

## **Growth of the Albino Rat with Special Reference to the Influence of Environment.**

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## I. INTRODUCTION.

DETAILED investigations on the growth of the albino rat have been conducted by the author in attempting to solve some aspects of a general problem, namely, the influence of environmental factors on growth.

### THE GENERAL PROBLEM.

The influence of environmental factors on growth has long been a matter of speculation and controversy. Yet this is a problem of paramount importance—not only to the laboratory scientist but also to the practical biologist and to the stock breeder—and very especially so in South Africa.

### THE PROBLEM OF ENVIRONMENTAL INFLUENCE WITH REFERENCE TO THE SOUTH AFRICAN STOCK BREEDER.

South African breeders know only too well that stock imported from European countries deteriorates, even when the animals are well treated and fed. Breeders of Thoroughbreds, therefore, have to resort to importations at regular intervals to keep up the standard. It is also well known that South African bred horses cannot compete with imported horses on the race track. According to Quinlan (1938), however, the main cause is the inferior blood of the South African bred horses, the mares used not being of the same standard as the imported stallions. As no data have yet been analysed, it cannot be stated definitely whether this is the only cause.

Some excellent herds of Friesland cattle have been established in South Africa as the result of the importation of very good bulls and heifers from Holland. But in spite of the excellent treatment which such herds receive, it appears to be necessary to import bulls to maintain the high standard. The same applies to the other dairy breeds and to the beef breeds. Now if these imported animals had had to subsist on natural grazing, then one could expect a very marked and quick decline in their productive and reproductive abilities, but stud herds of high excellence do not receive this inferior treatment.

The South African breeder is, therefore, faced with the problem of maintaining a high standard once it has been achieved. A necessary step in the attempt to solve this problem is the solution of the problem of environment. Environmental factors, it is certain, exert an influence. It is an established fact, for instance, that of those animals which are bred in a cold climate, those bred at high altitudes do better in a warm and semi-tropical environment than those bred at sea-level in the same cold climate (Duerst, 1931). Isolated facts like these, though in themselves helpful, do not solve the problem; they only point to the need for detailed analytical investigation.

THE PROBLEM OF ENVIRONMENTAL INFLUENCE ON GROWTH WITH  
REFERENCE TO THE WHITE RACE IN SOUTH AFRICA.

The effects of the specific environmental factors of South Africa are especially noticeable in the growth rate of the children. Scientists coming from Europe notice differences in South Africans as compared with the various European races. Grober (1937), for instance, commented on the large size of South African children at a relatively early age. It also struck him at the Olympic games that the South Africans were bigger than men of other nationalities.

These differences in growth and development which are noticeable in South African children, have also been commented on by scientists living in South Africa.

Skawran (1937) observed that young South Africans between the ages of 17 and 21 exceed European adults in all measurements and that a high percentage of them belonged to the athletic type. He, therefore, concluded that regeneration had taken place and not degeneration as contended by Leipoldt (1931).

This problem of the degeneration or regeneration of the white race in South Africa touches in the very core of human biological aspect of the general problem of the influence of environment on growth and development, but at present we have to suffice with general observations in South Africa, no analytical South African data being available as yet.

Thus Ross (1935) stated that he personally had seen no ill results from purely climatic reasons to anyone who lived in any part of Southern Africa under outdoor conditions and enjoyed a change occasionally. This more or less agrees with the findings of Franz (1937) who, after reviewing a large number of articles, came to the conclusion that even in a tropical environment degeneration need not necessarily take place.

A particularly interesting parallel example to the rapid growth of the South African children is that of the children in Kenya, as mentioned by the journalist MacKenzie (1938), who stated that, in order to check the rapid growth of the European children, these children have to be taken away to different countries during the holidays.

The study of the problem of growth leads to many and far-reaching results. Measuring scientifically the rapid rate of growth of the South African child is only the first step. More important even is the study of the consequences. If South African children do grow faster, then it is important to investigate whether the average ages and the life expectancy at different ages for South Africans are the same as or different from those of the inhabitants of Europe or America. It is also important to study and compare the different diseases or abnormalities from which South Africans suffer. McCay (1933), for instance, found that longevity in animals decreases with increased rate of growth. Such rapid growers escape many diseases typical in young animals but suffer from senile diseases at an earlier age than slow growers.

The very magnitude of the problem of the environmental effects on growth itself and of its related biological consequences insists on detailed analytical research. By referring to a limited number of such investigations on the problem of growth and on the effects of environmental factors on growth, we propose to illustrate some aspects of the problem which confronts us. In the subsequent sections dealing specifically with the growth of the albino rat, previous investigations on similar aspects of the problem of growth will be referred to more fully.

EXAMPLES OF THE TYPE OF RESEARCH WORK THAT IS BEING DONE, ILLUSTRATING DIFFERENT ASPECTS OF THE PROBLEM OF ENVIRONMENTAL INFLUENCE WITH REFERENCE TO GROWTH AND DEVELOPMENT.

(a) *Seasonal Variation in Growth.*

Nylin (1929) gives a comprehensive survey of the work which has been done by different investigators on growing children. Most of them, Nylin's included, found seasonal cycles in the increase in weight and height, although the maximum and minimum rates of increase in weight and height occurred at different seasons—basal metabolism, phosphate and calcium content of the blood, iodine content of the thyroids (in the case of sheep, cattle and pigs).

*Prenatal Development.*

It appears that environmental factors have some effect even before birth on the developing organism. This is indicated by the difference in the birth weights of children of different countries and also by the difference in the birth weights of animals during different seasons. Robertson (1923), for instance, found that South Australian infants weigh from 8 to 10 ounces more at birth than infants born in Great Britain.

The seasonal influence on birth weight and litter size has been studied chiefly in the case of the smaller animals, with which it is easier to control the nutrition throughout the year. Eaton (1932) found that the largest litters of guinea-pigs were born late in the summer and the smallest in winter. The primary seasonal influence on litter size occurs at conception about 67 days before the birth of the litters. Litters born late in the summer are conceived early in the summer or in mid-summer when conditions are favourable, and those born in winter and early in the spring are conceived late in the autumn or in the winter, when conditions are not so favourable.

Seasonal effect on birth weight on the other hand continues over the whole period from conception to birth. The influence is wholly through the mother and according to Eaton (1932) affects the young less than might be expected because nourishment of the foetus is entirely from the blood stream of the dam. Eaton found a strong argument in favour of his views in the researches of Eckles (1919) who showed that there was a strong tendency for the composition of the blood to remain constant, even under adverse conditions of nutrition. It therefore appeared to Eaton that the difference in

birth weight during different seasons was due rather to litter size than to direct seasonal influence. The results obtained by Hanson and Sholes (1924) also showed no variation in the birth weights of rats during the different seasons of the year.

But the question as to whether the season of the year influences prenatal development, is by no means satisfactorily answered. The results obtained by King (1935) were contrary to those obtained by Eaton. King's results pointed to seasonal variations in the birth weights of rats which were not dependent on litter size.

#### *Postnatal Development.*

Bertelli (1936) observed in his rabbit-breeding investigations that those born during October to December attained their complete development sooner than those born in March or April. The same was true for females, as they reached maturity a month at least before those born in the spring (March-April). Greenman and Duhring (1931), however, obtained only a slight deviation from a fixed standard of growth with rats, due to seasonal effect.

#### *(b) The Influence of certain Climatic Factors.*

We have drawn attention to the investigations of research workers who studied the influence of the season of the year on the growing organism. It may be of interest to show also how the influence of other environmental factors has recently been studied.

Mills (1932 a, b) made general observations on the influence of climate on the health of man in the United States, and then he and Ogle (1933) conducted experiments under controlled conditions which agreed with the general observations. It appears that temperature has a profound influence on the internal secretions of man and animals. The more stimulating climate (frequent changes in temperature) whips up the activity of the glands of internal secretion, especially the pancreas, thyroid, and adrenals. These glands exert the principal control over the body metabolism or the speed of living. Stimulation of them beyond their point of endurance results in very definite increases in the diseases that come with their failure to function. The sex glands are affected with the others. The onset of menses occurs at an earlier age in the more stimulating regions with fertility reaching an early peak and then declining more rapidly than in the less stimulating zones. Their experiments (1933) on rabbits showed that there were two temperature factors of importance which influenced the susceptibility to excessive heat:—

(i) *Mean temperature level* is perhaps of first importance: continuous high temperatures suppress heat production in the body and render the individual less sensitive to additional heat, while cooler environments so stimulate combustion as to make adaptation to higher temperatures difficult. As a result, individuals accustomed to continuous heat are able safely to endure degrees of excessive heat which in a few hours prostrate and kill those adapted to cooler conditions.

(ii) *Temperature variability* is the second factor of importance. Wide and frequent changes in temperature are so stimulative as to overcome the depressive effects of high heat levels completely. Individuals accustomed to a hot environment with wide daily variations, are very sensitive to slight rises in the temperature above that to which they are adapted.

In further experiments Ogle (1934) showed that temperature and humidity affected the growth and sexual activity of mice. Mice (males) adapted to a steady cool environment (64° F.) increased in body weight and body length at practically the same rate as control mice.

The mice from the cooler environment, however, had tails fully 1 cm. shorter than the control mice. Mice subjected to a continuous moist heat had exceptionally short slender bodies, the tails of each being 1 cm. longer than the body. These mice showed a low fertility in three ways, viz., a low percentage of matings that result in pregnancy, small litter size, and low viability of the offspring. The mice in the cool environment showed the most efficient sex functioning, a large number of matings and large litters of vigorous young. The onset of sexual life and fertility in the young came earlier than in the moist heat.

Ogle believes that there are gonadal, hypophyseal, and adrenal interrelationships, but the fine points of these interactions are by no means clear. The sexual functionings under the various conditions presented are believed to be dependent on changes in the interrelationships, indirectly affecting the sex glands, with the adrenals probably initiating the responses to changes in environmental stimulation.

With reference to the results of Ogle's (1934) investigations, namely, that mice developed short slender bodies in a moist warm atmosphere, it is interesting to compare Nylin's (1929) statements that he was not aware of any investigations which support the assumption that increase of height in man is affected by moisture in the atmosphere, by temperature, or by barometric pressure.

#### NUTRITION *versus* ENVIRONMENT.

There are, however, investigators who seem to ignore or minimize the influence of the external environment on the living organism. All the stress is laid on nutrition when they try to explain the differences between different nations or people from different parts of the same country. Heiser (1938) mentioned the work done by McCarrison on rats which received foodstuffs typical of different nations. The rats were considered to show the characteristics typical of those people who live on such foodstuffs.

Sherman (1936) would also appear to lay stress on the nutritional aspect of racial differences and of differences in growth and longevity.

Wolff (1935) was in favour of the view that improved conditions which resulted in improved nutrition, was the cause of the increased growth rate of children measured by him over a period of nine years.

The conclusions of Bennholdt-Thomsen (1938) agree with those of Wolff. He showed how the children of parents in good economic circumstances showed a more rapid growth rate and were sexually mature at an earlier age than children of the poorer classes.

Koch (1935), however, did not go accord with those who ascribed this accelerated growth rate as due to conditions being favourable above the average. He expounded the theory that the accelerated growth rate of children during recent years was due to the greater exposure of the body to ultra violet rays of the sun and lamps.

According to Hammond (1936), however, the scientific problem of how the environment shapes the breed is still to be solved. He takes it as proven that climatic and nutritional environment affect the physiology of the animal body.

As indicated above, a large amount of work has already been done on the influence of environmental conditions on growth and development. Though we are still far from a final solution of how these differences come about, the research work that is being done, brings us nearer to the final solution. It is hoped that the present study, too, will contribute to the solution of how environmental conditions affect growth and development.

## II. OBJECT OF THE INVESTIGATION.

We have indicated that the South African environment influences the growth and development of man and animals. A change takes place in the individual and in the races coming from the colder European countries. This change may be very slow and may only be noticed after many generations, but it is there, nevertheless. Which factor or what combination of factors exerts the greatest influence, is not yet known. Only the result can be seen. To conduct investigations on the influence of environment on man or on the larger domestic animals, will take a long time. Carrel (1936) is of the opinion that, in order to analyse the influence of the environment on man, many experiments should be conducted for a century at the least. The influence of environment on domestic animals could be satisfactorily analysed only after 25 years of research as it is essential to observe the influence over several generations. It was, therefore, more practical to carry out the preliminary investigations on an animal species which completed its life cycle in a much shorter time. For this purpose the albino rat was chosen. Thus important information could be obtained in a relatively short period. This information would be valuable in determining what factors affect growth.

The present work was therefore started, firstly to determine the normal growth rate for the albino rat under South African conditions, and secondly to establish the different factors which influence growth, production, and reproduction. When these factors have been established further experiments can be planned to find out in what way these factors work.

In order to establish which factors affect the growth of a certain species of animal, and, consequently, its life span and its susceptibility to different diseases, large numbers of this animal should be bred under controlled conditions for many generations. The feeding should remain constant throughout. When small laboratory animals are used for such experimental purposes, the conditions under which they live are not identical with those of our domestic animals. They may also react quite differently. But they nevertheless serve a useful purpose because the analysis of their growth rates leads to the establishment of the various factors affecting growth. When once these factors have been established, it becomes simpler to plan further investigations on the influence of environment on larger animals.

The present work is, therefore, devoted to the study of the normal growth curves of the albino rat under South African conditions and to the determination of the external factors which influence the growth and the development of the Albino rat. Research work on the actual problem of the influence of environment will follow. Rats will be bred for many generations under conditions where as many factors as possible (including light, temperature, and humidity) can be controlled and the effects of these factors will be analysed.

### III. EXPERIMENTAL DATA.

#### 1. ANIMALS.

In September, 1932, the Onderstepoort Veterinary Research Institute imported a number of albino rats. These were obtained from the Glaxo Research Laboratories, London. The latter again obtained them from the Wistar Institute, U.S.A. For a year these rats were used by Dr. Kellermann alone in nutrition experiments. Rats were bought for the other requirements of the Institution: This, however, proved to be unsatisfactory. In 1933 the author commenced to breed the rats for the requirements of the Institution and at the same time to make observations on the growth of the rats. In July, 1933, investigations were begun on a small number of males and females of the imported stock. The animals on which the observations were made were as uniform as can be obtained, since the Wistar strain had been closely inbred for many generations. The rats were also closely inbred at this Institution:—brother and sister or mother and son or father and daughter were often mated, mostly, however, brother and sister mating was practised. As will be discussed more fully at a later stage, not many females were allowed to produce more than one litter as the aim was to get as many generations as possible in order to see whether any change in growth and reproduction was taking place in the new environment during the four years that observations were made.

#### 2. HOUSING, MANAGEMENT, AND FEEDING.

##### (a) *Housing and Management.*

Throughout the four years that these investigations were carried out, the feeding, housing, and management of the rats were not changed.



