Genetic studies of ovulation rate in the mouse

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1. INTRODUCTION

The litter size of mice has been the subject of many genetical studies. These have shown that litter size (the number of live young born in a litter) is genetically variable in non-inbred strains and can be either increased or decreased by selection in at least two ways: by selection for litter size itself, or as a correlated response, by selection for body weight. Litter size is a 'complex' character in the sense that several component characters contribute to its variation. The two principle components are the number of eggs shed at ovulation (ovulation rate) and the proportion of eggs represented by live young at birth (intra-uterine survival). When litter size is changed by selection, these two components may react differently. Selection for increased litter size resulted in an increase of ovulation rate without any increase of intra-uterine survival, whereas selection for decreased litter size resulted in a decrease of intra-uterine survival without any reduction of ovulation rate (Falconer, 1963). The increase of litter size following selection for large body size, and the decrease following selection for small size, have however both been found to result from changes of ovulation rate (MacArthur, 1944; Fowler & Edwards, 1960). From the fact that litter size can be readily changed by artificial selection, the conclusion seems inescapable, though as far as we know this has never been directly demonstrated, that the existing intermediate litter size is optimal for the 'natural' fitness of mice in the laboratory. (Indirect evidence of litter size being optimal at intermediate values is provided by the observation (Festing, 1968) that the number of young weaned in a fixed period of time is not linearly correlated with the average litter size of the female during that time.) Obviously intra-uterine survival is optimal at the maximum, which accounts for the fact that it could be reduced but not increased by selection for litter size. Therefore, if litter size is adjusted by 'natural' selection in the laboratory to an intermediate optimum, the adjustment must be made through the ovulation rate. If the argument is correct, ovulation rate must therefore have an intermediate optimum and it should in consequence display at least a moderate amount of additive genetic variance.

The first object of the work described here was to find out how much additive genetic variance of ovulation rate was present in a genetically heterogenous strain

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of mice. This was done in two ways, by estimating the components of variance in a half-sib analysis, and by two-way selection for ovulation rate itself.

Ovulation rate, as a component of litter size, can itself be further divided into two components, the activity of circulating follicle-stimulating hormone (FSH) and the sensitivity of the ovary to FSH. These two components, again, behave differently in response to selection. The changes of ovulation rate following as correlated responses from selection for body size have been found to be mainly due to changes in FSH activity (Fowler & Edwards, 1960; Edwards, 1962), whereas changes in ovulation rate following selection for litter size were mainly due to ovarian sensitivity (McLaren, 1962). The second object of the present study was therefore to find out which of the two components would be changed by selection for ovulation rate itself.

Though natural ovulation rate may be subject to variation in its two components, FSH activity and ovarian sensitivity, variation in the number of eggs shed in response to exogenous gonadotrophins would be expected to be mainly or entirely in the one component, ovarian sensitivity. Ovarian sensitivity is thus open to genetic study through induced ovulation rate, and the contributions of the two components to any change of ovulation rate can be assessed.

The third object of the work was to study the genetic relationship between natural and induced ovulation rate, in order to find out if genetic variation affected both simultaneously, and if induced ovulation rate could be validly used for the comparison of natural ovulation rates in different strains. For this purpose selection, in different lines, was applied both to natural and to induced ovulation rate. This double selection programme allowed estimates to be made of the genetic correlation between natural and induced ovulation rates.

2. METHODS

The stock of mice used for all the experiments was the genetically heterogeneous Q-strain. This strain originated in 1957 from crosses between three strains selected for large body size and one selected for small body size. These four strains provided half the ancestry. The remaining half came equally from an unselected, non-inbred, strain (JC) and an inbred strain (JU) derived from it. About three-quarters of the ancestry of the Q-strain was the same as the strain on which Falconer's (1960a) work on the genetics of litter size was carried out. From 1960 the Q-strain was maintained by minimal inbreeding with 20 male and 40 female parents in each generation, and the mice used for the present experiment were taken from the tenth and eleventh generations. In some experiments the females used had not previously been mated or borne a litter: these are referred to as nulliparous females. In other experiments the females had previously borne one litter: these are referred to as primiparous females.

The exogenous gonadotrophins used for the induction of ovulation were 'Gestyl' (Organon Ltd.) pregnant mares' serum (PMS) and 'Pregnyl' (Organon Ltd.) human chorionic gonadotrophin (HCG).

The ovulation rate was scored as the number of eggs shed at natural oestrus, or in response to induction by exogenous gonadotrophins. Natural ovulation occurs during the night, close to the time of mating. Females, paired with males, were examined each morning and the occurrence of natural ovulation was identified by the presence of a copulatory plug in the morning. The timing of ovulation in response to exogenous gonadotrophins, on the other hand, is determined by the treatment used to induce the ovulation. The technique used was that described by Fowler & Edwards (1957), and consisted of the intra-peritoneal injection of PMS followed after 43 h by HCG. Ovulation takes place about 13 h after the second injection and optimal results are obtained if the times are adjusted so that ovulation occurs about 01.00 h, i.e. at the usual time of natural ovulation. This timing allows naturally and artificially ovulating mice to be scored at the same time, which is convenient.

When the eggs are shed from the ovary they are embedded in cumulus cells which are progressively broken down during the day following ovulation. If the female is dissected before this breakdown occurs, and the ovary with the fallopian tube and terminal part of the uterus are removed and examined under a dissecting microscope, the presence of eggs in the fallopian tube can be identified as a discreet swelling. The rupture of the fallopian tube at this point enables the eggs to be removed, the cumulus to be stretched and the eggs identified and counted. Females were always dissected before 13.00 h, at which time the cumulus was still in good condition. Only eggs embedded in cumulus were scored.

The artificial induction of ovulation was intended to provide a measure of ovarian sensitivity and the doses used were chosen with this end in view. The doses of exogenous hormone that would be most likely to reflect ovarian sensitivity to endogenous gonadotrophins are those that would induce the ovulation of a number of eggs similar to that shed at natural oestrus. From previous work (Land, 1965*a*) it was known that 4 i.u. of PMS followed by 3 i.u. of HCG was a suitable combination of doses. Edwards, Wilson & Fowler (1963) had, however, shown that the response to a low dose of PMS was affected by the stage of the oestrous cycle at which it was given. Consequently, as 4 i.u. of PMS is a relatively low dose, a preliminary experiment was carried out to examine the effects of the stage of the oestrous cycle on the response to this treatment. In addition, as it was necessary to use primiparous females in the main experiment (for reasons to be explained later), the effect of the interval between the weaning of the first litter and the start of the treatment was also examined. The preliminary experiment was a factorial design in which groups of primiparous females were treated with 4 i.u. of PMS when in oestrus, dioestrus, or metoestrus (as judged by vaginal smears), at 1, 3, or 5 days after the weaning of their first litters. There were altogether 101 females divided between the nine groups. No significant differences were found, and it was concluded that neither the stage of the oestrous cycle, nor the interval between weaning of the first litter and the treatment, had any important effect on the ovulation rate. Examination of the interval between the birth of the first and second litters in the first nine generations of the Q-strain

showed that the oestrous cycle is partially synchronized after weaning. In order to utilize this synchrony, and so eliminate any possible effects of the stage of the oestrous cycle, the treatment was given at a specific interval after weaning. This was on the first day after weaning, since it was not always possible to treat the mice on the day of weaning itself.

