from controls are expressed as percentage differences. Large mice were thus proportionately bigger than controls at all ages and small mice proportionately smaller. Instead of plotting the percentage deviation of small mice as negative values below the X axis, they were plotted, with the large mice, as unsigned deviations from controls.

On the same graph the deviation of the F control replicate from the C replicate is plotted at the several ages.

The resultant graphs show how the selected lines become progressively different from controls over the time period of one hundred and twenty days. and also by comparison with the inter-control (C · F) deviation, how much of the difference between lines might be ascribable to random effects.

Of interest is the period, during growth and development, when the differences noted among adults first become established. Especially the contribution of the maternal environment to the final difference is noted.

2. Effect of Selection on Body Size at Fixed Ages (Figs. 20 - 29)

During the suckling period both large lines demonstrate a period where their body weights approach the control value, that is to say the effects of selection on body weight become less apparent. This diminution in the effect of selection occurs earlier in the F replicate than the C replicate, at five days rather than fifteen days. Thereafter the response increases rapidly to reach a plateau, which is higher in the F than the C replicate, at forty days, the age of selection. Both large lines show little increase from forty till eighty days, whence the F, but not the C, replicate shows a gradual decline. At one hundred and twenty days the response is almost identical in both lines.

The large lines are similar in the respect that most of the response occurs in the thirty days which follow an initial decline in response during the suckling period, i.e. from about 18 to 48 days. They differ in the timing of this pre-weaning decline in response, it occurring about ten days earlier in the F replicate.

The downward selected lines, in contrast to the large lines, show increasing divergence from the control value during the entire suckling period, the degree of response being less at all ages following

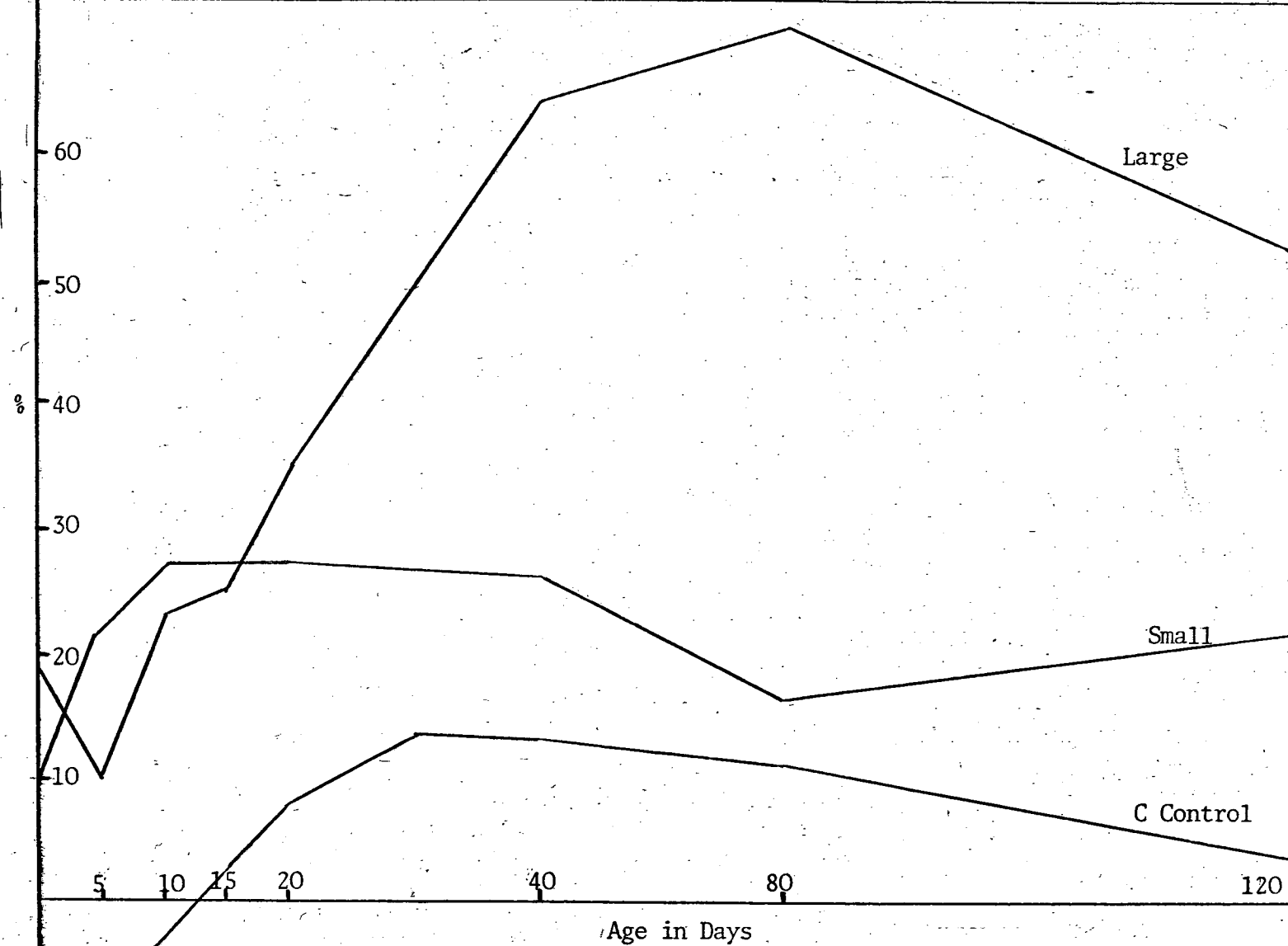


Fig. 20. F Replicate, Percentage Deviation in Body Weight From Control

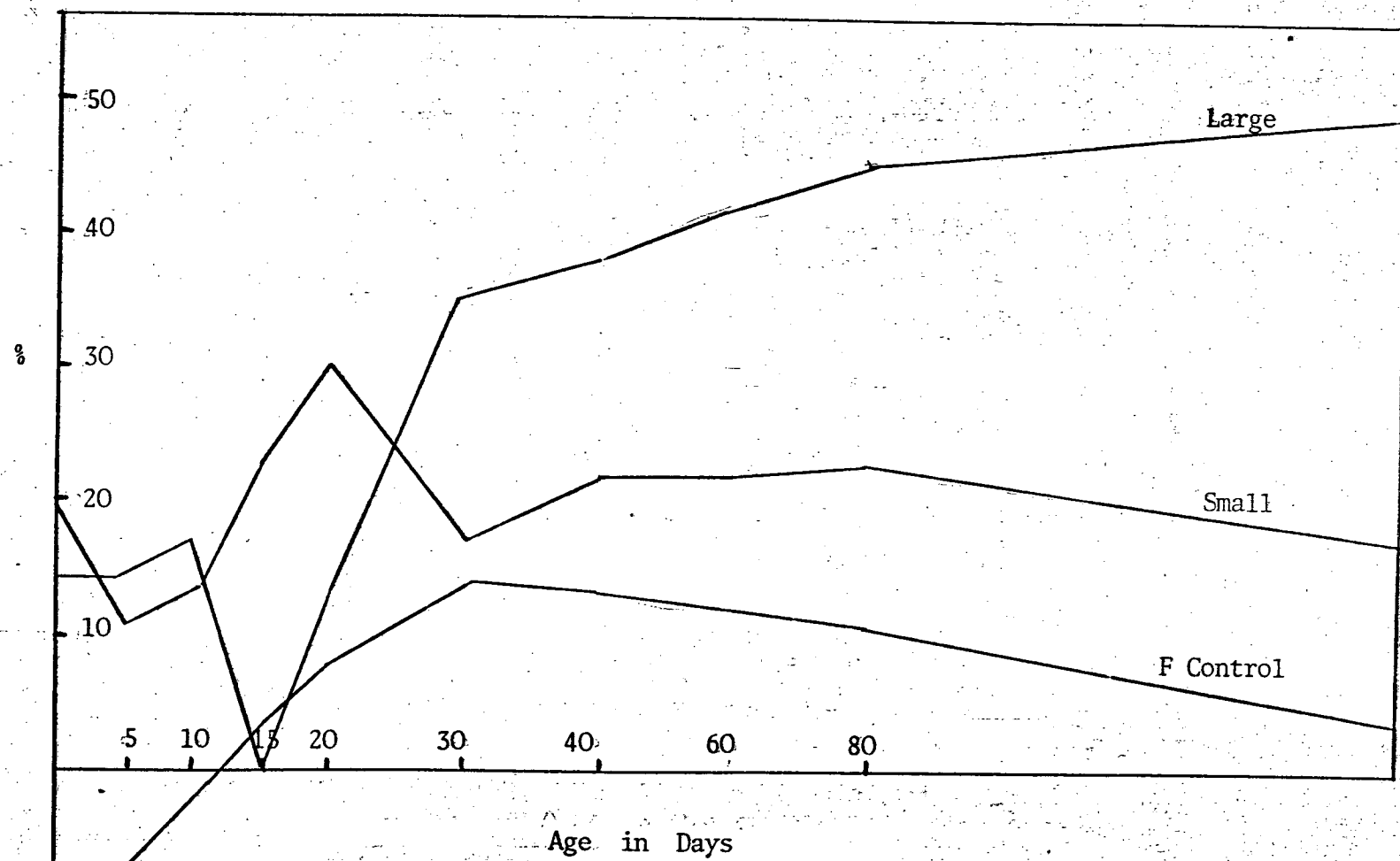


Fig. 21. C. Replicate, Percentage Deviation of Body Weight From Control.

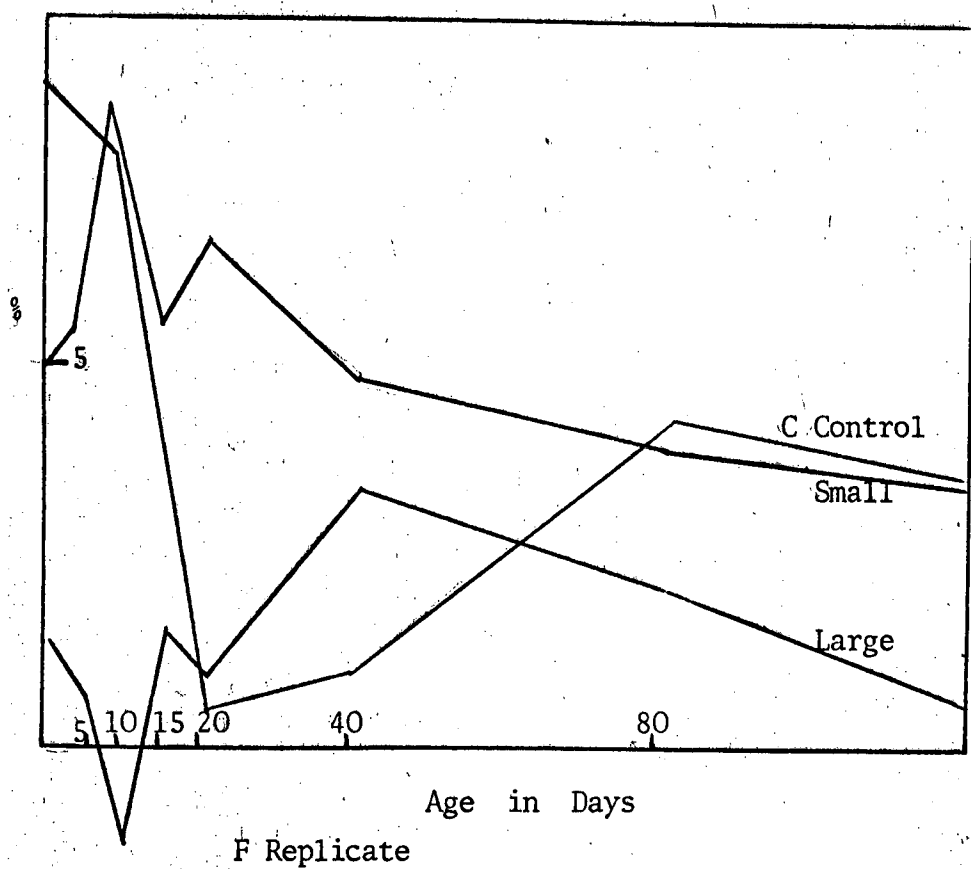
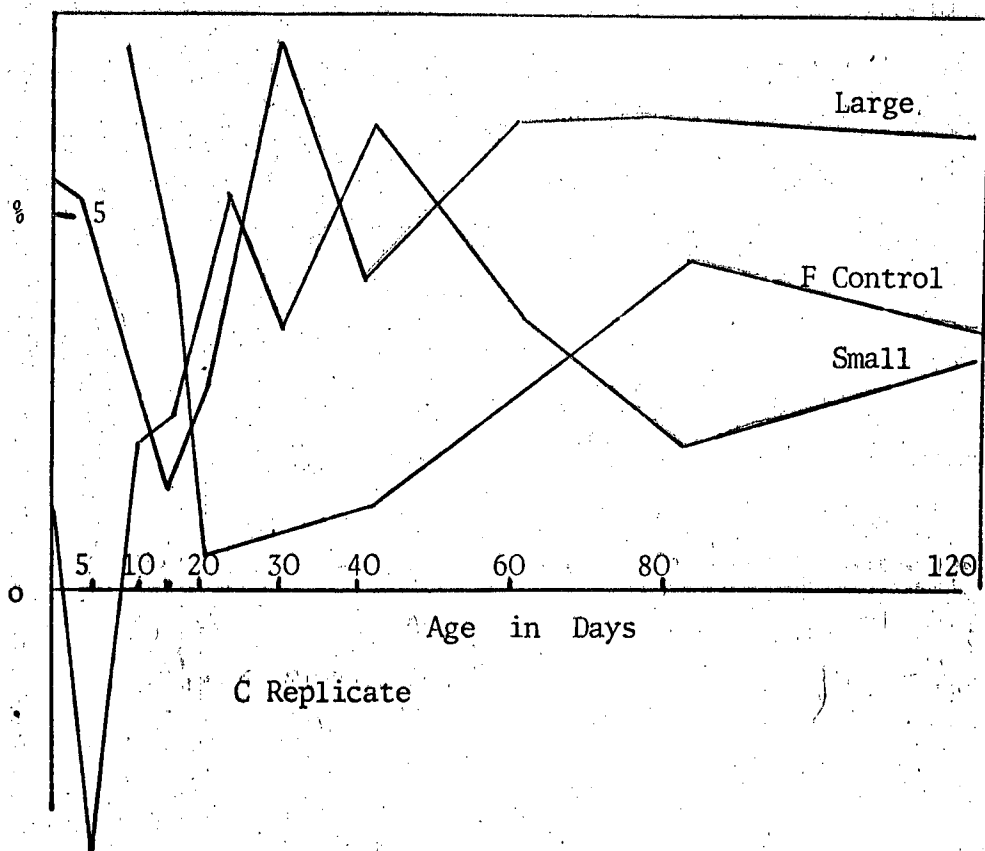


Fig. 22. Occipital Length, Percentage Deviation from Control.

