A GENETIC STUDY OF FERTILITY IN THE MOUSE

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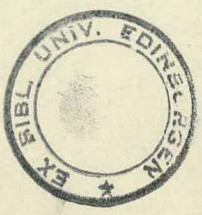

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TABLE OF CONTENTS

INTRODUCTION

The elucidation of the factors that affect mammalian fertility and their interactions is fundamental to the solution of problems associated with the increase in productivity of domestic animals, the control of human populations, and the relative importance of the different components of fitness. Most investigations of quantitative variation in reproductive performance, however, have been of characters which are relatively easy to score but difficult to describe in terms of their physiological components. It was therefore felt that it would be interesting to study a component of fertility which was dependent upon relatively few physiological variables, even though such a character might be difficult to score. In choosing a suitable character it is necessary to consider it in terms of its suitability for genetical investigation, and in terms of its association with characters previously studied. Bearing these factors in mind, it was decided to study ovulation rate in the mouse.

The primary aims of the study are to estimate the genetic parameters of ovulation rate, to evaluate the changes brought about by selection, and to examine the physiological factors underlying these changes. It was hoped that a study of this nature would give an indication of both the contribution of ovulation rate to gross fertility, and of the relative ease with which the components of ovulation rate could be changed by selection.

It is evident that such an investigation is dependent on both the feasibility of studying the physiological components of ovulation rate, and the existence of additive genetic variance of ovulation rate.

Consequently it is proposed to discuss these two factors in turn, and then to outline the work undertaken.

Changes in ovulation rate have, in the past, been produced as a result of selection for other traits, that is, as a correlated response, and attempts have been made to analyse these changes in terms of the physiological components of ovulation rate. The principle behind such analyses has been that the number of eggs shed by a polytocous species is dependent on the amount of follicle-stimulating hormone (FSH) secreted by the animal's pituitary, and the sensitivity of the ovaries to this hormone. Consequently it was argued that the changes in ovulation rate can be the result of changes in either or both components, and it has been the aim of these analyses to estimate the relative contribution of these two components to the overall change in ovulation rate.

An analysis of this type was carried out by Fowler and Edwards (1960), when they examined the differences in ovulation rate between lines selected for body weight by Falconer (1953, 1955, and 1960a). Ovulation was induced in adult females by the use of pregnant mares serum (PMS), which has FSH activity, and chorionic gonadotrophin (CGT), which has luteinizing hormone (LH) activity. The dose of PMS required to induce the ovulation of the same number of eggs as would be shed at natural oestrus was then estimated and called the PMS equivalent for the line. This PMS equivalent was then equated with the level of endogenous FSH activity. Their results suggested that changes in ovulation rate were mainly attributable to changes in the PMS equivalents of the different lines, rather than to changes in the sensitivity of the ovaries. The use of bioassay techniques for total pituitary gonadotrophin levels by Edwards (1962) corroborates this conclusion by showing that selection

had changed the size of the pituitary but not its activity per unit weight, which indicates that changes in ovulation rate had been mediated by changes in the level of endogenous gonadotrophins.

Lines selected by Falconer (1960) for high and low litter size were found to differ in ovulation rate and body weight, both correlated responses being in the same direction - body weight and ovulation rate were highest in the high line, and lowest in the control line, the low line being intermediate. From this it was concluded that upward selection had increased ovulation rate, and that downward selection had increased embryonic mortality. These lines were analysed by McLaren (1962) by a similar method to that of Fowler and Edwards, except that immature females were used, and body weight was confounded with the dose of PMS. Her results suggest that, by contrast to selection for body weight, selection for litter size had changed the ovulation rate by changing the sensitivity of the ovary rather than the PMS equivalent.

Throughout these analyses it has been assumed that changes in ovulation rate which cannot be accounted for by changes in ovarian sensitivity are the result of changes in the level of FSH activity. The possibility that FSH and LH may act synergistically, as they do in their effect on ovary weight, has not been considered. It has either been assumed that LH is solely responsible for the actual event of ovulation, or that FSH and LH are not secreted simultaneously when the ovary is in a state to react to them. If these assumptions are invalid it is evident that changes in the level of activity of either or both hormones could affect the number of eggs shed. Thus, even if a single dose of hormone can be compared with the endogenous situation where gonadotrophins are probably secreted for a longer period of time, the PMS equivalent could not be equated with the

level of endogenous FSH production, but with the total follicle stimulating activity of both FSH and LH. It would appear, therefore, that although the comparison of natural and induced ovulation rates provides a means of detecting changes in the sensitivity of the ovary to exogenous hormones, extrapolations from PMS equivalents to the activity of the FSH axis of the pituitary are only tentative. Consequently it was decided to make a further examination of the relationship between natural and induced ovulation, and to consider ways in which changes in the level of activity of FSH and LH could be differentiated.

The work described above, together with the correlated changes in ovulation rate observed by MacArthur (1944), shows that ovulation rate is genetically correlated with body weight, and that genetic variation for ovulation rate existed in the populations studied. However, the differences in ovulation rate obtained were the result of many generations of selection, and consequently before embarking on a genetical study of ovulation rate it is worthwhile making a further examination of the evidence for the existence of genetic variance of ovulation rate, and the mode of action of the genes involved.

The lines selected for body weight by Falconer (1955) were made up from four inbred lines, and consequently any segregating genes would be expected to be present at intermediate frequencies. The increase and decrease in ovulation rate as a result of selection is therefore not surprising. It is difficult to draw any conclusions regarding gene frequencies from the lines selected for body weight by Falconer (1960a), but in this case the problem is the absence of ovulation rate data for the control line. This means that it is impossible to assess the relative

change in ovulation rate in the two lines, and consequently inferences can not be made regarding the types of genes segregating, or about their frequencies in the base population. However, it is clear that both experiments provide evidence for the existence of 'correlated genes'.

Information regarding the nature of genes correlating body weight and ovulation rate is provided by the work of Falconer and Roberts (1960), when they studied the effects of inbreeding on body weight, ovulation rate, and litter size. It was found that as inbreeding progressed litter size decreased, but ovulation rate and body weight remained constant. From this they concluded that "the genes that influence ovulation rate without affecting body weight do not show directional dominance, but that genes that influence ovulation rate as a consequence of their effect on body weight may show directional dominance".

In conclusion, therefore, we may say that there is good evidence for the existence of genes segregating for ovulation rate in laboratory mouse populations, and that the evidence is sufficient to indicate that a genetical study of ovulation rate is a feasible proposition.

It has already been said that it was decided to make a preliminary study of the genetic parameters of ovulation rate, and to attempt to change ovulation rate by selection. It was hoped that a study of this nature would not only enable a comparison to be made between estimates of the parameters of ovulation rate, calculated from the two sources, but would also contribute to the solution of the problem of assessing the part played by ovulation rate in controlling litter size, discussed by Falconer (1960b and 1963). Furthermore, if any additive genetic variance was found, lines would emerge which had different ovulation rates, and which could,

therefore, be compared in terms of the physiological components of ovulation rate. It was decided that an appropriate way to study the genetic parameters would be to examine the relationships between half-sibs, that is, to carry out a half-sib analysis. In addition, it was decided to select not only for the number of eggs shed at natural oestrus, but also for the number of eggs shed in response to a standard dose of PMS. The reasons for selecting for induced as well as natural ovulation rate arise from the fact that induced ovulation is often used as a means of investigating natural ovulation, and it was felt that a comparison of the two procedures may help to assess the validity of this approach. Also the contrasting effects of selection for body weight and litter size on the PMS equivalents and ovarian sensitivities of the selected lines, as described above, indicated that the dual selection programme would be of interest. It was hoped that selection for natural ovulation rate would apply selection pressure to the intrinsic follicle stimulating system, and to the sensitivity of the ovary, while selection for the response to PMS would apply pressure solely to the sensitivity of the ovary. During the course of the selection programme a study was also made of any possible correlated responses (especially in the characters which had been selected for in the experiments described above) and on its completion the physiological components underlying the main traits were examined.

MATERIALS AND METHODS

(a) Materials

(i) Mice

The stock of mice used for all the experiments in the present work was a random bred strain designated Q. This strain bred well, and was easy to maintain. A good deal was already known about its reproductive performance and growth in body size from previous experiments, and it was related to the strain on which Falconer's (1960b) work on the genetics of litter size was carried out. The Q- strain was developed in 1957 from crosses between five non-inbred strains and one inbred. Since 1960 the stock has beenmaintained by 20 male and 40 female parents in each generation, and the mice used in the present work were taken from the progeny of the 11th generation.

(ii) Gonadotrophins

Throughout this work, Gestyl (Organon Ltd.) PMS, and Pregnyl (Organon Ltd.) CGT were used.

(b) Measuring the characters

The two characters to be studied are natural and induced ovulation rate, the former in its own right, the latter in the hope that it will be a measure of ovarian sensitivity to endogenous gonadotrophins. The measurement of both characters involves the identification of the time of ovulation, and the counting of the number of eggs shed. These two facets will therefore be described together. The induction of ovulation also involves the choice of doses of exogenous hormones that will reflect the ovarian sensitivity to endogenous gonadotrophins, and the estimation of the importance of various environmental sources of variation; as these problems are specific to induced ovulation, they will be described later.