An important question to be considered was whether the dose of PMS should be adjusted according to the body weight of the animal. If the induced ovulation rate was to be a measure of ovarian sensitivity then the number of eggs shed should be independent of body weight. It was found from experiments, to be described in detail in another paper, that when females were given a standard dose of PMS, the weight of the female had very little effect on the number of eggs shed. This showed that a standard dose would be satisfactory. In the light of these preliminary experiments and of previous knowledge, the treatment adopted for the measurement of ovarian sensitivity was the administration of 4 i.u. of PMS at 17.00 h on the day after the weaning of the first litter, followed by 3 i.u. of HCG at 12.00 h on the third day after weaning.

The breeding methods used in the experiments, and other experimental details, are described in conjunction with the results.

3. EXPERIMENTS AND RESULTS
Sib analysis

The opportunity was taken to utilize surplus mice from an experiment set up by Dr L. S. Monteiro for a study of growth (Monteiro & Falconer, 1966). Seventy-one males had been mated, each to three females, providing groups of full-sib

Table 1. *Analysis of variance of natural ovulation rate in nulliparous females, and components of variance estimated from it*

Source	D.F.	M.S.	Component
Between sires	70	10·360	$\sigma_S^2 = 0·305$
Between dams within sires	119	7·199	$\sigma_D^2 = 1·219$
Within dam progenies	368	3·901	$\sigma_W^2 = 3·901$
			$\sigma_T^2 = 5·425$

Interclass correlations (t)

Half sibs	$t = 0·056 \pm 0·049$
Full sibs (litter-mates)	$t = 0·281 \pm 0·042$

Genetic and environmental variances*

		% of total
$V_A = 4\sigma_S^2$	1·220	22·5
$V_{EC} + \frac{1}{4}V_D = \sigma_D^2 - \sigma_S^2$	0·914	16·8
$V_{EW} + \frac{3}{4}V_D = \sigma_W^2 - \sigma_S^2$	3·291	60·7
V_P (Total)	5·421	

* V_A = Additive genetic, V_D = dominance, V_{EC} = environmental common to full sibs, V_{EW} = environmental within full-sib families. Variance due to epistatic interactions is assumed to be negligible.

and half-sib offspring. Females from each group were obtained and were scored for natural ovulation rate when aged between 6 and 8 weeks, the average number scored per full-sib group being 2·9. There were insufficient mice available to allow an analysis of induced as well as of natural ovulation rate to be made. The analysis of variance and the components estimated from it are given in Table 1. The heritability of ovulation rate in nulliparous females estimated from the half-sib correlation was $0·22 \pm 0·19$. This estimate has a very large standard error and is of little value by itself except as an indication that ovulation rate might be expected to respond to selection. The dam-component of variance was much larger than the sire-component, which showed that causes of resemblance other than the additive genetic variance were probably contributing to the full-sib correlation. These may have been partly dominance variance, but were probably mainly environmental factors, or maternal effects, common to litter-mates.

Selection

Procedure

The measurement of ovulation rate requires the killing of the female. To select for ovulation rate one has therefore either to select individuals on the basis of the performance of their relatives, or to obtain offspring from the females before they are scored. The first alternative would allow selection to be applied to nulliparous females, but it would require the scoring of a prohibitively large number of females to obtain a reasonably reliable estimate of the mean performance of the relatives. The second alternative was therefore adopted, although it had the disadvantage that the ovulation rate selected for was that of primiparous females. Comparisons of nulliparous with primiparous females were made later to see what effect the selection had had on nulliparous ovulation rate.

The procedure of selection was as follows. Females were mated at the age of about 6–8 weeks. When their first litter had been weaned, 21 days after birth, their ovulation rate was counted, with or without treatment as appropriate. The females were then selected on the basis of their ovulation rate, the offspring of the selected females being retained and those of the others discarded.

The first selection, from the base population, was carried out as follows. In order to maximize the selection differential, the base population was made as large as possible and 200 females were scored for ovulation rate. In half of these, ovulation was allowed to occur naturally, females being paired with males when their litters were weaned. In the other half, ovulation was induced in the manner described above. A control line (C) was first formed from the offspring of eight females taken at random from the whole population. Selection was then applied to the remaining females, and four selection lines were established: lines HN, selected for high natural, and LN for low natural, ovulation rate; lines HI selected for high induced, and LI for low induced, ovulation rate. The selection was made on the basis of the females' individual ovulation rates. The eight highest and eight lowest females among the naturally ovulating group were selected and their litters used for continuation of the HN and LN lines. Similarly, the eight highest

and eight lowest females among the group with induced ovulation were selected and their litters used for continuation of the HI and LI lines.

In all subsequent generations selection was made within families, the best female in each set of full sisters being selected. This was done in order to increase the effective population size and to eliminate complications due to maternal effects. Each line consisted of eight full-sib families in each generation, bred from eight selected females and eight unselected males. The young untested females were mated to males from a different litter, and the matings made in successive generations followed a cyclic pattern designed to minimize inbreeding. The average number of females measured in each line in each generation was 32·5, the minimum being 24 and the maximum 43. The mean ovulation rate in each generation was calculated as the mean of family means. The selection differential on females was calculated as the mean deviation of selected females from their family means, and the net selection differential was half of this since males were unselected. The schedule of mating and selection could not always be strictly adhered to on account of occasional sterile matings and unisexual litters. When modifications had to be made the substitutes were chosen with the object of minimizing inbreeding and maximizing the selection differential expected in the next generation.

Only one control line (C) was maintained, bred from eight pairs in each generation, but both the natural and induced ovulation rates were measured in every generation. The females in each litter were divided at random into two groups, one of which was tested for natural ovulation and the other for induced ovulation. The generation means of the control line were therefore based on approximately half the number of measurements as were those of the selected lines.

The selected lines and the control were carried through 12 generations. Duplicate sets of matings were made in all lines in the 12th generation in order to provide additional females for the measurement of correlated responses and for other experiments on the physiological nature of the changes produced by selection.

Results

The mean ovulation rates in successive generations are plotted in Fig. 1. It is clear that selection was effective in separating all four selected lines from the unselected controls. Natural ovulation rate increased from about 16 to about 20 eggs, and decreased to about 14 eggs. Induced ovulation rate was more erratic and the extent of the response is not so clear. Starting at about 19 eggs, the high line increased to about 22 by the 9th or 10th generation and to about 30 in the 11th or 12th generations. The low line, in contrast, showed no clear decrease over the whole experiment. The control line, however, showed a clear increase of induced ovulation rate during the course of the experiment, and so the asymmetry between the upward and downward selected lines is probably not real. In the further analyses of the responses the high and low lines will not be considered separately and the responses will be judged from the differences between the high and low lines.

