MILK PRODUCTION IN MICE

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(With One Text-figure)

INTRODUCTION

The data reported in this paper are the outcome of an attempt to find a convenient method of measuring the milk production of mice; and, with this method, to investigate certain aspects of the inheritance of milk yield, in particular the extent to which differences of milk production were heritable. Mice, with their rapid and prolific breeding, possess many advantages for the experimental study of lactation, and allow genetical experiments to be made on a scale that would be impracticable with cattle. The logical analogy between mice and cows would, of course, require justification, and knowledge about lactation in mice could not be applied indiscriminately to the improvement of the milk production of cows. Nevertheless, if a plan for the improvement of milk production had been tried on mice and had failed, much faith and courage would be needed to put it into operation on a herd of dairy cattle. Experiments on the lactation of mice might, therefore, be of considerable prognostic value to the dairy-cattle breeder, and also supply more fundamental knowledge of lactation in general.

METHOD

Growth of the young. Of the several possible ways in which lactation may be studied quantitatively in mice or other small mammals, the only one that was considered worthy of trial in the present work was the measurement of the growth of the young mice during suckling. This method combined the advantage of allowing lactation to proceed naturally with that of being very simple to carry out. It had the obvious disadvantages of being indirect and of recording only part of the milk produced; for not all of the milk consumed by the young is used in growth. Nevertheless, the growth of the young was thought to provide a relative measure whereby the lactation of different mothers might be satisfactorily compared.

The validity of the method rests entirely on the condition that the amount of food obtained by the young is always the limiting factor in their growth. There is good evidence from the work of MacDowell, Gates & MacDowell (1930) that this condition is in general fulfilled. Thus, exchanges of litters between mothers of two inbred strains showed that the growth of the young was characteristic of the strain to which the foster-mother belonged: young mice circulated among a number of foster-mothers showed day-to-day changes of growth rate according to the foster-mother that suckled them. By removing young mice from the litters at chosen intervals the same authors were able to increase very greatly the growth rate of the single young mouse that remained; while Parkes (1929) found that young mice fostered under rats attained in some cases double the normal weight at 21 days. Finally, it is well known (Parkes, 1926; Crozier & Enzmann, 1935) that young mice in large litters grow more slowly than those in small litters. It seems, therefore, from this evidence that the growth rate of young mice is generally dependent on the amount of milk that they obtain, and measurement of the growth of the young may well provide an indication of the performance of the mother as a milk producer. Nevertheless, it must not be supposed that the growth of the young is completely uninfluenced by their own nature: in fact it was all too evident in the present work that infestation by mites and infection with an endemic intestinal disorder, both of which afflictions were rather prevalent in some stocks, had a very marked effect on the growth of the young. Inbreeding was also found to reduce the growth rate of the young as will be described in detail below. Thus it was apparent that the nature of the young themselves may introduce a source of uncontrolled error, but there can be no doubt that the amount of milk consumed is generally a major factor in determining the growth rate of the young.

The first object of the experimental work was therefore to find out if measurements of the growth of young mice would reveal differences that could be attributed to the milk production of the mothers that suckled them.

Period of measurement. The choice of the most suitable period over which to measure the growth of the young required some consideration. There seemed to be two possible courses open: to weigh the litters once only, and thereby to include as much as possible of the whole lactation as well as the antenatal nutrition; or to weigh the litters twice at a suitable interval, and thereby obtain a measurement of milk production over a restricted period. Each

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method had several points in its favour and, since it was not at once apparent which would be preferable, the litters were weighed twice, so that the double or the single measurement could ultimately be used according to which method was proved by the results to be the more satisfactory.

At about 13–15 days old, when their eyes open, young mice begin to take solid food. The second weighing was therefore made 12 days after birth, since this was considered to be the latest time at which a measurement would represent growth due only to the milk consumed. The age of 5 days was chosen for the first weighing partly for reasons of practical convenience, but mainly with the intention of reducing the effects of errors in estimating the age of the litter when first found, which in the present material may have amounted to about 1 day in either direction. These errors would have least effect when the period of measurement included the part of the growth curve of the young that was most nearly linear, and an examination of the growth curves of young mice obtained by Parkes (1926) and by Crozier & Enzmann (1935) led to the choice of 5–12 days as the most suitable period of measurement. The choice was supported by an examination of the data of Enzmann (1933) on the daily milk secretion of mice; the curve of milk secretion rose to a flat-topped maximum at about 8–10 days and was symmetrical within the period 5–12 days.

After all the records had been obtained in this way and the first analyses had been made, it was found that the single measurement at 12 days was subject to substantially less uncontrolled error than was the measurement based on the difference of weights. This was undoubtedly due to the fact that measurements of the weight of litters were subject to sampling errors caused by irregularities in the growth of the litters, and these errors entered twice into measurements of milk production based on a difference of weights but only once into those based on a single weight. Thus, although errors in estimating the age of the litter were expected to detract from the precision of the single measurement, this loss was in fact more than counterbalanced by the smaller sampling error of the single measurement. For this reason, only the weight of the litter at 12 days of age was used as a measure of milk production in the present work.

The use of a single measurement of the weight of the litter is, of course, open to the objection that the resulting measurement of milk production included the antenatal nutrition, which, according to the data of Parkes (1926), amounts to about one-quarter of the weight at 12 days. On the other hand, the 12-day weight has the greater general biological interest; for it gives probably the best indication that can be obtained of the physical endowment for independent existence that is provided by the mother, and it is therefore relevant to a study of natural selection if this were to be made.