(i) Timing and counting

Natural ovulation was identified by pairing the females with males, and examining them each morning for the presence of a vaginal plug. Ovulation is known to occur close to the time of mating. The timing of ovulation in response to gonadotrophins, on the other hand, is determined by the treatment used to induce the ovulation. The technique used was that described by Fowler and Edwards (1957), treatment being commenced by the intraperitoneal injection of PMS, followed by CGT 43 hours later, ovulation occurring approximately 13 hours after the second injection. Optimal results are obtained if the system is adjusted in such a way that ovulation occurs around 01.00 hrs., i.e. at the time ovulation would be expected to occur naturally. An additional advantage is that naturally and artificially ovulating mice can be scored at the same time. When the eggs are shed from the ovary, they are embedded in cumulus cells, which are progressively broken down during the day following ovulation. However, 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 in water under a dissecting microscope, the presence of eggs in the fallopian tube can be identified as a discrete swelling. The rupture of the fallopian tube at this point enables the eggs, still embedded in cumulus, to be removed, the cumulus stretched, and the eggs identified and counted. Although the degeneration of the cumulus is a gradual process, females were always dissected before 12.00 hrs., at which time it was still in good condition. Only eggs embedded in cumulus were scored.

(ii) The development of a technique for measuring ovarian sensitivity

The development of a satisfactory technique for measuring ovarian sensitivity necessitated the execution of several preliminary experiments, and in order to clarify the reasons for choosing the technique which was finally adopted, it is proposed to describe these experiments in this section.

It was felt that the doses of exogenous hormones which were most likely to reflect ovarian sensitivity to endogenous gonadotrophins were those which would induce the ovulation of a similar number of eggs to that shed at natural oestrus. In attempting to find such doses, it was realised that the number of eggs shed might be affected by three factors: (1) the dose of PMS, (2) the dose of CGT, and (3) the physiological state of the mouse at the time of treatment. Of these (1) is the main variable to be examined. Factor (2) has been shown by Fowler and Edwards (1957) to be relatively unimportant - above a minimal dose it did not affect the response. The effect of the stage of the cycle, part of factor (3), was examined by Edwards, Wilson and Fowler (1963), using several strains of mice. Only in one strain, and then only at low doses of PMS, was a significant difference found - when the response at metoestrus was greater than at oestrus or dioestrus. In view of the fact that low doses of PMS induce the natural response, the stage of the cycle as well as the dose had to be examined. This was done by constructing dose-response curves, that is, studying the response to various doses of PMS and CGT at specific stages of the cycle, and comparing the responses to that at natural oestrus.

Furthermore, as some workers (e.g. Fowler and Edwards 1960) gave a standard dose regardless of body weight, while others (e.g. McLaren 1962) gave a dose graded in proportion to body weight, it was decided to

examine the effects of body weight on the response to the dose of gonadotrophin to be used in the selection experiment. In addition, as post-lactational females, that is, females which had given birth to and suckled a litter, were to be used in the selection experiment (the reason for this will become apparent later), it was decided to compare their response to gonadotrophins with that of 6-8 week-old virgins, in the hope that generalisations could be extended from one to the other - the latter being easier to obtain.

In view of the limited time available for this work, it was necessary to answer as many of these problems as quickly as possible, and so a preliminary trial using post-lactational females was carried out. In this trial, the response to ¼, ½, 1 and 2 i.u. PMS followed by 2 i.u. CGT was examined at oestrus, metoestrus and dioestrus (the stage referring to the stage at which treatment was commenced). The mice were distributed between treatments in such a way that each had a range of body weights, and that the mean body weight was approximately the same for each group. The results shown in Table 1 are inconclusive, and suggest that a more systematic approach is necessary.

The responses of nulliparous and post-lactational females to 0, 1, 2, 4, 8 and 16 i.u. PMS followed by 2, 2, 2, 3, 4, 6 i.u. CGT respectively, together with the natural ovulation rate, are shown in Table 2, and Fig. 1. These females were all in dioestrus at the time of treatment, and distributed according to body weight. On the basis of the dose-response curves it was decided to use a dose of 4 i.u. PMS to induce ovulation in the induced selection lines, provided that body weight did not affect the response.

FIG. 1 The response of (a) Nulliparous and (b) Post-lactational Females to various Doses of PMS and CGT.

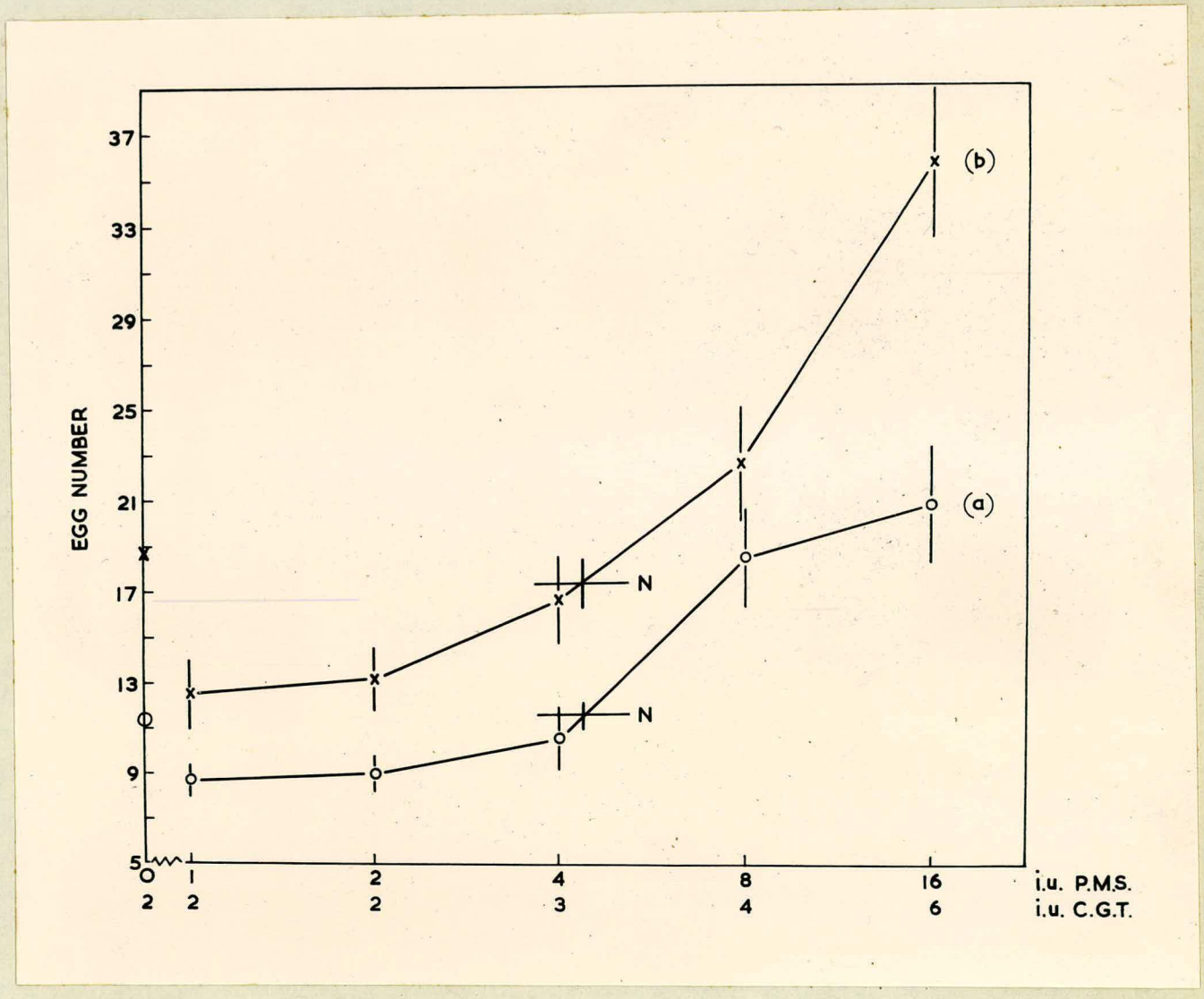

TABLE 1. The Responses of Post-lactational Females to various doses of PMS, given at Different Stages of the Oestrus Cycle.

Stage of cycle	Dose of PMS																Mean
	¼ i.u.				½ i.u.				1 i.u.				2 i.u.				
	No. treated	No. ovulated	Mean	S.E.	No. treated	No. ovulated	Mean	S.E.	No. treated	No. ovulated	Mean	S.E.	No. treated	No. ovulated	Mean	S.E.	
D	15	9	11.89	1.58	10	9	13.78	1.77	12	9	13.56	1.02	12	9	13.56	1.32	13.19
O	10	8	12.25	1.20	12	9	11.78	1.87	16	9	13.89	3.03	11	9	13.00	0.82	12.74
M	9	9	15.11	0.77	9	9	14.78	0.97	9	9	15.33	0.68	9	9	14.89	0.54	15.03
	13.11				13.44				14.26				13.82				

(Natural : No.treated = 18, No. ovulated = 14, Mean = 15.50, S.E. = 1.06)

Analysis of Variance

Source of variation	d.f.	SS	M.S.	U.R.
Stage of cycle	2	104.59	15.29	2.74 NS
Dose	3	19.31	6.44	
I	6	25.82	4.30	
Error	95	1814.17	19.10	
TOTAL	106	1963.89		

TABLE 2. The Responses of Nulliparous and Post-lactational Females to various Doses of PMS, given at Dioestrus, and followed by excess CGT.