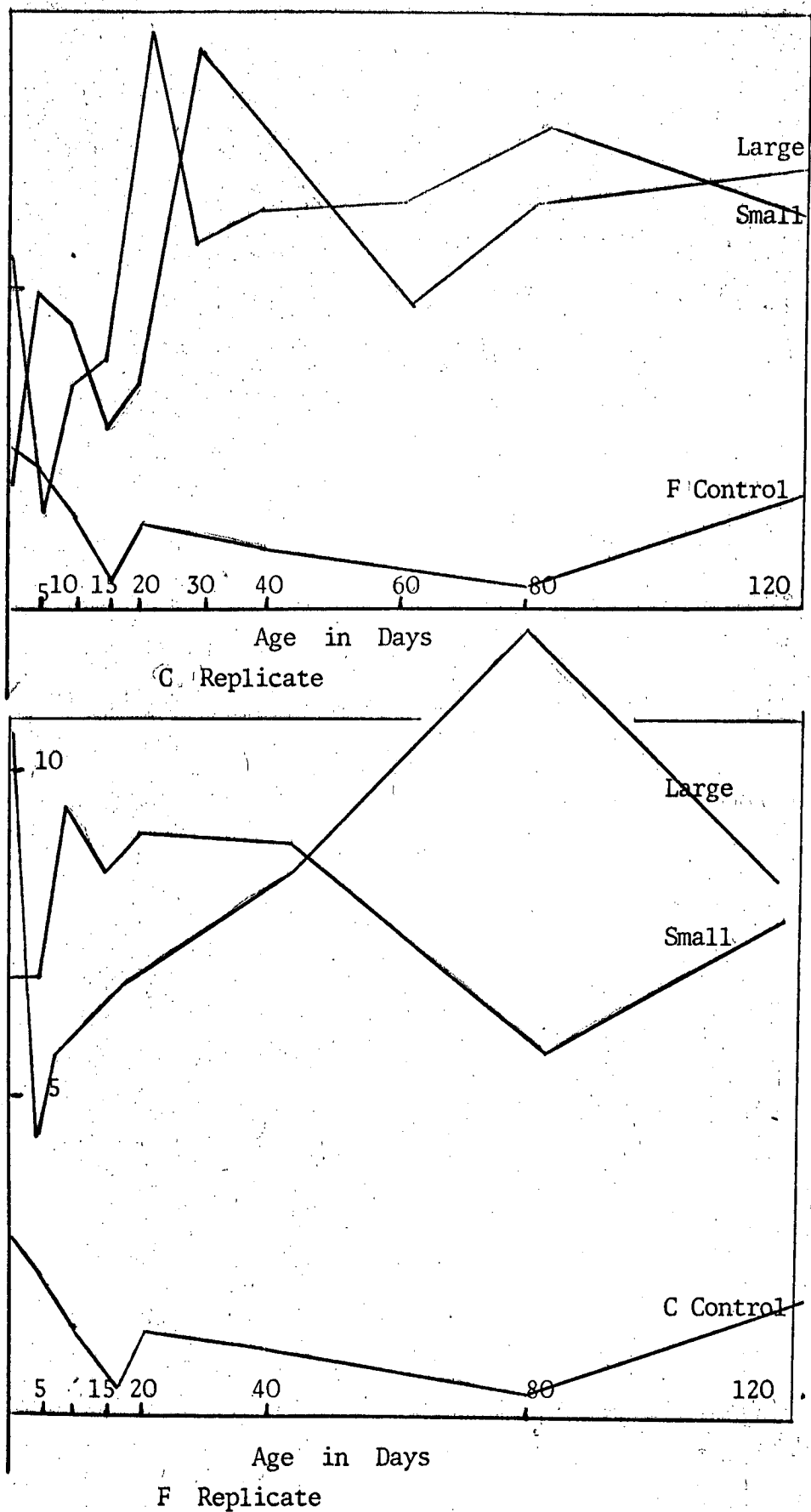
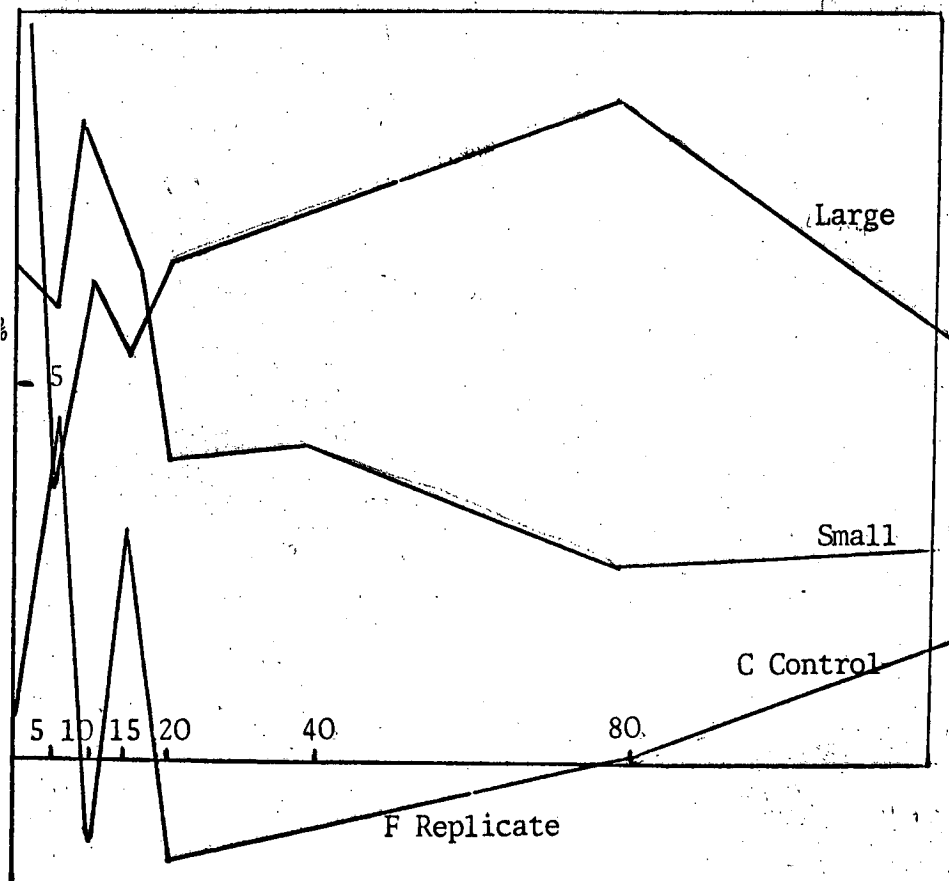
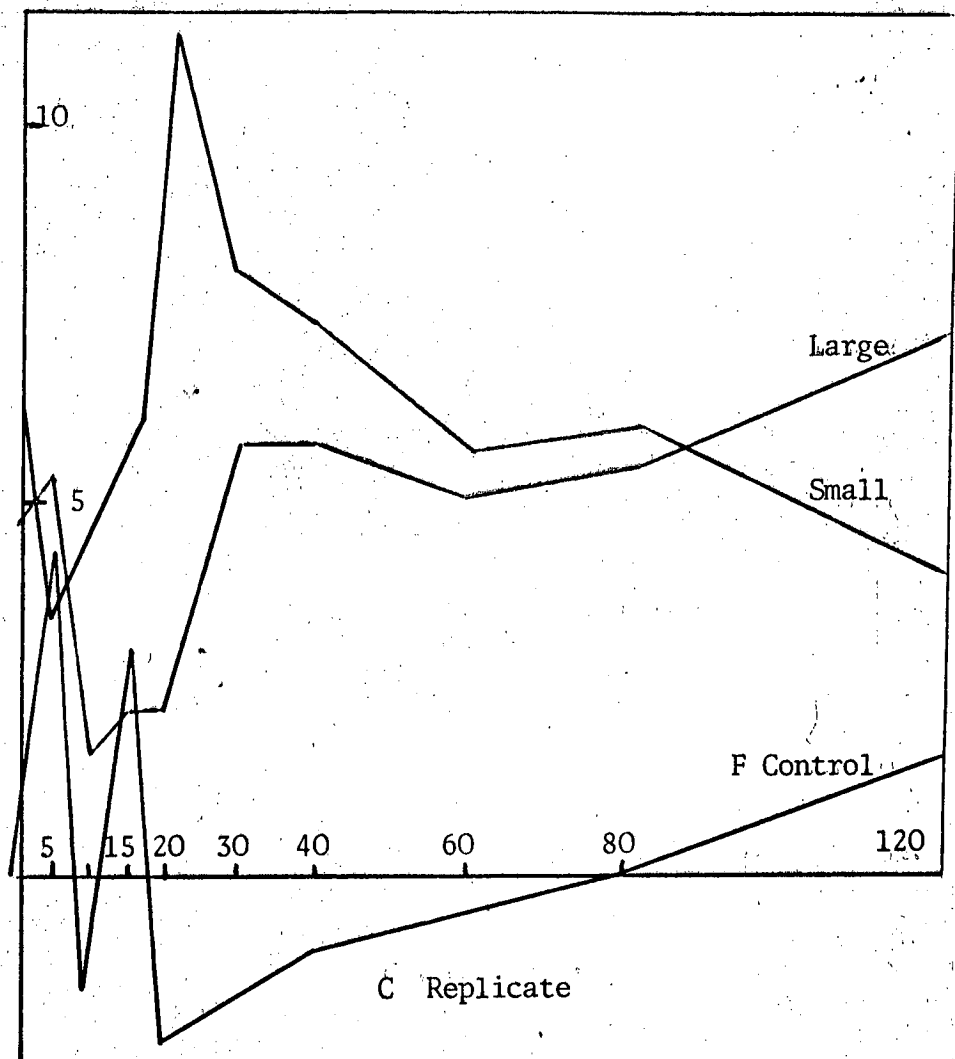


Fig. 23. Scapula Length, Percentage Difference from Control.



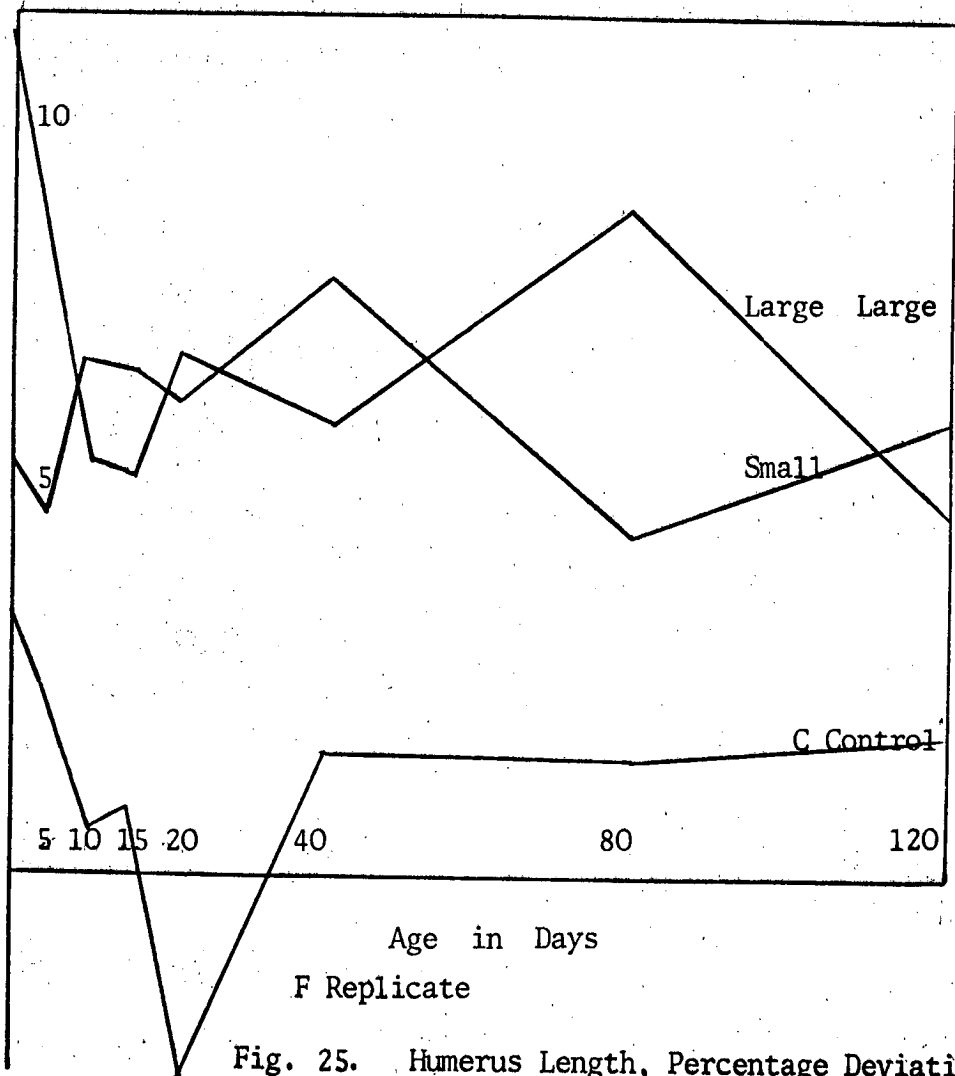
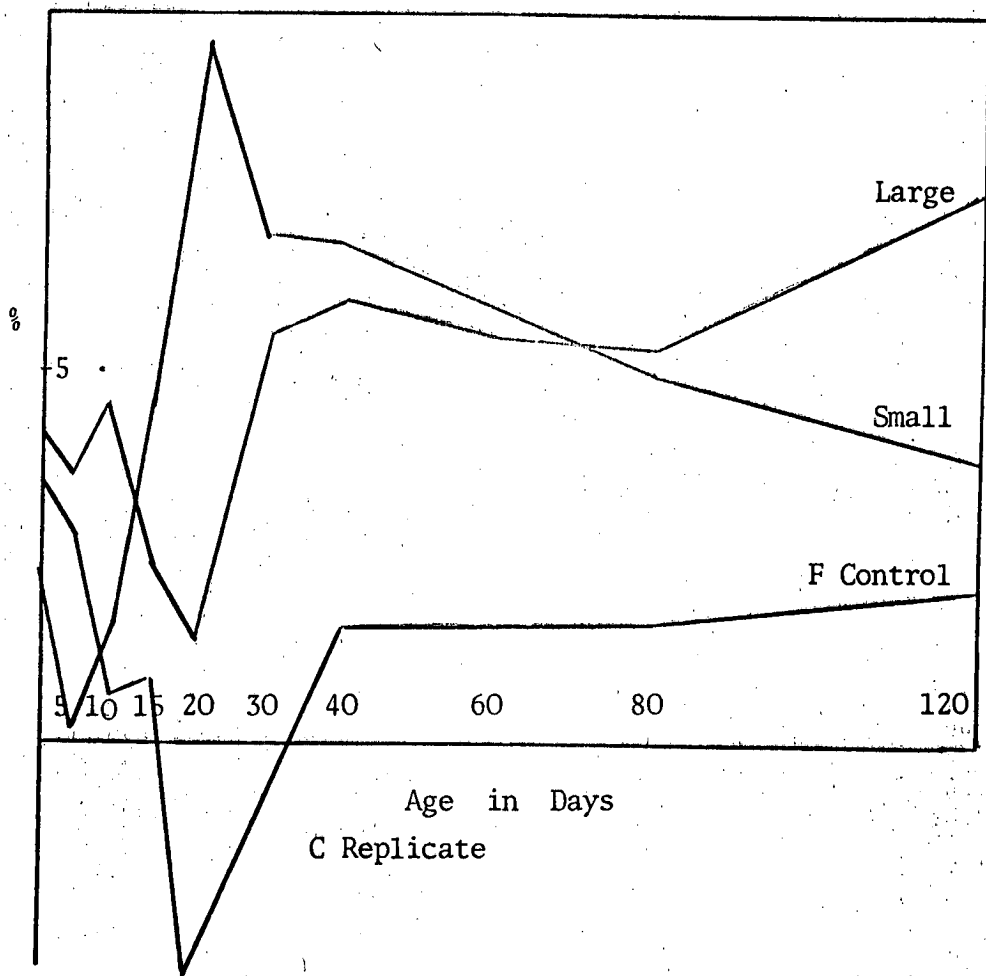