The rats were kept in a room measuring 21 by 18 feet. It had double doors, each 5 feet wide and 8 feet high, and one window, 4 by 5 feet. During the winter months the room was heated at night by means of tubular heaters in order to maintain breeding. Only on rare occasions was it necessary to leave heaters on during the day. During the summer months no fans were used. The double doors and window were thrown open to secure air circulation. At night the doors were closed, but the two 1 foot square holes at the bottom of the doors and the window remained open. Although the outside temperature (shade) sometimes went above 90° F. it never went above 90° in the room.

The rats were housed in wooden cages. For breeding purposes a cage measuring 14 by 12 by 24 inches was used. One male and three or four females were put into one cage. A male was never put with more than four females at a time. Usually only three females were given to a male. The date that the male and the females were put together, was recorded, as well as the number of each rat. When a female became pregnant she was put by herself into a cage of the same dimensions containing clean shavings and woodwool.

Every morning at 9 o'clock the author inspected all the cages. When any female had given birth to pups during the previous 24 hours, the pups were sexed and weighed individually. The weight of the mother was also taken. Up to two weeks after parturition the female and her pups were not handled. At two weeks every pup got a number which could be identified by means of a system of cutting slits into the ears. Up to weaning, and later, the pups were weighed and their tails measured every week. The mothers were also weighed every week until the litter was weaned.

The pups were weaned when they were between three and four weeks old. As the influence of age on weaning was being studied, they were weaned at different ages. At weaning the males and females were separated and kept apart. Rats of more or less the same age were put together, the number varying from 10 to 12 per cage. A larger cage measuring 21 by 18 by 24 inches was used for the weaned rats. The cages were cleaned once a week, wood shavings being used as absorbent material.

As mentioned above, the pups were weighed and their tails measured when they were two weeks old. This was repeated every week until they were 20 weeks old. Records were kept of the ages of all the rats when their eyes opened and afterwards of the ages of the females when their vaginas opened. When anything abnormal about a rat was noticed, a note of this was made. By means of the slits in the ears any rat could be identified at any time. Since the author had to breed rats for the other requirements of the Institution as well, most of the rats could not be retained for longer than 20 weeks. Only those required for breeding purposes were retained longer, and also a few others, which were weighed and measured for a longer period. Rats were often used for breeding purposes before they were 20 weeks old. In those cases only the males were weighed until they were 20 weeks of age.

*(a) Feeding.*

The rats received fresh milk every morning. A meal ration was given *ad lib.* Earthenware pots were used for the milk and the meal ration. The following meal ration was used for the breeding stock and for the young growing rats during the four years that observations were made:—

Yellow maize meal ... ..	75	per cent.
Linseed oil meal ... ..	15.5	per cent.
Lucerne meal ... ..	5	per cent.
Crude casein ... ..	2.5	per cent.
Bone meal ... ..	1	per cent.
CaCO <sub>3</sub> ... ..	0.5	per cent.
NaCl ... ..	0.5	per cent.

The protein content of this meal mixture is about 15.0 per cent.

The rats remained very healthy during the four years. The only ailment from which a small number of rats suffered was an infection of the lungs. The figures will be discussed in the appropriate section.

**IV. ANALYSIS OF THE DATA.****A. PRENATAL GROWTH AND GROWTH UP TO WEANING.***1. Prenatal growth.**(a) Oestrus and service.*

The investigations into the normal growth rate of the albino rat included observations not only on the development of the foetuses but also on the females before and during pregnancy. Therefore, before the females were put with the males, observations were carried out on their daily weights and on their oestrus cycles.

Their vaginas were inspected every day when they were weighed. It was observed that in most cases a clear, sometimes slightly milky fluid came from the vagina at regular intervals. In 40 rats under observation the frequency of this occurrence was as follows: every 3rd day in 9 cases, every 4th day in 23 cases, and every 5th day in 8 cases.

Since our observations on the oestrus cycle of the rat was more incidental to the prenatal growth we made no vaginal smears. This method was used by Long and Evans (1922), who were the first to describe accurately the sex cycle of the rat. They showed that the average length of the oestrus cycle is 4 to 5 days. Hemmingsen and Krarup (1937), however, considered that making observations on the psychical symptoms of the females were more reliable than the vaginal smear method, since a cycle may easily be upset if great care were not taken in collecting the mucus. The psychical method will also show when a female will take the male which will not always happen when the vaginal smear shows cornified cells.

At the time of ovulation fluid accumulates in the uterus and in the ovarian capsule. The fluid in the latter facilitates the passage of the ova down the tube. This clear fluid can therefore serve as an indication of the length and regularity of the oestrus cycles in the rat. In our observations the average length of the oestrus cycle in the majority of cases (60 per cent.), appeared to be 4 days. Cooley and Slonaker (1925) also found that in normal, healthy, young females the oestrus periods last approximately four days. In older rats the length of the period may be considerably increased. In nearly all instances this fluid or mucus in our rats is seen for only one day.

The time of service was noted for 15 females. Approximately 50 per cent., i.e., 7, were served on the day after the fluid was seen. Of the remaining 8 females, 3 were served 3 days after the fluid was seen, 5 were served 4 days after the fluid was seen, and only one female was served 5 days after the last appearance of the fluid.

Where the vaginas were inspected daily, the presence of a vaginal plug indicated that the female had been served. In some cases the plug was rather deep, and only a slightly bloody mucus was seen coming from the vagina. It was observed that the vaginal plug could be retained either for only about 6 to 12 hours or for as long as 10 days. In the largest number of females under observation the vaginal plug was retained for only about one to two days, after which period it was resorbed. After the disappearance or resorption of the plug, a creamy fluid could be seen in the vagina. This fluid was generally present for only one day, but in a few cases for two days. Cooley and Slonaker (1925) consider the presence of a vaginal plug to be essential for fertilisation. In every case where they found sperm in the vagina after mating, but no vaginal plug, gestation did not occur.

The females were served practically only during the night. At one time twenty females were put with males simultaneously and inspected every morning and afternoon for vaginal plugs. Only one of the females was served during the day. Cooley and Slonaker (1925) found that the oestrous period in rats began in the great majority of cases between 4 and 10 o'clock in the evening, and that the females were most receptive during the first three hours after the appearance of oestrus.

Hemmingsen and Krarup (1937) and Browman (1937) showed that the oestrus cycles and the activity of the rats were preferably confined to certain hours of the natural day—night rhythm. Oestrus and activity were at their maximum in the dark. When the rats were kept in the dark during the day and exposed to light during the night, then the cycle adjusted itself to the new rhythm, oestrus and activity being manifested during the dark period or day time. Browman also determined that temperature did not affect the cycles which he obtained during his light experiments. Lee (1926) has reported that the length of the oestrus cycles of rats varies roughly with that of their environmental temperatures.

The consistency of the fluid or mucus in the vagina of the pregnant rat changed at various stages of pregnancy and could therefore serve as a rough indication of the number of days the

female had been pregnant. Approximately a week after service a clear sticky mucus could be seen when the vagina was opened. This, however, could not be seen in all cases just by dilating the vagina. About 10 to 14 days after service this sticky mucus became dark and was quite black in some cases. This dark fluid had been called the "placental sign" by Long and Evans (1922). The fluid could become clear again at about 17 or 18 days, still remaining sticky. A few days later a discoloured and watery fluid might be seen.

There were, however, some variations of this sequence, especially in the case of old rats. In some old rats the fluid—which was dark and sticky in nearly all young rats—was watery and appeared to be mixed with blood. Sometimes fresh blood could be seen round the vagina without opening it. This appeared to be caused by the death of one or more of the foetuses. The uteri of females that were killed on the morning on which blood was noticed, always contained dead foetuses. The placentas were still quite firmly attached to the uterus. It appeared that the foetal membranes had been ruptured, with the result that blood escaped from the uterus and vagina. This bloody and watery discharge was not observed in any of the young females which were pregnant for the first time.

Long and Evans (1922) observed that blood could be detected in the vagina of rats from the beginning of the third week of gestation. In mice Parkes (1926) found blood at about the 12th day of pregnancy and Merton (1937-38) found blood in the vaginas of 37 out of 44 mice from the 11th to 13th day of pregnancy.

Another abnormality which was also seen only in old females, was a puslike discharge from the vagina. In some of the females this was seen for weeks at a time, and in some instances it seemed to be permanent. Although this puslike discharge came from the vagina, such females were rarely served. It was only after the disappearance of the puslike discharge and after the appearance of the clear mucus that such females became pregnant again. A number of females were killed while this puslike discharge was seen in the vagina. In all cases one or both horns of the uterus were filled with this puslike substance. It was quite watery in some cases, but thick and pasty in others. The ovaries in such cases were inactive and showed no recent corpora lutea nor any follicles. In one instance, however, some of the watery substance was present in the left horn and only a very small amount of the greyish material in the right horn. The right ovary had no recent corpora lutea nor any follicles, while in the left ovary about six to eight large follicles were present.

One old female that was served twice, showed a puslike discharge each time a few days after service. After she was killed, the right horn of the uterus was found to be about three quarters full of the thick pasty pus while the left horn seemed to be normal. There were several recent corpora lutea in the left ovary and two recent ones in the right. This showed that the ovaries could become active again and that the female could be served, before the pus disappeared completely from the uterus and the vagina. Such females, however, will not become pregnant.

Since this empyema of the uterus—or purulent metritis—was found only in females that had bred, it would appear that they became infected after parturition. On account of the two separate cervical openings only one horn of the uterus could become infected while the other remained normal. The female could therefore show oestrus and be served, but she did not seem to become pregnant. When pus was present in both horns, the females did not show oestrus. As long as the pus could escape, however, it was possible for the female to breed again. In some cases, however, all the pus could not escape with the result that it became pasty. In such cases the mucous membrane of the uterus may be damaged permanently.

(b) *Weight changes of the pregnant females.*

(i) *Different classes of females.*—The change in weight of the following three classes of pregnant females was studied: firstly, young females that had been put with the males as soon as their vaginas opened; secondly, old females, averaging more than 200 g., that were pregnant for the first time; and thirdly, old females that had had one or more litters. Altogether 83 pregnant rats were kept under observation. Of these, 5 belonged to the first class, 49 to the second class, and 29 to the third class.

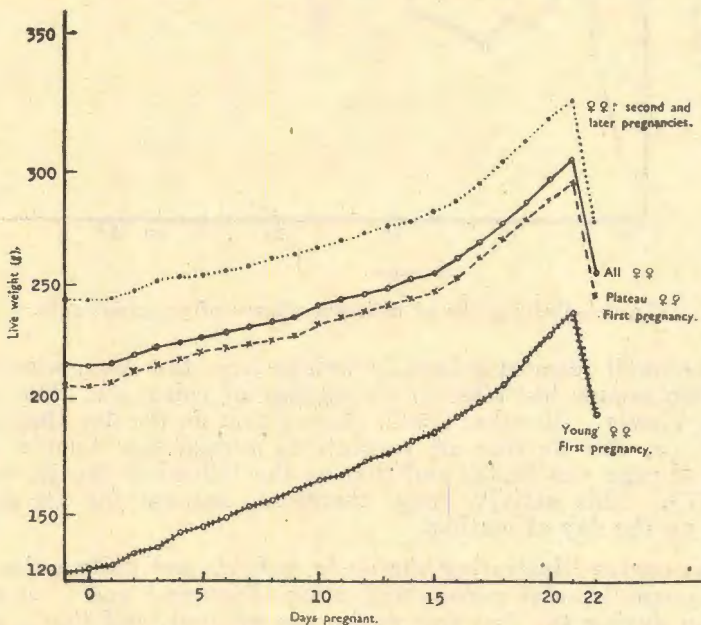


Fig. 1.—Daily weights of different classes of pregnant rats.

Daily weights were taken of these females during pregnancy. They had also been weighed daily for some time before they were put with the males. When they had been served, vaginal plugs were not seen in all cases. The duration of pregnancy was taken as 22 days (this will be discussed at a later stage), as it was found that most pregnancies (80 per cent.) lasted 22 days. Table 1 gives the average

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daily weights of these 83 rats as well as the average daily weights of each separate class. To get a better idea of the changes in live weight, the weight on the day before service, i.e., 23 days before parturition, was taken as 100 and the subsequent weights expressed as percentages. These weights, actual and relative, are illustrated in Figures 1 and 3.

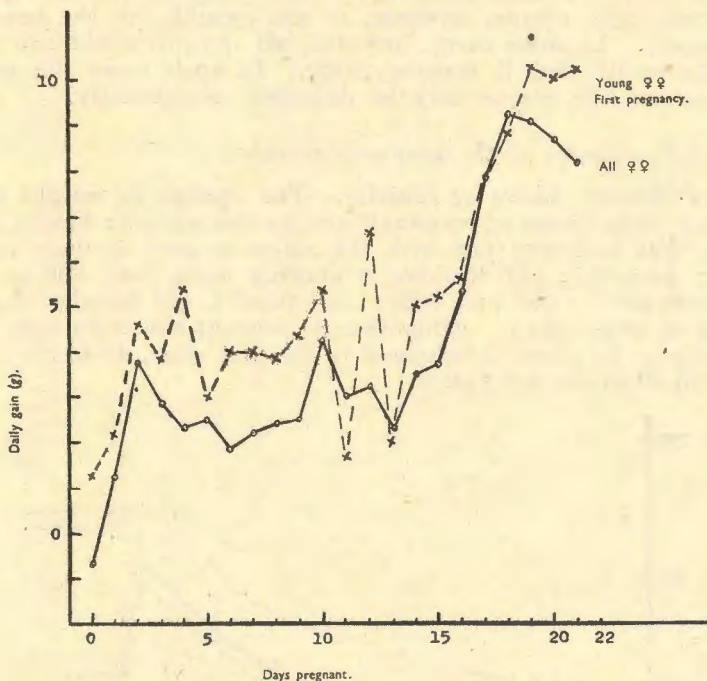


Fig. 2.—Daily gains of different classes of pregnant rats.

The small females gained in weight from the start, whereas the other two groups lost slightly on the day of coitus and then started gaining slowly. Slonaker (1925) showed that on the day that mating took place, the number of revolutions turned per female in the revolving cage was 16,352 and that on the following day the number was 2779. This activity may, therefore, account for the drop in weight on the day of mating.