Dose of PMS (i.u.)	Dose of CGT (i.u.)	Nulliparous Females				Post-lactational Females			
		No. treated	No. ovulated	Mean	S.E.	No. treated	No. ovulated	Mean	S.E.
0	2	12	9	11.33	1.64	10	9	18.6	1.69
1	2	15	15	8.73	0.67	11	11	11.73	1.49
2	2	13	12	9.00	0.85	10	10	13.10	1.39
4	3	14	14	10.79	1.36	10	8	16.75	2.08
8	4	13	13	18.69	2.26	11	11	22.64	2.71
16	6	15	15	20.87	2.58	10	10	35.70	3.41
Natural Oestrus		15	15	11.60	0.65	9	9	17.40	1.15
		Mean body weight = 20.73 g				Mean body weight = 32.56 g			

The effect of body weight was examined in both nulliparous and post-lactational females, and the regression of egg number on body weight was found to be 0.4 ± 0.1 eggs/gram and 0.2 ± 0.2 eggs per gram respectively. This suggests that there is a positive correlation between body weight and the response to a standard dose of PMS, and that this correlation is higher in nulliparous than post-lactational females. Now, the presence of a positive correlation indicates that the dose should be graded in inverse proportion to the weight, but in view of the insignificance of the relationship in post-lactational females it was decided to give a fixed dose regardless of weight. Furthermore, the insignificance of the difference between the two regression coefficients, together with the similarity of the shape of the nulliparous and post-lactational dose-response curves, indicates that it may be possible to extrapolate from one type of female to the other.

For the purposes of the selection experiment, the question of the effect of the stage of the cycle is now much more specific - does the stage of the cycle affect the response of post-lactational females to 4 i.u. PMS? In order to answer this problem, and to see if the number of days after cessation of lactation affects the response, the response of post-lactational females to 4 i.u. PMS at oestrus, metoestrus, and dioestrus, 1, 3 and 5 days after lactation was examined, and the results given in Table 3 show neither to be important.

The assessment of ovarian sensitivity was therefore based on the response to 4 i.u. PMS followed by 3 i.u. CGT.

Although the effects of the stage of the cycle and the number of days after weaning have not been shown to be large enough to warrant their determination, it was felt that it would be worthwhile looking for any natural

TABLE 3 The Response of Post-lactational Females to 4 i.u. PMS at Different Stages of the Cycle, and at Different Times after Weaning.

No. of days after weaning	Stage of Cycle								
	Oes			Met			Di		
	No. treated	No. ovulated	Mean response	No. treated	No. ovulated	Mean response	No. treated	No. ovulated	Mean response
1	13	13	14.54	12	12	14.25	14	13	14.54
3	14	14	15.93	10	10	13.70	12	12	11.50
5	5	5	15.80	11	9	17.00	15	13	14.75
Mean			15.42			14.98			13.60

Analysis of Means:

Source of variation	d.f.	M.S.	F	
Days	2	3.55	2.04	NS
Stage of cycle	2	2.73	1.57	NS
Interaction	4	1.74		
TOTAL	8	19.50		

synchronisation of oestrus following the cessation of lactation. In order to see if such an effect existed, the interval between the birth of first and second litters in the first 9 generations of the Q stock was examined, and the data given below:

Days between birth of 1st and 2nd litters.	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	>50
No. of females.	110	10	8	3	1	3	4	5	9	14	8	1	0	3	1	1	7

In interpretating these data, the length of suckling the first litter is taken to be 21 days and the gestation period of the second to be 19 days. Those females having a parity interval of more than 40 days therefore are those which mated after their first litter was removed from them, and the number of days above 40 corresponds to the number of days between weaning and oestrus.

The observation of a peak of births 44 days after the birth of the first litter suggests that oestrus is partially synchronised around the fourth day after weaning; that is, there is a relationship between the number of days post-weaning, and the stage of the cycle. It was decided to attempt to use this relationship to reduce environmental variation by treating the females on a fixed day after weaning. The choice of the day after weaning on which to start treatment is influenced by both the fact that for management purposes it is advantageous to keep the interval between weaning and scoring as small as possible, and that weaning did not always take place early enough for treatment to be started on the day of weaning. Consequently it was decided to start treatment on the day after weaning.

The measure of ovarian sensitivity finally adopted therefore was the response to 4 i.u. PMS given at 17.00 hrs. on the day after weaning, and followed by 3 i.u. CGT on the third day after weaning.

It was felt that the experiments described above provided sufficient information to justify the adoption of the technique described and the initiation of the selection programme. However, it was realised that the assumption that the induced response is an estimate of ovarian sensitivity to endogenous hormones needed further investigation. Although the results of such an investigation have a direct bearing on the interpretation of the work presented here, it was decided to preserve the continuity of the main theme of work, and present them in the form of an appendix.

(c) Estimation of the genetic parameters of natural ovulation rate.

If a trait is thought to have a low heritability, and to be subject to maternal effects, the most efficient way to partition its components of variance is to use a half-sib analysis. Consequently the heritability of natural ovulation rate, together with other genetic and environmental parameters was estimated by such a technique. (There were not enough mice to allow both natural and induced ovulation to be studied on an adequate scale, and since natural ovulation seemed to be of more general interest, this alone was studied).

Offspring from 71 sires, each mated to 3 dams, were obtained from Dr. L.S. Monteiro, who was using the half sib analysis to study growth (Monteiro, 1964). These females had either been weighed each week, 0-8 weeks of age, or had been discarded after the third week. The 'three week discards' were weighed at 6 weeks of age, and all were weighed at the time of scoring - between 8 and 10 weeks of age. The calculation of the heritability and the genetic and environmental correlations between ovulation rate and the various body weights was facilitated by the use of a computer programme provided by Dr. B. Woolf.

(d) Selection programme

In order to select for ovulation rate it is necessary to obtain offspring from females before scoring them, or to select individuals on the basis of the performance of their relatives. Of the latter systems, sib selection and progeny testing were both considered unsuitable; the former because of the long time that would be required to obtain large enough families, and because ovulation rate is probably subject to a maternal effect, and the latter because of the increased generation interval. A further drawback to the use of these systems in small laboratory populations is the high rate of inbreeding associated with them. It was decided therefore to score females on the basis of their own performance, mating them, and weaning the resultant litter before scoring. Furthermore, in order to avoid problems associated with maternal effects, and to increase the effective population size, it was decided to select within families.

Selection for increased natural ovulation rate was made in one line, referred to here as HN (high natural), and for decreased natural ovulation rate in another, referred to as LN (low natural). Two further lines were established, one selected for high induced ovulation rate, HI, and the other for low induced ovulation rate, LI. These, together with a control line, C, were all started from the same base population, which was one generation of random mating removed from the 11th generation of the Q strain. Each line consisted of eight full-sib families per generation.

In order to increase the selection differential, the base population was made as large as possible, and selection in this generation was carried out on an individual basis (with the proviso that females were not selected if their sisters had already been selected). In one half of the population, ovulation was allowed to occur naturally, females being

paired at the time of weaning; and in the other half, ovulation was induced in the manner previously described.

The control line was formed from the offspring of eight females taken at random from the whole population. The first generations of the selected lines, HN and HI, were then formed from the offspring of the eight highest females remaining in each of the two halves of the population. Similarly, lines LN and LI were formed from the offspring of the eight lowest females in each half of the population. Within each line, females of one sibship were all mated to males of one other sibship. Selection was then carried out within sibships, and the subsequent matings followed a cyclical pattern, designed to minimise inbreeding.

The selection differential on females was calculated as the mean deviation of selected females from their family means. The net selection differential was half of this, as males were unselected.

There were some situations in which the procedure described above was not adhered to. These were as follows. If all matings in a sibship were sterile, a female was chosen from another sibship in such a way that the mating of her progeny to those of her sisters was delayed for as long as possible. If two females of a sibship each had the same score, the one with the largest number of female offspring was chosen as this would enable greater selection pressure to be applied in the next generation. If the female to be selected had only one or two female offspring, the difference in selection differential between choosing her and the next best female was compared with the estimated increase in selection differential in the next generation. This comparison was made by multiplying the selection intensity expected in the next generation, obtained from Table 11.1 of Falconer (1960), by the within-family standard deviation in the present generation, and summing the

selection differentials over the two generations for each female. The female with the larger total selection differential was then chosen. This latter exception was further modified after generation 3, when, if the chosen female had only one or two daughters, or no sons, the possibility of taking offspring of one sex from one female, and the other from the second-best female was considered.

(e) Correlated characters.

As was outlined in the Introduction, part of the interest in this work was to observe the effects of selection for ovulation rate on various other characters, and consequently during the course of the experiment several characters were scored.

The three-week weight of all individuals was recorded at weaning. This was necessary as it was not known at this time which were to be selected. The six-week weight of selected individuals was also taken, as an estimate of mature weight. The females weight was recorded at the time of scoring, as another estimate of adult weight, and the number of young born and weaned was scored as a character of the female.