The first generation of selection was not directly comparable with the later generations because it was individual selection instead of within-family selection. In order to render the first generation comparable with the others, and to allow the accumulation of selection differentials from the base population onwards, the selection differential in the first generation was converted to the equivalent differential for within-family selection. That is to say, the selection differential was calculated which would have yielded the same response if the selection had
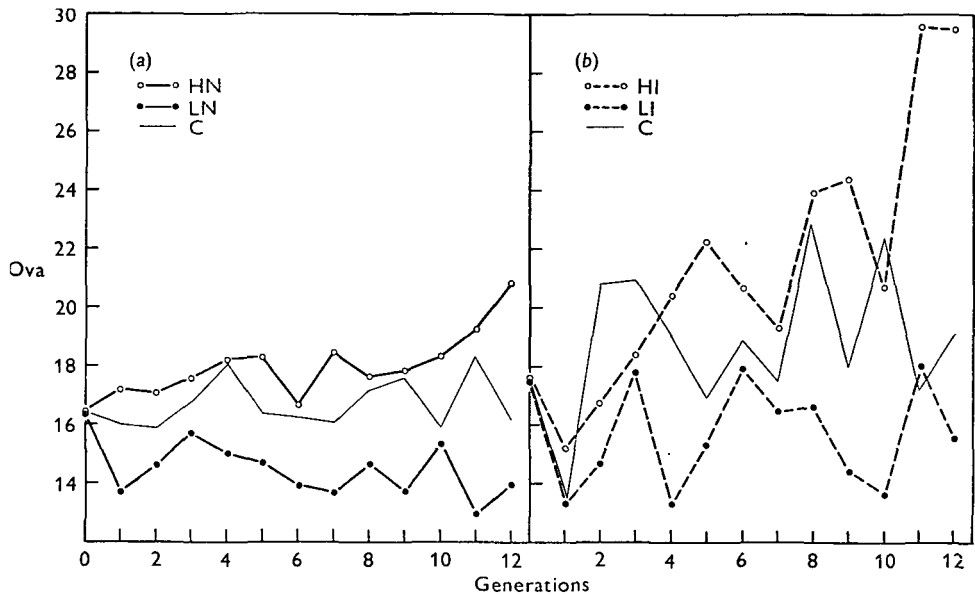

Fig. 1. Responses to selection for natural and for induced ovulation rate. Generation means plotted against the number of generations of selection. (a) Natural ovulation, (b) Induced ovulation.
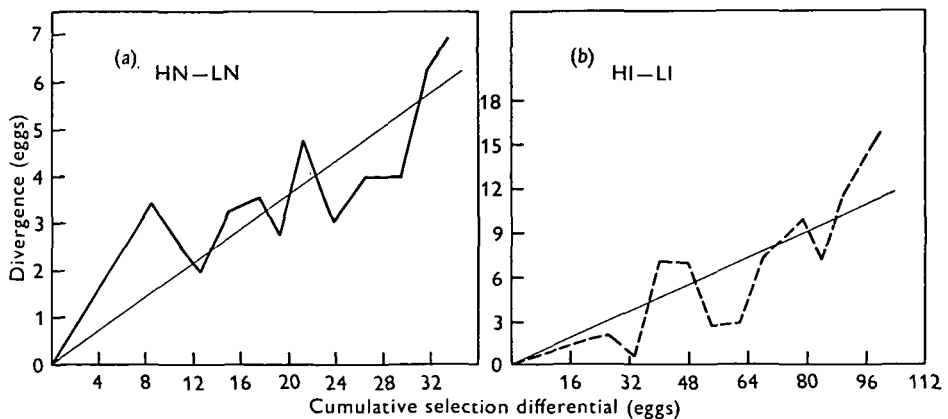

Fig. 2. Differences between upward and downward selected lines plotted against the cumulated selection differential. The straight lines are the fitted linear regressions estimating the realised heritabilities. (a) Natural ovulation, (b) Induced ovulation.

been within families. The convertion was made by means of the relationships given by Falconer (1960 b, p. 234). An estimate was needed of the phenotypic correlation, t, between full sisters. This was obtained from the components of variance between and within families in the base population, which are given in Table 2. The realized heritability from within-family selection was then estimated as the linear regression of divergence on accumulated selection differential over all generations, the regression line being constrained to pass through the zero point. The estimates so obtained were 0.18 ± 0.013 for natural ovulation, and 0.11 ± 0.038 for induced ovulation. The divergence of both the Natural and Induced lines, together with the fitted regression lines, are illustrated in Fig. 2.

Table 2. Components of phenotypic variance, within (σ_W^2) and between (σ_B^2) litters in the selection lines (degrees of freedom in parentheses), and the phenotypic correlation (t) between full sibs

Generation and lines	Natural ovulation			Induced ovulation		
	σ_B^2	σ_W^2	t	σ_B^2	σ_W^2	t
0 (Base popn.)	1·61 (34)	7·87 (35)	0·17	< 0 (52)	38·36 (29)	0
1–11 High	1·37 (77)	6·20 (252)	0·18	2·93 (77)	84·73 (289)	0·03
1–11 Low	0·31 (77)	6·19 (282)	0·05	5·53 (74)	42·79 (257)	0·11
0–11 Pooled	0·95 (188)	6·30 (569)	0·13	1·63 (203)	63·65 (575)	0·02

The over-all, or individual, heritabilities were estimated from the observed within-family heritabilities and the components of variance between and within families. The over-all heritabilities so obtained were: for natural ovulation rate, $h^2 = 0.31$; for induced ovulation rate, $h^2 = 0.22$. For this conversion the estimate of t was based on the components of variance (Table 2) in the selected lines from generations 0 to 11, with High and Low lines pooled. The estimate of 0·31 for the heritability of natural ovulation in primiparous females is not very different from the value of 0·22 for nulliparous females obtained from the analysis of variance given in the previous section.

Correlated responses to selection

Correlated responses to selection can be used to assess the closeness of the genetic relationship between two characters. Three relationships were examined in this way; between natural and induced ovulation, between ovulation in primiparous and nulliparous females, and between ovulation rate and fertility as judged by the numbers of young born and weaned. The genetic and phenotypic relationships between ovulation rate and body weight were also studied in this experiment, but these will be the subject of another paper.

The animals needed for the study of the correlated responses were obtained from the surplus litters which would normally have been discarded after the selection of the females for continuation of the lines had been made. The surplus litters were taken at random from all of those available, but since the litters of the selected females were not available for this purpose the surplus litters were

subjected to a small amount of reversed selection. The correlated responses were compared with the direct responses of the characters selected, measured in the most nearly contemporaneous generation, and the genetic correlations were estimated from the equations given by Falconer (1960*b*, p. 318), modified to make allowance for the difference of cumulated selection differentials.