Fig. 25. Humerus Length, Percentage Deviation From Control,

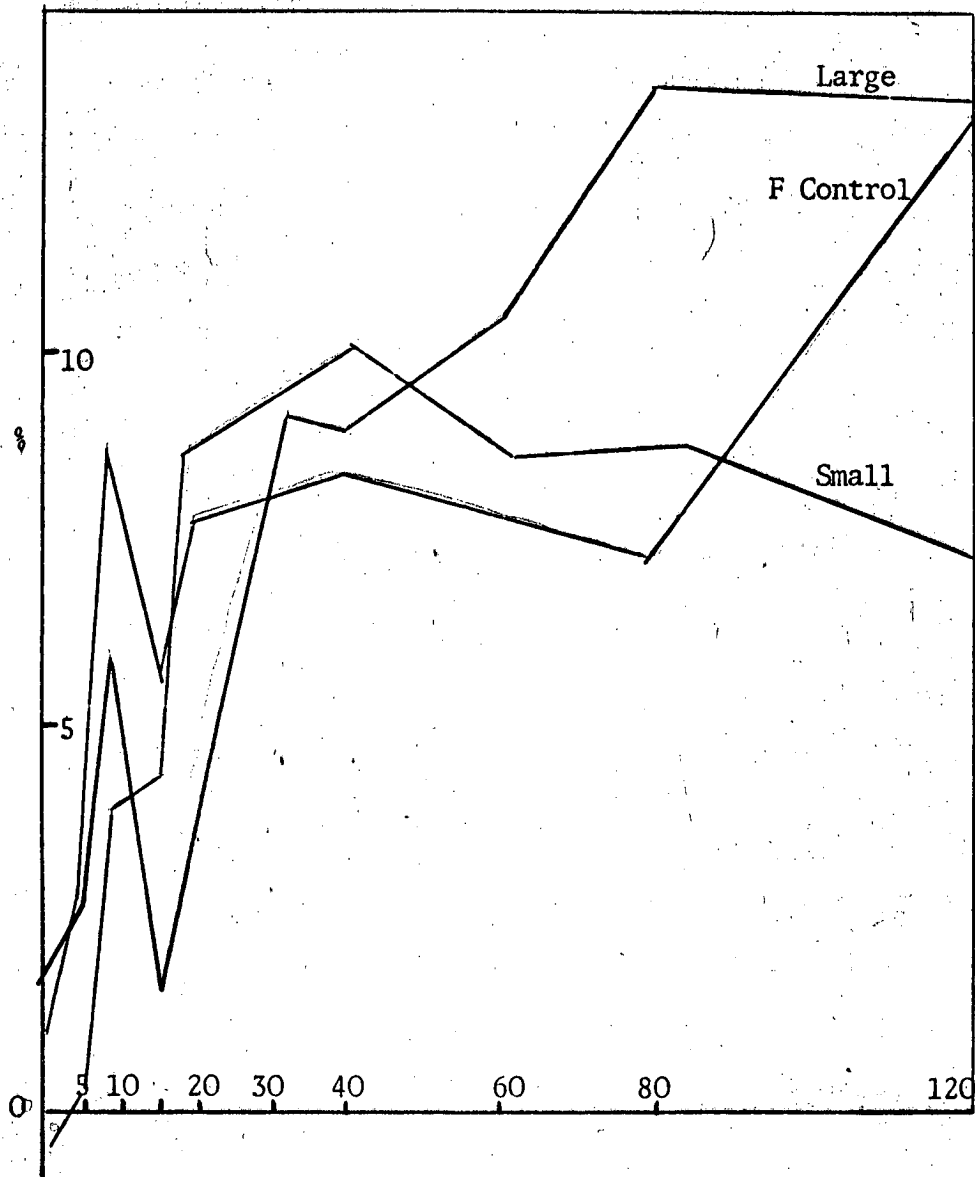


Fig. 26. C Replicate, Humerus Diameter Percentage Deviation from Control,

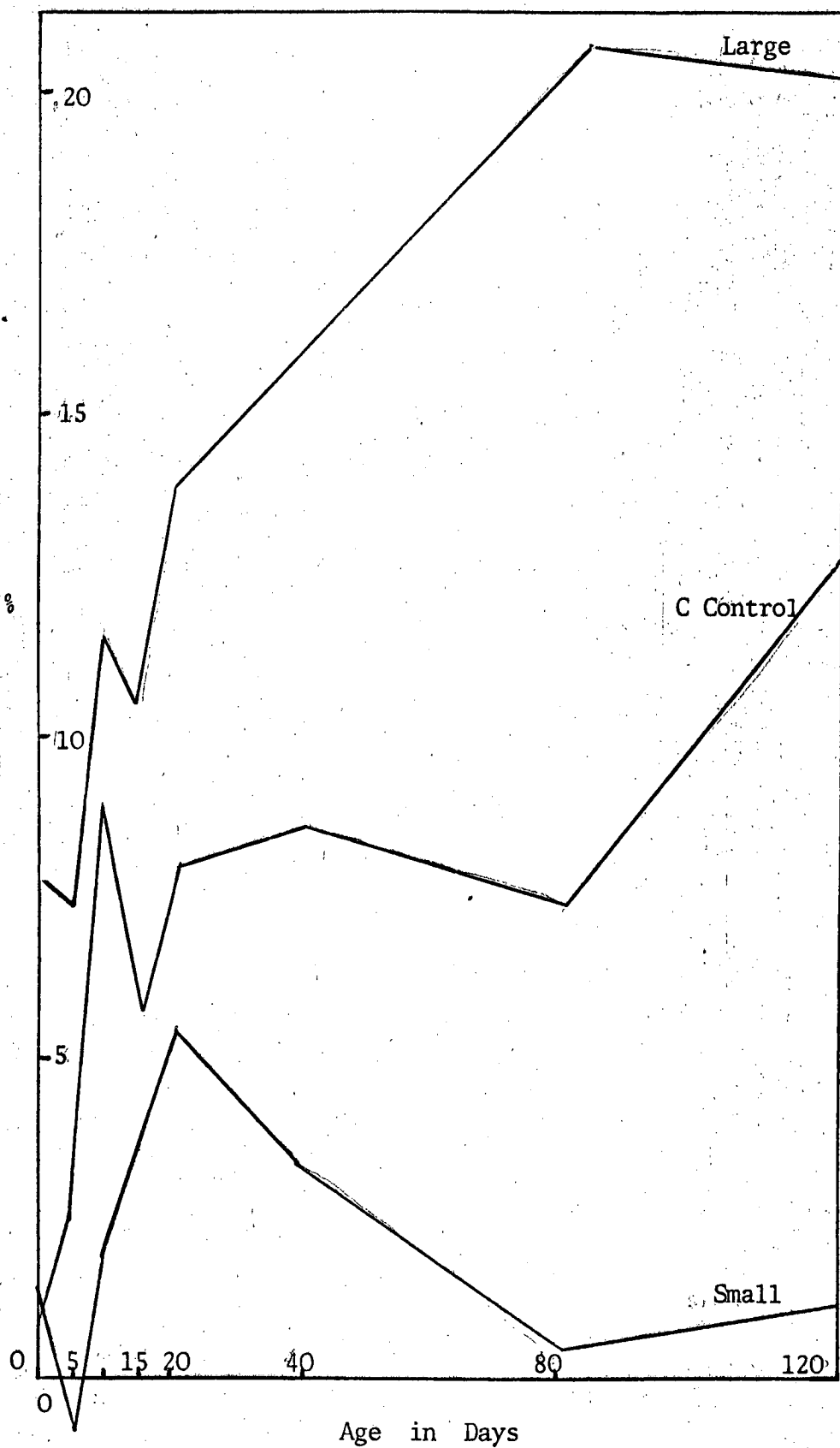


Fig. 27. F Replicate, Humerus Diameter Percentage Deviation from Control.

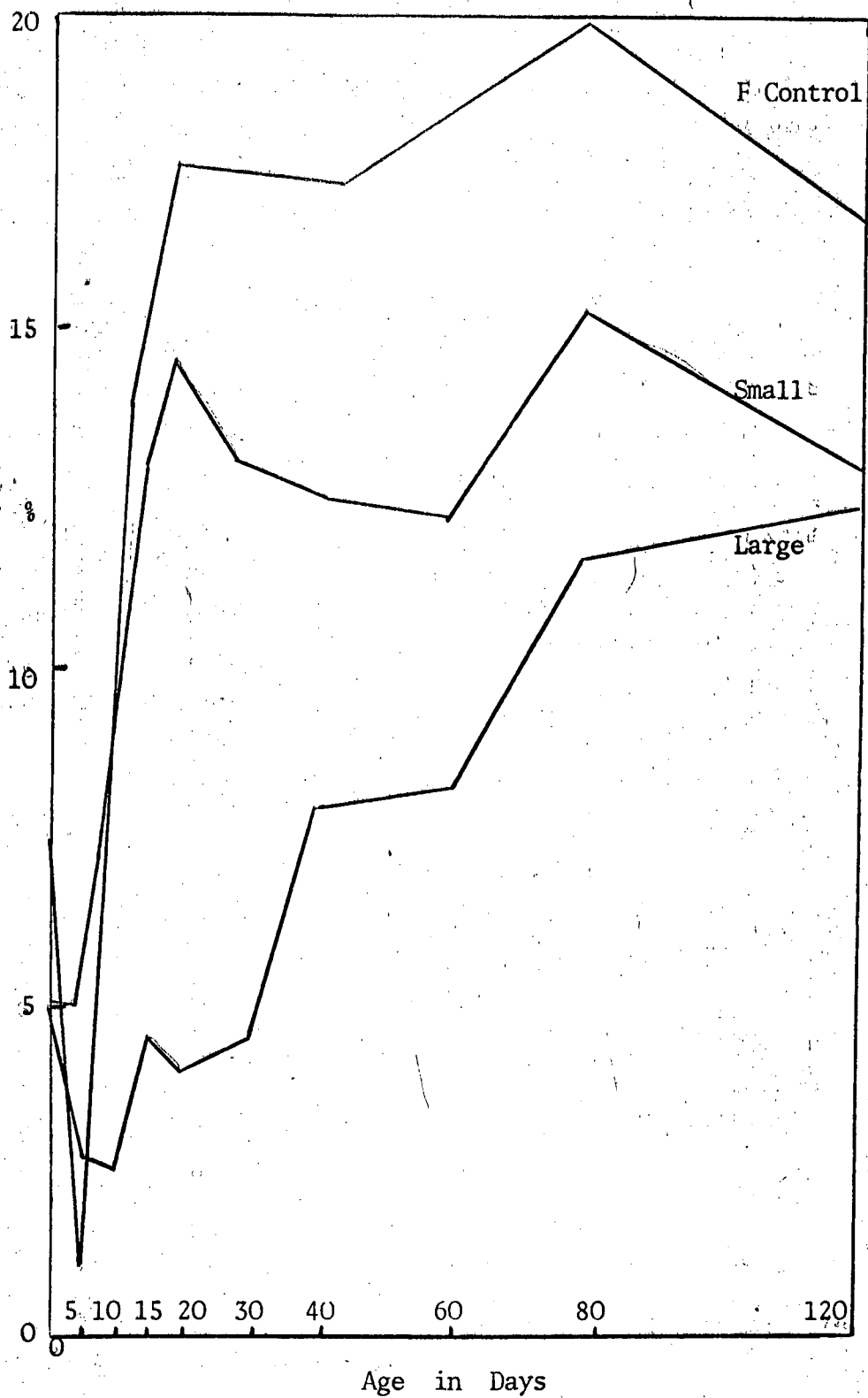


Fig. 28. C Replicate, Femur Diameter Percentage Deviation from Control.

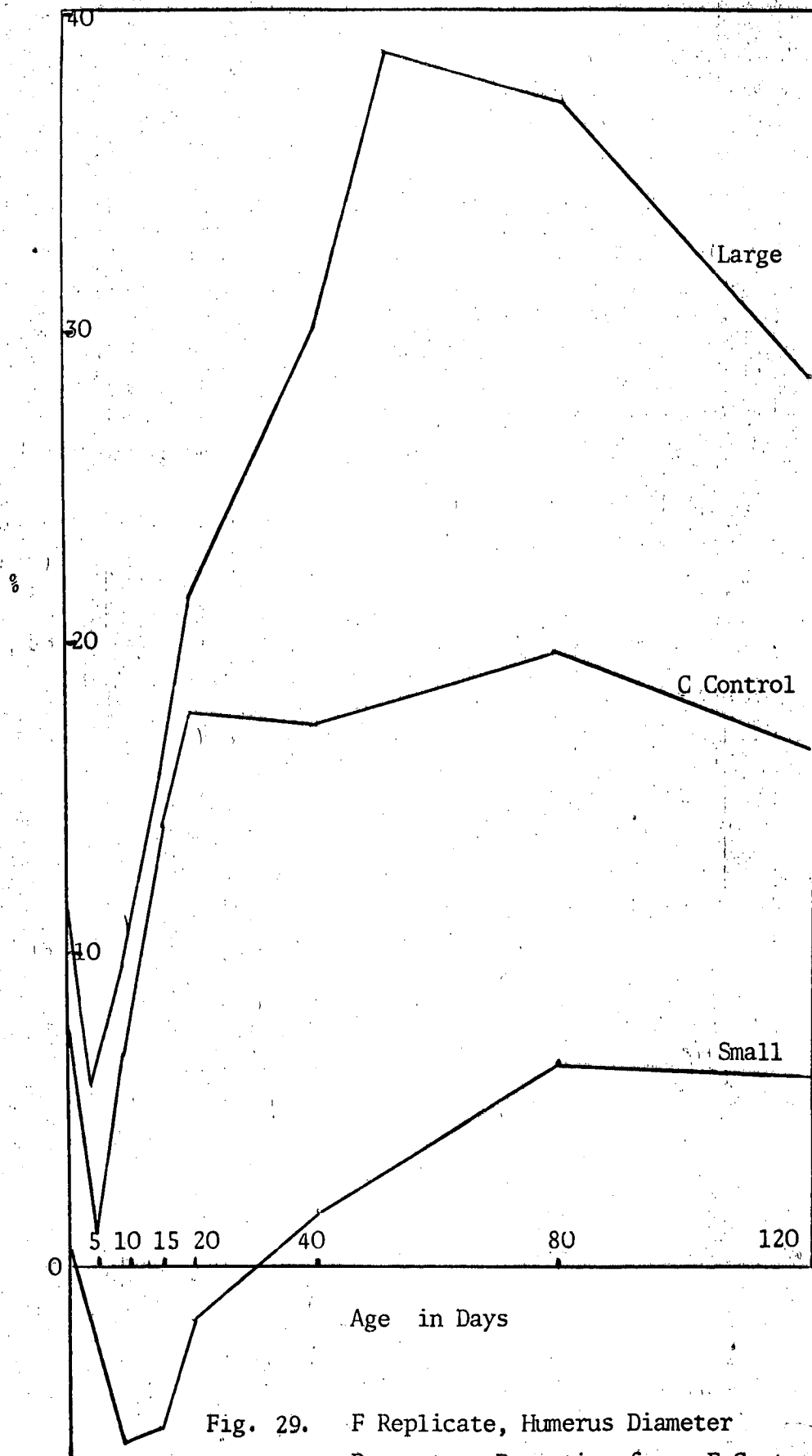


Fig. 29. F Replicate, Humerus Diameter
Percentage Deviation from F Control.

weaning. As in the large lines, however, the responses are not greatly different during most of the adult period, and are almost identical in both replicates at one hundred and twenty days. The replicates differ in the same respect as do the large lines; the maximal response occurs about ten days earlier in the F replicate than the C replicate, i.e. at ten days rather than twenty days.

The direct response in the F large replicate is much greater than in the C large replicate although both are very similar at one hundred and twenty days. Both the direct (40 day) and final, indirect, (120 day) responses are very similar in the small lines.

Conclusions

Selection for large size has the indirect effect of improving pre-natal performance, although post-natal performance is not immediately capable of sustaining this improvement. This relative inadequacy of the suckling performance occurs earlier in the F than the C replicate. Since body weight during suckling reflects mainly maternal performance, it appears likely that these differences reflect difference between mothers in the two replicates. Mothers in the large C replicate are poorer during the second week (10 to 15 days) of suckling than F mothers. After fifteen days most of the large mice of both replicates would have access to alternative sources of food since both incisor and molar teeth would be in occlusion. Therefore one would expect the selection response to occur independently of the mediation of maternal performance. Since C mothers have performed worse than F mothers up until this age (fifteen days) the mice from the C replicate are lighter, i.e. more resemble control mice. They therefore enter the peri-pubertal period of growth acceleration at a lower weight and at the end of this period do not reach as high a body weight as F mice.

In the C replicate, selection for small size appears to operate indirectly upon the maternal performance up to fifteen days, since it is during this period that the greatest response begins. Since small mice only become capable of making use of solid foods a day or two later than large mice, due to later eruption of molar teeth, the fall-off in weight gain for these lines is prolonged until the end of the suckling period.

In the F replicate, the indirect effect of selection operates upon maternal performance from the day the litter is born. Weight-gain diminishes compared with controls for the first ten days, reaching a plateau of response not encountered by the small C mice until the end of the suckling period. Since the peak of response, about 30%, is not exceeded earlier or later in either line, it may represent a limit to the amount body-weight may be reduced without threatening viability.

The relative importance of pre- and post-natal maternal performance is also suggested here. Post-weaning performance is not much worse in the small lines than the difference occurring randomly between two control lines. Most of the response in the adult size is brought about during the suckling period and may to some extent be ascribed to changed post-natal maternal performance.

Pre-natal performance in the large lines is greatly altered, but the degree of alteration diminishes at first during the suckling period, indicating that post-natal performance during the first week or so is not greatly affected by selection. Maternal performance during the second suckling week is different between the two large lines and differently affected by selection. During the last week of the suckling period mice are progressively more independent of maternal performance and the maximal selection response occurs during

this second two-week period. That is to say, the total selection response is less during the first three weeks of growth in the large line than in the small line, and more during the later period of independent growth following weaning.

Effect of selection on skeletal dimensions.

As was the case with body weight, the percentage difference between selected and control lines was compared with the percentage difference between the two replicate controls at each of the ages chosen. There was great variation among the different longitudinal skeletal measurements and also between the replicates; moreover, the results for bone diameters differed considerably from all the other results. Despite the differences, however, there are certain consistencies in the patterns of difference which distinguish large from small mice in both replicates.

The skeletal dimensions may be classified according to their selection response:

- 1 Occipital lengths.
- 2 Post-cranial lengths.
- 3 Bone diameters.

1. Occipital lengths. The differences between the control lines from the two replicates is considerable at birth, 8-9 per cent, falling to less than 1% at twenty days, whence it rises slowly to 4% at eighty days. This gradual change reflects quite closely the change in time in body weight between the two control lines, being greater at birth than at weaning and rising thereafter slowly.

The selected lines are more similar to their own control value at birth than the control lines are to each other. During the period studied the lines behaved inconsistently, both small lines, for instance, ending up very similar to their controls at one hundred

and twenty days. No line of selection exceeded six per cent in the size of its response, neither do they resemble the pattern of response in body weight.

2. Post-cranial bone lengths. Tibia, scapula and humerus show the same general pattern of response to selection and, although the replicates differ, the direction of selection operates similarly in both. All of these dimensions reflect the changes seen in the body-weight comparisons.

The differences between the two control lines do not exceed four per cent at birth and, following weaning, seldom exceed two per cent.

In the large lines the greatest response is about eight per cent in both lines and for all skeletal measurements. As for body-weight, most of this difference from control size occurs during the ten days following weaning. Also, during suckling period the maternal performance in the large lines shows a decline earlier in the F replicate than in the C replicate.