For all characters, the mean of family means was taken as the mean for a particular generation.

(f) Analysis of the lines.

After 5 generations of selection, it was decided to consider the possibility of using the males as an inbuilt bioassay of the lines. The use of males to bioassay gonadotrophins is outlined by Loraine (1958), and while it is realised that the use of the animals to bioassay themselves is not the ideal procedure, it was felt that they could contribute to the analysis of the lines. The males from the matings of generations 5 and 6

were removed 17 days after pairing, weighed, and their testes and seminal vesicles removed and weighed. Care was taken to remove the two vesicles as a unit and to dissect off the coagulating gland without puncturing the vesicles; after weighing, the seminal fluid was removed by gently squeezing the vesicle between filter papers, and then it was reweighed. It was hoped that the testis weight would be related to the amount of FSH produced by the pituitary, and that the seminal vesicle weight would be related to the rate of production of LH. Two seminal vesicle weights were taken for comparison - the intact and the squeezed weights. The intact weight is subject to variation from recent ejaculations, and from damage during the rather difficult process of removal. The squeezed weight is subject to variation from differences in the pressure used for squeezing. It was thought that these experimental errors would be minimized by consideration of both weights. No difficulty was encountered in the removal of the testes, and consequently the testis weights were subject to very little experimental error.

After the completion of 6 generations of selection, which was as many as were possible, the offspring of discarded females were used to examine the sensitivity of the ovaries of the different lines to exogenous gonadotrophins, and to compare this sensitivity with the natural ovulation rate. Although the females used for this purpose could not be taken at random from the lines, owing to the desirability of continuing the selection procedure, they were selected genetically, not phenotypically, i.e. they were discarded because of their mothers' performance, rather than their own. None the less the differences, if any, between the lines will be the result of an accumulated selection differential part way between the ultimate and penultimate generations.

It was hoped that data from this source, together with information from the males, would provide sufficient evidence to enable changes in the

sensitivity of the ovary to be distinguished from changes in the level of activity of the intrinsic follicle stimulating system; and that the contribution of changes in FSH and LH levels to the latter could be assessed - at least on a presence-or-absence basis.

RESULTS

(a) The parameters of Natural Ovulation Rate

The results of the half-sib analysis were analysed, and the heritability of natural ovulation rate, together with its various correlations with body weight at six weeks of age, and at the time of scoring, are given in Table 4. There were 274 degrees of freedom, 70 between sires, and 119 between dams.

A comparison of the sire and dam heritabilities shows that there is a maternal effect for ovulation rate, and that this is too large for the joint heritability to give a true estimate of the proportion of additive genetic variance. Similarly, the large maternal effect means that the dam, and sire and dam, genetic correlations between ovulation rate and these two body weights do not give true estimates of the genetic correlation between ovulation rate and body weight. Consequently these parameters have not been included in the table. The presence of a positive environmental correlation between these two body weights and ovulation rate indicates that the maternal effect on ovulation rate may be mediated via the maternal effect on body weight.

From the table it is evident that the heritability of natural ovulation rate is about 25%. and that there is a positive genetic correlation between ovulation rate and body weight. A subsidiary analysis, which only included those females for which all the 0-8 week weights were known, showed that the genetic correlation between ovulation rate and body weight increases with age, and that this increase is probably a reflection of the low heritabilities of the early body weights.

TABLE 4 The Parameters of Natural Ovulation Rate. The Sire and Dam Heritabilities are the Estimates of the Heritability of Ovulation Rate derived from the Sire and Dam Components of Variance respectively.

	Sire Heritability	S.E.	Dam Heritability
Ovulation rate	0.23	0.19	0.96

The correlation between ovulation rate and:-	$r_{(phen.)}$	$r_{(genet.)}$	$r_{(env.)}$
6 wk. Wt.	0.47	0.45	0.32
Scoring Wt.	0.46	0.33	0.34

Now, as selection from the first and subsequent generations of the selection programme was carried out within families, it is interesting to compare the within family and overall heritabilities. The above analysis gave an estimate of 0.305 for the component of variance of ovulation rate between sires, and 3.901 for the within full-sib phenotypic variance, thus giving an estimate of 16% for the within family heritability. The ratio between the within family and individual heritabilities is therefore 0.69. This means that 0.69 units of selection differential between individuals would be expected to produce the same response as 1 unit of selection differential within families.

(b) The effects of selection on:

(1) Ovulation rate

In view of the error associated with individual generation means, it was decided to represent the response to selection for both natural and induced ovulation rate in the form of regressions. Now, the response can either be regressed on generations, or on the cumulative selection differential, and whereas the former is sometimes clearer, the fact that the regression of response on selection differential is an estimate of the heritability makes the latter more meaningful. Consequently, the generation means are plotted against generations in Figs. 2 and 3, and, as there is no marked asymmetry in the responses, the deviations between the two pairs of lines are plotted against the cumulated selection differential in Figs. 4 and 5. In preparing these data, allowance was made for the fact that, whereas mass selection was conducted in the base generation, selection in subsequent generations was made within families. This meant that either the selection differential in the base population had to be converted to

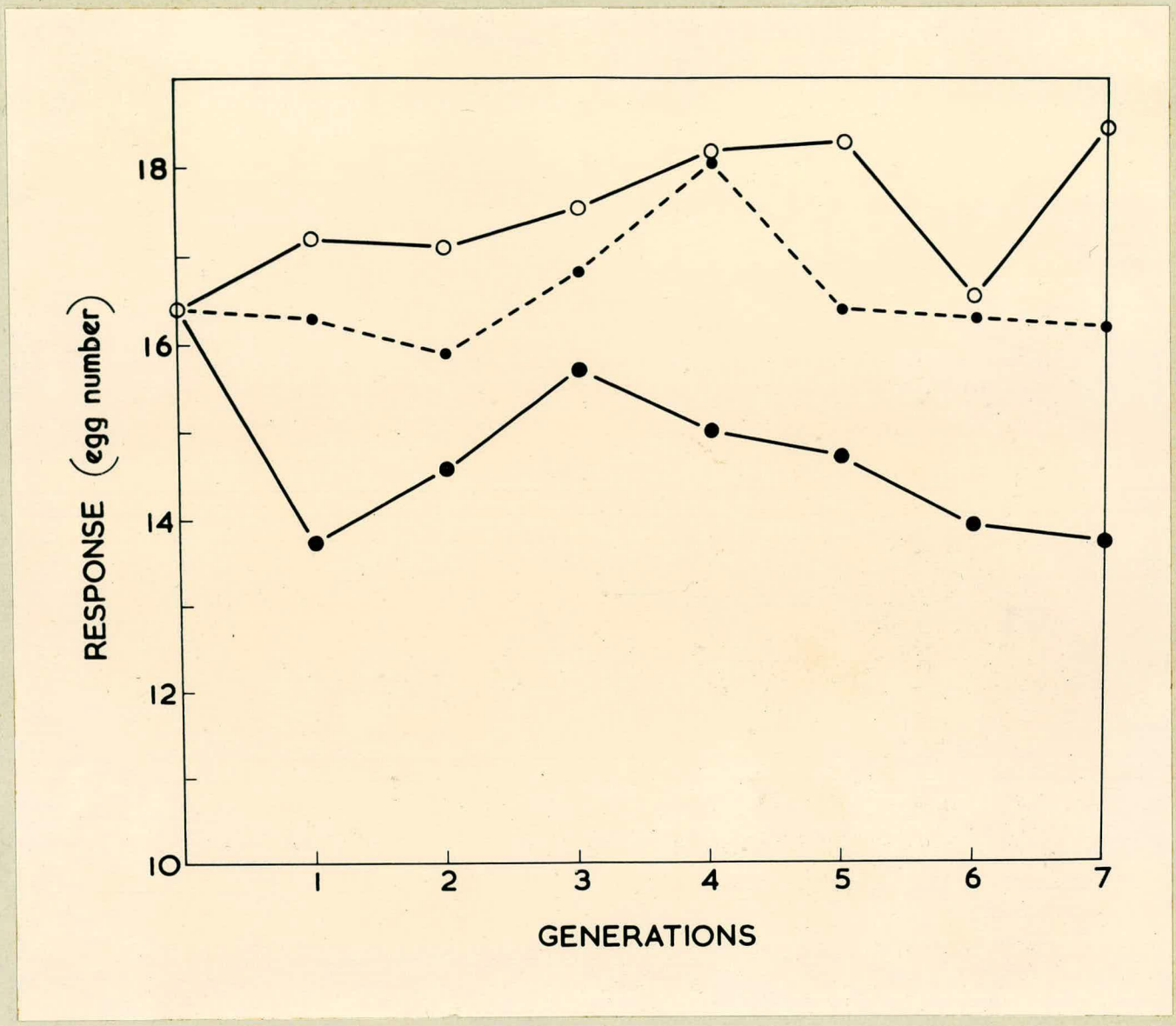

FIG. 2 The ovulation rates of the lines selected for high (open circles) and low (solid circles) natural ovulation rate, together with the natural ovulation rate of the unselected control line (broken line).