The method of measuring milk production from the growth of the litter over a limited period has been adopted independently by Cowie & Folley (1947) in their studies of the lactation of rats, though with a 5-day period from 6 to 11 days. The method appears to be well suited to the physiological study of lactation, and since the simplified method used in the present work would probably have only a limited application in such studies, both methods have been described above and will be examined in detail below.

Relation of the measurement to actual milk-production. Reasons have been given above for believing that measurements of the growth of young mice provide a satisfactory means whereby the milk production of different mothers may be compared. Though an absolute measure of the amount of milk produced was not essential, it seems desirable to consider how closely the weight of a litter at 12 days reflects the actual amount of milk produced by the mother. Since the measurement is in terms of the weight of the young mice it reflects the nutritive content of the milk rather than its weight or volume: but not all of the nutritive content is represented because a part is used by the young mice for maintenance. If the weight of the material that is lost by excretion, in its widest sense, were measured, an estimate of the total weight of the milk consumed by the young might be made by adding this amount to the weight of the young mice. Enzmann (1933) has estimated the daily loss of weight from excretion, in its widest sense, of young mice when not feeding. From his data and those of Crozier & Enzmann (1935) on the weights of young mice of the same strain, it appears that a single young mouse in the first 12 days after birth loses by excretion an amount equivalent to about 30 % of its weight at 12 days. Thus about 30 % would have to be added to the 12-day weight for material lost by excretion. But, as was mentioned above, about 25 % of the 12-day weight represents antenatal nutrition. Therefore the best estimate of the total weight of milk given during the first 12 days of lactation would be obtained by adding about 5 % to the weight of the litter at 12 days. The estimate would, of course, be extremely crude, and other methods such as that employed for mice by Enzmann (1933) and for rats by Brody & Nisbet (1938) would be preferable if measurements of the actual amount of milk produced were required.

MATERIAL

The mice that were used in this study of milk production were chosen from a large colony maintained for genetical work at the Department of Genetics, Cambridge. The colony consisted chiefly of a number of families in process of being partially isolated from each other, and within which inbreeding by brother-sister matings was frequently practised. Thus records were obtained from a number of mice that had been inbred in this way for a few generations. The majority of the mice, however, were outbred, though, owing to the partial isolation of the families, the mating system of these was not strictly random, and for this reason the material was not suited to a very exact quantitative treatment of the inheritance of milk yield.

Observations were also made on one strain of mice that had been inbred for many generations and should, according to mathematical theory, have contained negligible genetic diversity apart from the segregation of genes at the agouti locus. Approximately half of the mice tested from this strain carried the dominant gene yellow ($\mathbf{A}^{\mathbf{Y}}$), but this gene appears to have had little influence on the lactation of the mother.

All the mice in the colony were provided with a constant supply of food and water, and those that formed the material for the present study received no preferential treatment. The food was a commercial 'rat cake' made by The North-Eastern Co-operative Society, Aberdeen. This diet, though probably sufficient in itself, was supplemented by 'Yeastrel', bread, and cod-liver oil, given once a fortnight, and by green food (usually cabbage leaves) when available in the summer. Because food was unlimited, the measurement of milk production that was obtained gave no indication of the efficiency of the mice in converting food into milk.

Since all the mice formed part of a stock kept for other purposes, it was not possible to control the litter size, and a system of adjustment described below was made necessary. Nor was it possible to exclude from the material mothers that suffered from one or more physical defects due to genetic factors. It was to be expected that the more severe of these defects would impair the milk production, and an analysis was made in order to find out whether these defects were likely to have been an important source of variation in milk production. The number of mice that suffered from these defects was, however, rather small, and the analysis failed to reveal significant differences of milk production due to this cause. Nevertheless, there can be little doubt that the milk production of some of these mutants was impaired, though the number of mutant mice included in the material was too small for this source of variation to have been of great importance. Many of the litters, the growth of which was used as a measure of milk production, contained individuals handicapped by one or more of these defects, but the effects of mutant genes in the litters was not examined and must have contributed something to the uncontrolled error.

Altogether records were obtained from about 180 mice, of which seventy-three had two or more lactations, forty-one had three or more, and twenty-six had four or more. Litters that had suffered depletion at any time between 4 and 12 days after birth were excluded from the records.

RESULTS

Effect of litter size on milk yield

Calculation of an 'index of performance'. By far the most important influence on the total milk yield of a mouse is the number of young suckled; for the amount of milk produced increases very nearly in proportion to the number of young in the litter (Parkes, 1926; Crozier & Enzmann, 1935). It was therefore necessary to find some means of adjustment whereby the milk production of mice with different sizes of litter would be rendered comparable. The most satisfactory form of adjustment seemed to be to express the milk production of a mouse at a particular lactation as a percentage of the mean production of all mice that suckled litters of that size. Thus an 'index of performance' was obtained which was independent of the size of the litter suckled. For example, if the weight of the litter at 12 days represents the milk yield, the index of performance of a mouse with a litter of, say, six would be given by

$$\frac{\text{Observed weight of litter suckled}}{\text{Mean weight of litters of 6}} \times 100.$$

Indices of performance were calculated in this way for all lactations observed, and these were used as the measure of milk production throughout the analyses. For convenience, the index of performance of a mouse at a given lactation will be referred to simply as its performance, and will be quoted as a percentage.