The curves illustrating the *daily weights* and *daily gains* of all the pregnant females under observation (Figures 1 and 2) show that the gain during the first two weeks was gradual, and that a sudden increase in weight took place only from the 15th day of pregnancy. This increase in weight reached a maximum of .9·2 g. per day per rat at 18 days. Then there was a decrease to 8·3 g. gain per day at 21 days. The curves illustrating the daily weights of the separate classes of pregnant females show that the increase in weight was not the same for the three classes. The curves for the two older groups are more or less similar, while that of the five young females shows a more rapid increase from the beginning of pregnancy without the

sharp rise after 15 or 16 days. The curves of the *relative weights* and gains, which were obtained by taking the average weights of the females the day before they were served as 100 and expressing the subsequent weights as a percentage of it, in Figures 3 and 4, however, show better how much more the young females gained in weight and

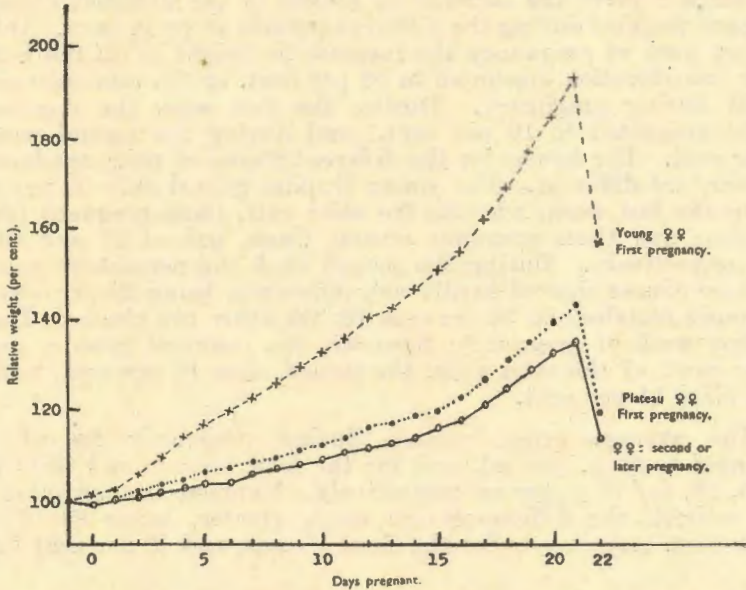


Fig. 3.—Relative daily weights of different classes of pregnant rats.

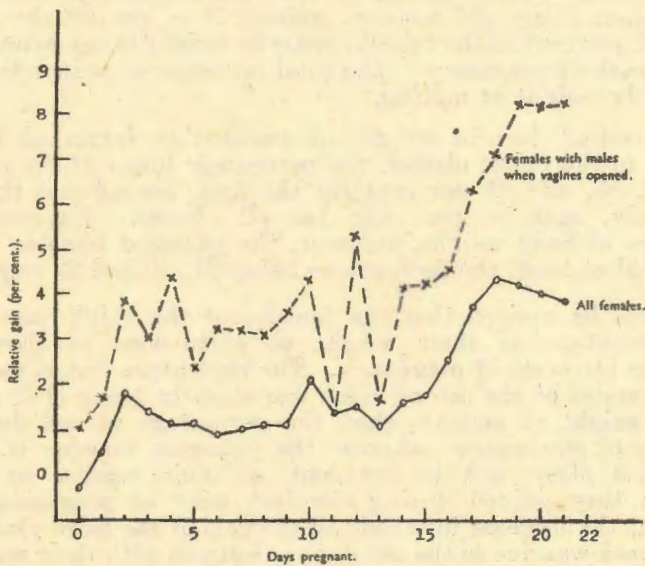


Fig. 4.—Relative daily gains of all rats and rats put with males when vaginas opened.

## GROWTH OF THE ALBINO RAT.

how this gain increased at 16 days after insemination. The relative weights of the two older and heavier classes lie fairly close together, although the third class—the 29 old females which had had one and more litters—gained relatively less than the second class—the 49 old females that were pregnant for the first time.

Table 2 gives the increase in weight of the different classes of pregnant females during the different periods of pregnancy. During the last week of pregnancy the increase in weight of all the females under consideration amounted to 58 per cent. of the total increase in weight during pregnancy. During the first week the increase in weight amounted to 16 per cent., and during the second week to 26 per cent. The figures for the different classes of pregnant females, however, are different. The young females gained only 50 per cent. during the last week, whereas the older rats, those pregnant for the first time and those pregnant several times, gained 58 and 60 per cent. respectively. During the second week the percentage gains of the three classes showed hardly any difference, being 29 per cent. for the young females and 26 per cent. for the other two classes. During the first week of pregnancy, however, the youngest females gained 21 per cent. of the total gain, the second class 16 per cent., and the third class 14 per cent.

The average gross increase during pregnancy for all rats amounted to 90 g. per rat, and for the first, second, and third class to 115, 88, and 85 g. per rat respectively. Expressed as percentages of body weight, the differences are much greater, being 93, 43 and 36 per cent. respectively for the three classes, and 42 per cent. for all rats.

More or less the same percentages were obtained by Hain (1933), who found that rats weighing between 150 and 200 g. on the day of mating, i.e., fairly old females, gained 71 g. during the gestation period, 54 per cent. of the total increase in weight being gained during the last week of pregnancy. The total increase comprised 41 per cent. of the body weight at mating.

The actual loss in weight at parturition increased from the youngest to the oldest classes, the percentage losses of the total gain being 39, 55, and 61 per cent. for the first, second and third class respectively, and 56 per cent. for all classes. Expressed as a percentage of body weight, however, the youngest females lost most and the oldest least, the percentages being 37, 23 and 22 respectively.

It will be noticed that the females of the third class lost the same percentage of their weight at parturition as they gained during the last week of pregnancy. The percentage lost at parturition by the females of the second class was slightly lower (1.2 per cent. of their weight at mating) than the percentage gained during the last week of pregnancy; whereas the youngest females (i.e., those of the first class) lost 10 per cent. of their weight (at mating) less than they gained during the last week of pregnancy. This shows that the increase in weight of the rats of the third class during the last week was due to the developing foetuses with their membranes and fluids, whereas the youngest rats retained some of the increase in weight. The increase in weight of the first two weeks was retained



by all the females, the oldest class being 14 per cent. heavier after parturition than at mating, the second class 19 per cent., and the youngest class 56 per cent. The actual gains (i.e. the difference between the total weight gained during pregnancy and the weight lost at parturition), averaged 70, 40 and 33 g. per rat for the rats of the youngest, second, and third classes respectively.

This increase in weight was much above that of virgin rats of the same weights. Some females of the colony having the same weights as the females of the first class had at mating, viz., 124 g., were weighed every week. After three weeks their increase in weight averaged only 31 g. per rat. Virgin rats weighing 200 g. gained only about 5 g. during three weeks. Pregnancy, therefore, caused an increase in weight which was not found in virgin rats of the same age or weight. In the case of the older rats, this increase took place only during the first two weeks of pregnancy before there was any marked increase in the weights of the foetuses. Slonaker (1931 f) showed that during gestation the increased food intake was relatively small and almost uniform in amount. As will be shown in another section, we obtained the same results. Slonaker, however, found that there was a great reduction of spontaneous activity during pregnancy. It therefore appears that the energy was used for fat deposition which resulted in the permanent increase after parturition. Whether this is the only cause of the increased weight of the old females, is not certain. Nevertheless, the amount of internal fat that was observed in many of the rats that were killed, was remarkable. This was the case with rats over 200 g.

According to Hain (1933), however, it is possible that the non-specific metabolic stimulant in the gonad, which is at the height of its activity during pregnancy, together with the general increase in the size and weight of the whole reproductive system resulting from the pregnant condition, accounts in some measure for the increase in weight of the pregnant animal over the virgin, during the same period. From our results it does not appear, however, that the increased size of the reproductive organs of parous and non-parous females could have influenced the weights of the rats to any marked extent, as the non-pregnant uterus of the rat weighs less than one gramme and only slightly more when filled with fluid. The weights of the uteri will be discussed at a later stage.

The young rats did not show the same amount of fat deposition as the old rats. In their case some of the increase was due to the development of the body, as was shown by the fact that the loss at parturition was less than the gain during the last week of gestation.

The difference between the weight of the young at birth and the loss of weight of the mother at parturition is due to the birth adnexa. The ratio of young to adnexa averaged 74 to 26 per cent respectively for all classes of females; 82 to 18 per cent. for the youngest rats; 74 to 26 per cent. for the second class; and 75 to 25 per cent. for the third class. Slonaker (1931 f) found a ratio of only 64 to 36 per cent. of young to adnexa. It is well known that the rat eats all the foetal membranes and usually also the young that are born dead. This will therefore increase her postpartum weight. This, however, does not

appear to be the cause of the difference in the percentages of young and of adnexa between the youngest and the oldest females. As is shown in Table 7 the average number of young per litter in the youngest class was 7.8, in the second class 7, and in the third class 7.5. As shown in the same table, the ratio of young to adnexa increased up to 80 to 20 per cent. as the litter size increased, then remained constant, and decreased again for the largest litter size. Ibsen (1928) found that the percentage distribution at term in the guinea pig was 81 per cent. young and 19 per cent adnexa. In the pig Warwick (1928) found that the ratio of young to adnexa was 83 per cent. to 17 per cent. In a small number of observations on sheep after 140 days of pregnancy, Cloete (1939) found that the ratio of foetus to membranes and fluids was 66 to 34 per cent. Hammond (1927) found that at 8 months' pregnancy the ratio of foetus to membranes and fluids in cattle was 62 to 38. This ratio, however, is higher than the one obtained at 7 months' pregnancy (55:45). It is therefore possible that the ratio at birth is higher still. This means that the ratio of foetus to membranes and fluids may be equally high in cattle and in sheep or slightly higher in cattle. In man (Lusk, 1928) this ratio is quite different, the loss at parturition being 20 per cent. of the weight of the mother. Of this the child comprises 5.9 per cent. and the birth adnexa 13.1 per cent., i.e., approximately  $\frac{1}{3}$  foetus and  $\frac{2}{3}$  adnexa.

(ii) *Influence of weight at time of mating.*—In order to ascertain what factors were responsible for the difference in the weight changes of the three different classes of females during pregnancy, the influence of the two most likely factors was analysed: viz., weight of female at time of mating, and litter size. For this purpose the weight changes of primiparous and of multiparous rats were analysed separately.

The average daily weights of the pregnant females and their relative weights expressed as percentages of their weights at mating are given in Table 3. The actual gains in grammes during the different periods of pregnancy as well as the gains expressed as percentages of the gross increase in weight during pregnancy are given in Table 4. These results are illustrated in Figures 5 and 6.

The relative weights of the females pregnant for the first time, i.e., their weights expressed as percentages of their weights at service, as illustrated in Fig. 5, indicate the trends of the different weight groups better than their actual weights do. The lightest group (110-139 g.) gained most in terms of its relative weight. The next heaviest group (140-169 g.) followed. The second and the third (170-199 g.) groups showed more or less the same increase up to eight days, after which the second group (140-169 g.) increased in weight more rapidly. The increases of the third group (170-199 g.) were practically the same as the increases of the fourth group (200-229 g.) for the whole duration of pregnancy. The daily weight increases of the primiparous and multiparous rats of the same weight were about the same, showing only slight differences in favour of the primiparous rats.

A clearer picture of the gains during different periods is given in Table 4 and Figure 6. The lightest group of rats pregnant for the first time (110-129 g.) gained 50 per cent of the gross increase in weight during the last week of pregnancy. The other groups of rats pregnant for the first time all gained relatively more, the highest percentage (59 per cent.) being gained by the 170-199 g. group. But no consistent trend was clear. The same was the case with the rats that had had several pregnancies. Their relative increases during the last week were, however, all above the relative increases of those pregnant for the first time.

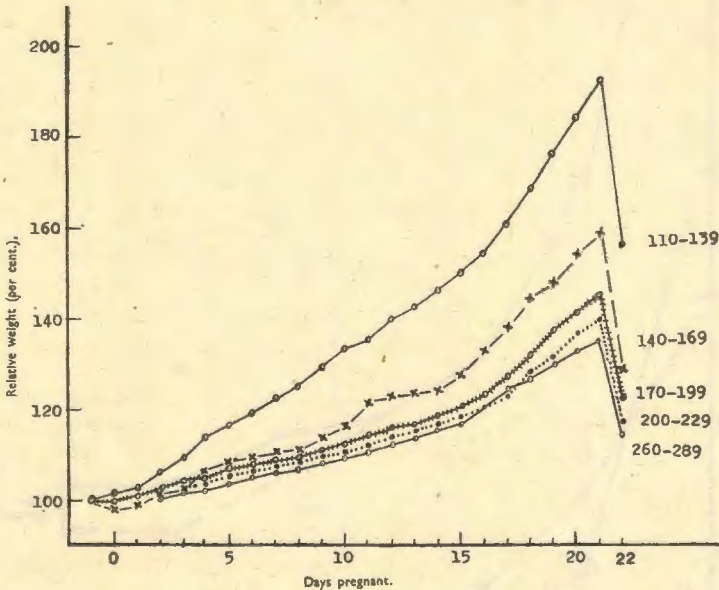


Fig. 5.—Relative daily weights of rats of different weights at mating during first pregnancy.

The percentage gain of the gross increase during the first week of pregnancy was the highest (21 per cent.) in the lightest group of primiparous rats, whereas the other groups of primiparous rats gained 16-17 per cent. of their gross increases. During the second week the two heaviest groups and the lightest group gained more or less the same percentages ( $\pm 29$ ) of their individual gross increases, whereas the remaining three groups gained lower percentages (25-26), which differed only slightly. The percentage gain of females pregnant for several times decreased during the first week and increased during the second week from the lightest to the heaviest group.

The gross increase during pregnancy was greatest (114.8 g.) for the lightest group. No consistent trend towards an increase in weight was shown.

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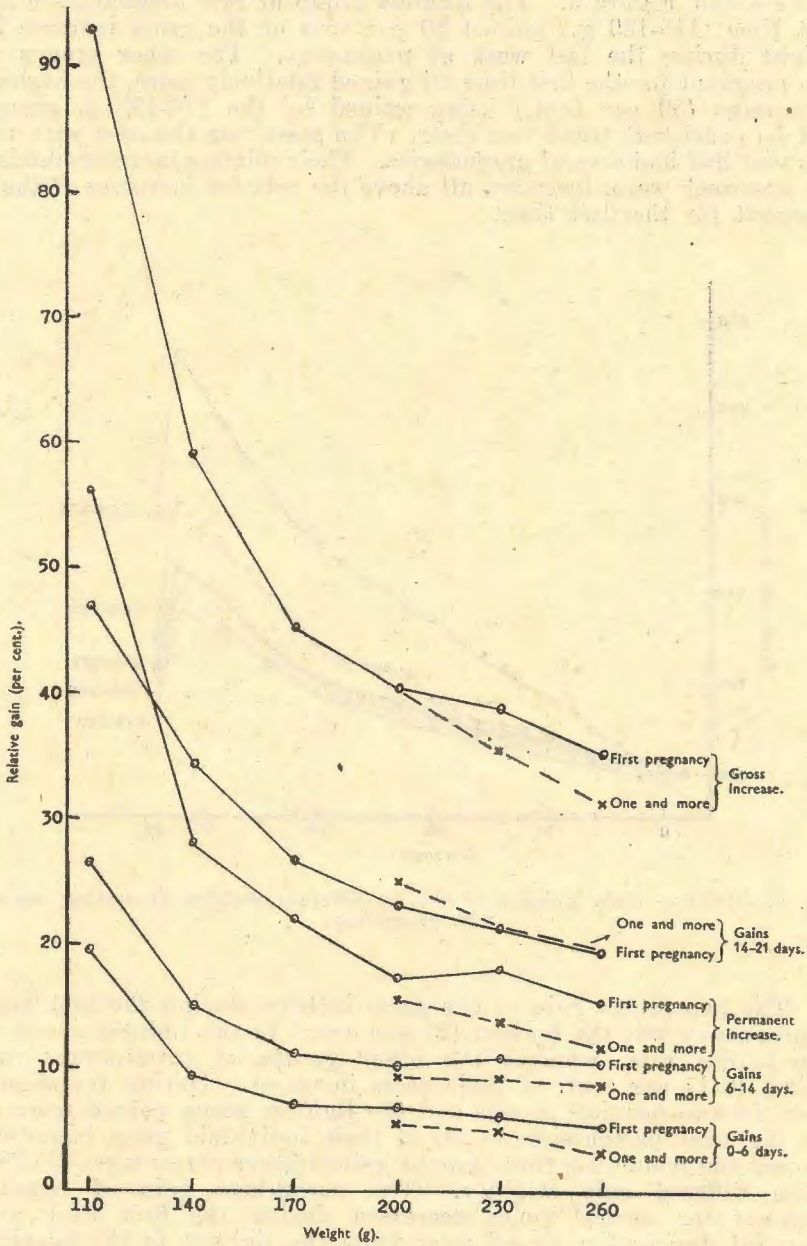


Fig. 6.—Relative gains of rats of different weights during different periods of the gestation period. (To obtain the relative gains the subsequent live weights of the rats were expressed as percentages of the weights at mating).