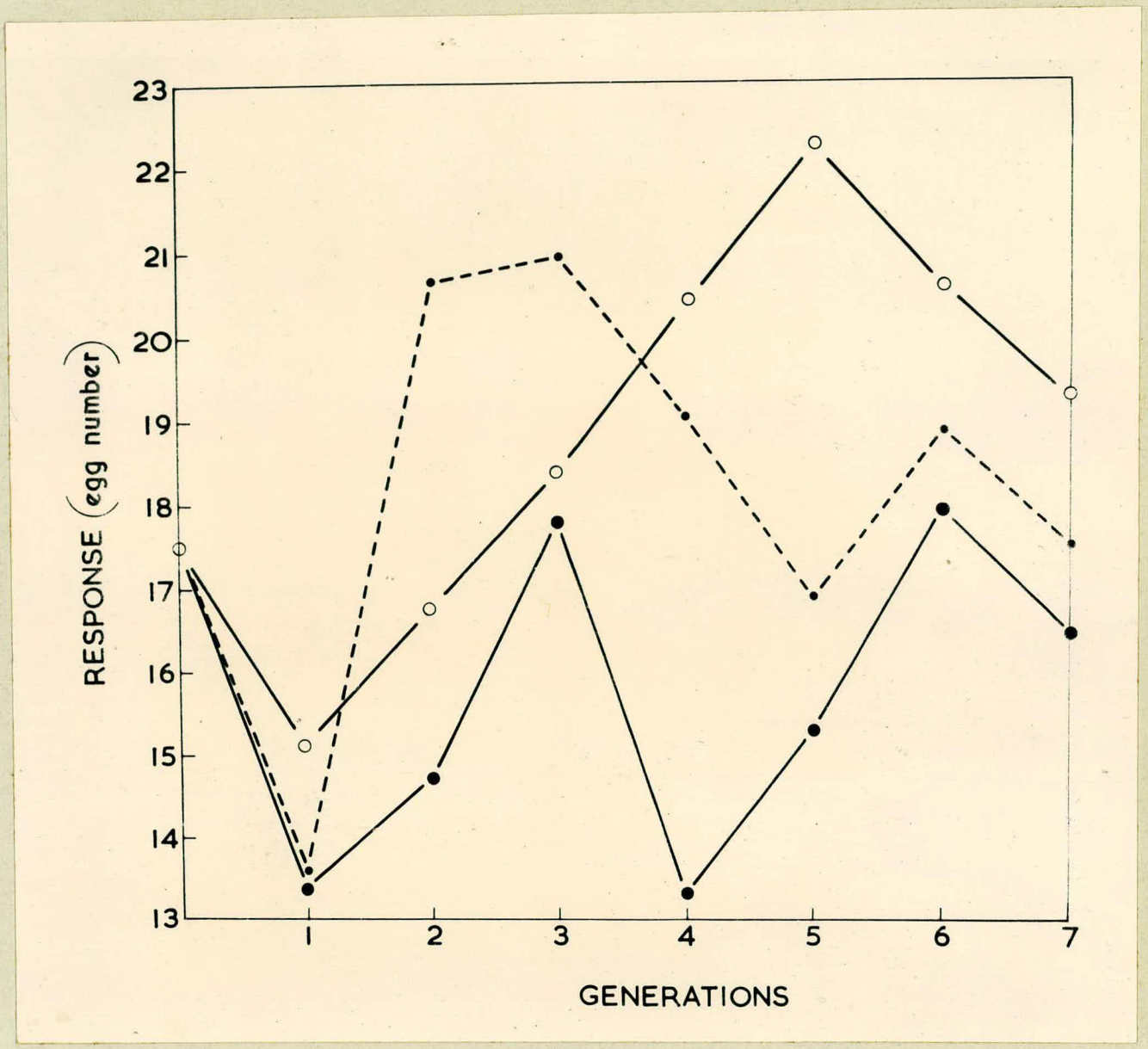

FIG. 3 The ovulation rates of the lines selected for high (open circles) and low (solid circles) induced ovulation rate, together with the induced ovulation rate of the unselected control line (broken line).

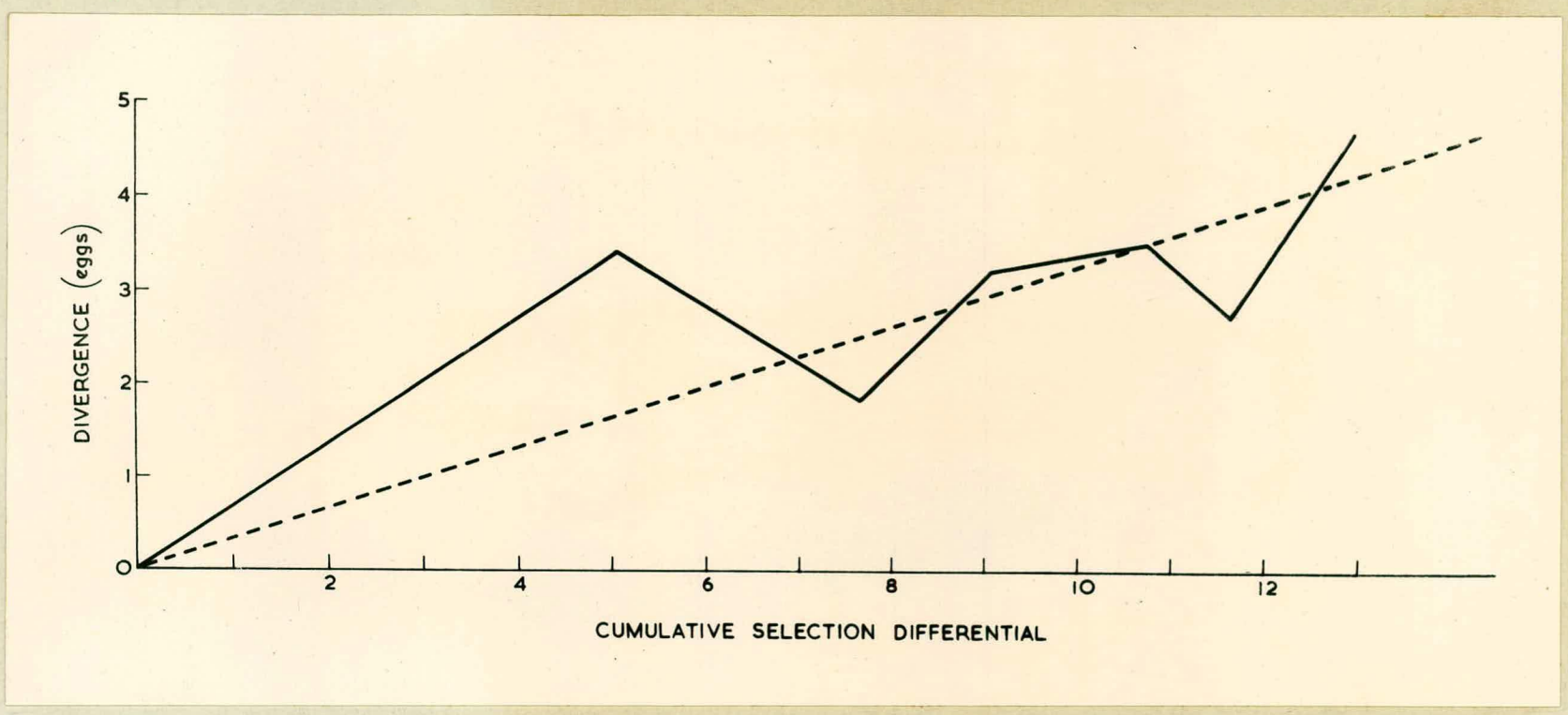

FIG.4 The divergence (solid line) between the lines selected for high and low natural ovulation rate, together with the fitted regression of the divergence on the cumulative selection differential (broken line).