The data that formed the basis for calculating the indices of performance are set out in Tables 1 and 2. The mean values of the milk production were calculated from the records of all litters measured, with the exception of those of the highly inbred strain, the inclusion of which would have given undue weight to one particular genotype. In Table 1 milk production is measured as the growth of the litter during the period from 5 to 12 days, and in Table 2 it is measured as the weight of the litter at 12 days. The calculation of indices of performance was not based directly on the observed means, since these were somewhat irregular, and it was thought that the results would be more reliable if the irregularities were first smoothed out. This was done by plotting the observed means on a graph and drawing a smooth curve to fit. No theoretical assumption was made about the nature of the relation between milk yield and litter size except that it was regular. 'Smoothed means' were then read from the curve, and on these the calculations of performance were based. Curve *A* in Fig. 1 is the graph constructed from the data in Table 2, milk production being based on 12-day weights. The graph appropriate to Table 1 is not given, but the 'smoothed means' obtained from it are included in Table 1.

Table 1. *Growth of litters from* 5 *to* 12 *days of age in relation to the number of mice in the litter*

Litter size	No. of litters	Growth of whole litter: Observed mean (g.)	S.E.	'Smoothed mean'
1	5	2·78	0·559	3·0
2	15	6·70	0·507	6·5
3	22	9·85	0·496	9·6
4	21	11·60	0·794	12·3
5	21	14·99	0·532	14·8
6	36	17·18	0·620	17·0
7	39	18·42	0·646	19·0
8	41	20·15	0·847	20·9
9	35	23·26	0·902	22·8
10	17	24·32	1·535	24·6
11	10	20·09	1·456	26·4
12	11	26·99	3·029	28·0

formed the bulk of the present material (curve *A*), give no indication of approaching their upper limit even with the largest litters recorded. This observation is of importance in connexion with the meaning to be attached to the index of performance, which is discussed in the succeeding paragraphs.

Meaning to be attached to the index of performance. The difficulty of comparing the milk production of mice that suckled different numbers of young was overcome by the calculation of indices of performance as described above. Yet this procedure was far from ideal because the meaning to be attached to the resulting measure of milk production was thereby severely restricted. The index of performance measures only the response of the mouse to a standard stimulus, and gives no indication of the maximum capacity of the mouse. Moreover, it is uncertain how just are the comparisons, given by the index, of the performances of mice with different sizes of litter. For, if there is any association between fertility and milk production (as has been claimed by Smith & Donald (1937) to be the case in pigs) mice that suckle large litters will in general give a higher response to a standard stimulus than mice that suckle small litters: and since the index of performance is based on a comparison of mice with the same size of litter, any such differences of real performance will escape detection in the index of performance. This disadvantage would be overcome if all litters were artificially reduced at birth to a standard size, when the need for the calculation of indices of performance would not arise.

Table 2. *Weight of litter at* 12 *days old in relation to the number of mice in the litter*

Litter size	No. of litters	Total weight of litter: Observed mean (g.)	S.E.	'Smoothed mean'	Weight of one mouse: Observed mean (g.)	S.E.
1	5	5·38	0·727	5·7	5·38	0·727
2	15	12·84	0·839	12·0	6·42	0·420
3	21	19·18	0·844	18·0	6·20	0·281
4	21	23·67	0·765	24·0	5·92	0·191
5	23	29·46	1·694	30·0	5·69	0·339
6	34	36·36	0·981	35·0	6·06	0·164
7	38	38·67	1·069	40·5	5·52	0·153
8	41	45·03	1·119	45·5	5·63	0·140
9	33	51·79	1·335	50·5	5·75	0·148
10	18	52·91	2·313	56·0	5·29	0·231
11	11	63·91	1·793	61·0	5·81	0·163
12	11	61·10	3·538	65·5	5·09	0·295
13	1	68·8	—	69·5	5·29	—
14	2	69·0	—	73·0	4·91	—
15	1	83·3	—	76·0	5·55	—

Three other curves derived from similar data for different stocks of mice are shown also in Fig. 1 for comparison. Curve *B* refers to the highly inbred strain used in the present investigation: curve *C*, drawn from the data of Crozier & Enzmann (1935), refers to another highly inbred strain, the Bagg albinos: curve *D* is drawn from measurements made by Parkes (1926) on a stock stated to show a 'fair degree of homozygosity'. It is clear from a comparison of these four curves that the relation of milk production to litter size differs to some extent according to the nature of the stock. In particular, it is seen that, whereas the inbred stocks appear to approach the upper limit of their milk yield with litters of about nine, the outbred stocks, which

Since the maximum capacity of the mouse is not reflected in the index of performance other means would be required for its measurement. The only satisfactory way in which maximum capacity could be measured would probably be by increasing artificially the size of the litter suckled beyond the limit at which the mouse could respond to the increased stimulus. There is one circumstance, however, that suggests that the actual yield is often in fact close to the limit of the mouse's capacity, for the practice of infanticide seems to be rather widespread among mice. Crozier & Enzmann (1935) state that mice that give birth to more young than they can adequately suckle generally kill a proportion of the litter, and the present writer observed that the majority of newborn litters suffered depletion in this way. Moreover, the observation that milk production in the outbred stock increased up to the largest litters observed points to the conclusion that few of these mice suckled litters that taxed their production beyond capacity.