The permanent increase in weight after parturition, i.e., the difference between the weight increase during pregnancy and the loss in weight at parturition, was greatest for the lightest group pregnant for the first time, while the permanent increases in weight of the other groups fluctuated. The same was the case with the loss in weight at parturition.

The relative gains, however, i.e., the increases in weight expressed as percentages of the weight at mating, showed quite regular trends, as is illustrated in Fig. 6. The same trends were manifested by primiparous and by multiparous rats, the relative gains of the latter in most cases being slightly below those of the former.

The relative gains of all females during the first and second weeks are parallel, the lightest group, pregnant for the first time (110-139 g.) gaining most, 19.5 and 26.5 per cent. of the body weight during the first and second weeks respectively. The relative gain of each of the following two weight groups (140-169 g. and 170-188 g.) showed a decrease on the relative gain of the weight group preceding it. The relative gains of the weight groups of 200-229 g. and above were the same. The relative increase during the last week of pregnancy was also highest (46.6 per cent.) for the lightest group and decreased with every weight group, being only 19.5 per cent. for the heaviest group. The same type of curve is obtained by plotting the relative gross increases; i.e., there is a continuous decrease from the lightest to the heaviest group. The lightest group gained 92.6 per cent. of its body weight during pregnancy while the heaviest group gained only 35.3 per cent. The relative weight retained after parturition decreased with weight, so also the weight lost at parturition. Here again it is interesting to note that the weight lost at parturition, being equivalent to the weight of the young, membranes and fluids, was practically the same as the increase during the last week of pregnancy in the three heaviest classes. It was, therefore, only the gain in weight of the first two weeks that was retained after pregnancy by the three heaviest classes. The three lighter groups gained more during the last week than they lost at parturition, the difference being the greatest for the lightest group.

(iii) *Influence of Litter Size.*—The influence of litter size on the gains during the different periods of pregnancy is shown in Tables 5 and 6. The weights of the primiparous and the multiparous females are given separately.

The gross increase in weight of rats pregnant for the first time, showed an increase with an increase in litter size. Those pregnant several times showed some fluctuation, but on the whole the gross increase in weight of the pregnant female also showed an increase with an increase in litter size. On account of the variation in live weights of the different groups, the relative increases, i.e., the increases in weight expressed as percentages of the weight at mating, give a better indication of the different tendencies (Fig. 7).

The gains during the first two weeks of the two classes of rats (first and several pregnancies) showed the same trends. There was no increase with an increase in litter size. During the last week of pregnancy, however, there was a continuous increase in the gains

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of the females with an increase in litter size. The loss in weight at parturition practically equalled the increase in weight during the last week for all litter sizes except the largest in both classes. This, therefore, shows conclusively that the weight of the pregnant female was not affected by the weight of the developing foetuses during the first two weeks of pregnancy. Only during the last week of pregnancy was the weight of the female influenced by the weight of the developing young. The increase in weight during the first two weeks was retained after parturition. The gross increase of the rats pregnant for the first time showed a regular increasing trend with increase in litter size. That of the rats pregnant more than once showed some fluctuations.

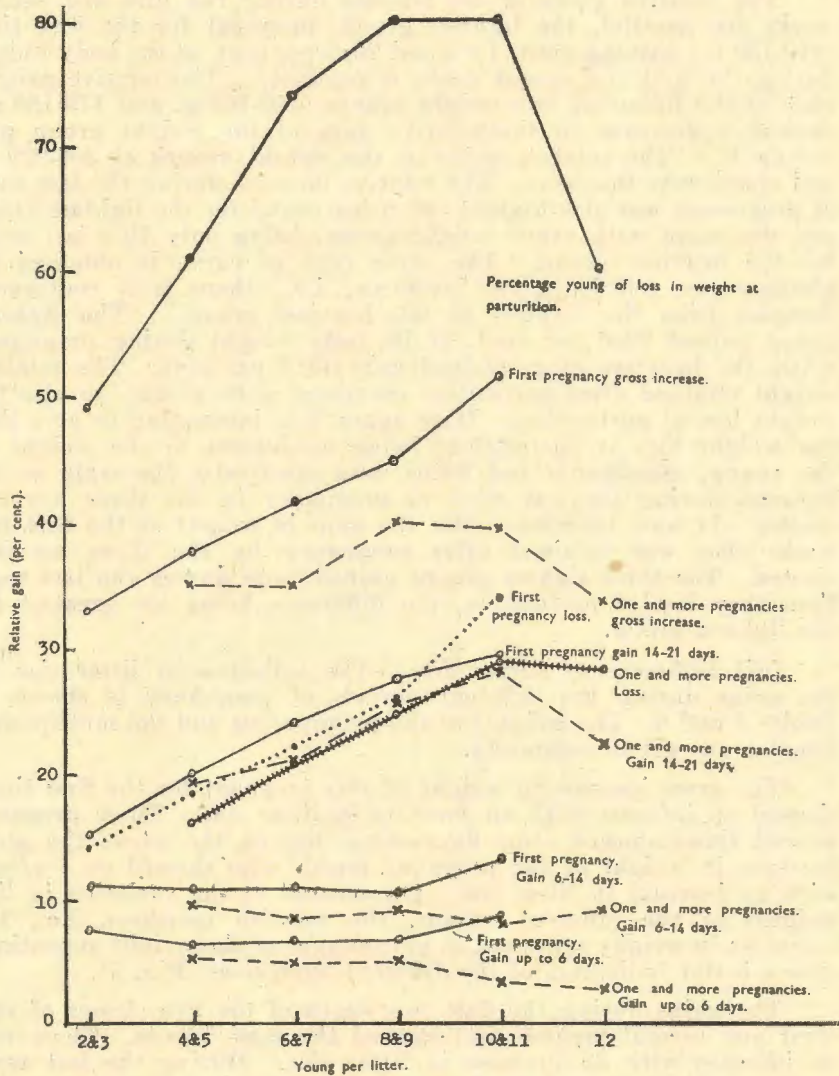


Fig. 7.—Relative gains during pregnancy of rats with different sized litters.

In Table 7 the weight of the young and the weight of the adnexa are expressed as percentages of the weight of the mother after parturition and as percentages of the total loss of weight of the mother at parturition. In the smallest litter size (2 and 3 pups), the ratio of young to adnexa was 49 per cent. young to 51 per cent. adnexa. This ratio increased up to 80 per cent. to 20 per cent. for litter sizes 8 and 9, remained the same for litter sizes of 10 and 11, and again decreased to 60 per cent. to 40 per cent. for a litter size of 12. As mentioned earlier, the actual ratios may be slightly different because the rat habitually eats the foetal membranes. It is unlikely, however, that the trend will be affected.

Our data show a higher ratio of young to adnexa (74 : 26) than Slonaker (1931 f) found with his rats (64 : 36), but this can be explained by the larger litters of our rats, and, consequently, by the higher ratio of young to adnexa in a larger litter.

Our litters averaged 7.2, whereas Slonaker's litters averaged 5.7 in his highest group and only 4.0 in his lowest group.

(c) *Pseudopregnancy.*

Mirskaia and Crew (1930-31) defined pseudopregnancy as follows: "An animal is said to be pregnant when the genital tract exhibits certain structural modifications which are regarded as being characteristic of the condition of pregnancy, and when in the uterus there are fertilised ova. If there are no fertilised ova, but the genital tract exhibits these pregnancy changes, then the female is said to be pseudopregnant."

Pseudopregnancy appears to be fairly common in rats. Cooley and Slonaker (1925) had females served three hours after the commencement of oestrous (early breeding) and three hours before the end of oestrous (late breeding). They found that in early breeding 33 per cent. of the pregnancies were pseudopregnancies, and in late breeding 57 per cent. They also found that the average duration of pseudopregnancy was 15.2 days. They did not attempt to give an explanation of the cause, but offered the following suggestions. There seems to be a critical period in the gestation of the rat about 15 days after coitus. Slonaker and Robertson (1925), for instance, found that when pregnant rats were forced to exercise, abortion or death of the mother occurred most frequently about the 15th day after coitus.

This may indicate that actual fertilization had taken place, and, that for some reason or other, the foetuses were absorbed at about the 15th day. According to them it seems more probable, however, that coitus produces some sort of inhibitory reaction which produces a delay in ovulation. This is not borne out by what takes place in the rabbit where copulation stimulates the production of the lutealizing hormone by the anterior pituitary and ovulates regularly 10 hours after copulation. That it is only a sensory stimulus is proved by the fact that ovulation takes place as well with sterile matings. Slonaker (1925) also found that it was quite likely that oestrus could occur at about 14 to 15 days of pregnancy. Gestation might, therefore, be disturbed causing resorption of the foetuses or abortion.

Long and Evans (1922) thought that pseudopregnancy could be produced by mechanical stimulation of the cervix, but Slonaker (1925) could not produce this condition when care was taken not to cause any injury. When injury occurred, the oestrous cycle was disturbed.

Later workers have shown conclusively that true pseudopregnancy can be produced by electrical or mechanical stimulation. Shelesnyak (1931) obtained 82 per cent. of pseudopregnancies by electrical and 47 per cent. by mechanical stimulation of the cervix. Harris (1936) obtained pseudopregnancy in 68 per cent. of the rats which were electrically stimulated through the head during oestrus. According to Haterius (1933) sterile mating gave uniformly positive results and he suggested that there is a "psychic factor" present in the copulation, which is missing in the different forms of artificial stimulation. The average duration of the pseudopregnancies obtained by the different workers were just over 14 days, the shortest period being 7 days and the longest 23 days.

We found seven females among those that were served, that first started to gain weight and afterwards lost weight again. Three out of these seven were nulliparous, the other four had had one litter. One female lost weight on the 10th day after she was served, three lost weight on the 11th day, and three on the 12th day. The weights are given in Table 8. It will be noticed that during the first ten days the relative gains of both the females pregnant for the first time and of those that had reared one litter, equalled the relative gains of pregnant females of the same weight (see Table 3). The females pregnant for the first time retained 61 per cent., and those that had had one litter retained 46 per cent. of the maximum increase in weight. Two to four days after the females had started to lose in weight, mucus was observed in their vaginas, which indicated that the regular oestrous cycles had recommenced.

Whether coitus produces some sort of inhibitory reaction which produces a delay in ovulation in some cases, as suggested by Cooley and Slonaker (1925) does not appear likely. In the cases we observed, however, it appears fairly certain that the females had been fertilised and that the foetuses were resorbed. The proof of this is found in the increase in weight of all these females. In ten days they gained an average of 25 g., which is far more than much lighter females will gain in the same time. A number of virgin rats with an average weight of 191 g. were put with vasectomised males. They were served, and abnormal cycles of the appearance of mucus in their vaginas were observed. Some cycles lasted five days, while others ten and eleven days. These females gained only 13 g. in 30 days, whereas the other females in the colony of the same weight gained an average of 8 g. in 28 days. The virgin rats served by the vasectomised males were not stimulated to put on weight to the same extent as the females served by the fertile males.

Another rather significant point in our observations is that in all instances the females started to lose weight from 9 to 11 days after they were served. This is, therefore, much earlier than the critical period of 15 days after coitus observed by Slonaker and Robertson (1925).



*(d) Weights of uteri and foetuses at different stages of pregnancy.*

(i) *Procedure.*—In order to study the weights of the uteri and the foetuses at different stages of gestation, a series of observations on different classes of females was systematically carried out, as follows: young virgins 2½-3 months old; old virgins, 8 months old; and females that had had two litters, 11 months old. The observations on the 3 classes of females were made during the months of February, March and April, 1938. During July and August a mixed lot of females was available of which the exact ages were not known. None were, however, younger than 4 months or older than 12 months.

The observations on these females (Table 15) were included to obtain more data in determining the average weights of foetuses at different periods.

The females of the above 3 classes were put with males and weighed and inspected for vaginal plugs every morning. Every female that was served, was removed from the male and placed in another cage. She was weighed daily until she was killed. The females were killed at different stages of pregnancy. At the time they were served, the average weight of the young virgins was 171 g., of the old virgins 245 g., and of the females that had had two litters 277 g.

Every morning after the females had been weighed, those to be killed that day were taken to the laboratory where they were killed in an atmosphere of chloroform. The females were always killed between ten and twelve o'clock in the morning. When several females were killed on one day, all observations on one female were first completed before the next one was opened.

The uterus was removed and immediately weighed. The uterus with all its contents is termed the "dressed uterus". The uterus was not dried before it was weighed. The foetuses were then removed and placed on a piece of filter paper that had been previously weighed. Those from the right and the left horns were always kept separately on the same piece of filter paper. The weight of the filter paper with all the foetuses was read. All weights were read to the nearest hundredth of a gramme. Those from the right horn were then removed and the weight read again. The difference in the two weights was that of all the foetuses in the right horn. The foetuses of the left horn were removed and only the weight of the wet filter paper read. The weight of the foetuses of the left horn was then determined by subtracting the weight of the wet filter paper from that of wet filter paper plus foetuses of the left horn.

Only from the 6th or 7th day of pregnancy can the number of foetuses in the uterus, that is, the number of swellings where the placentas are growing, be distinguished by the naked eye. Only from the 13th day of pregnancy was it possible to weigh the foetuses by the procedure we followed. Stotsenburg (1915) also stated that rat foetuses could only be weighed from the 13th day. MacDowell, Allen and MacDowell (1928) using a much finer technique than we did, weighed mice foetuses that were seven days old. It took the three workers up to three hours to complete the observations on and the weighing of one litter only.

(ii) *Weights of non-pregnant and pregnant uteri.*—The dressed uteri of young females of two to three months old are lighter than those of old virgins and of old females that have had two litters. According to the few observations the uteri of old virgins seem to weigh more or less the same as the uteri of those females that have had two litters (Table 9).