Natural and induced ovulation rate

The correlated responses were estimated from the selection lines at the end of the experiment. Eight surplus litters from among the progeny of generation 11 in each line were retained for this purpose. These formed a duplicate set of females genetically equivalent to generation 11 but contemporaneous with the females whose ovulation rate established the generation 12 means in the selection lines. The duplicate females, like the line females, were tested when primiparous, but

Table 3. *Mean ovulation rates* ($\pm$ S.E.), *natural and induced, of primiparous females of generation* 12, *showing direct and correlated responses to selection.*

Direction of selection	Lines selected for natural ovulation		Lines selected for induced ovulation	
	Mean Natural ovulation	Mean Induced ovulation*	Mean Natural ovulation*	Mean Induced ovulation
High	20·87 ± 0·54	18·19 ± 1·20	16·84 ± 0·73	29·48 ± 1·83
Low	13·98 ± 0·54	13·77 ± 1·01	14·41 ± 0·50	13·59 ± 0·92
Diff. (H − L)	6·89 ± 0·76	4·42 ± 1·57	2·23 ± 0·88	15·89 ± 2·05
Control (unselected)	16·13 ± 0·45	—	—	19·10 ± 1·55

* Correlated responses.

were tested for the character not selected in their line. That is to say, the induced ovulation rate was tested in the lines selected for natural ovulation, and the natural ovulation rate in the lines selected for induced ovulation. The control line was duplicated in the same way, so that roughly the same number of control females were measured for each character as were measured from the selection lines. The mean egg numbers in these groups, and the differences between high and low lines, are given in Table 3.

In respect of the characters selected for, the high and low lines selected for natural ovulation differed by 6·9 eggs in natural ovulation, and the lines selected for induced ovulation differed by 15·9 eggs in induced ovulation. The lines selected for natural ovulation, however, differed by only 4·4 eggs when their induced ovulation was tested; and the lines selected for induced ovulation differed by only 2·2 eggs when their natural ovulation was tested. In other words, the correlated responses were both much less than the direct responses to selection. The rather small correlated responses suggest that selection for natural ovulation and selection for induced ovulation have led to different genetic changes.

The degree of genetic similarity between the two characers can best be expressed

as the genetic correlation. Two estimates of the genetic correlation can be obtained, one from the correlated response of induced ovulation in the Natural lines and the other from the correlated response of natural ovulation in the Induced lines. The data needed for these calculations are set out in Table 4. The estimates of the genetic correlation (within litters) are 0·26 from the lines selected for natural ovulation, and 0·42 from the lines selected for induced ovulation. The two estimates are probably consistent within the limits of sampling error, and the combined estimate from the responses in both pairs of lines is 0·33. This rather low value of the genetic correlation suggests that natural and induced ovulation are to a large extent under the control of different genes.

Table 4. *Genetic correlations between the various traits, based on the differences between High and Low lines at generation* 12.

(The parameters entered in each column were estimated from the lines indicated in the upper column heading and refer to the type of ovulation indicated in the lower column heading. Correlated responses (CR) are in bold. The parameters and genetic correlations refer to within-litter variation.)

	'Natural' lines (HN − LN)			'Induced' lines (HI − LI)		
	Primiparous		Nulli-parous	Primiparous		Nulli-parous
	Natural	Induced	Natural	Natural	Induced	Induced
Response (R or CR)	6·89	**4·42**	**2·35**	**2·23**	15·89	**12·87**
Selection differential (S or S′)	33·58	32·40	33·58	85·06	100·34	96·80
Square root of heritability (h)	0·423	—	0·395	—	0·336	0·336
Phenotypic standard deviation (σ)	2·51	—	1·98	—	7·98	8·20

Estimated genetic correlations (r_A)

Between natural and induced ovulation in primiparous females:

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Estimated from 'Natural' lines	0·26
Estimated from 'Induced' lines	0·42
Combined estimate	0·33

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Between primiparous and nulliparous ovulation:

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Natural ovulation (estimated from 'Natural' lines)	0·46
Induced ovulation (estimated from 'Induced' lines)	0·82

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Ovulation rate in nulliparous and primiparous females

The ovulation rate of females, aged 8 weeks, which had not previously borne a litter, was measured three times during the course of the experiment for comparison with that of primiparous females on which selection in the lines was based. The nulliparous females used on the first two occasions came from the surplus litters that were genetically equivalent, except for a little reversed selection, with the selection lines at generations 6 and 8. The females used on the third occasion came from the litters of the females measured for primiparous ovulation rate in generation 12, supplemented by a few from the duplicate matings made in parallel with generation 12. The tests of nulliparous females in generations

6 and 12 were made primarily for dose-response experiments to be described later. Groups of about 20 (in generation 6) or 30 (in generation 12) nulliparous females from each of the four selection lines and the control were tested for ovulation rate after treatment with difference doses of PMS. Here we are concerned with the groups in which ovulation was natural, and with the groups in which ovulation was induced by the dose used in the selection, i.e. 4 i.u. of PMS. In these dose-response experiments nulliparous females from all the lines were tested for both natural and induced ovulation. In generation 8, however, the only tests of nulliparous females were of natural ovulation in the Natural and Control lines, about 20 females being tested in each group.
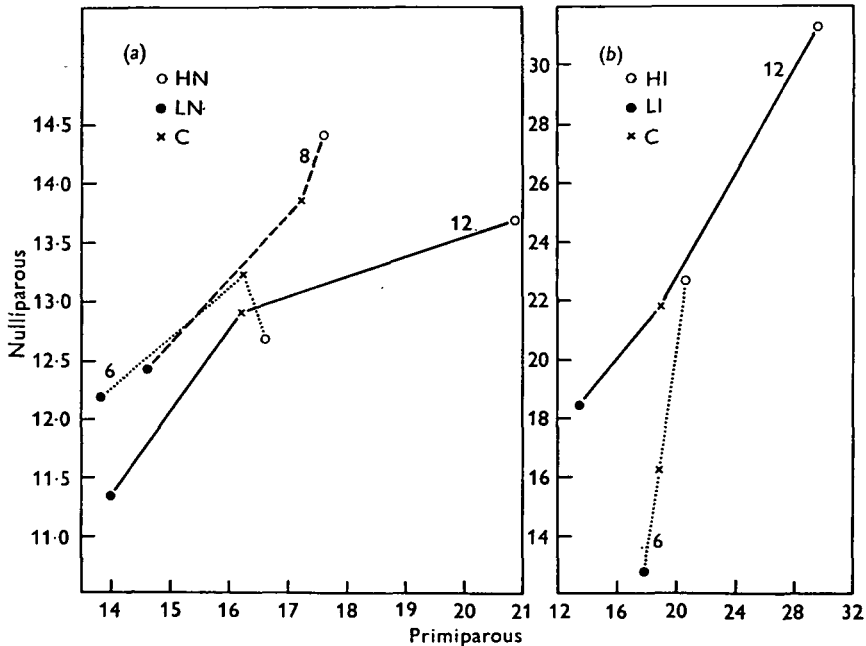

Fig. 3. Mean ovulation rates of nulliparous females of the five lines plotted against the mean ovulation rates of contemporary primiparous females. Comparisons were made at generations 6, 8 and 12, as indicated. (*a*) Natural ovulation, (*b*) Induced ovulation.