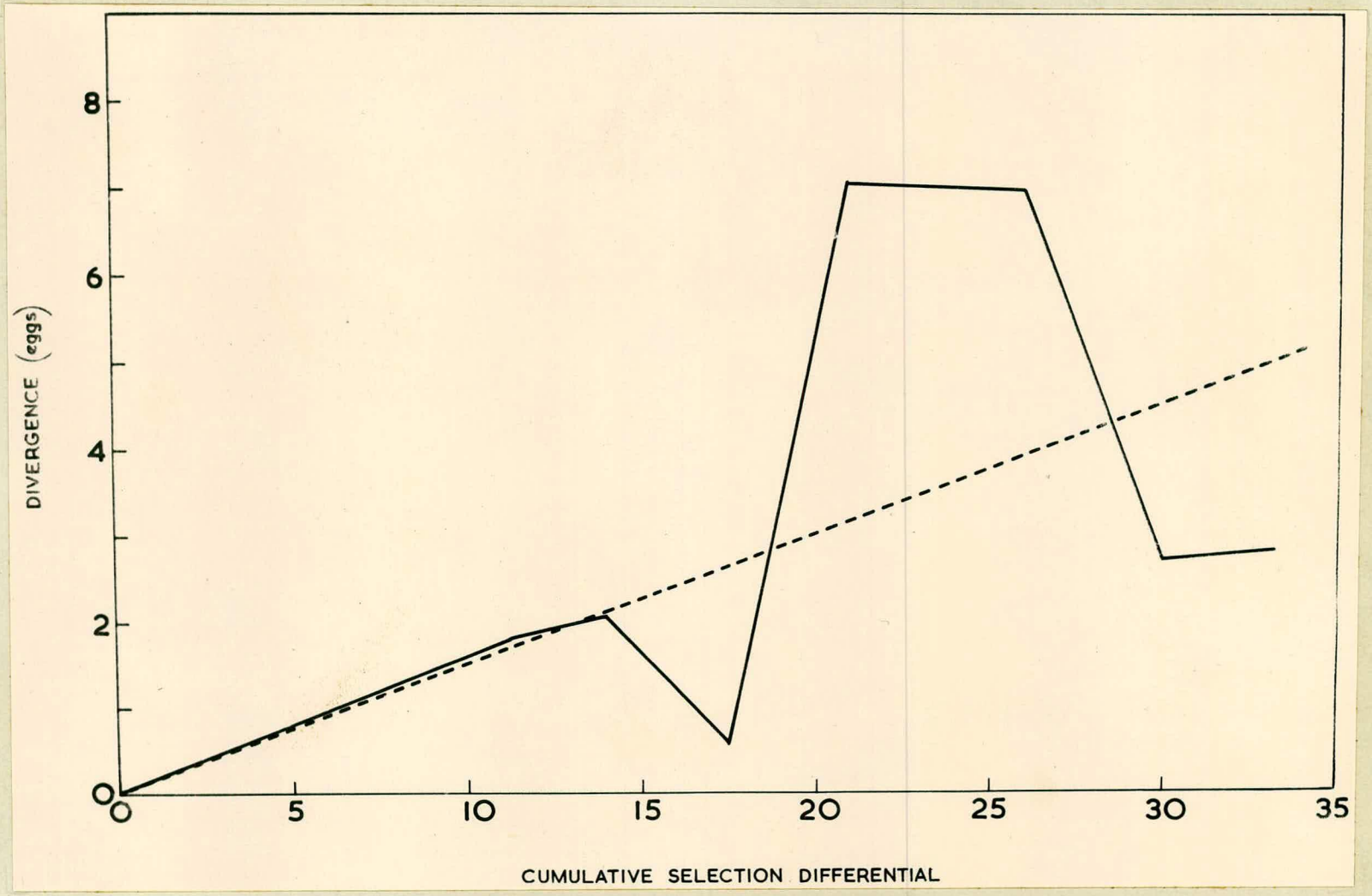

FIG. 5 The divergence (solid line) between the lines selected for high and low induced ovulation rate, together with the fitted regression of the divergence on the cumulative selection differential (broken line).

that which would be expected to produce the same response had it been within families or vice versa. In view of the more general interest of the overall heritability relative to the within family heritability, the latter was adopted and the within family selection differentials converted in the following way.

The response to selection differential (Sw) within families equals $S_w h_w^2$, when h_w^2 is the within family heritability.

Similarly the response to selection differential (S) within individuals equals Sh^2 when h^2 is the individual heritability.

Therefore, for the responses to be the same, $S_w h_w^2 = Sh^2$.

Therefore, 1 unit of selection differential within families would be expected to produce the same response as h_w^2/h^2 units of selection differential within individuals.

Now, h_w^2 is related to h^2 as follows:

$h_w^2 = \frac{1 - r}{1 - t} \cdot h^2$ (Falconer 1960c), when r is the coefficient of relationship and t is the intra-class correlation. As we are dealing with full-sibs, $r = \frac{1}{2}$ and $t = \frac{\sigma_B^2}{\sigma_B^2 + \sigma_W^2}$

$$h_w^2 = \frac{\frac{1}{2}}{1 - \frac{\sigma_B^2}{\sigma_B^2 + \sigma_W^2}} \cdot \frac{\sigma_A^2}{\sigma_B^2 + \sigma_W^2} = \frac{\sigma_A^2}{2\sigma_W^2}$$

$$\frac{h_w^2}{h^2} = \frac{\sigma_A^2}{2\sigma_W^2} \cdot \frac{\sigma_B^2 + \sigma_W^2}{\sigma_A^2} = \frac{\sigma_B^2 + \sigma_W^2}{2\sigma_W^2}$$

This correction factor was therefore calculated for each line each generation, and is given in tables 5 and 6, together with the other data used in the preparation of Figs. 2-5. The mean of these correction terms for the natural lines is 0.61. The ratio between the within family and individual heritabilities is therefore 0.61, which agrees well with the estimate of 0.69 from the half-sib analysis.

The regression coefficients of the deviations between the two pairs of lines on their respective cumulative selection differentials were calculated on the assumption that the regression lines pass through the origin, that is, that selection from the base generation was not biassed by sampling. The heritabilities of natural and induced ovulation rate, calculated by the above procedure, were found to be 33.4 $\pm$ 3.7% and 15.2 $\pm$ 4.1% respectively.

(2) Other characters.

The body weights at three weeks of age, six weeks of age, and at the time of scoring are given for the females of the five lines in Fig.6. The litter size, number weaned, and the percentage of infertile matings are given in Fig.7.

The increase and decrease in body weight in the lines selected for high and low ovulation rate respectively is indicative of the presence of a positive genetic correlation between ovulation rate and body weight. By contrast, it is evident from Fig. 7 that there has not been a change in the litter size of any of the lines. The apparent increase in the number of infertile matings in the LI line is of interest, and if it proves to be real may provide information regarding the nature of the physiological changes which have taken place in this line. Unfortunately however it was not possible to study this phenomenon further.

TABLE 5. The Response to Selection for Natural Ovulation Rate.

	High Line					Low Line						
Generation	Mean	s.e.	S	$\frac{\sigma_B^2+\sigma_W^2}{2\sigma_W^2}$	Corr.S	Mean	s.e.	S	$\frac{\sigma_B^2+\sigma_W^2}{2\sigma_W^2}$	Corr.S.	Cumulated combined S	Deviation in next gen.
0	16.39	0.36	2.50	-	2.50	16.39	0.36	2.57	-	2.57	5.07	3.46
1	17.17	0.51	0.86	0.56	0.48	13.71	0.84	1.79	0.58	1.05	6.60	2.49
2	17.07	0.57	0.77	0.70	0.54	14.58	0.43	0.79	0.64	0.50	7.64	1.86
3	17.55	0.54	1.16	0.61	0.71	15.69	0.43	1.03	0.67	0.69	9.04	3.24
4	18.22	0.66	1.39	0.50	0.70	14.98	0.68	1.31	0.72	0.95	10.69	3.57
5	18.29	0.44	0.86	0.50	0.43	14.72	0.75	0.79	0.59	0.47	11.59	2.74
6	16.65	0.64	1.21	0.65	0.79	13.91	0.64	0.92	0.56	0.52	12.90	4.74
7	18.44	0.89				13.70						

TABLE 6. The Response to Selection for Induced Ovulation Rate.

	High Line					Low Line						
Generation	Mean	s.e.	S	$\frac{\sigma_B^2-\sigma_W^2}{2\sigma_W^2}$	Corr.S	Mean	s.e.	S	$\frac{\sigma_B^2-\sigma_W^2}{2\sigma_W^2}$	Corr.S	Cumulated combined S	Deviation in next gen.
0	17.52	0.67	6.43	-	6.43	17.52	0.67	4.76	-	4.76	11.19	1.82
1	15.17	1.20	3.04	0.54	1.64	13.35	1.18	1.45	0.81	1.17	14.00	2.04
2	16.73	1.23	4.12	0.50	1.06	14.69	1.32	2.66	0.58	1.54	17.60	0.60
3	18.38	1.37	3.32	0.53	1.76	17.78	1.39	2.83	0.53	1.50	20.86	7.08
4	20.38	2.80	5.37	0.65	3.49	13.30	1.61	2.65	0.59	1.56	25.91	6.98
5	22.26	1.57	4.80	0.50	2.40	15.28	0.91	2.08	0.52	1.08	29.39	2.70
6	20.64	1.91	3.52	0.50	1.76	17.94	1.42	3.78	0.50	1.89	33.04	2.85
7	19.29	1.46				16.44	2.20					