It is possible, therefore, that the live weight of the rat is more important in determining the weight of the uterus than the circumstance whether the female has produced a litter or not. In some cases the uteri were heavier at the time the rat was served—on account of the clear fluid which was still in the uterus—than it was a few days later. This fluid is present when ovulation takes place. As were shown above, rats are in some cases served a day after mucus appeared in the vagina and in other cases three to five days after the mucus was last seen. In a number of cases no fluid was present in the uterus the morning after the female was served.

In our observations the weight of the uterus was lower a few days after service than it was at service. The weight of the uterus showed practically no change until the 6th or 7th day after service: Then an increase in weight was noticed. Only from the 7th day could the swellings be seen where the placentas were implanted and where they were commencing to increase in size. After the 7th day the weight of the uterus increased gradually. This increase in weight was influenced by the number of foetuses. Only after the 12th day after coitus was there a marked and sudden increase in the daily gain in weight of the uterus.

Hammond (1935) has shown for the rabbit how the weights of the uterine horns increased with the increase in the number of embryos. The increase in weight with increasing number of embryos was, however, not constant for one added, but obeyed the law of diminishing returns.

According to Hammond (1937) the muscular growth of the pregnant uterus has a twofold origin. Firstly, there is some internal secretion affecting its growth, and secondly there is a contact stimulus to growth caused by the attachment of the embryo. This causes a growth of the uterus in the neighbourhood of the attachment so that, even though the embryos should not be spaced evenly throughout the whole length of the horn when they become attached, they would towards the later stages of pregnancy be distributed equally along the length of the horn.

After the 8th day of pregnancy it was observed that these swellings were indeed evenly spaced along the length of each horn. Sometimes, however, the space between two embryos was twice as large as the space between any other two embryos. It is most likely that such spaces corresponded to the positions of embryos which had died and had been resorbed together with their placentas. From about the 14th day these spaces could not be seen since the foetuses and their placentas more or less filled the horns. According to Hammond (1937) this increase in size of the uterus to accommodate any number of embryos, prevents too great crowding in the uterus so that the nutrition of the embryo is not inhibited.

(iii) *Weight and number of the foetuses at different stages of pregnancy. Weight of foetuses.*—On account of the rather limited number of observations on the weights of foetuses at different ages, the three classes of rats will not be discussed separately. The total and average weights of the foetuses at the different ages are given in Table 10. As mentioned previously, the foetuses could only be weighed from the 13th day of gestation. The average weights have been plotted in Figure 8. It will be seen that a very smooth curve is the result. The average weights per foetus at 13 days' and 14 days' gestation are probably slightly high. A foetus of 13 days old appears to weigh 0.06-0.07 g. by our method. The average weight of 0.13 g. per foetus which we obtained for 13 days gestation in one female is more likely the weight of a 14 day old foetus. The same appears to be the case with one observation in the 14 day old foetuses. The average weight at 14 days appears to be 0.13 to 0.16 g., so that the one observation of 0.25 g. appears to be that of 15 day old foetuses. We explain later how such discrepancies could have arisen.

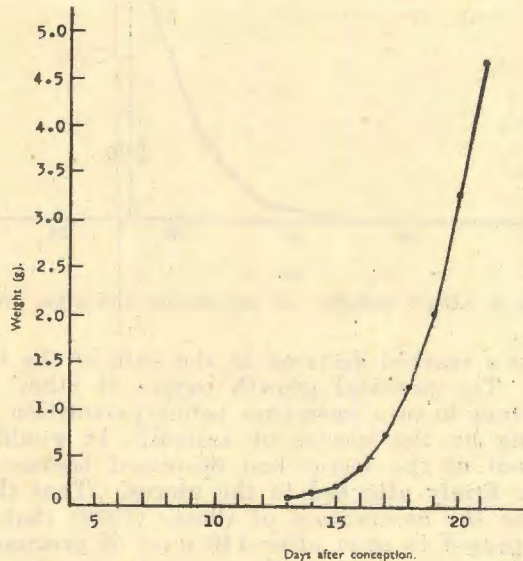


Fig. 8.—Pre-natal growth.

In Table 11 the daily weights of the young from birth to three weeks of age are given in order to show the effect of birth on the growth of the rat. Figure 9 illustrates the prenatal and postnatal curves. The daily weights of the young rats from birth to three weeks of age were obtained in experiments where the influence of pregnancy and lactation on food intake was studied. The weights of these young rats were very close to the weights obtained for the whole population. The average weights of the whole population at birth, at two weeks, and at three weeks were 5.07, 21.8, and 32.9 g., whereas the following average weights were obtained for young rats that were weighed daily: 5.09 g. at birth, 23.1 g. at two weeks, and 35.0 g. at three weeks. The growth of these rats therefore represents the growth of all the rats in the colony.

GROWTH OF THE ALBINO RAT.

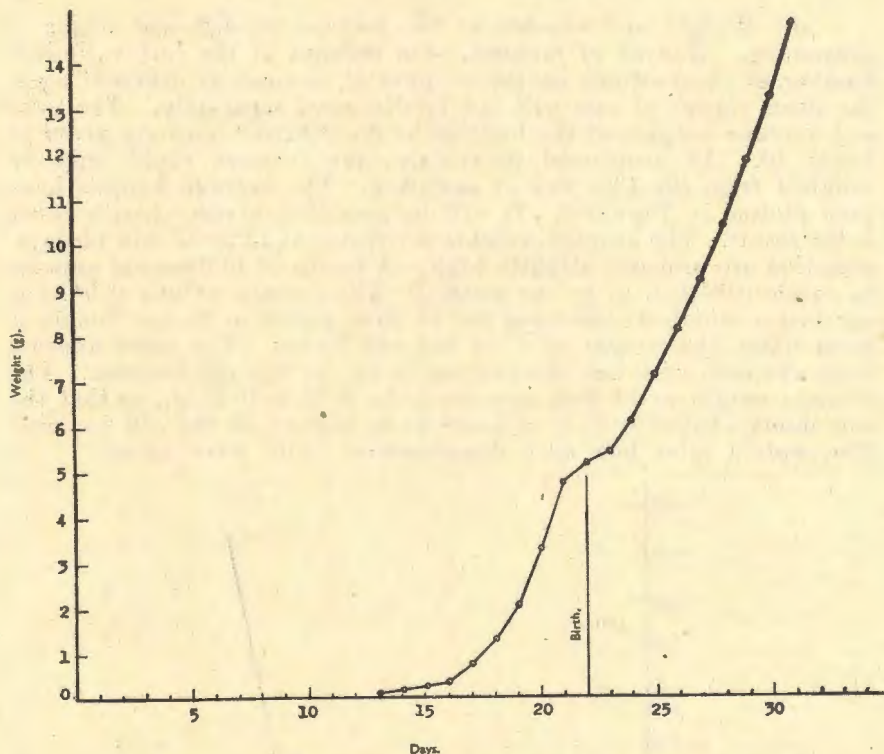


Fig. 9.—Daily weights of rats before and after birth.

There was a marked decrease in the gain of the foetus the day before birth. The prenatal growth curves of other animals show the same tendency to turn some time before parturition, the length of time depending on the species of animal. It would appear that the nourishment of the foetus had decreased because the placenta was no longer firmly attached to the uterus. That this may be so is indicated by the observation of Cloete (1939) that in sheep the cotyledons appeared to open after 140 days of pregnancy.

During the first 24 hours after birth only a small increase in the weight of the rat was registered. On the second day the increase was more than twice as much as on the first day. As will be shown at a later stage, new-born rats usually maintained a constant weight for about 12-18 hours after birth, but 24 hours after birth the new-born rat was slightly heavier than at birth. The relatively long period (12-18 hours) that the weight of the new-born rat remained constant accounted for the very small gain (0.28 g.) during the first day after birth. Figures 66 and 67 show the average daily gains and relative daily gains after birth to weaning. From the 2nd day the daily gains increased steadily till about 12-13 days after birth. The daily gains then decreased until 16 days after birth. From 18 days after birth onwards the daily gains showed a steady increase with only a few fluctuations. The percentage gain, however, only increased up to 3 days after birth, after which it decreased again.

Even before birth the percentage increase had decreased at a steady rate but showed a sudden drop only on the day before birth. During the first three days after birth a stimulus probably accelerates the growth rate to overtake the break, after which the percentage gain decreased again. The daily gain of rats from birth to weaning will be discussed more fully at a later stage.

In Tables 12, 13 and 14 the weights and numbers are given of the foetuses of the three classes of females:—young females (2½-3 months), old females (8 months) pregnant for the first time, and old females (11 months) that had several litters. Here we also see some discrepancies in the average weights of the foetuses. In Table 12, for instance, the average weights of foetuses at 14 and 15 days' gestation are the same. In Table 14 again the average weights at 13 and at 15 days' gestation show very little difference.

There are two possible explanations of these discrepancies. The presence of a vaginal plug was taken as an indication of the time of insemination. The vaginal plug could, however, have been very deep and could have been missed on the day of insemination and only seen on the day after. In some rats we noticed that the vagina was wet on the day of coitus and that the fluid showed traces of blood, but no plug could be seen at the time. A day later a plug might protrude. Failure to detect the vaginal plug on the day of insemination and hence postdating the beginning of gestation by one day, may be the reason why the average weights at 14 days in Table 12 and at 13 days in Table 14 are on the heavy side.

But even when the vaginal plugs had been visible on the day of coitus and their presence noted, there still remains another factor which may bring about a discrepancy in the average weights of the foetuses at different stages of pregnancy, namely the fact that coitus may take place at different hours. Cooley and Slonaker (1925) showed that oestrous could last from 12 to 18 hours. Some rats could be served at the commencement and others towards the end of the oestrous period.

In tabulating the average weight per foetus for each day from the 13th to the 22nd day of pregnancy inclusive in Table 10, we included the weights found by Angulo (1932) and Stotsenburg (1915) for the sake of comparison. At every age our weights are higher than their weights. The different methods of drying and weighing the foetuses may have influenced the weights at the earlier ages. After the 18th or 19th day of pregnancy, the technique should, however, not matter much. Nor is it likely that litter size played a part, as the following examples will show: the litters that we examined on the 18th, the 19th, and the 20th day of pregnancy, were below the average size for all litters, whereas the litters we examined at 21 days averaged 8.8 per litter (which is above the average), and still our weight is more than one gramme above the average found by Angulo and .84 g. above that found by Stotsenburg. The very smooth growth curves obtained for the foetuses also indicates that their growth could not have been influenced much by litter size at the different ages. Whether some influence of litter size on the weight before birth will be found when the weights are arranged according to litter size, is a question which can only be

answered when many more observations are available. Angulo (1932), however, found that the effect of litter size became more pronounced as the age of the foetus advanced. It slightly affected the growth of the foetus from the 17th to the 19th day and showed a marked effect toward the end of the gestation period.

The same was found in the guinea-pig (Ibsen, 1928) and the rabbit (Hammond, 1935). In the former it was found that up to the 55th day of pregnancy there was no difference in the growth rate of foetuses of large and small litters. With the rabbits Hammond found no difference up to the 24th day, but after that there was a distinct and ever increasing difference in the rate of growth of the foetuses in small as compared with those in large litters. Hammond (1935) has shown why the number of foetuses only affect the growth rate toward the end of pregnancy. He showed how the foetal placenta reached its maximum rate of growth earlier (20th to 24th day of pregnancy) than the foetuses (from 24th day). If the number of foetuses decreases, i.e., nutrition is increased, then the effect during the early part of gestation is only seen on the early developing foetal placenta, while the foetus is only affected towards the end. This is due to the following: The amount of increase per day that the foetus is capable of making during the later stages of pregnancy will depend on its nutritive supply, that is, on the scale and size to which its foetal placenta has developed. Hammond's results, for example, showed no correlation between weight of the foetus and the weight of the foetal placenta at the 16th day of pregnancy, that is, before the foetal placenta had come to its maximum growth rate. There was, however, a very marked correlation between the weight of the foetus and the weight of the foetal placenta at the 32nd day of pregnancy, when the growth rate in the foetal placenta had entered the second (decreasing) phase of growth and the growth rate of the foetus itself was still in the first (increasing) phase.

*Number of Foetuses.*—The average number of living foetuses per female at the various stages of pregnancy was 8.1 for all classes of females, but there was a different average for each class: (i) the number of foetuses of young females pregnant for the first time averaged 9.4; (ii) the number of foetuses of old females pregnant for the first time averaged only 4, and (iii) the number of foetuses of old rats pregnant several times averaged 5. The large proportion of young females with their large number of foetuses made the average for all rather high. Out of a total of 190 foetuses only 2 dead foetuses were found in the young females. The uteri of both the old females pregnant for the first time and of the old females that had had several litters contained 38.5 per cent. of dead foetuses. When the number of dead foetuses are included in the number of foetuses, the number of foetuses per female would be 6.5 for old females pregnant for the first time and 8.1 for old females pregnant several times. These numbers are still below those of the young females. McDowell, Allen and MacDowell (1928) found that the prenatal mortality in mice was the lowest in first litters. Our results, however, indicate that it is not a question of litter rank (parity) but one of age. This agrees with King's (1916) investigations on rats. In her opinion the age of the mother is more important

than the serial number of the pregnancy in determining litter size. In another section it will be shown that the average litter size is at its highest in the case of the youngest females and that the litter size decreases continuously with an increase in age.

For various animals a number of investigators found an increase in litter size to a maximum as the mother's age increased to a certain age, varying for each species, after which the litter size decreased; this was found to be the case for: the rabbit (Hammond, 1914), the rat (King, 1916), the guinea-pig (Keith, 1930 and Eaton, 1932) and for the pig (Morris and Johnson, 1932 and Krallinger and Gruhn, 1938). The age of the females when the maximum litter size was reached, was 12-16 months in the guinea-pig, 4½-5 years in the pig, and 4-5 months in the rat.

Contrary to the observations of these investigators we found that the average number of living foetuses at the various stages of pregnancy decreased as the age of the mother advanced. It is quite probable, however, that the number of ova shed followed the normal trend, i.e., first an increase to a maximum and then a gradual decrease as the age of the mother advanced. Hammond (1933) showed that the ova shed in young animals shortly after puberty is less than later in life. Hence it is possible that more ova were shed in the older rats but that the embryos were resorbed fairly early or that some did not become attached to the uterus. From Tables 13 and 14 it will be seen that dead foetuses were found at all stages of pregnancy from 11 to 20 days, with perhaps a slight indication of an increase as the age of the foetuses increased. If about the same had died before 11 or 12 days as after that age, then the average litter size would have been 10 in the case of old females, i.e., slightly higher than the average litter size actually observed in the young females. This can, however, only be settled by determining the number of ova shed.

In studying the factors controlling fertility and fetal atrophy, Hammond (1921) found that seasonal changes affect the number of ova shed from the ovary. As the number of ova shed increases the percentage which develops normally decreases. According to Hammond this is probably due to production outstripping nutrition. The seasonal factor, however, could not have influenced the ova shed in the rats under observation, since all the rats in the three classes were served and killed during the months of February, March and April.