The present state of knowledge does not allow anything more precise to be said about the relation of milk production to litter size. It seems probable, however, that if selection for milk production (in respect either of response or of maximum capacity) were to be made between two mice with the same mean index of performance, preference should be given to the one that suckled the greater number of young.

Weight of individuals in litters of different sizes. Though the growth of the litter as a whole is the only measure relevant to a study of milk production, it would be of general interest to know the weight of single young mice from litters of different sizes. For the weight of the individual young at weaning will be subject to natural selection, and mice from large litters will thereby be at a disadvantage. The optimum litter size will, therefore, be that for which the opposing selective values of quantity and quality are exactly balanced. The relationship between individual weights and litter size cannot be determined with any exactitude from the present data on account of the great variability. The mean individual weights with their standard errors are given in the

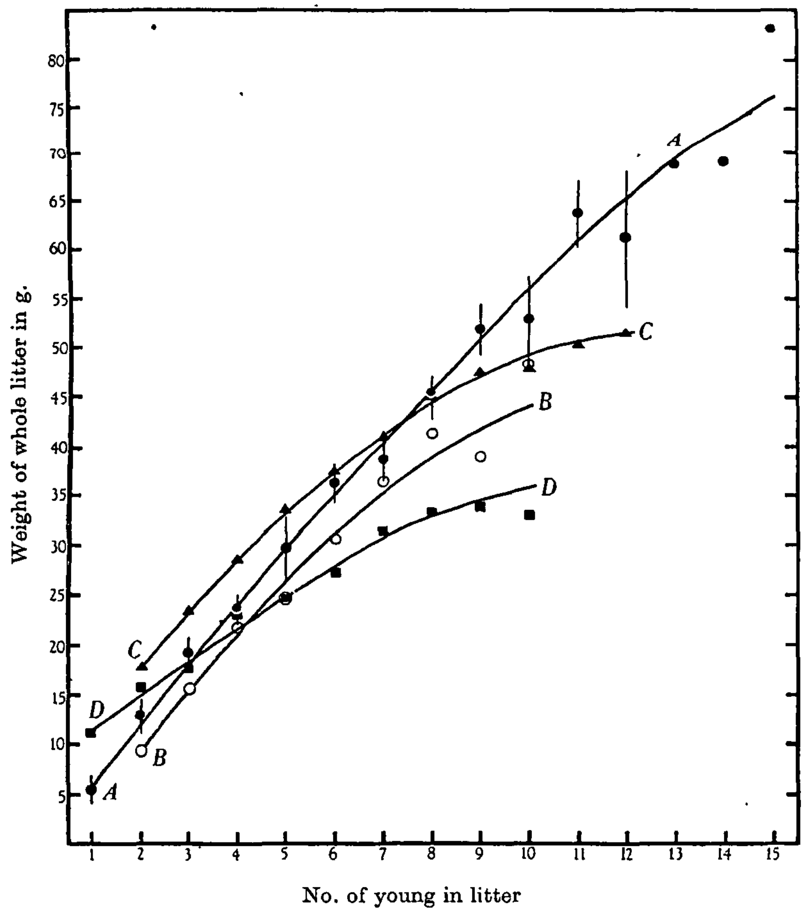

Fig. 1. Relation of milk production to litter size in different stocks of mice. Ordinate: weight of whole litter at 12 days old. Abscissa: number of young in litter. Curve *A* (full circles): outbred stocks of present material. (The vertical lines extend to $\pm 2 \times$ standard error). Curve *B* (open circles): highly inbred strain of present material. Curve *C* (triangles): from data of Crozier & Enzmann (1935). Curve *D* (squares): from data of Parkes (1926).

last two columns of Table 2. These means might be plotted on a graph and the best-fitting curve drawn through them. But, to the present writer at least, such a curve would give little conviction, for almost any curve might be drawn to fit the data without exceeding the customary limits of probability. All that can usefully be said, therefore, is that individual weights at 12 days were greatest in litters of two or three or perhaps four, and that they fell off gradually with increasing litter size, the reduction being about 0·075 g. for each additional mouse in the litter. Thus mice from small litters weighed about 6·0 g., while mice from litters of twelve weighed about 5·5 g.

Analysis of variation within and between individual mothers

The relationship between milk production and litter size has now been examined and the means whereby the performance of a mouse as a milk producer may be judged independently of the size of the litter suckled has been explained. When indices of performance had been calculated for every lactation measured, the material was ready for analysis. The first matter to be examined was the magnitude of the differences between mice relative to the error variation; for the data would be valueless if real differences between individual mice could not be shown to exist. The error variation, which appeared as differences of performance in successive lactations of the same mouse, was due to a number of different causes, the most important of which were probably the following:

(*a*) Sampling errors of the measurements, due to irregular growth of the litters.

(*b*) Errors in estimating the age of the litters when first found.

(*c*) Environmental factors such as exhausted water-bottles, excessive disturbance by handling, infestation by mites, etc.

(*d*) Different reactions of individual mothers to the demands made by differently sized litters, i.e. all individuals would not follow exactly the curve in Fig. 1 from which performances were calculated.

(*e*) Ensuing pregnancies: impregnation in mice often occurs immediately after parturition; some lactations will therefore have run concurrently with the first 11 or 12 days of the ensuing pregnancy, whereas others will have had no concurrent pregnancy.