The small mice also show the same pattern of change in bone dimensions as was the case for body-weight. The final difference is between three and eight per cent, most of this response occurring before weaning. Moreover, the pre-weaning peak in selection response occurs earlier in the C replicate than in the F replicate, as was the case for body weight.

3. Bone Diameters. Diameters of bones show no consistency either between lines of selection or between replicates. The two control lines differ markedly during most of the period measured, both in the femur diameter and humerus diameter. The replicate controls differ little at birth but show a considerable difference, 9%, after ten days, which increases to 13% at one hundred and twenty days.

Despite the dissimilarity of the control bone diameters to the

body-weights of these same mice, there are some similarities in the response to selection between body weight and bone diameters. As noted above, so also here, upward selection produces a response most of which is accomplished during the period following weaning. Downward selection, on the other hand, produces a greater response during the suckling period than is measurable afterwards.

Within these patterns of similarity, however, profound differences in degree of response are noticeable. In humerus diameter the response is much greater in the F large line, 21%, than in the C large line, 14%. For this dimension in the small line, the response of the C replicate, 9%, greatly exceeds that of the F replicate, 3%. These differences are also present in the femur diameters, the F replicate showing an upward response between 30% and 40% compared with about 12% in the C replicate, and the small line showing a greater response in this replicate, 14%, than in the F replicate, 6%.

The greater response in the large F replicate than the C replicate reflects the greater body-weight of the F large mice. The decline in response during the last forty days is also reflected in the bone diameters.

The association between body weight and bone diameter is not seen in the small line. Body weight response differs between adults of the two replicates, but their one great difference is the response of bone diameters, 10% - 14% in the C replicate against 5% - 6% in the F replicate.

General Conclusions

With some exceptions, body weight and skeletal dimensions show a similar pattern of response. The expected change in bone lengths can be calculated using the formula $\frac{W^3}{L}$ which expresses the constancy of the relation between weight and linear dimensions. Using the data from new-borns, when the difference in weight and skeletal

response was smallest, the ratio of (body weight)³ to scapula length was calculated using this formula for the control population of the F replicate. The constant was 15.1. Body weight had been increased in the large line and decreased in the small. On the expectation that the ratio would not change, the lengths of scapulae in the selected lines were calculated - Table 33. Skeletal lengths have not responded proportionately with body weight, there being a twenty-one per cent lag in the response of large mice scapulae and a twenty-five per cent lag in response in the small line.

TABLE 33. Comparison of Ratio Between Lines Based on Constant (Weight)³/Length Ratio

LINE	LARGE			SMALL		
	Expected	Observed	% Diff:	Expected	Observed	% Diff:
Scapula length	0.38	0.30	21	0.20	0.25	25
Ratio $\frac{(\text{Weight})^3}{\text{Length}}$	15.1	19.6		15.1	12.0	

This discrepancy increased with the passage of time.

Conclusions.

1. Body weight responds to selection more readily than skeletal measurements with the exception of some bone diameters.
2. Selection for weight gain has a greater total effect than selection for weight loss.
3. Among bones, occipital measurements respond less, and in a more unpredictable way, than long bone measurements, which generally reflect body weight.
4. Upward selection has a greater effect on the pre-natal maternal environment than downward selection.
5. Downward selection has a greater effect upon post-natal maternal performance than upward selection, most of the final selection responses occurring during this period.

6. Upward selection has a greater effect upon the growth of mice post-weaning than downward selection, most of the final selection response occurring during the days following weaning.

7. The replicates differ in the timing of the responses to selection, divergence from control mice occurring earlier in the F replicate than in the C replicate for both large and small mice.

8. It is possible that the timing of this response in the large line may be related to the size of the final response, a response occurring earlier in the suckling period engendering a greater peak in response in early adulthood, but not necessarily a greater final response at one hundred and twenty days.

3. Selection Response on Skeletal Length Measured at Fixed Weight

The previous section deals with comparisons made at fixed ages and shows how differences between the lines become established during growth. The different lines reach maturity, however, at different speeds, therefore it is necessary also to make the comparisons at the same fixed body weight in order to see whether the differences in size present at fixed ages are reflected in the skeleton at fixed weights.

Since animals were sacrificed at the same age rather than the same weight, the estimation of their sizes and ages at similar weights had to be obtained from a comparison of mean weight-gain curves. A smooth curve was drawn connecting mean weights from birth to a hundred and twenty days. The curves for the three selected lines of each replicate were drawn on the same page and four weights from the Y axis were chosen for comparison of the three lines. A similar set of mean curves was drawn where the Y axis was weighted, but the X axis was the lengths of the occiput and tibia. A horizontal line was drawn through the weights 10g, 19g and 27.5g (the last weights in the small line) and 33.1g (the last weight measured in the control line). The ages

TABLE 34

Measurement Error in Interpolating Graphs-

<u>% of Mean</u>	
Tibia	1.8%
Occipital	1.0%

Direct Error in Measuring Bones-

<u>% of Mean</u>	
Tibia	0.18%
Occipital	1.9%

TABLE 35

Percentage Differences Between Lines at Fixed Body Weights

<u>C REPLICATE</u>		<u>Control Com- parison</u>		<u>F REPLICATE</u>	
	<u>Large</u>	<u>Small</u>		<u>Large</u>	<u>Small</u>
<u>AT 10g</u>					
Age(days)	13.8	57.2	3.6	21.4	78.5
Tibia(cm)	4.0	16.0	1.0	9.2	27.6
Occipital(cm)	0.5	6.7	3.7	4.3	6.4
<u>AT 19g</u>					
Age(days)	12.9	22.2	14.9	25.8	58.0
Tibia(cm)	1.0	1.0	7.6	7.0	7.0
Occipital(cm)	0.5	0.0	6.4	4.8	3.9
<u>AT 27.4g</u>					
Age(days)	34.9	179.0	20.9	46.0	131.0
Tibia(cm)	9.6	0.0	0.1	10.0	3.8
Occipital(cm)	5.7	4.2	2.9	9.6	2.9
<u>AT 33.1g</u>					
Age(days)	71.7			72.0	
Tibia(cm)	8.9			7.1	
Occipital(cm)	8.4			11.3	

were read off the X axis corresponding to the points of intersection of the drawn line and the mean curves. Similarly, from the bone length/body weight curve, the X axis yielded the mean lengths of the tibia and occiput at these weights. The curves were all drawn twice, on different sheets, and the values estimated separately for each set of curves. The differences between the two independent sets of estimation were insignificantly small - Table 34.

Controls

Comparison was first made between the two control lines - Table 35. During the pre-weaning period the F replicate gains weight slightly faster than the C replicate, but after weaning this situation is reversed. The C replicate reaches mature body weight up to 20% faster than the F replicate. The control lines differ, therefore, in their speed of maturation in body weight, though very little in final weight; the difference in body weight at 120 days is slight, 3.7%.

The consequences of the difference in rate of maturation in body weight are measurable in the skeleton. At any post-weaning age the C replicate is heavier than the F replicate. Measured at the same weight, however, the slower-gaining replicate has a longer skeleton than the faster growing. Both skeletal measurements show this difference although they are located in widely separate parts of the body, one being very close to the central nervous system, the other being distant from it, in the limb furthest from the central nervous system. As is the case with body weight, the differences in the skeleton associated with speed of weight gain become trivial once adult sizes are reached, and are almost immeasurable in comparisons with the C line at 120 day weight.

The control lines, then, differ in their rate of maturation, the slower-growing replicate being longer in its skeleton during immature

ages. As adults they differ very little in either body weight or skeletal size. Other evidence identifies maternal factors as most important in determining rate of maturation, so it may be the case that differences in growth between these lines primarily reflect differences between the replicates in their maternal environment.

Selected Lines

The large lines gain weight faster than their respective controls, the F replicate diverging from its control more than the C replicate. The F replicate large line reaches mature weight faster than the C replicate, although as adults they differ little in selection response. In as much as speed of weight-gain is determined by early environment, the F replicate experiences a greater selection response in its maternal environment than the C replicate.

The small lines, different to the large lines, show bimodality in selection response. The response during the suckling period is much greater in the small lines than the large, but around the period of sexual maturity, when a peak in growth rate is experienced in all lines (see below), the differences between small and control lines diminish. As adults, however, the response increases greatly, differences between small and control lines in time taken to reach body weight 27.4g being 179% and 131% for the two replicates.

Comparing the large with the small line it is evident, as was shown above, that the post-natal maternal environment is more seriously influenced by selection in the small line than the large. In the latter the effect of selection is more measurable after weaning.

The influence of rate of weight-gain on skeletal dimensions is generally the same as that occurring in the control comparisons. Fast weight-gain produces skeletal dimensions which are smaller at a given weight than dimensions of slower growing mice. In the large line these

differences diminish slightly during the period of puberty and increase during the adult period. Conversely, the small line has larger skeletal dimensions than control mice measured at the same weight. The response is much greater at immature ages than it is at later ages, and larger in the tibia than the occipital. The size of the difference, again, reflects the differences observed in the control comparison, the F replicate showing a larger selection response than the C replicate during immature ages, but little difference at 33.1g. At this weight skeletal dimensions are between 8% and 11% smaller in large mice than controls. At 19g, differences between selected lines and controls are smaller than at other weights, being all less than 8%. This body weight approximates the stage of puberty in these mice though it is reached respectively earlier and later in the large and small lines.

The selected lines differ, then, in the degree to which the skeletal response follows the response in body weight. In the large line the skeletal response reflects in a general way the response in body weight, which is also rather less at immature ages than mature ages, though some measurements are inconsistent in this respect. The small line, on the other hand, shows a considerable response at immature weights both in body weight and skeletal dimensions. This response diminishes after weaning, at 19g. Following puberty, however, the response in body weight increases greatly, while the skeletal measurements become more similar to controls.

Conclusions

These comparisons yield different kinds of information depending upon whether the period before or after puberty is being compared. At immature ages the comparison informs us about differences between maternal environments. The maternal environment of small mice delays weight-gain considerably, with proportionately less effect on the

skeleton. The maternal environment of large mice is affected considerably less and although therefore body weight-gain is less affected than small mice, the rate of growth of the skeleton is more affected than that of small mice, indicating that the relationship between body weight and skeletal development is different between the large and small lines.

Following puberty, differences between control and small mice in body weight and size reflect the fact that small mice almost cease to grow, while control and large mice are still putting on weight steadily. Therefore at older ages the response in small mice appears very large in respect of body weight. The skeleton, however, much more closely resembles control mice at equal mature body weights than at immature sizes.

Large mice show a response that steadily increases during the entire period. Control mice require seventy-seven days to increase body weight from 27.4g to 33.1g. The larger mice accomplish this in only six days.

In conclusion, the response in body weight in small mice is associated with severe impairment of the maternal environment and an earlier termination of growth, with a proportional change in the skeleton of about 5%. In large mice there is enhancement of the maternal environment and a considerable increase in the rate and duration of weight-gain following weaning and a proportional change in the skeleton between 5% and 10%. The changes produced are considerably different between the two replicates. However, it is consistent among all the lines that fast weight gain is associated with a smaller skeleton at standard weight than slow weight gain.

4. Selection Response in Skeletal Lengths Measured at Fixed Tibia Lengths

It was shown that the proportional change brought about in the

TABLE 37 Percentage Difference From Controls at Fixed Tibia Lengths

<u>Tibia Length</u>	<u>Replicate C</u>		<u>Inter- Control</u>	<u>Replicate F</u>	
	<u>Large</u>	<u>Small</u>		<u>Large</u>	<u>Small</u>
<u>0.75cm</u>					
Age(days)	0.0	7.9	0	3.4	15.9
Occipital(cm)	0.0	0.0	11.5	4.4	6.4
humerus(cm)	5.8	0.0	1.0	3.8	9.4
<u>1.00cm</u>					
Age(days)	0.0	18.4	4.8	8.9	10.4
Occipital(cm)	0.0	0.0	2.9	0.0	5.6
humerus(cm)	8.2	4.5	0.5	0.0	3.0
<u>1.25cm</u>					
Age(days)	4.5	30.6	3.6	10.9	8.7
Occipital	0.0	0.0	5.3	2.5	5.3
humerus(cm)	10.5	9.5	9.2	0.0	3.7
<u>1.50cm</u>					
Age(days)	9.5	40.5	2.7	18.9	21.0
Occipital(cm)	3.3	0.0	3.6	2.9	2.9
humerus(cm)	13.3	10.0	12.2	5.9	3.6

skeleton due to changed body weight differed at different stages of growth and was greater in the large line than the small.

In this section differences in the proportional change among three skeletal measurements were estimated by comparing the lengths of two bones at a fixed size for the third. Here, the lengths of the occiput and humerus were interpolated from growth curves plotting bone lengths against time. The lengths of these bones were estimated at ages corresponding to the following lengths in tibia length, 0.75cm, 1.0cm, 1.25cm and 1.5cm. Curves were all drawn twice and the interpolated values measured twice to obtain an estimation error - Table 36.

TABLE 36 Measurement Error in Interpolations From Graphs

<u>% of Mean</u>		
Occipital	..	1.9
Humerus	..	0.95

RESULTS - Table 37

Controls. The replicates differ by no more than 5% in the rate at which fixed tibia lengths are reached. This difference in speed of growth of the tibia is associated with a greater difference in rate of growth of occiput at immature ages, up to 11.5%, than in adult life 3.6%, and the reverse condition in the humerus which differs little at immature ages, 1%, and more during the adult stage, 12.2%.

Selected Lines. The large and small lines in both replicates show a difference from controls in rate of growth in tibia length which increases with body size, i.e. with maturity. The differences are greater in the small lines than in the controls, indicating that the skeleton is affected more in the small than the large line at a

fixed skeletal size.

Conclusion

The proportional change in occipital and humerus length reflect the differences shown in the inter-control comparison. But, since they do not exceed the inter-control difference at any tibia length it can be concluded that selection for six week body weight has had no significant effect on the skeletal body proportions, at least in respect of occipital and humerus length. Since these bones are respectively dermal and cartilaginous bones in origin and, moreover, widely separated in the body, it is a reasonable expectation that other bones would likewise show no proportional change in response to body weight selection.

5. Comparison of Incremental Growth Among Selected Lines

The differences observed by making comparisons between groups of animals at fixed ages or weights can be given prominence by making comparisons not of absolute quantities but of incremental growth.