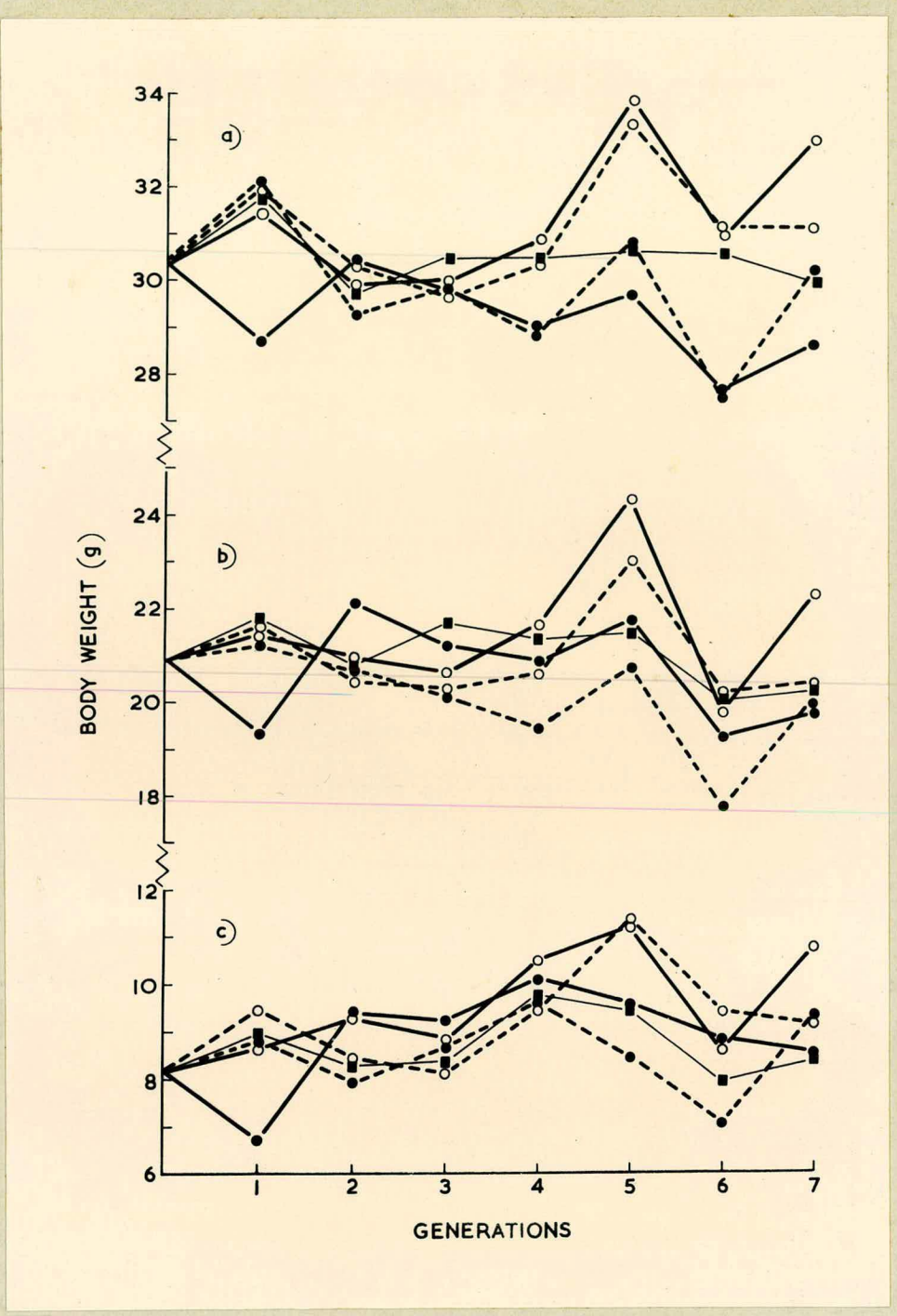

FIG.6 The body weights of the females of the lines selected for high (open circles) and low (solid circles) natural (solid line) and induced (broken line) ovulation rate, together with those of the unselected control line (squares), at (a) the time of scoring, (b) 6 weeks of age, and (c) 3 weeks of age.

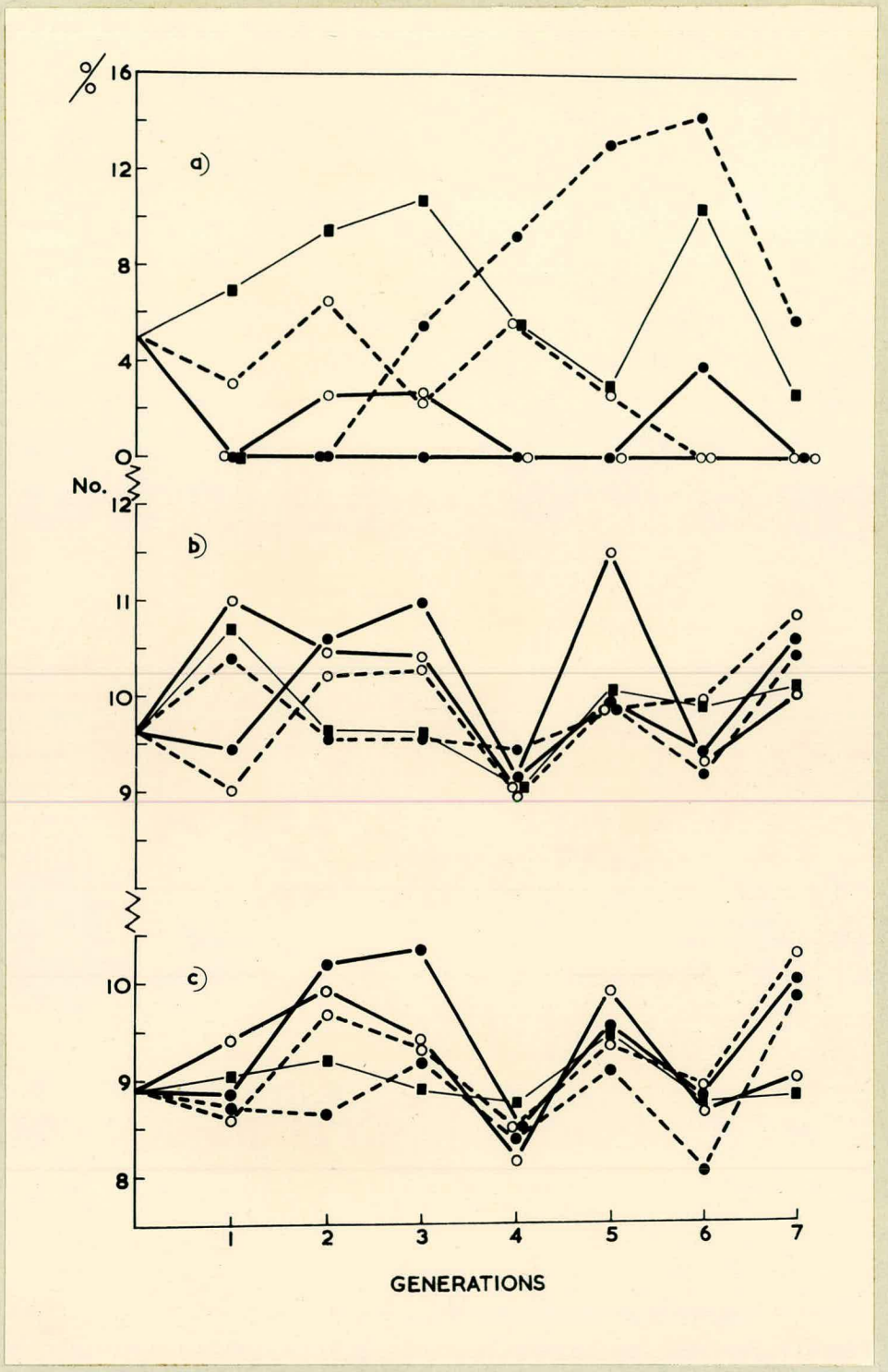

FIG. 7 The reproductive performance of the lines selected for high (open circles) and low (solid circles) natural (solid line) and induced (broken line) ovulation rate, together with that of the unselected control line (squares). (a) The percentage of infertile matings, (b) The litter size at birth, and (c) The litter size at weaning.

(c) Analysis of the Lines

(1) Testis and seminal vesicle weights

The mean weights of the testes and seminal vesicles of the males which formed the matings of generations 5 and 6 are given in Table 7, together with their weights at 3 and 6 weeks of age, and at the time of scoring (that is, at the time of dissection). Now, some of the differences between the mean weights of these organs in the five lines could be the result of differences between the body weights of the lines. Consequently, it was decided to correct the organ weights for differences in body weight, and the corrected weights are also given in Table 7. The organ weights were corrected by substituting the relevant weights and regression coefficients into the following equation:

$$\bar{O}_c = \bar{O}_{uc} + b(\bar{SW} - \bar{\bar{SW}})$$

when $\bar{O}_c$ is the corrected organ weight, $\bar{O}_{uc}$ is the uncorrected organ weight, b is the regression coefficient of the particular organ weight on scoring weight, $\bar{SW}$ is the mean scoring weight of the particular line, and $\bar{\bar{SW}}$ is the mean of the mean scoring weights of the five lines. The regression coefficients were calculated from data obtained from 96 ♀ males. They were found to be 0.0013, 0.0089 and 0.0027 gm./gm. for testis weight, seminal vesicle weight and squeezed seminal vesicle weight respectively. The body weights, together with the corrected and uncorrected organ weights were analysed, and the means compared by Tukey's method (Snedecor, 1962). Differences which are significant at the 5% level are given in Table 8.

In interpreting this table, differences in corrected testis weight are taken as being primarily indicative of a difference in the FSH

TABLE 7 The Body and Organ Weights of the Males from Generations 5 and 6.

	3 wks.Wt.	6 wks.Wt.	Scoring Wt.	Seminal Vesicles	Corr. Seminal Vesicles	Squeezed Seminal Vesicles	Corr. Squeezed Seminal Vesicles	Testes	Corr. Testes
HN	9.20	26.46	31.42	0.277	0.216	0.096	0.091	0.247	0.243
LN	9.59	25.32	29.49	0.190	0.186	0.084	0.083	0.214	0.213
C	8.43	24.35	28.80	0.197	0.197	0.078	0.078	0.203	0.204
HI	9.88	25.77	30.29	0.235	0.224	0.087	0.083	0.222	0.221
LI	7.65	22.26	25.87	0.171	0.199	0.068	0.076	0.188	0.192

TABLE 8 Significant Differences between the Body and Organ Weights of the Males from Generations 5 and 6.