The analysis of variance was the most suitable method by which the differences between mice could be compared with the error variation due to these various causes. Accordingly the performances of thirty-three mice of the outbred stocks, each of which had given records of three or more lactations, were examined by this means. In order to compare the relative merits of the two methods of measuring milk production, i.e. by the growth of the litter from 5 to 12 days old and by the weight of the litter at 12 days old, indices of performance appropriate to each method were calculated and analysed separately.

Milk production measured by the growth of the litter from 5 to 12 days of age. The analysis of variance of performances appropriate to this method is shown in Table 3. The reality of the differences between mice is indicated by the fact that the variance between

Table 3. *Analysis of variance of the performances of thirty-three mice, each with three or more lactations. Milk production measured by the growth of the litter from 5 to 12 days*

Source of variation	D.F.	Sums of squares	Variance	S.D.
Between mice	32	26,694	834	28·9
Between litters	11	4,184	380	—
Within mice	101	48,686	482	21·95
Total	144	79,564		

$F = 1{\cdot}73$; $P \simeq 0{\cdot}01$.

mice is significantly greater than the error variance. The variance ratio is 1·73 and has a probability of about 0·01. Differences between mice were therefore detectable by this method of measuring milk production, but the error due to the variation of individual mice in their successive litters and to the sampling errors of the measurements was rather large. The standard deviation of this error variation was 21·95 %, and this statistic probably indicates most clearly the accuracy with which the real performance of a mouse may be judged from the measurement of a single lactation. Thus records of single lactations must be expected to differ by about 22 % from the mean performance of the mouse, if this were judged from a large number of lactations; and single lactations cannot be relied upon to indicate the real performance of the mouse within limits closer than about ± 44 %.

The variation 'between litters' shown in Table 3 represents differences of performance due to litter order; that is to say, systematic differences between first, second, third, etc., lactations. The variance ascribed to this source is less than the error variance, and the data therefore give no indication that litter order had any influence on performance.

Milk production measured by the weight of the litter at 12 days old. The analysis of variance of performances appropriate to the measurement of milk production by 12-day weights is shown in Table 4. The differences of performance between mice are now highly significant. The variance ratio is 3·27 and has a probability of less than 0·001. There is again no indication that litter order had any influence on performance. If the analyses of variance applied to the two methods of measuring milk production are compared it will be seen that the 12-day weight method is subject to very much less error variation. The variance ratio for differences between mice is consequently higher, and differences of performance between mice may be more readily detected by this method. The smaller error variation means that differences of performance between successive lactations of the same mouse are less, and single observa-

Table 4. *Analysis of variance of the performances of thirty-three mice each with three or more lactations. Milk production measured by the weight of the litter at* 12 *days*

Source of variation	D.F.	Sums of squares	Variance	S.D.
Between mice	32	15,753	492	22·2
Between litters	11	1,522	138	—
Within mice	108	16,263	151	12·5
Total	151	33,538		

$F = 3{\cdot}27$; $P < 0{\cdot}001$.

tions will therefore give a more reliable indication of the true value of the mouse as a milk producer. The standard deviation of the error variation was 12·5 %, which means that observations of single lactations must be expected to differ from the mean performance of the mouse by about 12·5 % and may be relied upon to differ by less than about 25 %.

In assessing the relative merits of the two methods of measuring milk production it is seen that the loss of precision expected from errors in estimating the age of the litter, when milk production was judged from a single measurement, was more than offset by the smaller sampling error of the single measurement in comparison with that of the double measurement. For this reason the weight of the 12-day litter was adopted as the measurement of milk production throughout the analyses which follow, and all indices of performance were based on this measurement.

The foregoing analysis has shown that the technique employed was capable of revealing differences of performance in lactation between individual mice. With this essential point established, attention may now be turned to the investigation of the causes of these differences. Interest is, of course, centred primarily on the relative importance of genetic and environmental factors in determining the performance of individual mice. There are, however, two other possible causes of variation that must be examined first. These are the weight of the mother, and the degree of inbreeding both of the mother and of the litter suckled.

Effect of the weight of a mouse on her milk production

It is well known that in general the size of an animal influences the quantity of milk produced, the milk yield being roughly proportional to the 0·7th power of the body weight (see Brody, 1945, p. 856). Mice that are mated soon after weaning produce their first litter before they are fully grown: those that formed the material of the present work increased in weight up to their fourth or fifth litter. For this reason considerable attention was given to the problem of adjusting the observed milk production to compensate for differences of body weight.

All the mice that gave records of milk production were weighed, as a matter of routine, 5 days after parturition, and it was hoped that these data would allow the relationship between milk production and body weight to be calculated. This hope, however, was not realized. The difficulty lay in the fact that mice continue to suckle their litter until the next is born, and it was impossible to weigh a mouse without weighing also the tissues and contents of her mammary glands. In these circumstances adjustment of the observed milk yield to compensate for body weight was obviously impracticable, because a 'heavy' mouse would then be penalized for having well-developed mammary glands and so, presumably, for having a high milk yield.