Two correlations will be considered—between nulliparous and primiparous ovulation when ovulation is natural, and between nulliparous and primiparous ovulation when ovulation is induced. The relationships between the changes of nulliparous ovulation and the changes of primiparous ovulation resulting from the selection are shown in Fig. 3, in which the mean nulliparous ovulation rate is plotted against the mean primiparous ovulation rate in the comparable generation of each line. Though the three generations are not very consistent in the relationships shown, it is clear that the ovulation rate in nulliparous females did change as a result of selection in primiparous females, and that the change was less with natural than with induced ovulation. With natural ovulation, the correlated

3-2

response in nulliparous females was roughly 40% of the direct response in primiparous females. Upward selection, however, produced less change in nulliparous ovulation than downward selection. With induced ovulation, the correlated response differed in the two tests. In the tests at generation 6 there was apparently very little difference between the lines in the ovulation of primiparous females, and the nulliparous females differed much more. In the tests at generation 12 the correlated response of nulliparous females was about 80% of the direct response in primiparous females.

Approximate estimates of the genetic correlation were calculated from the differences between High and Low lines at generation 12. The mean ovulation rates of the nulliparous females, with their standard errors, will be found in Table 5, in the rows designated 'Natural Ovulation' (lines HN, LN and C) and '4 i.u. PMS' (lines C, HI and LI). These are to be compared with the means of primiparous females given in Table 3. The parameters needed for the calculation for the genetic correlation are given in Table 4. The two genetic correlations, i.e. for natural and for induced ovulation, can each be estimated only once—from the correlated response in nulliparous females—because there was no selection for ovulation rate in nulliparous females to give a correlated response in primiparous females. The estimates obtained were 0·46 for natural ovulation and 0·82 for induced ovulation.

These estimates are only approximate because the heritability and standard deviation in nulliparous females were not known or known only approximately. The values taken for the calculations were obtained as follows. The heritability and standard deviation of natural ovulation in nulliparous females were taken from the results of the sib-analysis as described earlier, the heritability within litters being calculated from the components of variance. The heritability of induced ovulation in nulliparous females was entirely unknown and was assumed to be the same as that of induced ovulation in primiparous females as estimated from the selection response. The phenotypic standard deviation of induced ovulation was taken from the observed variance in the groups of nulliparous females in the tests at generations 6 and 12. The over-all variance (σ_T^2) was taken as the square of the unweighted mean of the standard deviations in all five lines in both tests. The within-litter variance was calculated from this on the assumption that the full-sib correlation was equal to half the heritability, and that the heritability was the same in nulliparous as in primiparous females.

The values obtained for the genetic correlation between nulliparous and primiparous ovulation (0·46 for natural, and 0·82 for induced ovulation) seem to indicate that natural ovulation is to a large extent under the control of different genes in nulliparous and in primiparous females, whereas induced ovulation is genetically more alike in nulliparous and in primiparous females. This, however, can only be a very tentative conclusion since the sampling errors of the genetic correlations are not known and some of the parameters used to calculate them were not reliably estimated.

Fertility

The litters produced by all the females of the selection and control lines before their ovulation rates were tested provide measures of the fertility of the different lines. The mean litter sizes at birth and the differences between High and Low lines in each generation are shown in Fig. 4. The litter sizes at weaning showed substantially the same picture and need not be further discussed. All these litters
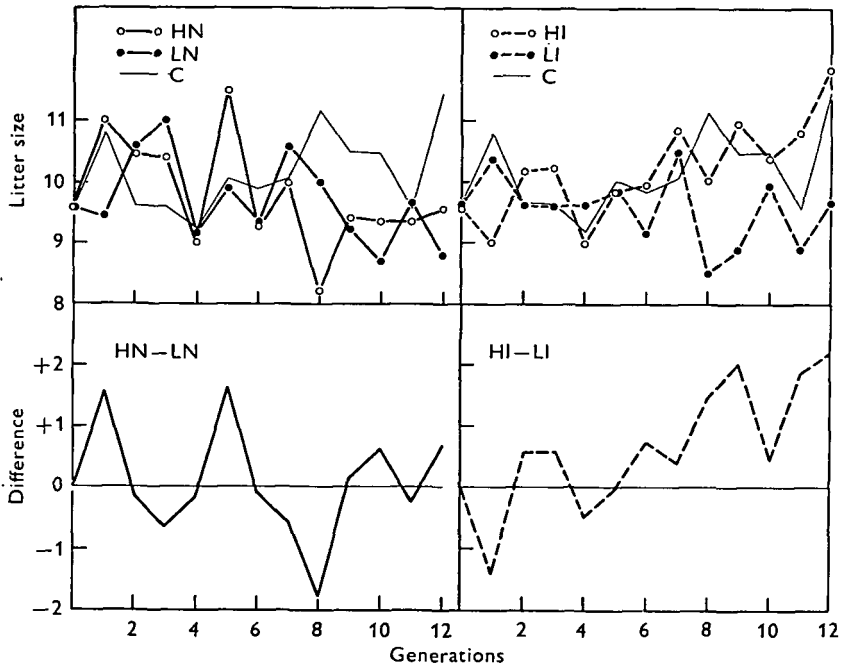

Fig. 4. Fertility, as measured by the number of live young in first litters. Above, means in successive generations. Below, differences between High and Low lines.

were, of course, the product of natural ovulation in nulliparous females. The measurements of ovulation rate of nulliparous females, estimated from the 'natural ovulation' groups in the dose-response experiment (see Table 5), showed that if all the difference in ovulation rate were reflected in a difference in the number of young born, the lines selected for natural ovulation should differ by about two young at the end of the selection and the lines selected for induced ovulation by about three young. This expectation was approximately fulfilled by the Induced lines which showed a clearly increasing divergence and differed by about two young per litter at the end of the experiment. The High and Low Natural lines showed no differentiation in litter size during the course of the selection, and no clear difference at the end. On the other hand, they both had a lower litter size than the Control over the last five generations of the experiment. This suggests that the Low Natural line declined in litter size in accordance with its reduced ovulation rate, but that the High Natural line increased in prenatal

mortality to an extent that more than offset its increased ovulation rate. In the absence of embryo counts, however, this conclusion can only be tentative.

Throughout the present experiment the time allowed for mating to take place was the same for all lines, and the proportion of pairs that failed to mate within this period was noted. In contrast with the lines selected for body weight, reported by Fowler & Edwards (1960), none of the present lines showed any consistent changes in the proportion of infertile matings.

FSH activity and ovarian sensitivity

One of the main questions of interest in the selection responses was whether the changes in ovulation rate were the result of changes in the level of endogenous FSH activity or of changes in ovarian sensitivity.