The effect of selection upon rate of growth may be compared in two ways:

1. by making a comparison between the increments added to successive measurements over the same time span, in which case the comparison is between absolute quantities, or

2. by comparing the amounts added in successive periods, related to the size at the beginning of each period; this is the relative growth rate. Brody used the following equation to estimate the Instantaneous Growth Rate, K:

$$K = \frac{\log W_t - \log W_o}{\text{days}_{t-o}}$$

The second method enables comparisons to be made while, at the same time, taking account of differences in scale which might

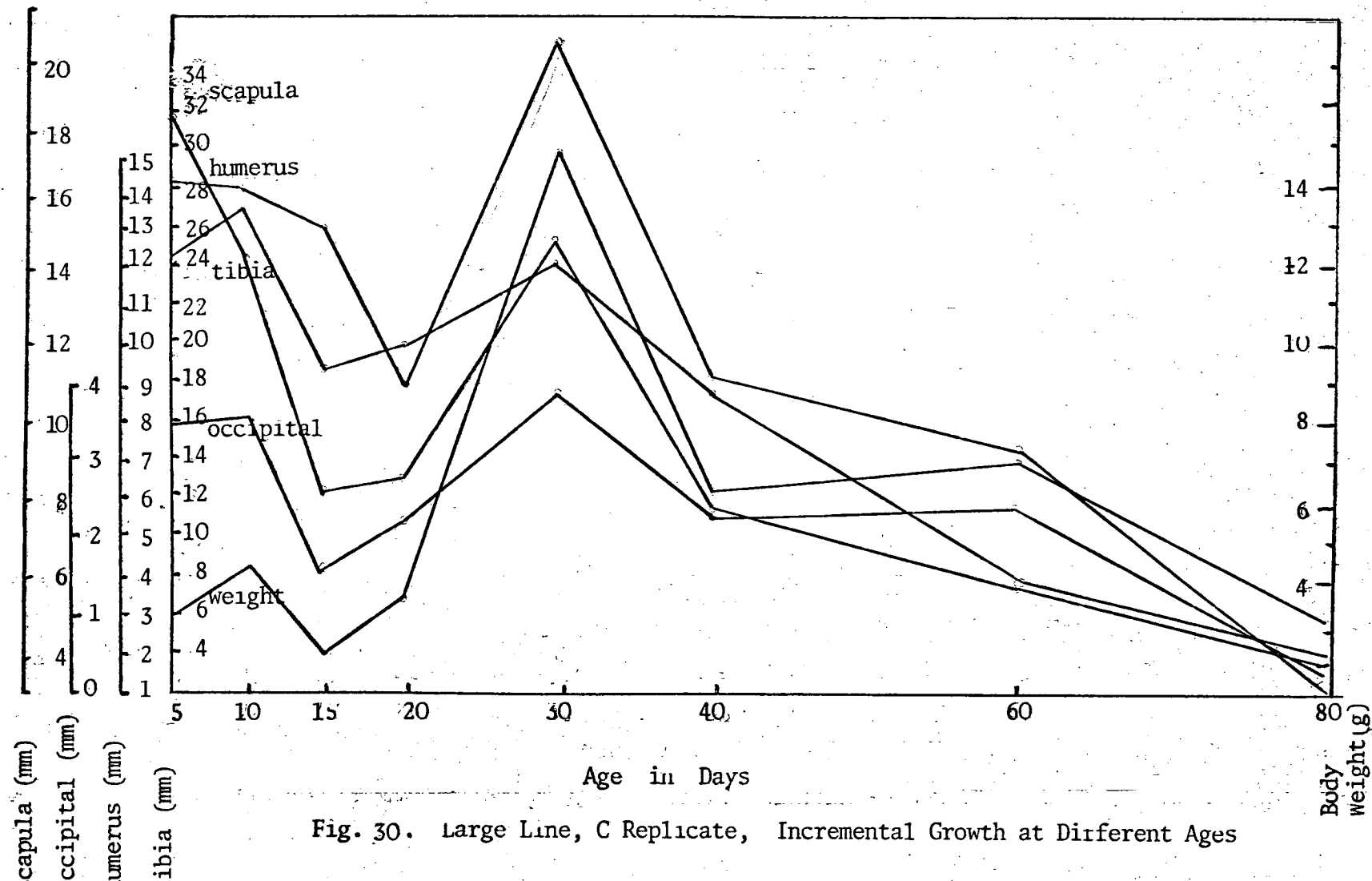


Fig. 30. Large Line, C Replicate, Incremental Growth at Different Ages

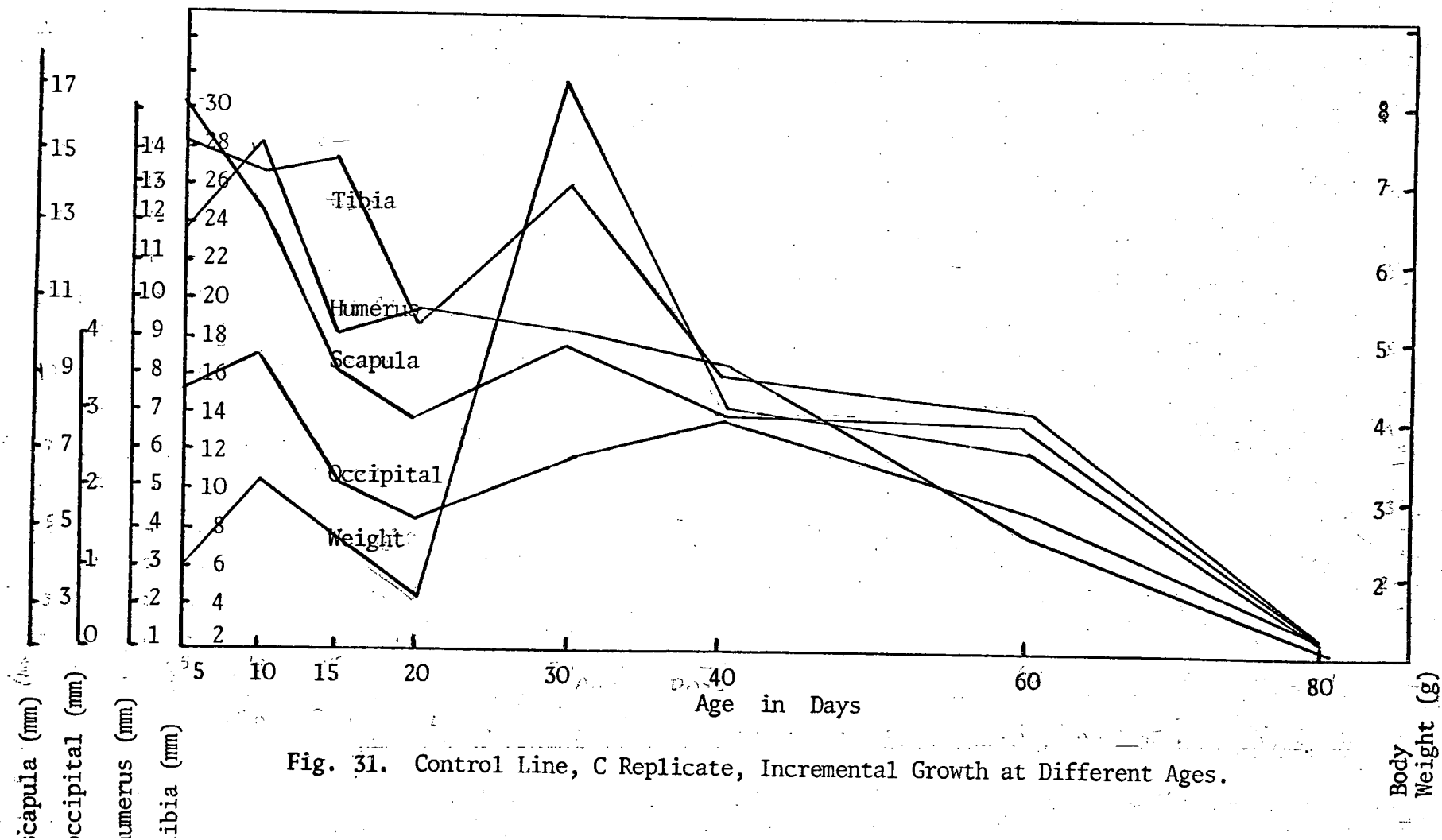


Fig. 31. Control Line, C Replicate, Incremental Growth at Different Ages.

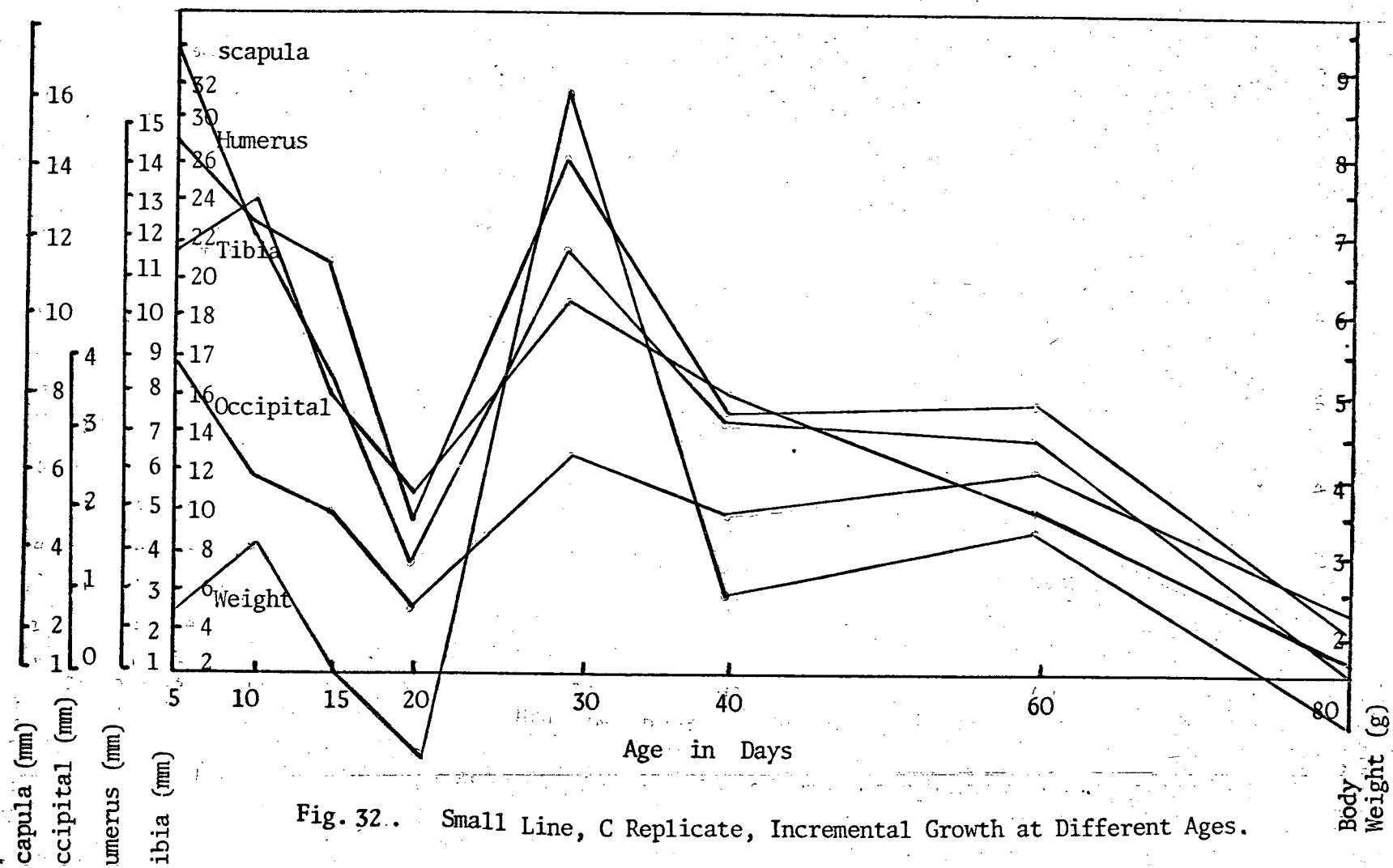


Fig. 32.. Small Line, C Replicate, Incremental Growth at Different Ages.

influence the comparison.

Comparison of absolute incremental gains.

The accompanying figures - Figs. 30 to 32 - show the increments, for weight in grams and for bone measurements in millimetres, added between the age in days for successive periods, for replicate C. The pattern of incremental additions over time is evidently very similar in all lines, both for body weight gains and skeletal enlargement.

Gains made in the first five days after birth establish a peak for most skeletal measurements, which falls off during the remainder of the suckling period to a lowest value during the second fourteen days. Following weaning there is a dramatic increase in incremental gains which reaches a second peak at about thirty days. After this, incremental gains fall to reach a second low point at the end of the period of examination, 120 days.

The skeletal increments demonstrate a slightly different pattern to that shown by body weight. During the period of puberty the second peak in incremental gains shown in body weight greatly exceeds that found during the first few days following birth. The skeleton, however, although showing a substantial increase in size at the time of puberty, does not increase in size by such large increments as were recorded at the beginning of the suckling period. For body weight, therefore, most of the weight gained during life is put on after weaning, while skeletal elongation occurs to a great extent during the suckling period. The changes in body weight following suckling might, therefore, be expected to register changes mainly in extra-skeletal components of the body.

Selection has had very little effect on the pattern of incremental gains over time, or on the relative degree of response among different skeletal measurements. Notable, however, is the difference

between the lines in respect of the effect of the maternal environment on growth. The period of smallest incremental gain in the skeleton is the five days before weaning in the control lines.

In the large line of the C replicate this occurs earlier, during the ages 10 to 15 days, and thereafter increments increase. The small line of the C replicate shows the low point in incremental gains occurring at the same time as the control line, but the low point is lower in the small than the control line. Correspondingly, the peak following this trough is higher in the small line than the control or large lines. All three lines of the F replicate show the lowest incremental gain occurring at the end of the suckling period, but, here also, the lowest period of growth in the small line is lower than that in the control. In the large line of the C replicate (information is not available for the F large) the size of the increase in incremental gain during puberty greatly exceeds the control .

Conclusions

Two features emerge from the comparison of incremental gains. First, that both body weight and skeletal dimensions show two peaks in growth associated with the maternal environment and the event of puberty and, second, that there are differences in the way these two characters demonstrate the response.

During the suckling period body weight in control mice shows increased increments during the second five days, after which increments decline during the rest of the nursing period. Tibia and occipital length reflect the same changes, but the other bone measurements show a steady decline during suckling which is greater during the second half. Body weight and skeletal increments are greatly increased during puberty.

Selection for large size both accelerates the timing of changes

in growth rate and increases the size of the pubertal growth acceleration. Selection for small size retards the timing of changes in growth rate and has the particular effect of depressing growth during the later part of the suckling period. Thus upward selection, while it undoubtedly has the effect of enhancing the maternal environment, exerts its main influence on the growth potential after suckling. Downward selection, on the other hand, exerts its main effect through its alteration of growth during suckling.

CHAPTER V

MORTALITY AND FERTILITY

It was suggested earlier that unselected mice develop from embryos to adults at a speed determined primarily by rate of weight gain. The mechanism by which this operates is one which links a particular stage of development to a particular body weight. Mice putting weight on fast reach these 'threshold' weights earlier and so develop faster. The reverse is the case for slow growing mice. Limits exist to the amount that development time can be thus advanced or retarded. These limits are narrower earlier in development when viability is critical and widen in later development when reproductive success is synonymous with biological fitness.

The effect of selection is to alter the rate of weight gain so that the limits which demarcate the association between growth rate and development time are encountered. In the large line the effect is to cause individuals to put on weight at the maximum speed so that the earlier time limit is encountered. Subsequently increasing weight gain is no longer associated with reduction in development time. The effect of reducing speed of weight gain is to cause the later time limit to be encountered. Progressive reduction in body weight without correlated longer development time is impeded by the proximity of low body weight (and slow development) to another 'threshold', that of viability.

The expectations regarding viability were explored by examining

the records of mice reared in all three lines. The body weight and speed of development of all mice which died after the first day of life were separated from those which survived and their statistics were compared. The results are shown in Table 39. Mice which subsequently die are 'non-survivors'.

At birth non-survivors from the selected lines are lighter than those which survive. In all three lines they are also shorter from nose to rump than those which do not die. At birth, then, mice which will not survive are already distinguishable from the normal population. Their progressive disadvantage thus must begin in utero.

Although they begin life smaller in size they are not necessarily developmentally retarded at the time of incisor eruption compared with normal mice. They are all, however, considerably lighter than surviving mice in the selected lines although the difference is very slight in the unselected population.

The passage of four more days brings mice to the stage of eye-opening. It is at this stage that stress from inter-litter competition begins to operate with more severity since milk supply no longer increases. The casualties from this vigorous environment are evident from the Table. Animals which subsequently die are three-quarters of a day retarded in development and considerably underweight at the time of eye opening. The differences between non-surviving and surviving control mice are in the same direction but much smaller in degree.

After a further three or four days in this environment the effects become evident at the stage of molar eruption. Mice from the selected lines are retarded by half a day in the large line and a day and three quarters in the small line behind the normally developing survivors. In body weight they are lighter than normal individuals half their age. Once more the control comparison is rather different. Non-survivors are neither retarded nor lighter than survivors.

Following weaning there was no further mortality until reproduction

TABLE 39 Development of Mice which died before Weaning

<u>B i r t h</u>		<u>Incisor Eruption</u>		<u>Eye Opening</u>		<u>Molar Eruption</u>	
<u>Body</u> <u>Weight(g)</u>	<u>Body</u> <u>length(cm)</u>						
<u>LARGE</u> 1.58	3.30	10.6	4.6	14.8	5.1	17.0	4.8
<u>CONTROL</u> 1.54	3.20	11.6	6.2	14.0	6.8	16.5	7.9
<u>SMALL</u> 1.32	3.10	11.0	4.4	15.7	4.8	18.7	4.3

Development of Mice which survived until 21 days

<u>LARGE</u> 1.72	3.35	10.7	6.9	14.1	8.8	16.5	9.6
<u>CONTROL</u> 1.53	3.30	11.4	6.3	14.2	7.5	16.8	7.9
<u>SMALL</u> 1.4	3.20	11.7	5.8	15.0	6.6	17.0	6.8

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Following weaning there was no further mortality until reproduction

occurred, when a few females died.

Although the numbers involved in this analysis are extremely small they do conform with predictions made on the basis of earlier analyses. It was anticipated that the end of the suckling period would be one where viability was, like the period of birth, under particular hazard.

The population consisted of 63 large mice, 99 control mice and 82 small mice. In these lines respectively 14 large, 8 control and 21 small mice did not survive until weaning.

The overall mortality for the entire population of 244 is as follows - Table 38:

TABLE 38 Mortality During Suckling

	<u>% died be- tween birth & incisor eruption</u>	<u>% died be- tween in- cisor er- uption and eye opening</u>	<u>% died be- tween eye opening and molar eruption</u>	<u>% died be- tween molar eruption and weaning</u>	<u>Total %</u>
Large	16	0	3	3	22
Control	5	0	1	2	8
Small	21	0	1	4	26
All lines	13	0	1.6	2.9	17.6

From these sparse records two conclusions can be made. First, there are two peaks in mortality corresponding to the two periods of transition of environment and nutrition, the first being substantially greater than the second. These periods are separated by an interval when stress is minimal in all lines and no deaths were recorded. This interval lasted from a day or two after birth until about the sixteenth day.