From the same work of Hammond (1921) it appears that ovarian nutrition is a very important factor in determining the number of atrophic foetuses. The percentage of atrophic foetuses is high in pigs and tame rabbits where the number of eggs shed is high, and low in wild rabbits and sheep where the number of eggs shed is low. A production of a large number of eggs per ovary, whether due to breeding, time of year, domestication or other causes, frequently outstrips the nutrition and leads to atrophy—either as follicles, newly fertilised ova, or as partially developed embryos. The nutrition is more likely to be outstripped as regards quality. It does not appear probable, however, that a difference in the number of ova shed could have caused such drastic results to the effect that the living embryos

were decreased to less than half the number found in the young females. Angulo (1932) found that the albino rat produced the largest litters between 110 and 130 days of age, but he kept no rats younger than 110 days of age under observation. According to the investigations of King (1916) on albino rats from 3 to 16 months old the average litter size was 6.9 at 3 months and 7.9 at 4 months. The average remained high until the seventh month, when the decline became more marked. If the number of ova shed in our rats followed the same trend as found by King (1916) and Angulo (1932) for their respective rats, then the number of ova shed should increase with an increase in age up to a certain age and then decrease. From the results obtained by the two above workers the maximum appears to be round 4 and 5 months of age. The old rats we used were already 8 and 11 months old so that they were probably past their maximum.

Other factors that may cause foetal atrophy are inbreeding, fatness, and genetic differences (lethals). It is obvious, however, that inbreeding and lethal factors could not have played any rôle in the large number of dead foetuses we found in our material. Not only has the albino rat been inbred for so many generations without any decrease in fertility but also hardly any (2 out of a total of 190) dead foetuses were found in the very young females. As to fatness, some of the old females that were killed, were rather fat. The only exercise the breeding females got, was running about in their cages. No exercising cages were used. As many old females, however, had no excessive fat but their uteri nevertheless contained large numbers of dead foetuses. Young rats appear to be much more active than old rats. They run about and play even in quite small cages. Whether the old females were, consequently, in a poorer physical condition than the young rats and consequently could not supply the embryos with the necessary nourishment, can only be settled by conducting controlled experiments with old females, which have regular exercise in exercising cages against those that have no exercise.

Benedict and MacLeod (1929), however, found no difference in the activity of four females 4 to 8 months old as compared with four females 20 to 24 months old. This question of activity should, therefore, be studied at the same time to determine at what age activity decreases.

The metritis from which some of the rats suffered, probably explains why they did not breed, but it does not seem to stand in relation to the atrophy of the foetuses. This takes place at any time during the gestation period. Even if the metritis had not completely damaged the uterine wall so that fertilisation and perhaps imperfect implantation could have taken place, this does not explain the presence of the atrophied foetuses in the old females pregnant for the first time in which no metritis was observed.

## 2. Birth weights.

### (a) Procedure.

The cages of the pregnant females were inspected every morning at 9 o'clock. The litters that had been born during the preceding



24 hours were then weighed. These weights were registered as the birth weights. It is, however, possible that the ages of the young varied from 0 to 24 hours at the time when they were weighed and that they had even been suckled for some time. But it appeared from general observations that most of the litters were born during the night or early in the morning. The cages of pregnant females were often inspected at four o'clock in the afternoon and it was only seldomly found that a litter had been born between nine in the morning and four in the afternoon.

Parkes (1926) found that practically all litters of mice were born during the second half of the night. He therefore estimated that when he first found his litters in the morning their average age was about six hours. In order to investigate the time of day (or night) when mice litters were born, Merton (1937-38) used self-recording balances which recorded the exact times of birth on the drum of a kymograph. He found one peak between 4 and 8 p.m. and another between 12 (midnight) and 4 a.m. The number of parturitions recorded between 4 p.m. and 4 a.m. and between 4 a.m. and 4 p.m. gave a ratio of 2:1, whereas practically equal numbers were recorded for parturitions between 8 p.m. and 8 a.m. and between 8 a.m., and 8 p.m., viz., 81 and 83. On account of the relatively small number of observations Merton (1937-38) could not determine whether the decrease between the two maxima, i.e. from 8 p.m. to midnight, would disappear when larger numbers were used or whether a decrease between the two maxima was a peculiarity of his stocks. It is of interest to note, however, that the results of Parkes and of Merton as well as our own observations agree on an important point, viz., that most litters are born after 4 p.m. and before 9 a.m. When our litters were therefore weighed at 9 a.m. their ages could not have exceeded 17 hours.

In this respect it is interesting to compare the observations of Rademacher (1936) on cattle. He found that the number of births recorded during the day differed only slightly from the number recorded at night. The largest number (38.4 per cent.) was born during the day, i.e. between 8 a.m. and 4 p.m., whereas a slightly lower number (34.7 per cent.) was born between 8 p.m. and 4 a.m. The remaining 27.4 per cent. were born between 4 a.m. and 8 a.m. and between 4 p.m. and 8 p.m.

In recording the birth weights no distinction was made between those litters that had suckled their mothers. Most workers, however, take it for granted that there is an increase in the weights of newborn rats and mice after suckling, that, therefore, when they are weighed after they had suckled, the weights thus recorded as their birth weights will be higher than their true birth weights. To test out this point, ten litters, comprising 76 young that were born during the night, were weighed at 9 a.m., at 4 p.m., and at the same hours on the following two days. On the day of birth the average weight per pup was 4.87 g. at 9 a.m., and 4.89 g. at 4 p.m. The next morning at 9 a.m. the average weight was 5.16 g. At 4 p.m. this weight showed no increase. At 9 a.m. on the third day, i.e. when the young were just over 48 hours old, the average weight was

5.48 g. From then onward the average weight showed an increase at each morning and afternoon weighing. During this period only three litters (30 young) were born after 9 a.m. and before 4 p.m. These litters were weighed for the first time at 4 p.m. on the day they were born. The average weight was 4.58 g. The next morning at 9 a.m. the average weight was 4.47 g. It remained constant throughout the day. On the third day at 9 a.m., i.e. 41 hours after the first weighing, the weight was 4.96 g. That afternoon it also showed an increase.

It therefore appears that the birth weights of young born at night tend to remain constant during the first day and to show an increase only after some time, viz., 24 hours after the first weighing. During the daytime of the second day, i.e., when the age of the young increased from just over 24 hours to just over 31 hours, the weights also remained more or less constant. After that the weights increased continuously. The young born during the day showed the same tendency. Their weights remained constant or could even show a slight decrease when they were weighed for the second time (9 a.m.). Their weights further remained constant during the daytime of the second day. Thus when they were weighed 24 hours after they were first weighed, their weights were still below those read at the first weighing. It was only after 41 hours after the first weighing that the young born during the day showed an increase in weight, i.e. somewhat later than those born during the night. The latter showed an increase 24 hours after the first weighing.

Schneider (1936) made a study of the birth weights and postnatal weights of various species of domestic animals. He found that most of them lost weight after birth. The loss in weight was regained at different periods by the different species, the larger species regaining the loss in weight more slowly than the smaller ones.

It therefore appears that even though the litters were weighed when their average ages amounted to 6 to 9 hours, or slightly more, and these weights registered as their birth weights, the actual weights read could not have exceeded their true birth weights, regardless of the circumstance whether they had already suckled or not. This, however, does not agree with the results obtained by Freudenberger (1932). He found that when rats with empty stomachs were compared with those that had suckled, the presence of milk apparently increased the weight only 0.15 to 0.4 g. But he made no mention of the length of time the young rats were allowed to suckle.

There are several factors—as different investigators have found—which influence the birth weights of multiparous animals. Of these the more important are: litter size, age of mother, weight of mother, and seasonal and nutrition factors. There is, however, no general agreement on the effect of the different factors and the degree of influence of each. In this section only the influence of the first three factors will be discussed, while the seasonal influence will be considered at a later stage. Since the nutrition remained constant, its influence can be excluded.

*(b) Mortality.*

Only during the last two years of this research were the still-born rats weighed and their sexes determined. During the previous two years the number of dead per litter alone was recorded. Altogether 29 pups were born dead, a total which amounted to 1.4 per cent. of all pups at birth. The actual percentage may be slightly higher since some of the females devoured the young that were born dead. This percentage is slightly higher than that (1.3 per cent.) found by King (1921). The average size of those litters in which stillborn pups were found was 8.8, whereas the average of all litters was 7.4. In guinea-pigs Eaton (1932) found that litters of 4 and 5 and litters of 1 show greater prenatal mortality than litters of 2 and 3. In the two years during which the weights and the sexes of the stillborn pups were determined, 11 males and 5 females were born dead, their average weights being 4.88 g., and 4.8 g. respectively. The average weights of 988 males and 1,046 females born alive amounted to 5.19 g. and 4.96 g. respectively. King (1935) also found that the young born dead in the gray Norway rat were higher than those born alive. She found a difference of 15 per cent. in the weights of those born alive and of those born dead, whereas in our data the difference amounted to only 5 per cent. Similar results have been recorded for the pig (Carmichael and Rice, 1920), the guinea-pig (Haines, 1928, 1931), and the rabbit (Hammond, 1934). According to Eaton (1932) this difference may be due to more available nutrition for those that remained alive from the blood stream of the dam after the death of the sibs.

King (1935) came to the conclusion that "aside from the action of unfavourable genes that may reduce the vitality of embryos to the point where survival at birth is not possible, the main factor that seems responsible for death in utero or at birth in rats is inadequate nutrition of the young due either to the age and physical condition of the mother, to the presence of more embryos than can be adequately nourished, or the fact that the gravid female is suckling a litter of growing young which require the greater proportion of nutriment that the mother is able to supply". As will be shown at a later stage most of the females were used to produce only one or two litters. No one was served while nursing litters, so that the main factor causing the stillbirths was probably the presence of the larger number of embryos present in the uterus. This conclusion is supported by the fact that the stillbirths occurred in litters of appreciably larger size (8.8) than the average size of all litters (7.4).

*(c) Birth Weight and Litter Size.*

The birth weights of the males, of the females, and of both sexes together are arranged according to litter size in Table 16 and illustrated in Fig. 10. The weights of the stillborn rats are not included here.

The males are always heavier than the females except at three points. The average birth weight of all the males in our data is 5.19 g. and that of all the females 4.96 g., i.e., there is a difference

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of 0.23 g. In the gray Norway rat King (1935) found a difference of 0.4 g. in the average birth weights of the males and the females. In various strains of the albino rat the average birth weight of the males was found to be higher than that of the females (Jackson, 1913; King, 1915; Cooley and Slonaker, 1925; Greenman and Duhring, 1931).

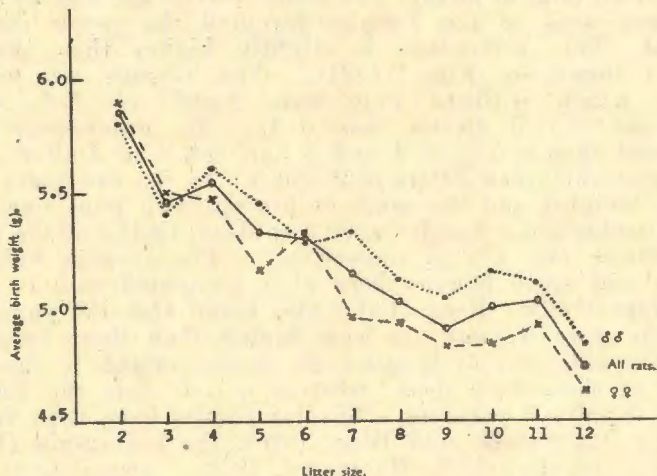


Fig. 10.—Litter size and average weight of young at birth.

It has long been recognized that the two sexes of animals differ both in weight (at birth as well as at maturity) and in growth rate. In most mammals the male is the heavier. Thus Carmichael and Rice (1920) found that the males of the pig were on the average 0.08 pound heavier at birth than the females, and Murray (1934) found a difference of 0.21 pound in favour of the males. According to Haines (1931) the average difference in the birth weights of male and female guinea-pigs amounted to 3 g. in favour of the males.

Figure 10 also shows that the average birth weights tend to decrease with increase in litter size, also that the curve tends to flatten out for litter sizes above 9. This flattening out, however, is masked by a sudden drop from litter size 11 to litter size 12. But a litter size of 12 was recorded only four times. Hence this small number of observations may account for the rather big difference in the average weights of pups in litters of 11 and 12.

When moving averages are determined three litter size classes taken each time to obtain one point then a smooth and regular curve is obtained (Fig. 11). The decreasing tendency of birth weight with increase in litter size can then be seen more clearly. The curve flattens out after litter sizes of 9 or more have been reached. If there is a constant internal secretion or metabolic product produced by the mother, then one can expect such a curve. In a litter of 1 and 3 young at birth the difference in the amount of substance obtained by each young in the litters is  $\frac{2}{3}$ , while in litters of 9 and 11 young at birth, the difference is only  $\frac{2}{9}$ , which may have a negligible influence when the gestation period is so short as in the case of the rat.

This diminishing difference agrees with Wishart and Hammond's (1933) results on the influence of litter size on the birth weight of rabbits. The linear curve they fitted was regarded only as a first approximation to the truth since, it appeared as if the law of diminishing returns was in operation.

It has long been known that there exists a negative correlation between litter size and average birth weights of the young of multiparous mammals. The relationship has been shown to hold for rats (King, 1915, 1935; Stotesenburg, 1915), for pigs (Carmichael and Rice, 1920), for guinea-pigs (Wright, 1922; Eaton, 1932), for rabbits (Kopec, 1924, 1927; Hammond, 1934), and for mice (Gates, 1915; Parkes, 1926). The domestic cat, however, appears to be an exception. Hall and Pierce (1934) found that the average birth weight of kittens born in litters of five is significantly greater than that of kittens born in smaller or larger litters.

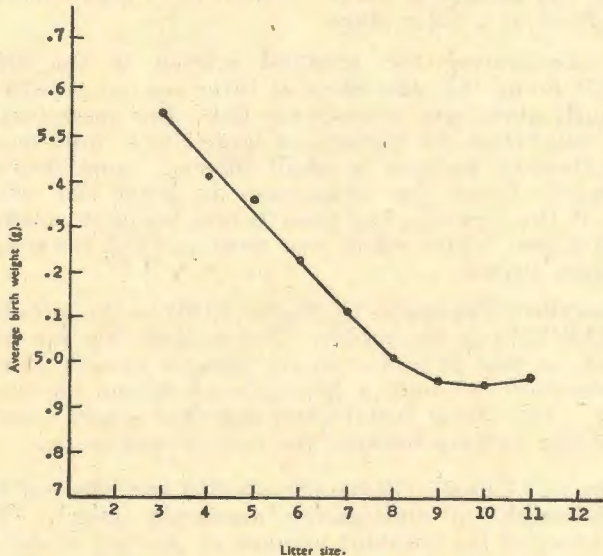


Fig. 11.—Litter size and average birth weight of all rats. (Curve smoothed by means of moving average—3 classes for 1 point.)