Evidence on this question was looked for in the responses of mature nulliparous females of the selection lines to different doses of exogenous FSH in a dose-response experiment. Immature females were also tested in another dose-response experiment, with the object of finding out whether the method previously applied by McLaren (1962) would lead to similar conclusions.

Mature females

The supposition underlying the dose-response experiment was that if two strains with different natural ovulation rates shed different numbers of eggs in response to induction by the same dose of exogenous FSH, then the sensitivities of their ovaries must be different; whereas if they shed the same number of eggs in response to the same dose, their ovarian sensitivities are the same and their different natural ovulation rates must be the result of different levels of endogenous FSH activity. The situation is, however, complicated in two ways. First, one strain may shed more eggs than the other at a low dose but fewer at a higher dose, as found by McLaren (1962). Secondly, when the dose of exogenous FSH is reduced, the induced ovulation rate declines, and reaches a minimum at a dose of about 1 i.u. of PMS. At doses below this value the ovulation rate increases again and reaches the natural level at zero-dose, i.e. in response to HCG alone (Land, 1965b). For these reasons it is necessary to compare the strains over a range of doses, and the interpretation of the result is even then not always clear.

Two dose-response experiments were carried out; the first, at generation 6 and the second at generation 12. The females used were all nulliparous and aged about 8 weeks. In the first experiment the doses used were 2, 4, 8, 16 i.u. of PMS, followed respectively by 2, 3, 4, 6 i.u. of HCG. About 20 females were tested on each dose and in addition another similar group of females were allowed to ovulate naturally. In the second experiment there were about 30 females in each group and the same doses were used with the addition of a dose of 1 i.u. of PMS followed by 2 i.u. of HCG. The results of the second experiment are given in Table 5, and the mean ovulation rates at each dose in both experiments are plotted in Fig. 5.

The conclusions to be drawn about the lines selected for induced ovulation are

very clear. At all doses the High line shed more eggs than the Control and the Low line shed fewer eggs than the Control. All doses were therefore consistent in showing that lines differed in ovarian sensitivity. The lines selected for natural ovulation are less straightforward. Consider first the Low and Control lines. In the first experiment they were almost identical at all doses, but in the second experiment the Low line shed fewer eggs than the Control at all doses except the highest. From this it must be concluded that selection for low natural ovulation rate led to a decrease in ovarian sensitivity. The High Natural line, however, behaved quite differently. The shape of its dose-response curve differed markedly

Table 5. *The natural ovulation rate of the nulliparous female offspring of generation 12, together with the mean number of eggs shed in response to exogenous gonadotrophins*

(n = the number of females which ovulated. L.S.D. = least significant difference for $P < 0.05$.)

		Line									
Treatment i.u. of		HN		LN		C		HI		LI	
PMS	HCG	n	Mean ± s.e.	n	Mean ± s.e.	n	Mean ± s.e.	n	Mean ± s.e.	n	Mean ± s.e.	L.S.D.
Natural		30	13·7 ± 0·54	31	11·4 ± 0·26	29	12·9 ± 0·11	31	14·7 ± 0·49	29	11·5 ± 0·11	1·60
1	2	28	12·7 ± 1·03	30	9·5 ± 0·55	28	11·3 ± 0·60	31	12·1 ± 0·80	26	9·1 ± 0·65	2·93
2	2	28	12·6 ± 0·97	30	10·7 ± 0·96	28	12·7 ± 1·27	30	16·0 ± 1·78	28	10·0 ± 1·03	4·40
4	3	28	14·1 ± 1·30	30	17·7 ± 1·92	28	21·8 ± 1·67	30	31·3 ± 2·76	28	18·4 ± 1·69	7·69
8	4	26	22·6 ± 1·96	30	28·3 ± 2·80	25	32·7 ± 2·77	30	46·8 ± 2·53	23	20·2 ± 1·81	9·70
16	6	7	12·3 ± 1·82	30	30·5 ± 3·53	25	28·5 ± 3·88	30	30·9 ± 3·60	10	11·1 ± 4·31	—

from those of the other lines, particularly in the second experiment. The 'dip' was much shallower, and the minimum response occurred at a dose of 2 i.u. instead of 1 i.u. as in the other lines. At doses below 2 i.u. it shed more eggs than the Control, but at doses above 2 i.u. it shed fewer, which seems to indicate that its ovaries were more sensitive to low doses but less sensitive to high doses. Similar results were obtained by McLaren (1962) with a strain selected for high litter size.

The conclusion from the dose-response experiments so far is that all four selected lines had changed in ovarian sensitivity. The question then remains whether there is any evidence of changes in FSH activity or whether the changes in ovarian sensitivity are sufficient to account for the differences in ovulation rate.

Fowler & Edwards (1960) introduced the idea of the 'PMS-equivalent' as a means of assessing the FSH activity in natural ovulation. The PMS-equivalent is the dose of PMS required to induce the number of eggs shed in natural ovulation. The interpretation of the PMS-equivalent is made a little uncertain by the dip in the dose-response curve, but, at least when the dose-response curves do not differ in shape, the PMS-equivalent would seem to provide a meaningful comparison of the natural FSH levels. The PMS-equivalents estimated from the second dose-response experiment are given in Table 6. All the lines except the

High Natural line are very nearly the same, with a PMS-equivalent of about 2·0 i.u., but the High Natural line, with a value of 3·8, is very much higher. The PMS-equivalents thus show that the changes in ovarian sensitivity are enough
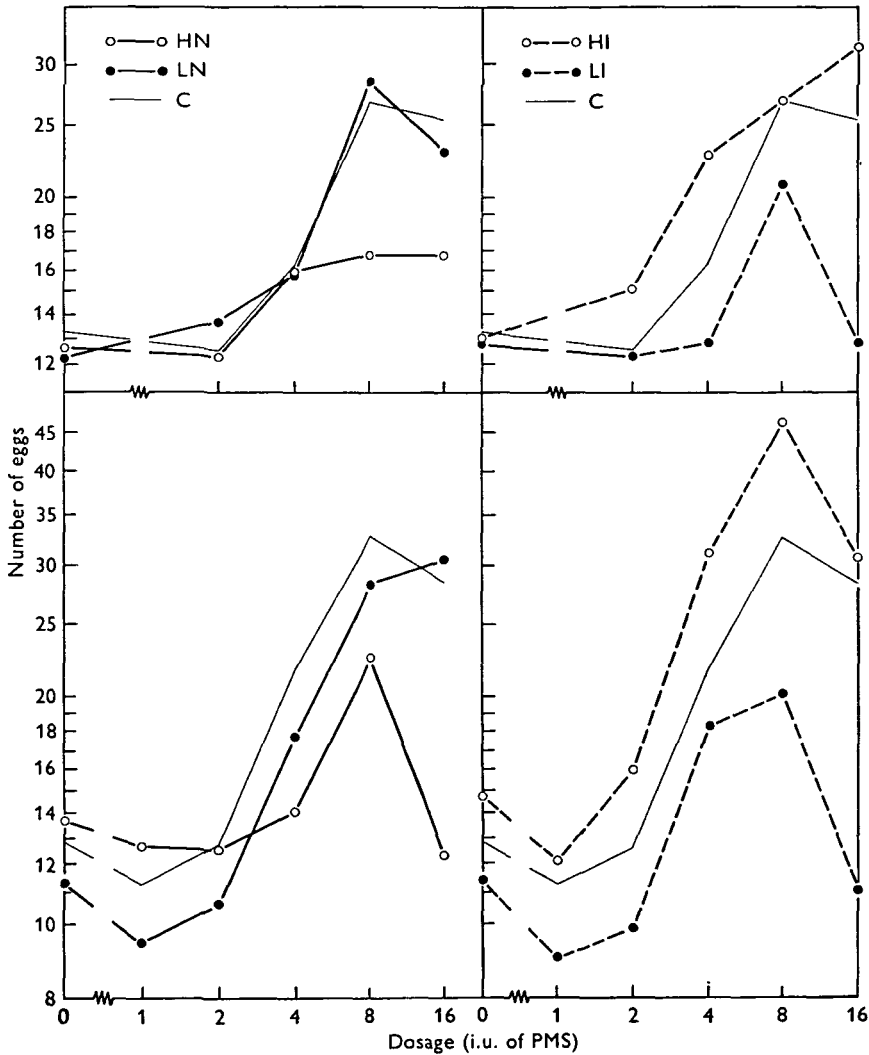