Second, the two selected lines are more susceptible to these environmental stresses, experiencing greater mortality at both periods. The

TABLE 39 Development of Mice which died before Weaning

<u>B i r t h</u>		<u>Incisor Eruption</u>		<u>Eye Opening</u>		<u>Molar Eruption</u>	
<u>Body</u> <u>Weight(g)</u>	<u>Body</u> <u>Length(cm)</u>	<u>Day</u>	<u>Weight(g)</u>	<u>Day</u>	<u>Weight(g)</u>	<u>Day</u>	<u>Weight(g)</u>
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<u>SMALL</u> 1.4	3.20	11.7	5.8	15.0	6.6	17.0	6.8

small line is the worst affected.

It was pointed out that the effect of selection would particularly alter the development of mice during early stages when viability was critically dependent upon body weight. Later on in life the stage of precarious viability will have passed and those most seriously impaired would have died.

Those which did not die, however, were none the less measurably afflicted by their prenatal environment. It has been shown that time taken to reach puberty is importantly influenced by body weight attained during suckling, i.e. by weaning weight. Mice suffering most seriously from poor maternal environment would be most seriously delayed in reaching puberty, that is, in reaching the stage of sexual maturity. They would, under natural conditions, be expected to show low (because development was delayed) biological fitness.

Under the experimental conditions prevailing in the selection experiment involving the C strain, an artificial "natural selection" was imposed by the method. Each generation consisted of forty-eight matings. Ideally, individuals were mated at 42 days of age. Assuming that fertilization occurred immediately, young would be born when parents were about sixty-three days old. This litter would be weaned when the parents were eighty-four days old. This age was the cut-off point for that generation. Parents not producing offspring during the three weeks preceding the day when their age reached eighty four days would not have their genes represented in the next generation. From the point of view of the experiment they were biologically unfit. Selection, therefore, chose only from among the relatively fast-breeding individuals in the generation.

Sometimes it was found that the interval of time allowed for a new generation to be born was insufficient. On these occasions a

further week was provided to allow numbers to be made up. When this further week was insufficient, the random system of mating was complemented by the use of substitute matings from the previous generation.

The selection experiment had been proceeding for twenty-one generations when an analysis was made of the number of times it had been found necessary to delay selection for one week, and the number of substitute matings used in the three lines.

The data was divided into the first and second halves of the selection experiment. During the first ten generations a total of four extra weeks were required in addition to the substitutions noted in the Table (Table 39). During the second ten generations a total of eleven extra weeks were necessary. Performance was more seriously impaired as selection proceeded.

TABLE 39

Cens:	LARGE		CONTROL		SMALL	
	Mean No: per gen:	% in each half	Mean No: per gen:	% in each half	Mean No: per gen:	% in each half
I - X	3.2	(32)* 19%	1.5	(15)* 16.6%	4.5	(45)* 19%
XI - XXI	9.8	(137)* 81%	7.0	(75)* 83.3%	13.6	(191)* 81
Total		169		90		236

* Numbers in brackets

From this data the mean failure rate of matings set up in each generation can be compared (Table 40).

TABLE 40

Gens:	LARGE		CONTROL		SMALL	
	Mean failure per gen:	% of 48	Mean failure per gen:	% of 48	Mean failure per gen:	% of 48
I -X	3.2	6.7%	1.5	3.1%	4.5	9.4%
XI -XXI	9.8	20.4%	7.0	14.6%	13.6	28.3%

The distribution of failures between the first and second part of the experiment is very similar in all three lines. Among all mice some factor was in operation which acted independently of the effects of selection and quadrupled the failure rate after ten generations of random breeding.

A calculation was made to discover whether this general lowering of performance might be due to inbreeding. The minimum amount of inbreeding provided by the system if it ran perfectly for ten generations was 0.15*. The figure is in fact higher than this since in the large, control and small lines, respectively 6.7%, 3.1% and 9.4% of matings were made with parents probably more closely related than was desired by the system. At the end of the second ten generations the coefficient of inbreeding was, at its lowest value, 0.28. Since the percentages of unfavourable matings were 20.4%, 14.6% and 28.3% during this period it is highly likely that this figure is low. Therefore the population after twenty-one generations was at least as inbred as

* Twenty Generations
8 pairs, N = 16

Ten Generations

For minimum inbreeding

$$N_e = 32$$

$$F = 1 - \left(\frac{63}{64}\right)^{10} = 0.15$$

$$F_t = 1 - (1 - F)^t$$

$$F = 1 - \left(\frac{63}{64}\right)^{20} = 0.28$$

the offspring deriving from brother-sister matings. It is probable, therefore, that the increase in failure in all three lines reflects the expression of deleterious recessive genes.

The data shows, however, that the selected lines were more seriously impaired than the control line, and the small line was particularly affected. In order to represent the differences in susceptibility of the selected lines, the number of substitutions was calculated in excess of those required in the control line, i.e. as in previous comparisons the deviations from control were calculated. The number of deviations and the deviations from control are shown in Figs. 33 and 34. In Fig. 35 the cumulative frequency of deviations was plotted against generations. While the large line has shown a steady trend in deviations over the entire period, it is clear that the small line suffered particularly as time and generations passed, 50% of the total number of substitutions required (in deviations) occurring in the last eight generations.

1. Conclusions

The system of mating used in this experiment has interfered with normal development in all three lines, causing an overall increase in infertility measured at eighty-four days. The large and small lines had suffered to a greater degree than the unselected line and this difference must in some measure be a consequence of the selection imposed.

A comparison of the mean data for reproductive performance in the experiment indicates where this may operate. These mice were not subjected to selection for body weight at forty-two days, so each sample represents the entire range of values for each line. Table 41 illustrates the relative physiological maturity and functional maturity

of the different lines.

TABLE 4.1

	<u>Age (days) at Vagina opening</u>	<u>Age (days) at first litter</u>	<u>Vagina-Litter interval (days)</u>
	<u>Mean</u>	<u>Mean</u>	<u>Mean</u>
Large	31.2	57.7	28.2
Control	35.2	59.4	23.8
Small	37.8	62.9	25.7

Comparing large with control mice, it is evident that the former reach sexual maturity four days earlier but take longer to reproduce since they are only a day and a half younger when the first litter appears. Assuming that gestation time is not different between lines, the difference in age at first litter must be due to the slowness of large mice to perform copulation and/or be fertilized. This is confirmed by a comparison of the mean interval between age at vagina opening and litter production for individual mice. Control mice require about four days less time to become fertilized than large mice. It is probable that this tardiness in reproducing among large mice is greater than that indicated here since the males used were unselected males of proven fertility. The selection operation mated selected females with selected males of unproven reproductive ability and unknown maturity. The large line, therefore, probably suffered seriously from infertility which was partly shared by both potential parents.

The small line is slowest to reach the stage of vagina opening and oldest when the litter is born. It is also more variable than the other two lines at these ages. Once past physiological maturity,

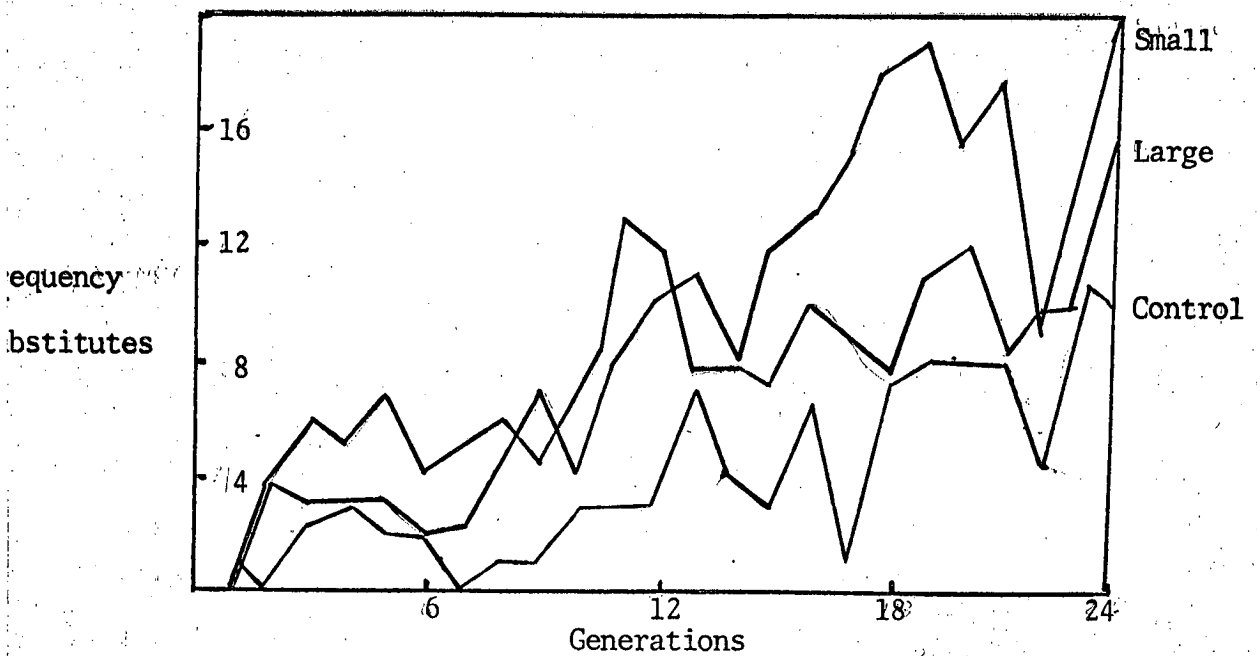


Fig. 33. Frequency of Substitute Matings Required Over Entire Experiment.

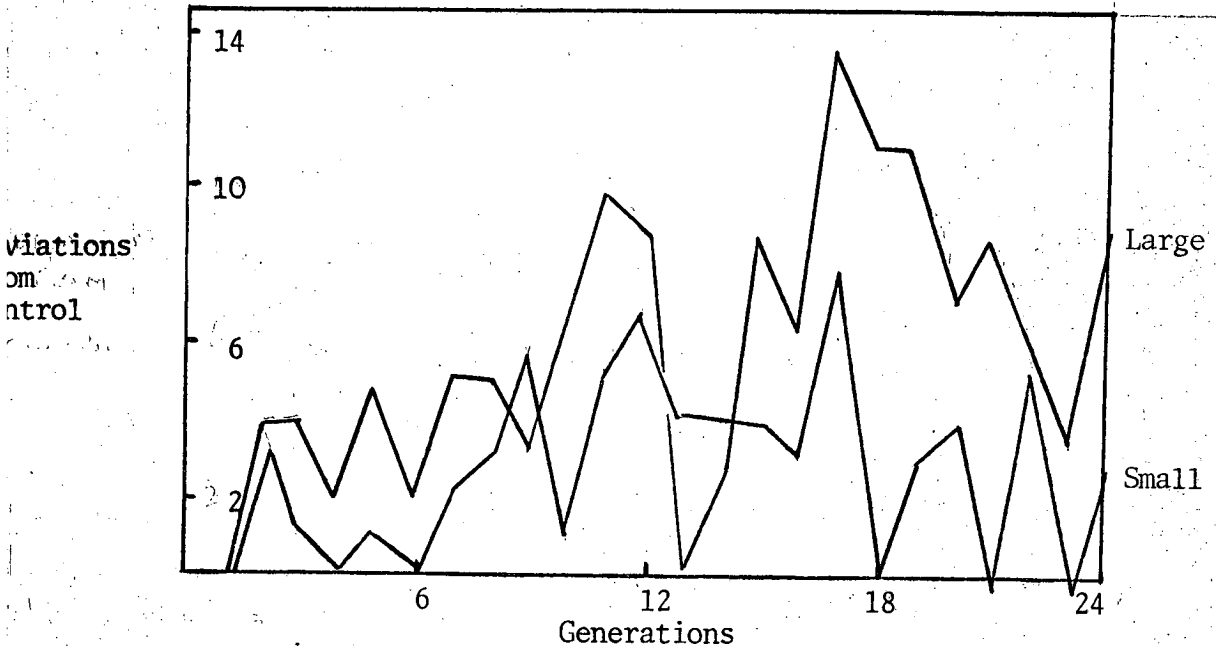


Fig. 34. Deviations from Control of Number of Substitute Matings Required.

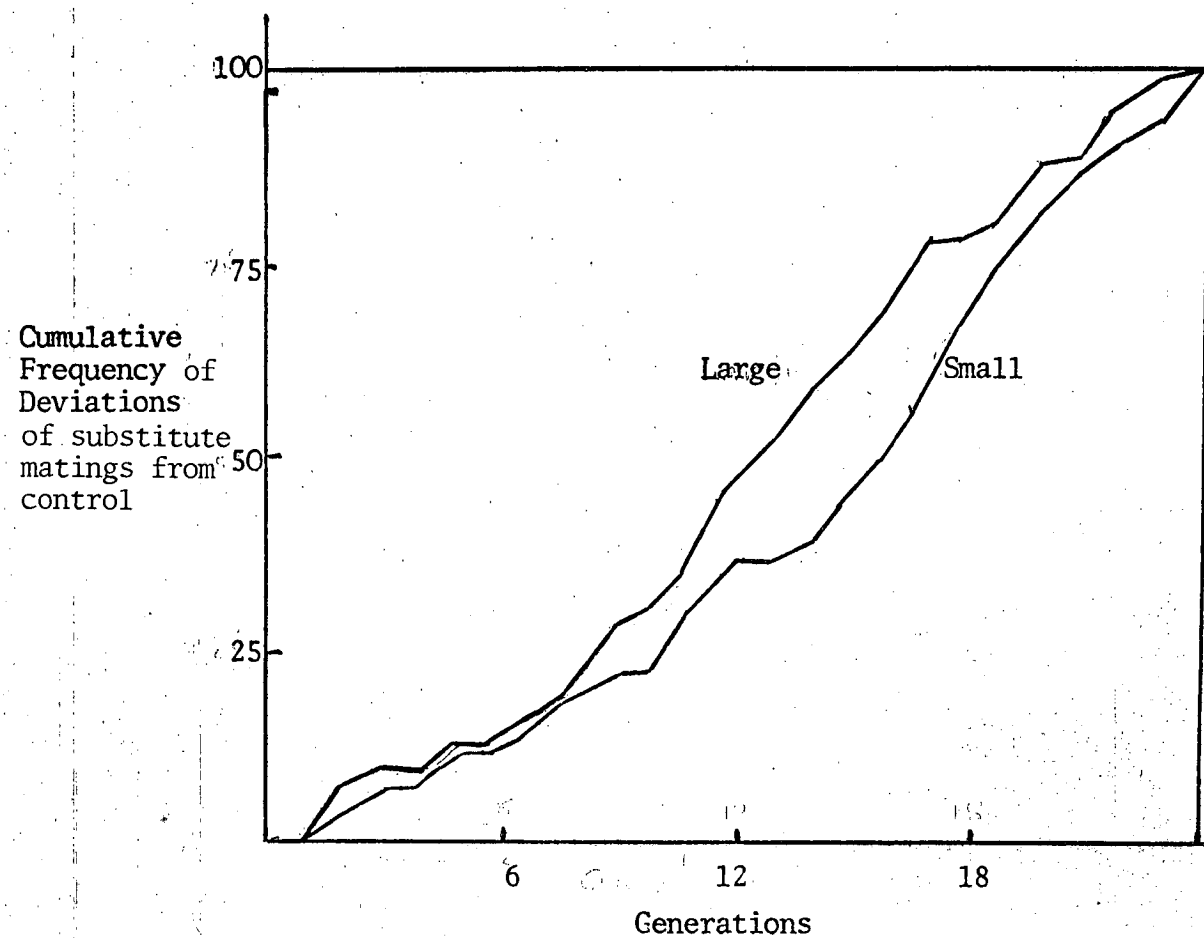


Fig. 35, Cumulative Frequency of Deviations From Control of Large and Small Lines Over Entire Experiment.

not so long is needed for small mice to become pregnant as for large mice. The delay in maturation is probably the chief cause of the older age seen at first litter production. Selection for low six-week intra-family body weight, therefore, would accentuate the effects of slow weight gain and be more likely to chose individuals whose genes would not be represented in the following generation due to slow development.

In the small line, therefore, there is a direct relationship between fertility measured up to age eighty-four days and the selection applied, which is not present in the large line. Among the latter the variance of development time was very low so that sexual maturity was reached in most mice within a few days of age thirty two days. Among the former the variance was large, many mice experiencing developmental delay of sexual maturity until they were more than six weeks old, that is until the date when selection was made had passed.

Small mice were, therefore, subjected to a selection index which was not so applied to the large line. In order to be selected for the next generation they had to be of low weight, and also of fast development time. Since the primary character selected was body weight, selection intensity was high for this character. Development time was not given any attention, yet, nevertheless, began to influence the following generation as soon as development time was delayed enough to spill a proportion of individuals past the forty-two day cut-off. The effect of this would be to disrupt the association between body weight and development time, i.e. change in development time would no longer be an accompaniment of change in body weight. The data comparing the ninth with the eighteenth generation illustrates this. The time taken to reach puberty was delayed by 3.9% in the first nine generations, but in the subsequent nine generations actually advanced by 0.1%.