Several theories have been proposed to account for the correlation between the litter size and birth weight of multiparous mammals. The most likely explanation seems to be that of Hammand (1934). He studied the birth weights of three different strains of rabbits, the one strain weighing 1,500 g. when mature whereas the other two weighed 3,500 g. Hammand came to the conclusion "that it is probable that the size of the young is controlled by the amount of some internal secretion or metabolic product of the mother, and that while no amount of food ingested will put up the weight beyond the limits set up by this substance the limitation of the number of young increases the amount available for each individual young greatly and thus leads to increase in their weight". Therefore, when the amount of food ingested is not below maintenance, it has only a

slight effect on the birth weight. This has been confirmed by Eckles (1916). He found that the calves from heifers fed heavy (energy value) rations were no larger than those from heifers fed light rations. Hammond further argued that a large doe should have more of this internal secretion or metabolic product than a small doe, that therefore with increase in weight of the mother the increase in weight per young with reduction in size of the litter should be greater still than when the litter size alone decreased.

This is actually borne out by the findings of Hammond. He found that in the large doe the difference in average weight per young born in litters of 2 and of 6 was larger than the difference in the average weights per young in similar litter sizes in the small doe. According to Hammond this difference could not be attributed to the genetic capabilities of the young for growth of the one strain being greater than that of the other. Our observations on the influence of the weight of the mother at parturition on the weights of the young will be discussed at a later stage.

When investigating the prenatal growth in the albino rat, Angulo (1932) found that the effect of litter size on growth rate was negligible until about the seventeenth day after insemination, i.e., the average weights of the foetuses of large litters were more or less the same as those of foetuses in small litters. From then onwards, however, Angulo found that differences in litter size affected the growth rate of the foetuses, the growth rate being inversely proportional to litter size. This effect was most marked towards the end of the gestation period.

The same effect was found by Ibsen (1928) in the guinea-pig and by Hammond (1935) in the rabbit. The explanation for this, given by Hammond, is that it is due to the greater growth of the foetal placenta when there are only a few embryos during the earlier part of pregnancy. The larger foetal placentas thus causes a greater rate of growth of the foetuses towards the end of pregnancy.

Enzmann and Crozier (1935) also studied the effect of litter size on the birth weight of multiparous mammals (mice). They also favour the theory of the constant amount of internal secretion of the mother available for the developing young, i.e., in large litters there is less available per individual than in smaller litters. They, however, favour this as only a partial explanation.

#### (d) *Sex Ratio.*

In Table 16 are given the various sex ratios which were obtained when litters of different sizes were considered separately and when all the rats were taken together. At birth the total number of females exceeded the number of males, the sex ratio being 94.5. King (1935) on the other hand found that in the gray Norway rat more males were born than females, the sex ratio obtained being 105.2. Slonaker (1931 e), however, obtained the same results with albino rats as we did, i.e., a larger number of females than males at birth. According to Slonaker this relatively low number of males at birth therefore suggested that the prenatal mortality of the males was greater than that of the females. He considered the normal sex

ratio to be 106 males to 100 females. A figure very close to this was found in pigs by Carmichael and Rice (1920) who obtained a sex ratio of 108 at birth.

Parkes (1925) determined the sex ratio of pig foetuses of different weights. He found that among 281 foetuses which weighed from 0 to 100 g., 59.1 per cent. were males; among 114 averaging from 101 to 300 g., 57.0 per cent. were males; and among 188 weighing over 301 g., 53.2 per cent. were males. In the total population 56.8 per cent. were males, i.e., the sex ratio was 131. The author concluded that as the foetal sex ratio of males was much above the post-natal ratio, the proportion of males had decreased during gestation. He estimated that the conception ratio is approximately 150 males to 100 females. Parkes concluded that the most logical hypothesis for explaining the cause of the excess males at conception was based on a difference in the ability of the X- and Y-bearing sperms to reach the ova. The greater male prenatal and postnatal mortality was explained as due to a relatively lower viability of males.

Since the birth weights decreased with an increase in litter size, the sex ratios for the different litter sizes have been determined in order to find out whether a lower birth weight had any influence on the proportion of males and females that were born alive. The sex ratio is highest (163) for a litter size of 3 and lowest (77) for a litter size of 9. (Fig. 12.) The number of males tend to decrease with an increase in litter size up to litter size 9, after which there

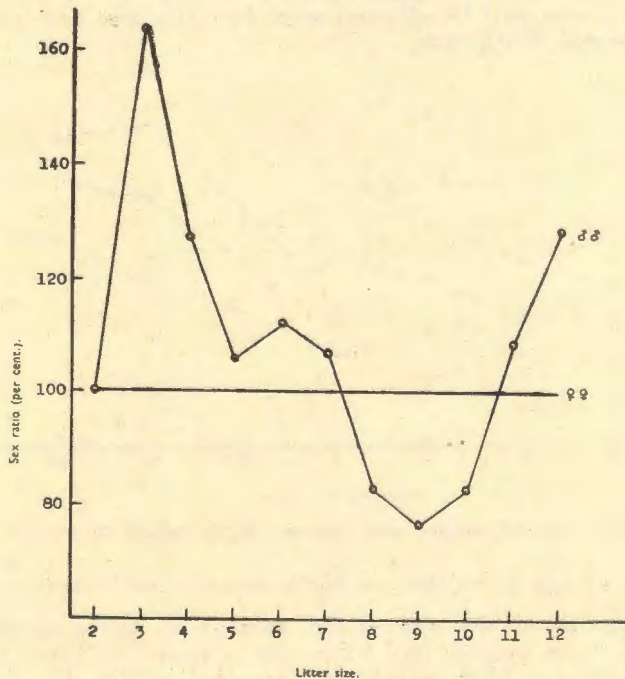


Fig. 12.—Litter size and sex ratio at birth.

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is an increase. King (1935) gave the numbers of males and females born in different litter sizes. When the sex ratios are calculated, rather wide fluctuations are observed, the highest being 116 and the lowest 66. No definite trend is shown. It is of interest to note the percentages found in the pig by Parkes (1923). Out of 2,020 litters, there were 50 per cent. or slightly more males in litters of 2, 3, 4, 12, 13, and 14, but less than 50 per cent. males in litters of 5 to 11 inclusive. In the guinea-pig Haines (1931), however, found no relationship between litter size and sex ratio.

From our results and from those of Parkes it would appear that litter size may have some influence on the sex ratio at birth. Our data would indicate that in litter sizes of 9 and less an increasing number of males died before birth as the litter size increased. When the litter size increased still further, the number of prenatal deaths decreased to a very low figure, therefore the number of males that died before birth also decreased. It would appear that the decrease in the number of males that died before birth had an increasing effect on the sex ratio at birth which now tended to approach the sex ratio at conception.

Whether the influence of litter size on the larger proportion of male deaths is through a decrease in weight and hence through a decrease in viability, is not certain. The lighter weight of rats born dead as compared to the weights of those born alive would suggest that weight may have some influence. The decreased weight may again be due to a decreased internal nourishment (this aspect will be discussed more fully at another stage). With their lower viability the males will be affected more by litter size and will show a higher prenatal death rate.

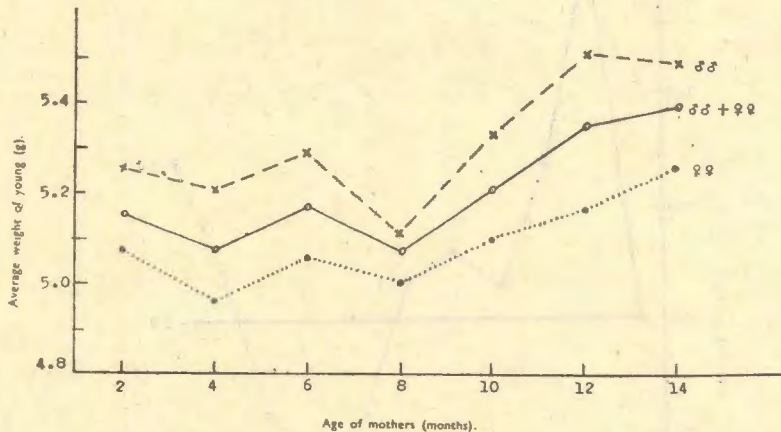


Fig. 13.—Age of mother and average birth weight of young.

(e) Influence of age of mother on birth weight and litter size.

The influence of the age of the mother on the average birth weight, total litter weight, and litter size is shown in Table 17. Its effect on the average birth weight is illustrated in Fig. 13. At the 4 and 5 months' age group and at the 8 and 9 months age group the



curve shows decreases on the respective preceding age class, i.e., the average birth weights of rats born of mothers of the 4 and 5 months' age group and of the 8 and 9 months' age group were lower than the birth weights of pups born of mothers of the 2 and 3 months' age group and of the 6 and 7 months' age group respectively. After that the trend of the curve is upwards, i.e., the average birth weights increased with the increase in age of the mother. At all points, the males are heavier than the females. King (1935), working with much larger numbers of the gray Norway rat, found an increase in birth weight as the age of the mothers advanced. (Correlation coefficients males  $+0.207 \pm 0.013$ ; females  $+0.256 \pm 0.0114$ .) But King also noted that the average birth weights of rats born of 4 months' old mothers showed a decrease on the birth weights of rats born of the mothers in the preceding age group. She could not explain this anomaly. The same trend, i.e., an increase in birth weight as the age of the mothers advanced, was obtained by various investigators for different animals: King (1915) and Cooley and Slonaker (1925) for the albino rat, Carmichael and Rice (1920) for the pig, and Eaton (1932) for the guinea-pig. The correlation coefficient is much smaller than it is in the case of litter size and birth weight. Wan and Wu (1931) also found that the birth weight increased with the age of the mothers when vegetarian and mixed diets were fed for many generations.

According to Hammond (1932) and several investigators cited by him, sheep and cattle have smaller young when they are immature and larger ones after they have reached maturity. According to Hammond it appears that when the dam is still growing she also requires some of the growth hormone so that there is less available for the developing young, while in the mature dam all the supply can go to the young.

Table 17 and Fig. 14 show clearly that age of mother and litter size at birth are strongly negatively correlated, i.e., as the age of the mothers advanced the litter size became smaller. There was a continuous decrease in litter size from 8.4 at 2 to 3 months to 4.7 at 12 to 13 months, then there was an increase at 14 to 15 months to 5.7.

Figure 15 shows that the total litter weight at birth follows the same downward trend as litter size with increase in age of the mothers. The curve shows an increase in litter weight only at the last point, i.e., at 14 to 15 months. This decrease in total litter weight with increase in age of the mothers shows clearly how the efficiency decreased as the mothers grew older. At 2 to 3 months a total litter weight of 43.3 g. was produced, while at 12 to 13 months the total litter weight produced amounted to only 25.0 g., i.e., there was a decrease of 43 per cent.

It has been shown that litter size and birth weight are negatively correlated (Fig. 11) and that litter size and age of mother are also negatively correlated (Fig. 14). To investigate the influence of the age of the mother on the average birth weight, the effect of litter size has been eliminated by keeping litter size constant. In Table 18 the birth weights in litter sizes of 6 to 7, 8 to 9 and 10 to 11 are grouped together and arranged according to age of mother. The results show

GROWTH OF THE ALBINO RAT.

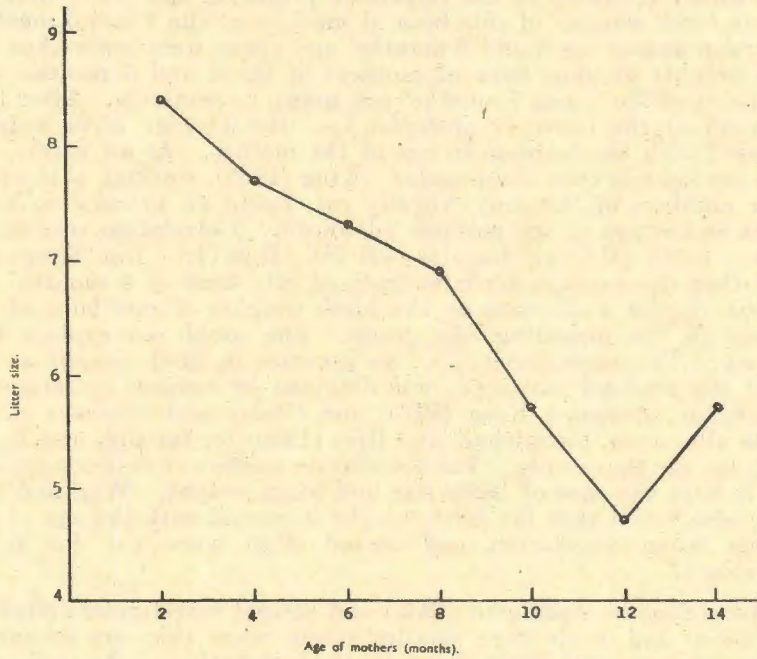


Fig. 14.—Age of mother and litter size at birth.

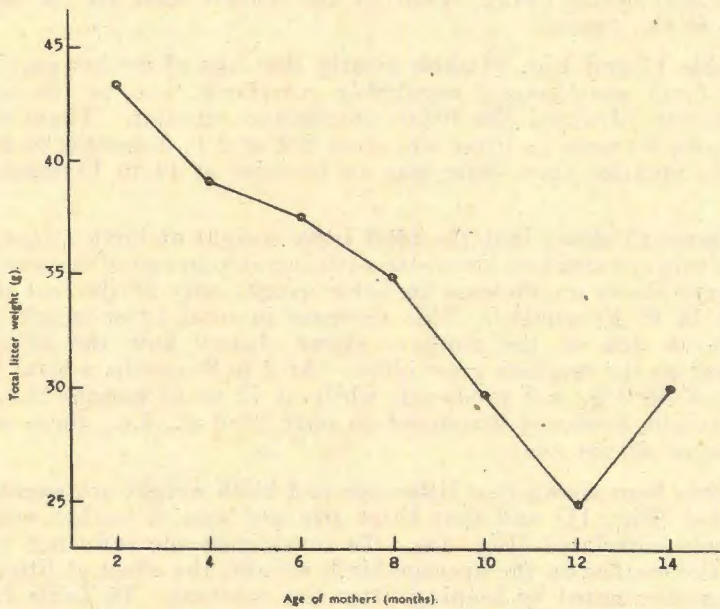


Fig. 15.—Age of mother and total litter weight at birth.

clearly that in none of the three litter size classes can any regular trend in the birth weights with increase in age of the mothers be seen. The different investigators referred to above did not show the influence of advancing age on litter size and hence it appeared that the increase in birth weight was due to advance in age of the mothers. Eaton (1932), however, stated that the dam's age had a greater influence in determining litter size than in determining the weight of the young. From these results it would appear that the internal secretions of the mother decreases with advancing age in spite of the fact that the average individual birth weights increase. If the same amount had been produced as the mothers grew older then there would probably not have been the very marked decrease in the litter weight at birth.