It was possible, however, to circumvent the difficulty of obtaining a valid measurement of body weight, and to discover whether the failure to adjust for body weight was likely to cause a serious error in the estimates of milk production when these were expressed as indices of performance. For, since the mice continued to grow till after they had had their third or fourth litter, a comparison of the mean performances of mice suckling their first, second, third, etc., litters would show whether body size influenced performance. The relevant analysis has already been given in Table 4, and it shows that the differences of performance 'between litters' were insignificant. Table 5 gives the mean performances

Table 5. *Mean performance of thirty-three mice according to litter order, and mean weight of the mice at each lactation*

Litter order	1st	2nd	3rd	4th	5th	6th
No. of litters	12	22	23	28	17	14
Mean performance	105	108	104	103	104	100
Mean weight (g.)	29·6	33·8	35·4	37·9	38·4	38·7
Litter order	7th	8th	9th	10th	11th	12th
No. of litters	10	6	4	3	2	1
Mean performance	105	103	114	115	108	121
Mean weight (g.)	38·8	37·1	38·5	37·8	36·4	31·6

classified according to litter order, among the thirty-three mice analysed, and it may be seen that there is no indication of a systematic increase of performance in successive lactations. The fact that body weight increased up to about the 5th lactation may also be seen from Table 5; for the increase of the observed weights is too great to be attributed entirely to an increased development of the mammary glands.

From the considerations outlined above it was concluded that, with the present material, differences of body weight contributed little or nothing to the variation of performance, and that adjustment for body weight was neither practicable nor necessary.

Effect of inbreeding on performance

Inbreeding without selection. The programme of research for which the mice used in this investigation were kept required the frequent mating of brother to sister, and some of the mice from which records of milk production were obtained had been inbred in this way for one or more generations. Since the choice of pairs depended entirely on their genetical suitability and not at all on their health or fertility, the material may be regarded as quite unselected for any characters that might influence milk production. In this respect the material was very suitable for an investigation of the effect of inbreeding on milk production. But the number of mice that had been inbred beyond the first generation was rather small, and the milk production of these in particular was liable to disturbance by the presence of major mutant genes in both mothers and litters. For these reasons the data are insufficient for the detailed study that the relationship of milk production to inbreeding merits, though the results that they yielded are of considerable interest.

Milk production, when measured by the growth of the young, may be subject to two distinct effects of inbreeding. The lactation of the mother may be influenced by the degree of inbreeding in her own ancestry; and the young, if inbred, may suck less vigorously or utilize the milk less efficiently in growing, thereby diminishing the apparent milk yield of their mother. The available material made it possible to disentangle these two effects, for inbred mice were often outcrossed to unrelated mates, with the result that the mother was inbred but the litters were not. In these circumstances the observed milk production was subject only to the effect of inbreeding on the mother. On the other hand, when a mouse was mated to her brother the resulting litter was one generation further inbred than its parents, and in these circumstances the observed milk production was subject to the effects of inbreeding both in the mother and in the litter.

For the purpose of analysis the mice were divided into four groups according to the degree of inbreeding of the mother whose milk production was measured. In the first group, designated '0', the mothers were not inbred. In the next group the mothers were one generation inbred; that is, their parents were sibs. In the group designated '2' were mothers that had been inbred by two consecutive sib-matings, and in group 3 were those that had been inbred by three or more consecutive sib-matings. Each group was subdivided according to whether the mother was mated to her brother and the litter consequently one generation further inbred, or the mother was mated to an unrelated mouse and the litter consequently outbred. The mean performances of the mice in each group are given in Table 6, and show clearly that the milk production of the mice,

Table 6. *Performances of mice inbred to different degrees*

Inbreeding of mother (generations)	No. of litters	No. of mothers	Performance Mean	S.E.
		Young inbred		
0	120	39	104·6	1·42
1	22	18	87·6	5·13
2	4	3	77·8	2·65
3	5	3	74·8	4·37
		Young outbred		
0	28	14	104·8	3·01
1	27	12	98·4	6·83
2	8	5	91·1	4·71
3	No data			

both with outbred and with inbred litters, was reduced in accordance with the degree of inbreeding. The effect of inbreeding on performance was, however, smaller when only the mother was inbred than when both mother and young were inbred. Thus, inbreeding is shown to have impaired both the lactation of the mother and the sucking power or the intrinsic growth rate of the young.

These conclusions can be supported by statistical analysis. A comparison of the performances of the mice in the four groups with inbred young gave the analysis of variance shown in Table 7. The differences

Table 7. *Analysis of variance of the performances of mice representing four different degrees of inbreeding; inbreeding affecting both mothers and young*

Source of variation	D.F.	Sums of squares	Variance
Between groups	3	11,276	3759
Within groups	147	42,755	291
Total	150	54,031	

$F=12{\cdot}9$; $P<0{\cdot}001$.

of performance between the groups are highly significant. The analysis of variance applied to the three groups of mice with outbred young failed to show significant differences between the groups, owing to the large variance in group 1. However, when the performances of the mice in groups 0 and 2 were compared, the difference between the means was shown to be significant at the 5 % level ($t=2{\cdot}38$ with 34 degrees of freedom; $P=0{\cdot}02$–$0{\cdot}05$). Thus the reduction of the performance of inbred mothers, both with inbred and with outbred young, was verified. Finally, in order to verify the conclusion that inbreeding affected the growth of the young independently of the amount of milk supplied, a comparison was made of the performance of the mice in group 2 that had inbred litters with those in the same group that had outbred litters. The difference of performance was again found to be significant at the 5% level ($t=2\cdot46$ with 10 degrees of freedom; $P=0\cdot02$–$0\cdot05$), and there can be little doubt that inbreeding of the young did reduce their intrinsic growth rate.

Since inbreeding has been shown to have so marked an effect on milk production it is clear that, in the examination of the other possible causes of variation, differences due to inbreeding will have to be eliminated. Nevertheless, inbreeding probably contributed very little to the differences between the thirty-three mice of the outbred stocks, of which an analysis was presented in a previous section, because only six of the thirty-three mice were inbred, and only two of these suckled inbred young. There remain, therefore, only environmental and genetic factors to be investigated as possible causes of the observed variation.