This may be compared with the response in development time in the large line which was advanced by 10% in the first nine generations, i.e. the response was two and a half times as great in the large line. It is likely that this difference in response is due to the fact that in the large line a selection index involving development time was not imposed.

We may look at the behaviour of theoretical growth curves when intra-family selection is carried out for body weight at forty-two days. The mortality and fertility data suggest the existence of limiting factors to the transformation of these curves. The first is that associating body weight with viability in small mice during the suckling period. During this period weight-gain was slow, causing development to be delayed. At the end of the lactation period this developmental delay caused mice to be left without a sufficiently mature dental apparatus to enable them to seek out food independently. Consequently their body weight when weaned reflected both a poor maternal environment and a period of impaired development consequent upon it. The fact that weaning weight was reduced by 18% in the first nine generations and only 2.4% in the second nine confirms the existence of a lower limit to rate of weight gain during suckling.

The influence of maternal environment on age at puberty operates through body weight. The small line, therefore, due to their poor maternal environment, encountered a second limiting factor at puberty preventing development from being delayed beyond forty-two days. Since, if this limit did not exist, selection for body weight at forty two days might be more intense, it is not surprising to note that selection response in the small line for body weight at puberty is less in the second nine generations than the first, 8.6% compared with 13.2%. It is, nevertheless, greater than the response at weaning. It appears,

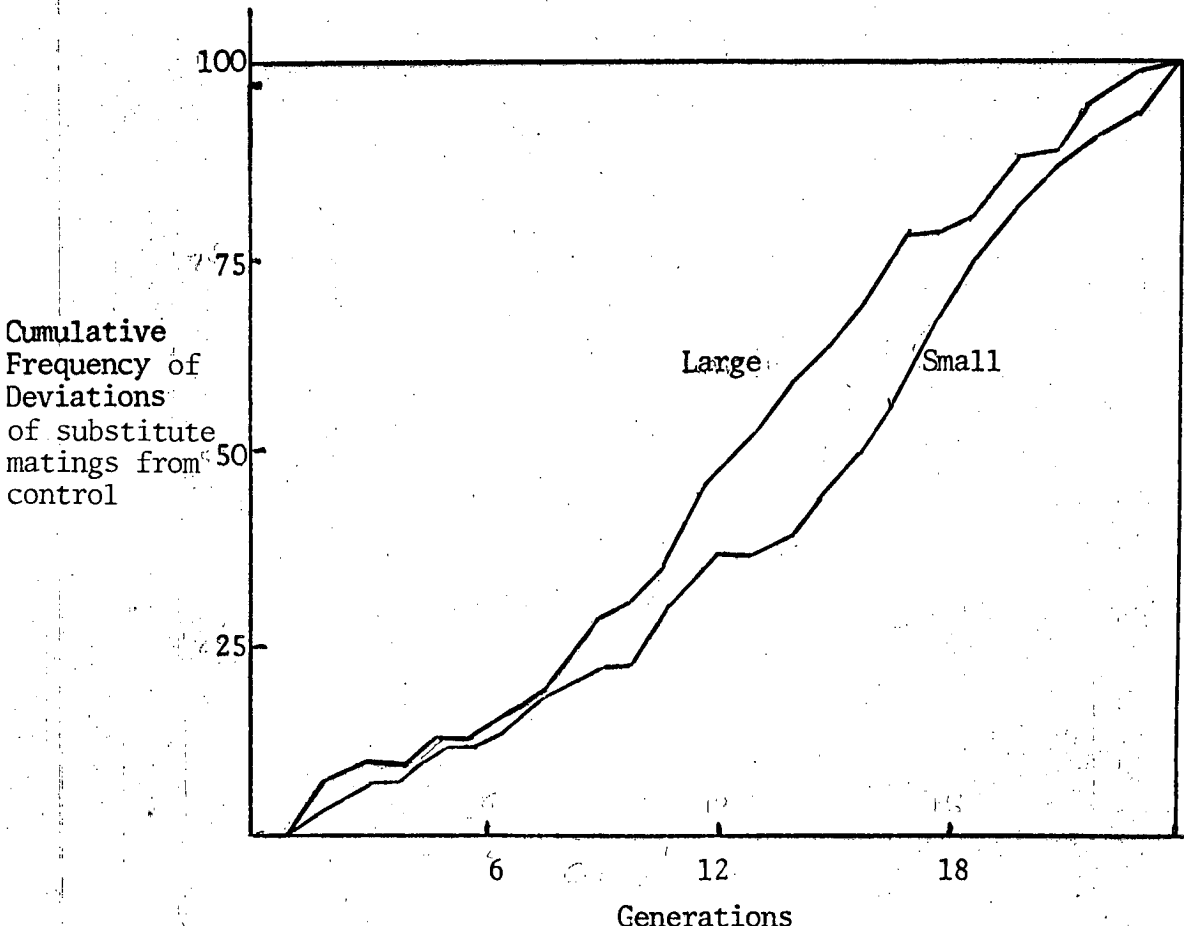


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therefore, that selection for low body weight operates primarily by depressing weight during the suckling period. Among those mice which survive this environment, selection gradually separates those individuals which show poorest recovery. It is on the growth from weaning till forty two days that selection secondarily operates.

Selection in the upward direction encounters other limiting factors. Although viability and mortality are worse than controls they are considerably better than small mice. The absence of a limit to the amount that suckling performance can be increased is reflected in the continuing response in weaning weight for large mice, 12% during the first nine generations and 18.6% during the second nine. The increase in weaning weight had the predictable consequence of reducing the age at which puberty was reached. However, as with the small line, no progress was made after the ninth generation. The correlated change in weight at puberty was rather more than the change in weaning weight during the first nine generations, 15.2% compared with 11.9%. During the next nine generations this relationship was greatly altered. The response during this period was far more for weaning weight than for weight at puberty, 18.6% compared with 4.9%. It appears that both large and small lines encounter limits in the amount that development time can be changed and they encounter these limits before nine generations of selection have been carried out. The direct response is not subject to these limitations, however, and proceeds faster in the large than the small line, 41.4% compared with 28.1% at generation eighteen.

2. Other Experimental Work

In the introductory chapter, the work of Clarke was mentioned and note was made of the two different kinds of response he found in the large line. Some replicates showed that increase in weight at

asymptote was associated with increase in time to reach asymptote. a situation predicted by the positive correlation between time taken to mature and mature body size, whereas other replicates showed that weight at asymptote could be increased with no change in time taken to reach asymptote. The second experiment used one replicate from each of these two groups and this difference was confirmed as shown in the body weight graph earlier. The F replicate encountered its point of greatest response (its plateau, or asymptote) earlier than the C replicate. This difference was associated with a difference in weaning weights, the response being 35% in the F replicate at generation eighteen and only 12% in the C replicate. The mean response among six replicates, therefore, conceals the presence of two (at least) different kinds of response to upward selection, which are associated with differences in response during the suckling period. Such heterogeneity of response does not appear in the small line.

The observations regarding Clarke's experiments were that upward selection for body weight produced a ready increase in weight at asymptote, but in only some of the replicates did it increase time to asymptote beyond the range found in control mice. Selection for low body weight had no difficulty in reducing time to asymptote beyond the range in controls, but encountered difficulty in reducing mean body weight below the range found in controls.

Earlier experiments with upward and downward selection showed that when the respective lines reached a plateau, additive genetic variation in the large line had been removed, while there was still additive variation in the small line.

Combining Clarke's data with the present study, it appears that selection for small size achieves a response which soon slows down due, perhaps, to the presence of the demonstrated lower limit to the

amount weaning weight can be reduced. The reason why additive genetic variation is still detected after the selected line has plateaued may be due to genetic variation for small body size being associated with poor maternal performance and impaired development which prevents such genes from being represented in the population selected at forty-two days.

With large mice upward selection appears either to be associated with a large correlated improvement in maternal performance, resulting in a high response in weaning weight, or to be associated with a prolonged development time and only a modest improvement in maternal performance. Selection for large size, then, produces a final response at, say, 120 days, which is similar whether or not it is accompanied by alteration of the maternal environment.

The explanation for the different kinds of response in large and small lines must, therefore, account for a response for large size which is somehow independent of the correlated improvement in maternal performance, and a response for small size which is somehow mediated, and limited, by correlated poor maternal performance.

CHAPTER VI

GENERAL DISCUSSION

The discussion that follows will develop from the conclusions which emerge from the experimental section.

These are as follows:

1. It is shown that a number of functionally unrelated but readily observable milestones may each or severally give a consistent impression of the process of growth and development. These stages of development are:

1. Eye opening.
2. Tooth eruption.
3. Vagina opening.
4. Bone ossification.
5. Skeletal/body weight ratios.

2. Environmental influences upon these characters are similar. Heavier mice develop faster at all stages.

3. Within a certain time range, state of development is closely associated with body weight in unselected mice, animals at similar stages of development having a similar body weight.

4. Limits to this association exist and these are narrower early in development than later. Increasing body weight causes faster development up to a limit, and decreasing body weight is associated with slower development up to a limit. These limits are, respectively, limits in time before which the developmental event cannot occur no matter what

body weight is, and limits in time beyond which delay in development will not be possible even though the individual may be losing weight.

5. Selection for body weight causes body weights at all stages of development to be altered in predictable directions.

6. The effect of selection for body weight on development time is different at different stages of growth. In small mice time taken to reach sexual maturity is increased, while time taken to reach asymptote is reduced; in large mice time taken to reach sexual maturity is decreased, while time to asymptote is either increased or unchanged. Variances are altered in opposite directions in large and small lines. Selection does not alter the limits in age between which development occurs.

7. The effect of selection on the weight association of development is to engender a negative association where in unselected mice there is a zero association. This occurs through the selected line producing more individuals developing at the extremes of development times and at altered weights. Individuals developing at intermediate speeds are not greatly altered in body weight.

8. Selection for body weight has no significant effect upon the ratio of one bone length to another.

9. Selection for body weight influences weight more than bone size, so that large mice have smaller skeletons than small mice when measured at the same weight.

10. The final response in body weight is brought about by means of alterations in growth rate which occur at different times in the two selected lines. The large line demonstrates moderate improvement in growth rate during the suckling period, followed by a pronounced increase in growth rate during the week or two following weaning, which is largely responsible for the final difference from controls. The small line shows

an impoverishment of growth during suckling and a greatly reduced growth rate during the peripubertal phase.

11. It is suggested that continued reduction in growth rate during the suckling period in the small line is hampered by the close association between body weight and viability at low body weights.

There are, therefore, two basic observations. First, that in unselected mice development up to sexual maturity appears to accommodate to environmental stress by altering the time variable and keeping body size at different stages of development constant. Second, that in selected lines this homeostatic mechanism does not operate. Unselected mice show zero correlation between body size and time taken to reach that size when estimated at a particular stage of development, whereas the association is negative in selected lines.

The difference between these two responses is explained in terms of the existence of thresholds in body weight associated with consecutive stages of development, and limits in time beyond which development cannot be varied.

The effect of selection for body weight is to alter development time in the same direction as if the mice had been subjected to a constant environmental influence during their growth, from generation to generation, that is to say, large mice develop faster and small mice slower. Since it is unlikely that a genetic change would produce an alteration in development time in the direction found here, it is much more likely that the source of the influence lies in the lineage of mothers within each line. Large mice provide better maternal environments which cause a small decrease in development time, and small mice are worse mothers, thus

prolonging development time. The lines differ, however, in the importance of the period of dependent growth, the final response being closely related to suckling growth performance in small mice, and more related to independent post-weaning growth in large mice.

The question of a casual relationship between the performance of the mother and the final size of the offspring must be explored at this point. Could the whole of the response in the selected lines be due to the correlated effect of body size on maternal performance? If this were the case, a selection experiment such as was carried out on the C strain would, in effect, not be an experiment altering the genetic control of body size, but rather an experiment gradually altering the environmental control over body size.

Brumby's experiments on transplanted ova leave no room for equivocation on this issue, (Brumby, 1960). Ova from large mice transplanted into small mice develop into adult mice very little different from large mice reared and suckled by their natural mothers. Similarly, ova from selected small strains transplanted into large mothers closely resemble small mice born and reared normally. The effects of selection for body weight, then, do not produce their alteration of selected lines through the mediation of maternal performance to any important degree. Most of the transformation in growth in selected lines is a consequence of alterations of the zygotes in the respective lines, which are presumably mainly genetic.

Nevertheless, if attention is confined to the first three or four weeks, the maternal environment exerts the major influence both on growth rate and development rate. Following weaning, genetic effects become more important, although effects of a purely environmental origin can continue to modify the effectiveness of selection at six weeks of age.

Briefly, the alteration in development time and growth rate found

in the selected lines is probably due to changed maternal environments, but is mainly independent of the final size achieved in the different lines.

How closely is the response to selection for body weight reflected in the skeleton? Quantitatively the response in skeletal dimensions was not nearly so extensive as it was in body weight. Moreover, the response was slightly different in different parts. Upward selection increased mean body weight by 50%. Long bones showed the closest similarity to this response but did not exceed 8% increase in the large line or 6% decrease in the small. The pattern of response for long bones was almost identical to that for body weight; in large mice most of the response was established during the pubertal growth phase, while in small mice the response was established during suckling.

Bone diameters also followed the body weight pattern of response, but the degree of response was inconsistent from line to line. This may reflect the fact that long bones increase in length inexorably and can only change their rate of increase, whereas bone diameters lay down bone by apposition and simultaneously remove it by remodelling and thus are influenced by a more complex set of factors.

Occipital length shows very little similarity to body weight in the degree of its response to selection. It does show some similarity to the other bones in the pattern of response, however, in that the response is less in the small line after weaning than before, and greater in the large line after weaning than before. The greatest response does not exceed 6% and does not differ from the amount by which one unselected replicate differs from the other.

It appears, then, that most of the response to selection is in the extra skeletal components of body weight, the skeleton showing proportionately less response, although the correlation is clearly high.

The correlation is lowest in occipital length. Since this is closely associated with the development of the central nervous system other evidence would lead us to expect a smaller response in this region.

In order to examine more closely the change in response in the skeleton throughout growth, the incremental increases within each period were compared with the response in body weight. This comparison reveals a fundamental difference between the pattern of growth of skeletal tissue and other constituents of the body. In both there are two peaks of growth, one during the suckling period and one during the peri-pubertal growth acceleration. They differ, however, in the relative sizes of the peaks; the second peak in skeletal growth is smaller than that during suckling. Body weight, however, increases far more in the pubertal period than during suckling.

The difference between the control and selected lines lies not in the distribution of incremental growth throughout time, the two peaks showing close coincidence in time in all lines. The chief difference produced by selection is in the incremental growth between the two peaks. The trough, or low point, in incremental growth is far lower in the small line and much higher in the large line than in controls.

From this comparison between the nature of growth in bone and soft tissue it appears that the major skeletal dimensions of the body are laid out during suckling and added to in only small degree during the rest of the growth period. Soft tissue, however, is mainly created during the period following weaning, that is, after the skeletal framework has been formed. Selection at six weeks alters particularly the transition between suckling growth and pubertal growth, either depressing or enhancing it, leading to a smaller or larger pubertal growth increase.

Due to the response in weight exceeding that in skeletal dimensions, comparisons between ratios of skeletal length to body weight reflect

mainly the differences in body weight. Slow growing mice have larger skeletons at standard weight than controls and fast growing mice have smaller skeletons. Or, contrariwise, the amount of weight put on to achieve a certain length of bone is less in small mice than controls and more in large mice. These differences become more pronounced as time passes and the response in the character directly selected increases. At birth the differences are less. The regressions for occipital length/body weight body weight ratio are very similar between all lines. When a particular weight common to all six lines was taken it was found that the ratios were practically identical. The difference in means at birth, then, is mainly a reflection of changed body weight, the populations having been moved along the regression line. These differences in ratios become much more pronounced later on, the peak differences reaching 50% before weaning in the small line and 50% in the large line after weaning.

The very small alterations among skeletal ratios is reflected in the comparisons between lines. Most of the differences do not exceed 2% throughout the entire period, which is very little different from the intra-control comparison. Thus, skeletal body proportions are virtually unchanged by body weight selection.

In this interpretation of the results, growth has been divided into two parts - the early part, until weaning, being almost entirely determined by the mother, and the second part, after weaning, being independent of the mother. This division separates the growth achieved in the maternal environment from that accomplished during the pubertal period. Final size and form is thus comprised of these two independent components.

There are two (at least) possible views of the way growth and development come about. According to the first, growth is a continuous process under the control of certain factors which operate throughout the entire period of growth. In many cases these factors can be represented by two

or three parameters which specify a mathematical curve, and this curve can represent the size of the organism at any point in time during its growth. When growth can, in this way, be represented by a mathematical curve, the final size or form may be predicted from any earlier stage if only the parameters of the curve are known.