Another point of interest is that the litter size decreased from the youngest age onward. This is contrary to expectation, as experience has shown that in different animals litter size first increases to a maximum and then decreases. Our results showing a continuous decrease in litter size therefore differ from the results obtained by various investigators for different animals: Hammond (1914) and Kopec (1924) for the rabbit, King (1916) for the rat, Keith (1930) and Morris and Johnson (1932) for the pig, and Eaton (1932) for the guinea-pig. These investigators all found an increase in litter size up to a maximum as the dam's age increased to a certain age, varying for each species, after which the litter size decreased. The ages at which the females reached their maximum litter sizes were as follows: 12 to 16 months for the guinea-pig, 4½ to 5 years for the pig, and 4 to 5 months for the albino rat.

In a previous section (Prenatal Growth) we pointed out that the prenatal mortality was much larger in the litters born by females that were 8 and 11 months old than in the litters of females that were 3 months old. Increase in prenatal mortality with advance in age of the mothers appears to have been the cause of the continuous decrease in the average litter size from the youngest age class onward. This point will be discussed more fully at a later stage.

In Table 19 the birth weights have been arranged according to parity. As the litter number increased the average individual birth weight tended to increase while the total litter weight tended to decrease. These tendencies are therefore the same as those obtained when age of mother increased when litter size did not remain constant. As would be expected, there is a high correlation between age of mother and parity. The average age of a female at the birth of her first litter was 4.8 months. Then the ages increased by 2 to 2½ months for every litter. Litter size remained constant from the first to the second litter, then gradually decreased with the birth of each litter.

King (1916) found that in the rat the second litter produced was the largest averaging 7.7. The first averaged 7.2. The numbers in the third to the seventh litters were somewhat below those of the second. Thereafter there was a steady decline. In King's opinion, the age of the mother is more important than the serial number of the pregnancy in determining litter size. This agrees with our observations with regard to the influence of the age of the female on the number of foetuses.

## GROWTH OF THE ALBINO RAT.

Freudenberger (1932) made observations on the Wistar albino rat and the Long-Evans hybrid strain of rat. He found that in the Wistar rat the first litter was the largest (first 8.9, second 8.7, third 7.8), while in the Long-Evans strain the second was the largest (first 6.2, second 7.0, third 6.9). Greenman and Duhring (1931) stated that the first litter is the largest if the female was not permitted to mate until she was 110 to 120 days of age.

In the guinea-pig Eaton (1932) found that there was a tendency for litter size to increase up to the third or fourth litter and then to decrease gradually. He found only a very small influence of parity on birth weight. When he corrected the figures for litter size he found no significant differences in the birth weights of first and later litters.

Wan and Wu (1931) fed albino rats on vegetarian and mixed diets for many generations. They found that the average birth weights increased with parity up to a maximum. The maximum birth weight was reached in the third litter on the mixed diet and in the fourth litter on the vegetarian diet. It should be mentioned here that the rats on the vegetarian diet grew at a slower rate than those on the mixed diet. The original article of Wan and Wu could unfortunately not be obtained. In the abstract the trend of litter size with increase in parity is not mentioned. Their results are, however, contrary to what one would expect.

The average age of the females that produced the sixth litter was 16.7 months and the average litter size 6. This litter size is therefore higher than the litter size of the age classes from 10 months onward. 5.7 for females 10-11 months old, 4.7 for 12-13 months and 5.7 for 14-15 months. This apparent discrepancy is due to the fact that only the best breeders were retained for three or more litters. The higher litter size for the old females that were selected as the best breeders is masked when the litter sizes are arranged according to the ages of the mothers but can ultimately be seen in the highest age class—14 to 15 months (Table 17)—where there is the increase in litter size. The increase in parity is also greater in the last age class than between the third and second last classes.

### *(f) Influence of weight of mother on birth weight.*

In Table 20 average birth weights and litter sizes have been arranged according to the weights of the mothers just after parturition. Fig. 16 and Fig. 17 illustrate the influence of the weight of the mother just after parturition on average birth weight and litter size respectively. The birth weights were fairly constant in all classes except in the two heaviest classes (320 g. and 340 g.). Here the birth weights exceed those in the other classes. These results indicate that there may be an increase in birth weight in the classes where the weight of the mother after parturition is heaviest. Litter size on the other hand showed the opposite tendency. It first increased and then decreased with increase in weight of the mother. But the curve (Fig. 17) illustrating the influence of the weight of the mother on litter size at birth does not show a regular trend.

When birth weights, and litter sizes of first litters only are considered (Table 21) it is possible to compare a number of litters, more or less equal in size—thus eliminating the influence of litter size—as the litter sizes of the first litters were practically the same for all weight classes except for those of the 200 to 219 g. and of the 300 to 319 g. weight classes. With increase in weight of the mother after parturition, the birth weights of the pups decreased slightly and then remained more or less constant.

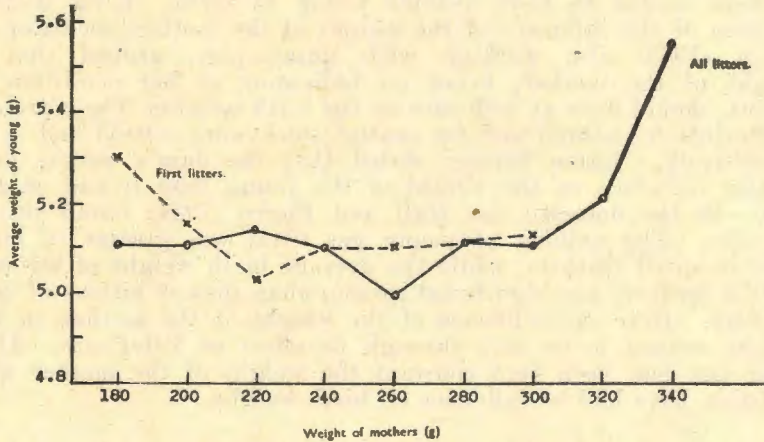


Fig. 16.—Influence of weight of mother on average birth weight of young—males and females taken together.

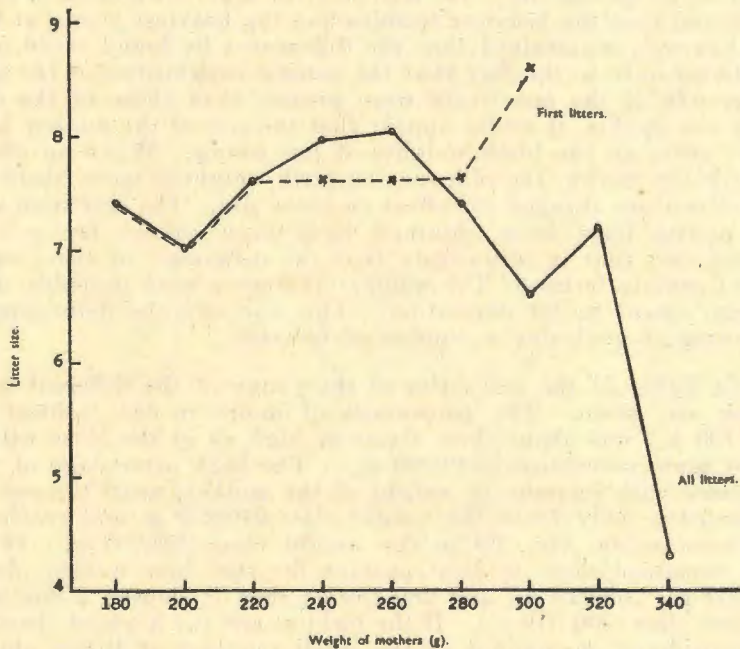


Fig. 17.—Influence of weight of mother on litter size at birth.

King (1935) stated that large females of the gray Norway rat as well as of the albino rat frequently had litters much above the average in size, also that the birth weights of their young were generally heavier than those of the offspring of females of like age but of a much smaller body size. She however stated that the age and the physical condition of the mother, and the genetic constitution of both parents influenced birth far more than the size of the mother. In guinea-pigs Ibsen (1928) found the same as King, i.e., that heavy mothers tended to have heavier young at birth. King made no mention of the influence of the weight of the mothers on litter size. Eaton (1932) also working with guinea-pigs, argued that the weight of the mother, being an indication of her condition and vigour, should have an influence on the birth weights. The correlation coefficients for inbred and for control stock were +0.35 and +0.44 respectively. Eaton further stated that the dam's weight had a greater influence on the weight of the young than it had on litter size. In the domestic cat Hall and Pierce (1934) found just the opposite. The number of young per litter was greater in larger, than in small mothers, while the average birth weight of kittens of smaller mothers was significant greater than that of kittens of larger mothers. Here the influence of the weight of the mother on birth weight seemed to be only through its effect on litter size. If the litter size had been kept constant the weight of the mother would probably have had no influence on birth weight.

As mentioned previously, Hammond (1934) maintains that with increase in the size of the mother there is an increase in the internal secretion of the mother. He worked with different strains of rabbits and found that the heaviest females had the heaviest young at birth. He, however, maintained that the differences he found could not be attributed only to the fact that the genetic capabilities of the young for growth of the one strain were greater than those of the other. From our results, it would appear that the size of the mother has no direct effect on the birth weights of her young. When an effect is nevertheless shown, the influence on birth weight is more likely to be an indirect one through the effect on litter size. The rats from which our results have been obtained have been inbred for so many generations, that it is unlikely that the difference in size could be due to genetic factors. The weight differences were probably due to a large extent to fat deposition. This can only be determined by dissecting or analysing a number of females.

In Table 21 the sex ratios of the young of the different weight classes are given. The percentage of males in the lightest class (180-199 g.) was about three times as high as in the class with the lowest male percentage (280-299 g.). The high percentage of males decreased with increase in weight of the mother, until the sex ratio amounted to only 73 in the weight class 240-259 g. and reached its minimum value, viz., 69 in the weight class 280-299 g. The sex ratio remained more or less constant for the three weight classes: 240-259 g., 260-279 g., and 280-289 g.; then it showed a rise at the heaviest class (300-319 g.). If the lightest and the heaviest classes are not considered on account of the small numbers of litters obtained in these classes, a regular trend is observed (Fig. 18). Whether a

large number of males die off before birth or whether there is a difference in the sex ratio already at fertilisation, cannot be seen from this work. More data are, however, still required to prove or disprove the influence of the weight of the mother on the sex ratio.

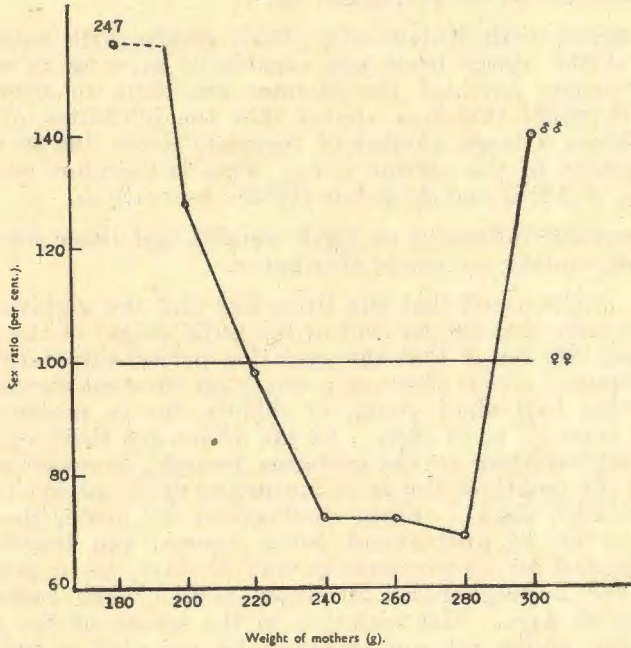


Fig. 18.—Influence of weight of mothers on sex ratio—first litters only.

(g) *Discussion of factors affecting weight at birth.*

We have shown and discussed the effects of litter size, age of mother, and weight of mother on the birth weight of the offspring. There are, however, other factors that may also influence the birth weight. Such factors are the length of the gestation period, seasonal factors, the physical condition of the mother, different types of diet, distribution of the embryo in the two horns, and hereditary factors.

In our data it is unlikely that different types of diet or the physical condition of the mother had an influence on the birth weights of the young as the feeding was not changed throughout the four years during which observations were made and only well grown and healthy females were used for breeding. It is further unlikely that hereditary factors could have exerted any marked influence because the rats had been inbred for many generations.

Stotsenburg (1915) and Angulo (1932) found that the distribution of the embryos in the two horns may be very uneven in the albino rat and that the embryos in the horn containing the smaller number may be heavier than those in the horn with the larger number. It however appears unlikely that the uneven distribution of foetuses between the two horns had any effect on the birth weights of the

young. Our observations on the prenatal growth of the rat showed no significant differences. One rat, for instance, was killed when she had been pregnant for 19 days; there were 7 foetuses in the one horn of the uterus and only one in the other. The weight of the one was the same as the average of the 7.

This agrees with Hammond's (1935) results with rabbits. He showed that the uterus itself was capable of growing to an almost unlimited extent provided the foetuses are there to stimulate its growth. It would therefore appear that the inhibition of growth, when there are a large number of foetuses, is not due to the large number present in the uterine horn. This is therefore contrary to Stotsenburg's (1915) and Angulo's (1932) observations.

The seasonal influences on birth weights and litter sizes will be discussed in another section of this paper.

Eaton (1932) stated that the litter size and the gestation period determined more than 60 per cent of the birth weight of the young in guinea-pigs. He found that the gestation period varied from 62-76 days. Hammond (1934) observed a small but constant increase in the weight of the individual young of rabbits due to prolongation of pregnancy from 31 to 34 days. In the albino rat there seems to be only a small variation in the gestation period. Stotsenburg (1915) found that the length of the gestation period of the albino rat varied from 21 to 22.5 days. From observations we made, the time of insemination of 42 pregnancies being known, the length of the gestation period for 33 pregnancies was 22 days, for 5 pregnancies 21 days, for 2 pregnancies 20 days, and for the remaining 2 pregnancies 23 days. The variation in the length of the gestation period of the albino rat can therefore be regarded as very small. Hence the effect of this factor on birth weight was probably only very small.

With regard to the influence of the remaining factors on birth weight, viz., litter size, age of dam, and weight of dam, the different investigators seem to agree only on the influence of litter size. In all multiparous animals it appears that litter size is probably the most important in determining birth weight. Wright (1922) and Eaton (1932) found in guinea-pigs correlation coefficients between litter size and birth weights of  $-0.66$  and  $-0.60$  respectively. The latter found a partial correlation between litter size and birth weight of  $-0.79$  when dam's weight remained constant.

With regard to the influence exerted on birth weight by the age and weight of the mother, there appears to be no agreement in the conclusions of different investigators. Eaton (1932) states that in the guinea-pig the dam's age has a greater influence in determining litter size than in determining weight of young, while on the other hand, dam's weight has a greater influence on the weight of the young than it has on litter size. King (1935) again, working with rats, found that age had far more influence on birth weight than the size of the mother had. In rabbits Hammond (1934) found that between breeds the size of the doe influenced the birth weights of the young. Within his different strains of inbred rabbits, size of doe did not affect the birth weights.