Performance of the highly inbred strain. Before proceeding with the analysis of the causes of variation in the outbred stocks, it will be convenient to present the observations of milk production in the highly inbred mice which formed part of the material. The records obtained from these mice are interesting in connexion with the effects of inbreeding, both on the mothers and on the young.

Records were obtained for fifty-one lactations distributed among thirty-one mice of this highly inbred strain. The mean performance was 85·2%, with a standard error of 2·10%. The performance was thus about 15% below the average for the outbred stocks, but in comparison with the mice that had been randomly inbred it was about equal to that of one generation inbred mothers with inbred young. The deterioration of milk production was thus far from being commensurate with the degree of inbreeding, a result that must presumably be attributed to selection during the formative stages of the strain.

Nine mice from this inbred strain had been outcrossed to unrelated mice and had consequently suckled outbred litters. Records of 16 lactations were obtained from these mice, and the mean performance was 103·8% with a standard error of 2·35%. These mice with outbred young were thus equal in performance to the average of the outbred stocks. This observation is interesting for two reasons. It shows that the poor performance of the inbred strain was due mainly, if not entirely, to the nature of the young. Thus the complete dependence of the growth of young mice on the amount of milk that they receive, found by MacDowell *et al.* (1930), is clearly not true of all inbred strains. Secondly, the milk production of the mice of this inbred strain, when suckling outbred young, was not at all impaired by the high degree of inbreeding. The same was true of the Bagg albino inbred strain investigated by Crozier & Enzmann (1935), the milk production of which is shown in Fig. 1 of the present paper, and is not worse than that of the outbred mice, at least with small and medium-sized litters. Thus inbreeding does not necessarily result in impaired milk production, though a fairly rigorous selection must presumably be practised if the ill effects of inbreeding are to be avoided.

Effect of environment on performance

A detailed discussion of the many effects of environmental conditions upon milk production would be out of place here, and it will be sufficient for the present purpose to decide whether the differences of environmental conditions that affected the stocks studied were important or unimportant causes of variation of milk production between mice. It was, of course, evident that there were environmental factors that influenced milk production and contributed to the uncontrolled error represented by differences within mice. The point at issue here is whether these or other environmental factors affected individual mice differentially and thereby contributed to the differences found to exist between mice. The data obtained from the highly inbred strain provided suitable material for the investigation of this point. Genetic differences within this strain may be considered to have been negligible, and environmental factors alone will have been responsible for any differences of milk production between the mice. Since these mice were kept under the same conditions as the rest of the stock, the extent of the differences of milk production between the mice of the inbred strain will give a reasonably good indication of the importance of environmental factors in causing differences between the mice of the outbred stocks.

The performances of thirteen mice of the highly inbred strain, each with records of two or more lactations, were compared by means of the analysis of variance shown in Table 8. This analysis showed

Table 8. *Analysis of variance of the performances of thirteen mice of the highly inbred strain*

Source of variation	D.F.	Sums of squares	Variance	S.D.
Between mice	12	1973	164	12·8
Within mice	20	4073	204	14·3
Total	32	6046		

$F<1$.

that the variation between mice was less than that within mice. There is, therefore, no indication that the mice of this strain differed at all from one another in their milk production, and it must be concluded that, under the conditions in which the stocks were kept, environmental factors were unimportant as a source of differences of milk production between individual mice.

The inheritance of milk production

The foregoing analysis has shown that environmental factors played little part in causing the differences of milk production that were observed in the stocks. Inbreeding, though it greatly influenced milk production, cannot have been the only cause of variation, since the mice that exhibited significant differences of milk production differed little in their degree of inbreeding. It appears probable, therefore, that genetic factors were the major cause of variation, and the importance of this source of variation must now be examined.

The most satisfactory way in which to assess the contribution of genetic factors to the determination of milk production was to compare the variation of performance within sisterhoods with the variation of unrelated mice. Since there were a few families from which records of three or four sisters were obtained, the material was more suitably dealt with by the analysis of variance than by the more usual method of correlating pairs of sibs. Because sisters in a randomly mating population have on the average half their germplasm in common, it is to be expected that the variance within sisterhoods will be only half as great as the variance in unrelated individuals, if the character concerned is entirely determined by the genetic constitution of the individual (Fisher, 1918). The less the importance of genetic constitution relative to other causes of variation, the greater will be the variance within sisterhoods relative to the variance in unrelated individuals. An analysis of the variance within and between sisterhoods will, therefore, indicate the extent to which milk production was genetically determined. Data from fifteen groups of full sisters were available for this analysis. In order to eliminate differences due to inbreeding from the comparisons, only mice that were not inbred were chosen, though those with once-inbred litters were not excluded. The performances of the majority of these mice were mean values based on records of more than one lactation. The average number of lactations per mouse was 3·4, and the mean performances of the majority of the mice must therefore have been estimated with a fair degree of accuracy. The analysis, which is given in Table 9, shows that

Table 9. *Analysis of variance of performance within and between sisterhoods*

Source of variation	D.F.	Sums of squares	Variance
Between sisterhoods	14	4550	325
Within sisterhoods	18	1085	60·3
Total	32	5635	

$F = 5{\cdot}4$; $P < 0{\cdot}001$.

the differences of performance between unrelated mice were significantly greater than the differences between sisters. The variance ratio is 5·4 and has a probability of less than 0·001. The variance between sisterhoods was, in fact, more than five times as great as the variance within sisterhoods, and sisters were therefore more alike in performance than was to be expected if milk production were wholly determined by genetic constitution. This greater similarity of sisters was, without doubt, due to the division of the stock of mice into partially isolated families with the result that the mating system was not random. Besides having rather more of their ancestry in common than would occur in a population mating at random, the sisters shared also, to some extent, the physical defects of which genetical study was being made. There were thus other causes, besides the sharing of parents, to which the great similarity of the sisters may be ascribed and, since the magnitude of their effects could not be evaluated, it is impossible to make an exact estimate of the part played by heredity in determining the milk production of the mice.