According to the opposite view, growth is the expression of immediate circumstances interacting with a continuously unfolding set of specifications. Since the entire genome is represented in all cells at all times a mechanism must exist which represses all those genes which are not currently being used and initiates their activities in an orderly fashion. Thus, genes relating to tooth function will be de-repressed some time after those responsible for bone ossification have been activated. Similarly, genes which initiate the function of reproductive organs will be activated some time after those dealing with tooth function. It is conceivable that the activities of these different genes, acting at different periods during growth, might be independent of each other. If this were the case it might be possible to select for particular genes when they become operative and alter the trajectory of growth during the different phases of growth. Alternatively, the environment may be altered during different phases and the growth trajectory altered during subsequent development. Under this interpretation the curve necessary to represent growth would have to be specified by as many parameters as there were environmental variables and sets of genetic alternatives. It does not exclude the possibility that growth might be represented by a simple curve with a small number of parameters. Indeed, it is the case that the growth of population or of organisms can sometimes be represented by a simple curve. However, these curves are usually fitted to data post hoc facto and do not generally yield further information about the nature of, or limits to, the growth of the organisms being studied. In particular,

the curve represents a mean growth path and does not enlighten us regarding the deviations from the curve which are the source of change or expression of limitation.

It was concluded from this experiment that the changes brought about in the large line affected the period of dependent growth, causing increasing growth rate, but to a much greater degree affected the growth occurring during the pubertal growth period. In the small line, growth during the period of dependence was greatly depressed and the pubertal increment also smaller. These conclusions do not fit easily into either of the interpretations outlined above. It is evidently not the case that by specifying final size and time to reach it, the shape of the growth curve can be specified. First, the 'final size' is never reached in mice and, second, even if some point could be chosen to represent 'final size' it is clear that the growth curve does not follow the pattern predicted by altering final size. That is to say, growth is not controlled by a small number of factors which operate throughout development.

On the other hand, neither can it be claimed that the different phases of growth are totally independent of each other. The growth achieved by large mice at weaning appears to enhance and accelerate later growth so that the pubertal growth acceleration is greatly increased, and it is this increase which is mainly responsible for distinguishing large adult mice from control adults. The suckling period in some way prepares mice to optimise growth thereafter, perhaps merely by increasing appetite and accelerating development.

The reverse is the case for small mice. Suckling growth is worse, particularly during the last ten days. Mice, therefore, receive a poorer maternal environment just prior to the stage when they become independent food seekers. This is clear from the section where incremental growth is compared among the lines. This situation releases them into the independent

phase both underweight and developmentally retarded.

This interpretation assumes that the shape of the growth curve is regulated primarily by the maternal environment. Yet, it was shown earlier that ova-transplantation experiments had demonstrated the greater importance of genetic constitution rather than environment in determining growth trajectory over the entire period till adulthood. A comparison between growth curves of mice selected for large and small size, and mice whose growth has been altered by dietary interference, strongly suggests, however, that the transformation of growth operates in the same way in both situations. Small mice which have been selected for low growth rate become small, not through being denied food during the suckling period but through transforming less of available food into biomass during this period. Whether the restriction in biosynthesis is imposed externally or resides in the metabolic pathways themselves, the final product at the end of suckling seems to be the same in small mice. Similarly, it appears that the character of growth after weaning is established in large mice identically by either environmental or genetic causes.

Although, therefore, the two phases of growth, suckling and puberty, are separate in time, there may be some inter-dependence between them. The first phase is dominated by environmental considerations. The second is controlled by the operation of a portion of the individual's own genome, associated with the establishment of sex differences in form and function. The evidence of these experiments suggests that the earlier period exerts control over the second period by exerting permissiveness for deviations above the mean and restriction for deviations below the mean. Small improvements in suckling performance are multiplied during puberty, poor performance during suckling is less likely to be compensated for, the worse the performance.

In essence this implied that body weight, not surprisingly since it

represents volume, behaves exponentially. Small increases early in growth have great effects later on, and vice versa. This expectation was tested by transforming all the data to logarithms. The mean growth curves for all six populations were then compared by the mini-max method. It was found that the growth curves of the selected lines did not differ significantly from controls. There was, however, a significant difference in the initial weight of the different lines. Once the difference in birth weight was accounted for, however, growth proceeded identically in the selected lines. The observed differences were the result of initial differences together with differences in scale.

Consideration of the changes brought about in the variance of development time at different stages of growth by selection of body weight suggest that variance also reflects exponential growth. In large fast-growing mice the variances for development time were reduced while the variances for slower developing mice were increased. It seems, then, that while chronological time is linear, physiological time behaves exponentially. This is expressed not only in the mean development path of a population but also of variation within the population.

The mean development path of a population expresses the rate at which the genes responsible for development are expressed. It is evident that the rate of expression is fastest at the beginning of development and slows down with the passage of time. Thus the de-repression of genes during embryogenesis occurs faster than during morphogenesis, and morphogenesis merges even more slowly into the phase of maturation.

Variation in development time within a population expresses the resultant both of environment variation and genetic variation. The major influence is environmental. The effect of environmental inclemency is to prolong physiological time so that a group of organisms growing slowly as a result of poor nutrition show increased variance in time taken to

reach a particular stage of development: that is to say to reach stages where the same portion of their 'developmental genome' is being de-repressed. The coincidence of exponential characteristics in both the entire period of growth and also environmentally induced variation in it, brings about the conditions found in the experiment and in reports elsewhere. When stress causes the growth of individuals to deviate from the mean value, the time, in a chronological sense, permissible for growth to return to normal is much less early in development than later on. Therefore, individuals whose growth is delayed initially responded by prolonging development time; when the limit in this is reached they suffer increasing degrees of permanent growth deficit and enter a zone when viability itself is endangered. Within a population under stress, casualties appear more frequently at early stages of growth and development than later.

A feature of growth and development which requires further attention is the constancy of body weight at similar stages of development and at intermediate speeds of development. What are the implications of this observation? The description 'same stage of development' operationally means when a certain milestone is reached, e.g. vagina opening, eye opening, etc. These external events, however, are the expression of a continuous process of de-repression of genes. The 'same stage', here, implies that in the reading of the DNA responsible for development, i.e. for synthesis of new biomass, different individuals from within a population will have synthesised the same quantity of tissue when genes appropriate to the next stage become accessible for transcription, translation, etc., although their ages may be different. Thus, the progressive de-repression of the 'developmental genome' is linked very closely with the mass of tissue already formed.

The term 'biomass' includes a wide variety of different tissues and

fluid content. But, at the simplest level, the mass of an organism expresses a quantum constituted of a certain number of cells of a certain size. Variation in biomass may be brought about either by altering the total number of cells while keeping their individual volume constant, or by altering the volume of some or all cells while keeping cell number the same. Altering cell number requires altering the process of replication, while altering cell size requires altering the process of transcription and translation. It is also possible that both alteration of cell number and cell size may occur, or that different tissues may react independently in this regard.

The comparison between bone length/body weight ratios showed great differences between the lines. The differences, however, mainly reflect the differences established in body weight rather than in bone size. The most notable feature of the section describing incremental growth was that, although like body weight there were two peaks of incremental growth, the skeleton differed in respect of the relative size of the peaks. For bone sizes the major contributions are made during the suckling period and lesser amounts added during the pubertal period. This is the reverse of body weight gain, where the latter period's contribution greatly exceeds the former. Although the skeleton showed the same pattern of response, it is clear that it demonstrates a much smaller response and shows insignificant alteration in body proportions. The absence of a phase of simultaneous hypertrophy in skeletal tissue, as mentioned below, probably accounts for this difference.

In this experiment, therefore, the major part of the response was in the cells constituting soft tissue rather than skeletal tissue.

Since the variation in time taken to reach a certain body weight (or stage of development) is primarily determined by environmental factors, at least in rodents, the nature of the effect that extreme variation in developmental time has upon the constituents of body composition is briefly reviewed.

Altering litter size, or interfering with maternal nutrition, has been widely used to vary the rate of weight gain of offspring. Alteration of body weight has been complemented by examination of the accompanying alteration in cell number and cell size of these experimental populations. Reduction of food intake after weaning causes reduction in body weight, but the normal level can be reached by restoration of an adequate diet. Cell size and number are not altered when normal weight is reached (Winick & Noble, 1967). If food deprivation is commenced at birth and restoration of a good diet is delayed until after weaning there is a permanent deficit in cell numbers, with no alteration in size, and this deficit in cell numbers is reflected in lower body weight.

The difference between food deprivation early and late in growth is due to the presence of three phases of development during the growth period. Prenatal development is associated with cell division; this is followed after birth by cell division accompanied by cell enlargement. Finally, after weaning cell division ceases and growth proceeds by cell enlargement alone. These three phases follow successively in all tissues except bone, but their timing is different in different tissues (Winick, 1969). Thus, brain tissue completes its phase of hyperphasia before muscle tissue; therefore if rehabilitation occurs after this phase has terminated a permanent deficit in brain tissue is brought about, while the same animal may show little difference in muscle cell number (Winick, Fish and Rosso, 1968). In general, differences in early nutrition between deprived and over-fed animals were reflected in lower and higher body weights when they became adults, and the difference in body weight was a reflection not of cell size but of cell number. One of the last tissues to enter its phase of hypertrophy is muscle tissue, and it is clear that most of the difference between groups is due to the deficit in cells in muscle tissue.

Summarising this data, we may refer to the behaviour of a cell or of a tissue during the growth period of an individual. A cell is produced by replication; increases to a certain size (primary critical size) by a process of transcription and translation then, after a certain interval, increases to a larger size (final critical size) by further transcription and translation. Regarding a particular tissue, it begins growth by a process of replication with limited transcription and translation and completes it by increasing its entire volume through a large increase in the rate of transcription and translation with no further replication.

It appears from Winick's work that the process of transcription and/or translation may be delayed for long periods without permanent loss of tissue. Deprivation of food during the process of replication, however, produces only a limited delay, then growth continues with a loss of cells due to replication not being completed. The normal hypertrophy of the cells which were formed during this period does not make up for the loss of total tissue due to foregone replications. This implies that cell size is held constant, although time taken to reach the 'correct' size may be varied. The amount of hypertrophy accompanying the early phase of cell proliferation is small and therefore the amount of delay permissible is small. The amount of hypertrophy occurring during the final phase, mostly during the peri-pubertal period, is great and thus the permissible delay is greater.

The constancy of cell size also may enlighten the observation mentioned repeatedly that similar stages of development occur at constant weight, or that body composition is constant at the same weight. The implication here is that at the same stage of development organisms are constituted of cells of similar numbers and size. Extremes of nutrition cause loss of cell numbers, not of cell size.

We may interpret the present data in the light of this. Unselected mice weigh the same at puberty but are of different ages. They, therefore, contain similar numbers and sizes of cells. Their differences in age reflect differences in time taken to ingest sufficient food to supply the processes of transcription and translation required for hypertrophy. During the suckling period the slower growing individuals may not reach the appropriate weight for a stage of development to appear, but nevertheless will develop to that stage. It is likely that in these individuals cell division is occurring before cell sizes have reached the primary critical size, which is reflected in their lower weight. The faster growing individuals at this stage, on the other hand, probably demonstrate replication occurring at cell sizes greater than the primary critical size. In both cases the same final critical size is reached, but by means of a longer or shorter time path.

The major difference between the material of this discussion and the experimental data is that the variation in growth and development introduced by Winick and his associates was, like that occurring in the control lines, environmental, whereas the selected lines had, in addition, been differentiated genetically. Had the differences between the large and small lines been due entirely to environmental influences, we might confidently predict that large lines would have more cells than controls, but of a similar size. The single experiment which examines this question in the published literature showed that a line selected for large size did indeed have more cells but they were smaller than controls. (Robinson and Bradford, 1969).

It appears from this, then, that making large mice out of normal mice occurs through the same mechanism, whether environmental or genetic methods are used. Since the larger number of cells must have been

produced early in development, that is before weaning, this gives some credence to the impression mentioned earlier that growth during suckling exerts a permissive influence over subsequent growth. In the large line it was found that development occurred with very little variation in time and with minimal delay. This suggests that during the phase of cell-multiplication the provision or consumption of nutrients exceeded the speed with which replication could transform it into new cells. Consequently the association of development with cells of a certain number and size ceased to exert an influence and more cells were formed. This larger number of cells increased by hypertrophy during the pubertal phase, producing the large increase in weight deviation of the large line at that time, but a smaller final size of the individual cells.

Whether a similar mechanism operates for the small line is not known. One might predict, however, that the extreme delay caused in the development of small mice might diminish their number and perhaps also size. This would result in a smaller pubertal phase of hypertrophy and produce adults with fewer cells. The major difference between the control and selected lines would thus be a consequence of the difference in cell number causing a magnified difference in cell hypertrophy and, inasmuch as this is reflected by pubertal weight, it is mainly in the non-skeletal, perhaps muscular, component.

Regarding the contribution of muscle to body weight, it was shown that both skeletal lengths and body weight showed peaks during suckling but that the peak during puberty was small in skeletal lengths and large in body weight. The difference here might be due to hypertrophy of muscle mainly being involved in increasing the width of muscle cells rather than their length, since bone length is little changed, for which there is some evidence, and the contribution made by fat cells which is very large at this stage (Robinson and Bradford, 1969).

Regarding the process of maturation, the selection experiment has described a zone around the mean weight gain curve reflecting variation in growth rate which is the consequence of environmental variation. Other evidence suggests that this variation in response indicates the direct effect of nutrient supply upon the process of transcription and translation rather than replication. Extreme environmental variation or genetic selection appear to have similar effects on this process, first exhausting physiological variations in transcription/translation time, then either increasing or decreasing replication. Whether the positive correlation between final size and development holds or not possibly depends upon the extent of alteration of cell number in selected lines with or without change in cell size.

Comparisons made between natural population of different mature sizes indicate that the major difference between them is in the time scale. Thus mice and elephants grow along identical shaped curves but in a shorter or longer period. Similar species might differ in total cell number and cell size. During growth similar events would then occur at earlier and lower weight in smaller animals and vice versa. Genetic differences between populations would then be associated with a positive correlation between development time and body size.

Within a population, environmental variation of moderate degree alters the process of cell hypertrophy, but not the final cell number, so that there is zero correlation between development time and body size.

Under conditions of extreme environmental interference the limits in time between which cell hypertrophy may occur are exceeded so that phases of cell replication are interfered with. This results in larger, or smaller, cells at successive stages of development and perhaps also more, or fewer, cells for favoured or deprived individuals. In

either case the result is a negative association between development time and body size. Where the cell number has been altered the difference in final size is greater.

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ABSTRACT OF THESIS

Name of Candidate MILLS, Norman Joseph.
Address Flat 18, 50 Cornwall Gardens, Kensington, London, SW7.
Degree Ph.D., University of Edinburgh. *Date* 3rd November, 1973.
Title of Thesis Developmental Studies on Lines of Mice Selected for Body Weight.

The creation of an adult organism from a fertilised zygote requires the orderly transformation of food into tissue. The growing tissue itself demonstrates a sequence of morphological rearrangements and changes during development which cease when the adult form is established. Thus increase in size goes hand in hand with differentiation and development.

A review of relevant literature shows that, taking for an end-point sexual maturity in mammals, or pupation in *Drosophila*, within a population there is zero correlation between development time and body size at that stage, since the stage occurs at approximately the same weight in all organisms, while age is variable. Extreme environmental variation engenders a negative correlation due to deprived individuals being stunted in size even when fully grown. Differences between sub-groups within a population can be ascribed mainly to genetic differences and here the correlation between size and age at the end-point is positive, larger organisms taking longer to mature.

Experiments were carried out to determine whether earlier stages of growth in mice were also, like sexual maturity, determined by body weight rather than by age. It was found that in an unselected population body weight was the major determinant of stage of development for eye opening, incisor eruption and molar

The effect of selection in both lines was mainly on the extra-skeletal component of body weight, the bones themselves showing a much smaller response. Thus at fixed body weights large mice had proportionately smaller skeletons and small mice larger skeletons than unselected mice.

The lines differed in the time during growth at which the final difference at one hundred and twenty days became established. In small mice the selection response was brought about mainly during the suckling period, while in large mice the pubertal growth period was mostly responsible for the final difference. Thus, six-week selection operated upon different parts of the developmental path of large and small mice.

The stability of body weight at fixed stages of development was examined in the light of experiments relating growth and development to the cellular constitution of the body. It was suggested that the fixed weight at a particular stage of development indicated that animals at that stage and weight contained the same number of cells of the same size. They differed mainly in the time taken to increase cells to that size, i.e. in the rate of transcription and translation rather than replication. Upward selection would increase cell numbers though diminishing development time variance, while downward selection would diminish cell numbers.

A model system was described which related the different correlations between development time and mature body size to the cellular constitution of the body.