Fig. 5. Dose-response curves. Numbers of eggs shed by nulliparous females in response to different doses of PMS. Above, females from generation 6; below, females from generation 12. Left, the lines selected for Natural ovulation and the Control; right, the lines selected for Induced ovulation and the Control.

to account for the changes in ovulation rate produced by selection in both the Induced lines and in the Low Natural line, but not in the High Natural line. If the PMS-equivalent can be accepted as an indication of the output of endogenous FSH despite the difference in the shape of the dose-response curve, it shows that the endogenous FSH was increased by about 90% by selection in the High

Natural line. These conclusions from the dose-response curves are supported by the estimates of the FSH potency of the pituitaries of these lines obtained by bioassay (Bell & Land, 1969). Although the differences were insignificant, the FSH levels were greater in the High Natural line than in the others.

Table 6. *PMS equivalent of the selected lines at generation* 12, *estimated from the dose-response experiment*

	Natural		Induced		
	High	Low	High	Low	Control
i.u. of PMS	3·8	2·2	1·8	2·3	2·1

Table 7. *Induced ovulation rate in immature females*

(N = number of females; W = mean weight of females (g); E = mean no. of eggs ovulated, $\hat{E}$ = mean egg number adjusted to weight of 10 g, $\pm$ s.e.; P = significance of difference. The standard errors of the unadjusted responses to low and high doses were approximately 0·2 and 4·0 eggs respectively.)

Dose	Line	N	W	E	$\hat{E}$	P
0·05 i.u./g	HN	20	10·8	8·0	$7·4 \pm 0·4$	0·7
	LN	28	9·4	6·7	$7·1 \pm 0·3$	
	C	19	9·6	7·9	$8·2 \pm 0·4$	
	HI	23	10·5	8·5	$8·1 \pm 0·4$	0·2
	LI	25	9·5	6·9	$7·3 \pm 0·3$	
0·2 i.u./g	HN	19	10·6	15·7	$14·7 \pm 1·9$	0·5
	LN	28	9·5	15·3	$16·3 \pm 1·6$	
	C	21	10·3	18·8	$18·4 \pm 1·8$	
	HI	27	10·3	22·8	$22·3 \pm 1·6$	$< 0·001$
	LI	25	9·2	11·0	$12·5 \pm 1·7$	

Immature females

McLaren (1962) used the induced ovulation rate of immature females to compare the ovarian sensitivity of strains selected for litter size. The immature females were given a dose of FSH proportional to their body weight at the time of treatment. In order to find out if this method would give the same results as were described above, immature females of generation 12 of the present selection lines were tested by McLaren's method as follows. Two groups of between 20 and 30 females aged between 21 and 25 days were taken from each line. One group from each line was treated with 0·05 i.u. of PMS per g of body weight, and the other group with 0·2 i.u. of PMS per g. The average weights were about 10 g, so the first group had a dose of about ½ i.u. and the second group about 2 i.u. In both groups the PMS treatment was followed by a dose of 0·2 i.u. of HCG per g of body weight. The results, given in Table 7, were as follows.

First, the regression of egg number on body weight was calculated within each group. Within each dose-level the groups (i.e. lines) did not differ significantly in their regression coefficients. Pooled regressions were therefore calculated for

each dose-level, the values obtained being $+0.77 \pm 0.09$ eggs/g for the low dose and $+1.82 \pm 0.38$ eggs/g for the high dose. The mean egg number of each group was then adjusted by the pooled regression to a standard body weight of 10 g. These adjusted egg numbers therefore estimate the responses induced by doses of $\frac{1}{2}$ i.u. and 2 i.u., when differences in body weight, and therefore of dose, are eliminated. With a dose of $\frac{1}{2}$ i.u. the differences between the lines were small and insignificant, though the two induced lines differed in the expected direction. With a dose of 2 i.u. the High and Low Induced lines differed markedly from each other and from the Control in the expected direction. The differences between the Natural lines and the Control, though not significant, were partially in the expected direction: the Low line shed fewer eggs than the Control, and the High line shed fewer even than the Low line. Thus, when tested at the higher dose, the immature females partially confirmed the conclusion based on the adults. They show convincingly that the Induced lines differed in ovarian sensitivity and that the high ovulation rate in the High Natural line could not be accounted for by increased ovarian sensitivity. The method of treating females with a dose graded according to their weight is, however, not a satisfactory one. It does not remove the variation associated with body weight at the time of treatment, and the effects of weight and of dose cannot be disentangled since the two variables are completely correlated.

4. DISCUSSION

We may now consider how far the objects of the work, as stated in the Introduction, have been achieved, and what additional conclusions of general interest can be drawn.

The first object was to find out how much additive genetic variation in ovulation rate existed. Here the conclusion is clear; the heritabilities of both natural and induced ovulation rate were fairly high—31 % in the first case and 22 % in the second. A considerable amount of genetic variation thus existed in the base population by which natural selection under laboratory conditions could have changed the ovulation rate in either direction. The persistance of additive genetic variance at this level argues in favour of ovulation rate having an intermediate optimum with respect to fitness. It was suggested in the Introduction that the adjustment of litter size to an intermediate optimum (if there is an intermediate optimum for litter size) must be through ovulation rate because the other component of litter size—foetal survival—is unlikely to have an intermediate optimum. The existence of a substantial amount of additive variance of ovulation rate supports this view. In this connexion it is interesting to note that domestication of the deer-mouse, *Peromyscus*, has led to litter size becoming more variable but without a change of mean, the inference being that in the wild natural selection favours litters of intermediate size (Price, 1967).

The second object of the work was to find out which of the components—FSH level or ovarian sensitivity—would be changed by selection for ovulation rate.