The part played by heredity was certainly very great, and there appears, indeed, to be no need to postulate any factor other than genetic constitution and degree of inbreeding as agents in the determination of the milk production of an individual mouse.

DISCUSSION

The investigation which forms the subject of this paper was undertaken chiefly with the object of exploring the possibilities of using mice as material for the experimental study of lactation, and the conclusions to which the results seem to point may be summarized here.

In the first place, an extremely simple technique of measurement may be employed if one is content with a measure that reflects lactation only comparatively, and if an absolute, quantitative measure of milk yield is not required. In the second place, the results have shown that if selection for milk production were to be applied to mice rapid progress could be confidently expected. No difficulty would be encountered in discarding a large proportion of the animals tested, since the high fertility of mice ensures a large number of offspring from which selection may be made. Furthermore, it appears that the genotype, or 'breeding value', of a mouse may be judged with fair accuracy from its performance in the test, because (*a*) the observed differences of milk production were in the main genetically determined, (*b*) environmental factors had little influence on the mean performances of mice, although no special measures for standardization were taken, and (*c*) the mean performance of a mouse may be estimated fairly accurately from a record of its first lactation.

There appears, therefore, to be no serious obstacle to the production by selection of strains of mice differing markedly in their milk production. Such strains might be of value for a number of purposes. A genetical study might be made of matters such as the possible existence of sex-linked genes that control milk production, of which there is some evidence in cattle (Smith, 1937). Breeding programmes for the improvement of milk production in cattle, particularly for the transference of the high milking capacity of European cattle on to the genetic background of certain native breeds, might be tried first on mice, and much time and effort saved in the event of failure. A comparison of strains of mice that differed in milk production might also throw light on many aspects of the physiology of lactation and on the development and functioning of the mammary glands.

Finally, measurements of milk production in mice might provide a useful tool for the study of problems related to inbreeding. The effects of inbreeding in animals are usually measured in terms of fertility, and milk production, since it has been shown by the present study to be greatly influenced by inbreeding, might serve as another index for measuring the effects of inbreeding. Milk production, being an expression of what may be vaguely termed 'physiological efficiency', might possibly evaluate better than fertility a quality in mammals that is comparable with the 'hybrid vigour' of plants.

SUMMARY

1. Lactation was studied quantitatively in a heterogeneous stock of laboratory mice, in order to find out whether the milk production of mice could be satisfactorily evaluated from measurements of the growth of the young; and, if individual differences were proved to be readily detectable, to find out to what extent these differences were heritable.

2. The total weight of the litter suckled, at 12 days of age, was found to provide a convenient measure of milk production.

3. Since the amount of milk given by a mouse is greatly influenced by the number of young suckled, allowance for differences of litter size was made by means of an index of performance which expressed the amount of milk given by a mouse at a particular lactation as a percentage of the average amount given to litters of that size. The index of performance thus evaluated the response of the mouse to a standard stimulus and gave no indication of the maximum productivity of the mouse.

4. The performance of individual mice was found to differ in successive lactations owing to sampling errors and other uncontrolled sources of variation. It was concluded that observations of single lactations must be expected to differ from the mean performance of the mouse by about 12·5 %, but may be relied on to differ by less than about 25 %.

5. Individual mice were found to differ greatly in their mean performance, and the causes of these differences were investigated.

6. The weight of the mouse had little or no effect on performance, nor had the ordinal number of the lactation. (Performances at first lactations did not differ from those at later ones.)

7. No differences of performance between the individuals of a highly inbred strain were found; from this it was concluded that environmental factors, which may have contributed to the variation within individuals, were not a cause of the differences observed between individuals of the outbred stocks.

8. Inbreeding without selection for a few generations was found to reduce substantially the observed performance, and the amount of reduction followed the degree of inbreeding with some regularity. This effect of inbreeding operated partly on the mother by impairing her lactation, and partly on the young by reducing their intrinsic growth rate; inbred mothers with outbred young recorded substantially higher performances than similarly inbred mothers with inbred young.

9. The performance of the highly inbred stock was consistently below that of the outbred stocks. This poor performance was, however, mainly due to the young; for highly inbred mothers suckling outbred young equalled in performance the average of the outbred stocks. The unimpaired lactation of these highly inbred mice was thought to have been due to selection during the formative stages of the inbred strain.

10. The extent to which milk production was genetically determined was investigated by a comparison of the variance within sisterhoods with that of unrelated mice, and it was concluded that genetic constitution was a fully adequate cause of the observed differences of milk production.

11. The results of the investigation led to the conclusion that mice would form suitable material for the experimental study of lactation, and that if selection for milk production were to be applied to mice, rapid progress could be expected.

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