The analysis of the lines selected for induced ovulation showed unequivocally that ovarian sensitivity had been changed by the selection, as was indeed to be expected when ovulation was induced by a fixed dose of gonadotrophin. The rate of response to selection for induced ovulation, indicating a heritability of 22 %, proves that there was a considerable amount of genetic variation of ovarian sensitivity in the base population. The conclusion from the lines selected for natural ovulation was not so clear. The high line had an increased ovarian sensitivity to low doses of PMS but a reduced sensitivity to high doses. The increased natural ovulation rate could not be accounted for by the altered ovarian sensitivity and was ascribed to an increased FSH activity. The Low Natural line, in contrast, had a reduced ovarian sensitivity which fully accounted for the reduced natural ovulation rate. Thus selection for natural ovulation rate led to different physiological changes in the two directions; upward selection led primarily to increased FSH activity, while downward selection led to reduced ovarian sensitivity. Qualitatively different responses in opposite directions were found also following selection for litter size (Falconer, 1963) and may be formally accounted for in terms of the gene frequencies in the base population. The asymmetrical response of FSH activity (an increase in the High line but no decrease in the Low line) suggests that alleles that increased FSH activity were at low frequencies in the base population, though why this should be so is not clear.

The changes of ovarian sensitivity and FSH activity discussed above were based on the induced ovulation rates of nulliparous females, whereas the selection which produced the changes was based on the ovulation rates of primiparous females. When the correlated changes in nulliparous females were compared with the direct responses in primiparous females, it was found that the two Induced lines and the Low Natural line had all changed nearly as much in nulliparous ovulation as in primiparous. Consequently the conclusions about ovarian sensitivity can be accepted as applicable also to the primiparous females on which selection was based. The high genetic correlation between nulliparous and primiparous females in respect of induced ovulation rate proves that the genetic variation in ovarian sensitivity is largely common to both first and second parities. The High Natural line, unlike the others, changed much less in nulliparous than in primiparous females. This suggests that FSH activity is not highly correlated in first and second parities.

The third object of the work was to find out how closely induced and natural ovulation rates are connected genetically, and how reliably natural ovulation rates in different strains could be compared by measurements of induced ovulation rates. The correlated responses to selection showed that the genetic similarity of the two characters was not very close, the genetic correlation being about 0·33. Consequently the induced ovulation rates could not be relied on as a valid comparison of the natural ovulation rates of different strains.

Finally we may consider how our results of selection for natural ovulation rate compare with the changes of ovulation rate following selection for other characters. The situation is complicated by the asymmetrical responses and,

unfortunately, no very clear pattern emerges. The changes of ovulation rate resulting from selection both for large and for small body size were attributed to altered FSH activity (Fowler & Edwards, 1960), and the altered FSH activity was attributed to the larger or smaller size of the pituitary rather than to its unit potency (Edwards, 1962). Thus it seems that ovulation rate responds to selection for body size simply through the correlated change in the size of the pituitary and its output of FSH. Presumably the size of the ovary is also correlated with body size. The absence of any change of ovarian sensitivity therefore suggests that the size of the ovary has little or no effect on the ovulation rate, a conclusion which follows also from studies on the correlation between the numbers of eggs shed by the two ovaries (Falconer, Edwards, Fowler & Roberts, 1961; McLaren, 1963).

Selection for litter size produced puzzling asymmetrical responses. High litter size resulted from an increase of ovulation rate, but low litter size resulted from an increase of prenatal mortality accompanied by a small increase—not a decrease—of ovulation rate (Falconer, 1963). The present experiment proved that ovulation rate can be reduced by selection and the failure of selection for low litter size to reduce ovulation rate remains unexplained. According to McLaren's (1962) experiment on immature females, the differences of ovulation rate resulting from selection for litter size were due to ovarian sensitivity rather than to FSH activity.

Our selection for natural ovulation rate produced asymmetrical responses with the same puzzling features as the selection for litter size. Selection upward and downward were both effective, but changed ovulation rate by different physiological means. Upward selection, though it changed ovarian sensitivity in a rather complicated way, was effective through an increase of FSH activity. Downward selection, however, was effective through a reduced ovarian sensitivity and did not alter FSH activity. It is not clear why ovarian sensitivity was not increased by upward selection or why FSH activity was not reduced by downward selection. It is possible, however, that the conclusions about ovarian sensitivity and FSH activity may be wrong since they are based on the responses to exogenous PMS, and these may not accurately reflect the sensitivity to endogenous FSH. The change in the shape of the dose-response curve after treatment with PMS is another puzzling feature of the response to selection for increased natural ovulation rate. McLaren (1962) found the same change in immature females after selection for litter size; in both cases sensitivity to low doses was increased but sensitivity to high doses was reduced.

In a recent paper, Bradford (1968) describes an experiment in which selection was applied to the size of litter born after ovulation had been induced by PMS. There was no response of the PMS-induced litter size and no correlated response of litter size after natural ovulation. Our results do not throw much light on this unexpected lack of response to selection. The high heritability of induced ovulation rate in our strain suggests, however, that the lack of response may have been due to a low phenotypic correlation between litter size and induced ovulation rate, rather than to an absence of genetic variability of ovulation rate.

SUMMARY

Genetic and physiological studies of ovulation rate, both natural and induced, in a random bred population of laboratory mice led to the following results.

(1) The heritability of natural ovulation rate in nulliparous females was $22 \pm 19\%$, estimated from the correlation between paternal half-sibs.

(2) Selection applied to natural ovulation in primiparous females led to a response in both directions, with a realized heritability of 31 % (h^2 within litters = $0\cdot18 \pm 0\cdot013$). The number of eggs shed by the High, Control and Low lines after 12 generations were respectively 21, 16 and 14.

(3) Selection applied to ovulation induced by 4 i.u. of PMS led also to a response in both directions, with a realized heritability of 22 % (h^2 within litters = $0\cdot11 \pm 0\cdot038$). The induced ovulation rates of the High, Control and Low lines after 12 generations were respectively 29, 19 and 14 eggs.

(4) PMS-equivalents were calculated from dose-response curves, and were found to be nearly the same in all lines except the line selected for high natural ovulation rate, which had a PMS-equivalent nearly double that of the Control. From this it was inferred that the increase of ovulation rate resulting from selection for high natural ovulation was due to an increased FSH activity, whereas the changes of ovulation rate resulting from selection for low natural ovulation and for both high and low induced ovulation were due to changes of ovarian sensitivity.

(5) Genetic correlations, estimated from correlated responses to selection, were: (i) between natural and induced ovulation in primiparous females, $0\cdot33$; (ii) between primiparous and nulliparous females in natural ovulation, $0\cdot46$; (iii) between primiparous and nulliparous females in induced ovulation, $0\cdot82$.

(6) The selection for induced ovulation produced changes in litter size following natural ovulation that were nearly equal to the changes in natural ovulation rate. The selection for natural ovulation, however, produced no clear changes of litter size.

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