	3 wks.Wt.	6 wks.Wt.	Scoring wt.	Testes	Corr. Testes	Seminal Vesicles	Corr. Seminal Vesicles	Squeezed Seminal Vesicles	Corr. Squeezed Seminal Vesicles
HN - C	=	=	>	>	>	>	=	>	>
LN - C	=	=	=	=	=	=	=	=	=
HI - C	=	=	=	>	=	>	>	=	=
LI - C	=	=	<	=	=	=	=	=	=
HN - LN	=	=	=	>	>	>	>	>	>
HI - LI	>	>	>	>	>	>	>	>	=
HN - HI	=	=	=	>	=	=	=	=	=
LN - LI	>	>	>	>	=	=	=	=	=
HN - LI	>	>	>	>	>	=	=	>	>
LN - HI	=	=	=	=	=	<	<	=	=

component of pituitary gonadotrophin activity, while differences in the corrected seminal vesicle weight are taken as being primarily indicative of a difference in the LH component. Differences in the squeezed, but not the unsqueezed seminal vesicles, and vice versa, are taken as an indication of a probable difference in the LH component.

It is evident, therefore, that the two natural lines and the two induced lines differ from each other in their levels of FSH activity, and probably in their levels of LH activity. Also, as neither of the low lines differ from the control, these differences would appear to be due to an increased level of activity in the high lines, rather than a decrease in the low lines.

(2) The response of non-selected females to PMS

The female offspring of females not selected in generation 6 were used to compare the ovarian sensitivities of the 5 lines.

The work presented in the Appendix indicates that the response to several small doses of PMS would give a better indication of the ovarian sensitivity than the response to a single large dose. However, in view of the fact that previous workers have used the response to a single large dose, and that single doses were used in the dose response-curves presented earlier, it was decided to use single doses for the inter-line comparisons. The doses used were 2, 4, 8 and 16 i.u. PMS, given at dioestrus, and followed by 2, 3, 4 and 6 i.u. CGT respectively. The responses, together with the natural responses and the mean body weights are given in Table 9 and Fig. 8. The differences in body weight, although confirming the trend shown in the data already presented, were not considered large enough to warrant their consideration when interpreting the responses.

TABLE 9 The Response of Nulliparous Females of the Selected Lines to Different Doses of PMS.

Line	Body wt. (g)	Treatment N: No. treated	N: No. ovulated	N: Mean	N: S.E.	2: No. treated	2: No. ovulated	2: Mean	2: S.E.	4: No. treated	4: No. ovulated	4: Mean	4: S.E.	8: No. treated	8: No. ovulated	8: Mean	8: S.E.	16: No. treated	16: No. ovulated	16: Mean	16: SE.
HN	22.81	23	21	12.67	0.66	24	23	12.35	0.69	26	24	15.96	1.41	27	26	16.77	1.23	26	24	16.83	2.67
LN	21.68	27	25	12.20	0.40	26	26	13.61	0.81	27	27	15.81	1.02	26	25	28.44	2.26	28	25	22.76	1.92
C	22.08	25	24	13.25	0.41	24	24	12.46	0.77	25	24	16.25	1.29	23	21	26.71	2.37	21	21	25.19	2.18
HI	21.46	16	16	13.00	0.57	15	15	15.07	1.61	15	15	22.73	3.06	14	14	26.71	2.96	13	12	31.42	3.82
LI	20.51	18	18	12.94	0.40	17	17	12.35	1.15	17	17	12.82	1.41	17	17	20.71	1.92	19	17	12.82	2.06
$F^{df_b}_{df_w}$ =		4 / 99	0.75 (N.S.)			4 / 100	1.23 (N.S.)			4 / 103	2.20 (N.S.)			4 / 99	6.72 sig.at 1%			4 / 94	6.89 sig.at 1%		

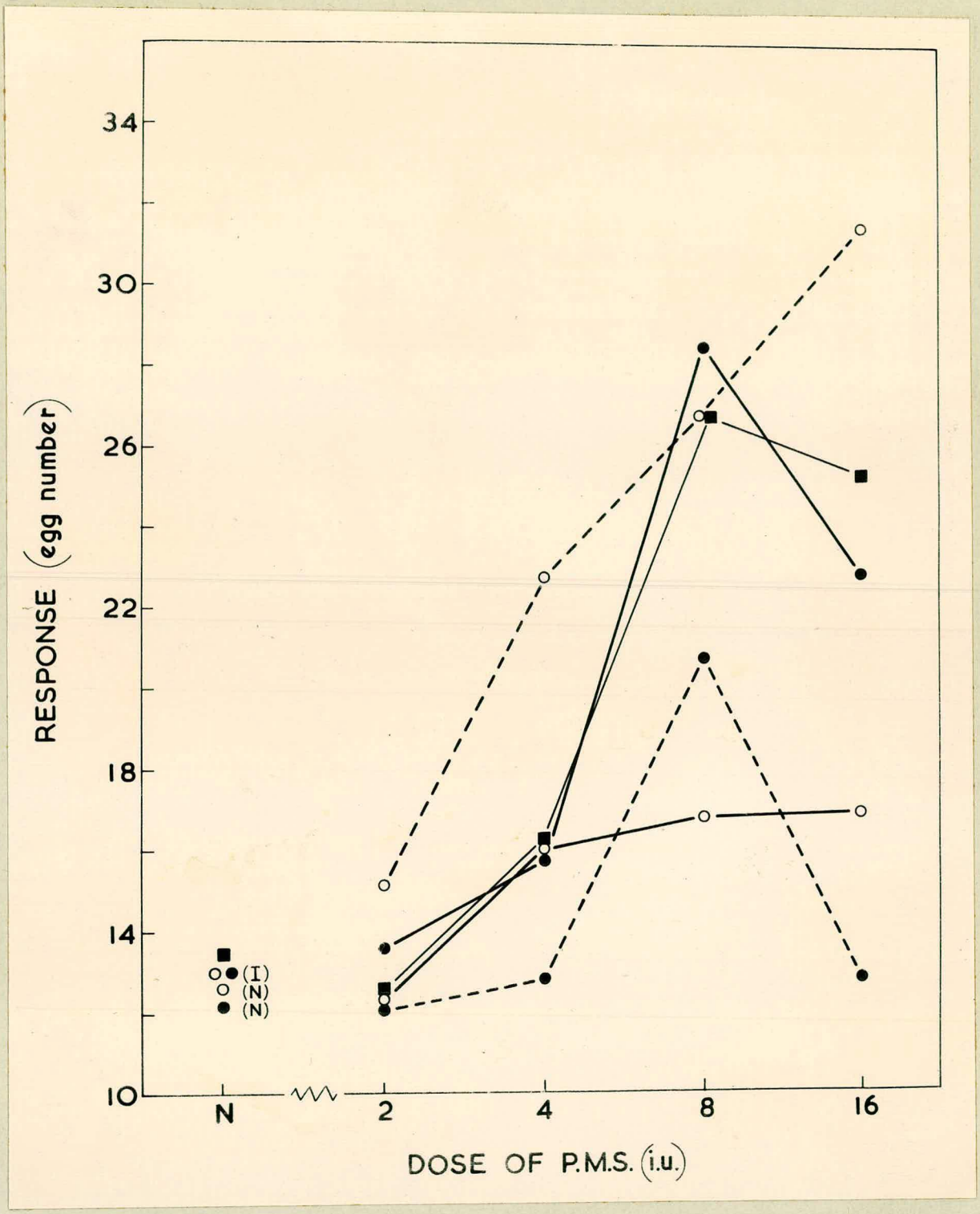

FIG. 8 The responses to different doses of gonadotrophins of nulliparous females of the lines selected for high (open circles) and low (solid circles) natural (solid line) and induced (broken line) ovulation rate, together with the unselected control line (squares). The natural ovulation rates are also given (N).

Before examining these curves, let us consider possible ways in which dose response curves may differ. They may differ in shape; be the same shape and give a higher response to all doses - i.e. be displaced vertically; or, the shape and maximum response may be the same, but the dose needed to induce this response may differ - i.e. they may be displaced laterally.

In view of the apparent differences in shape, it was decided not to fit a linear regression through the points, but to compare the response to each dose separately. The ratio of the between- and within-line mean squares, together with the degrees of freedom, is given for each dose in Table 9. The lack of significant differences between the responses to low doses suggests that the sensitivity of the various lines to endogenous gonadotrophins is the same. However, in spite of this, it was felt that these responses merited a further examination.

In order to detect lateral displacement, a reference point is needed, and if the overall natural mean is taken as this point, and the curves adjusted so that they all pass through this point, the lines can then be easily ranked in order of the dose needed to induce the natural response. This has been done in Fig.9 and it is evident that the ranking is: LI > HN = C > LN > HI.

The similarity of the natural and low dose responses suggests that there is no vertical displacement. Also, although it is difficult to draw any firm conclusions regarding the shapes of the curves, it would appear that they do differ, and that the differences are largely due to the decreased response of the LI and HN lines to high doses.

An alternative way of comparing the curves is to examine them in

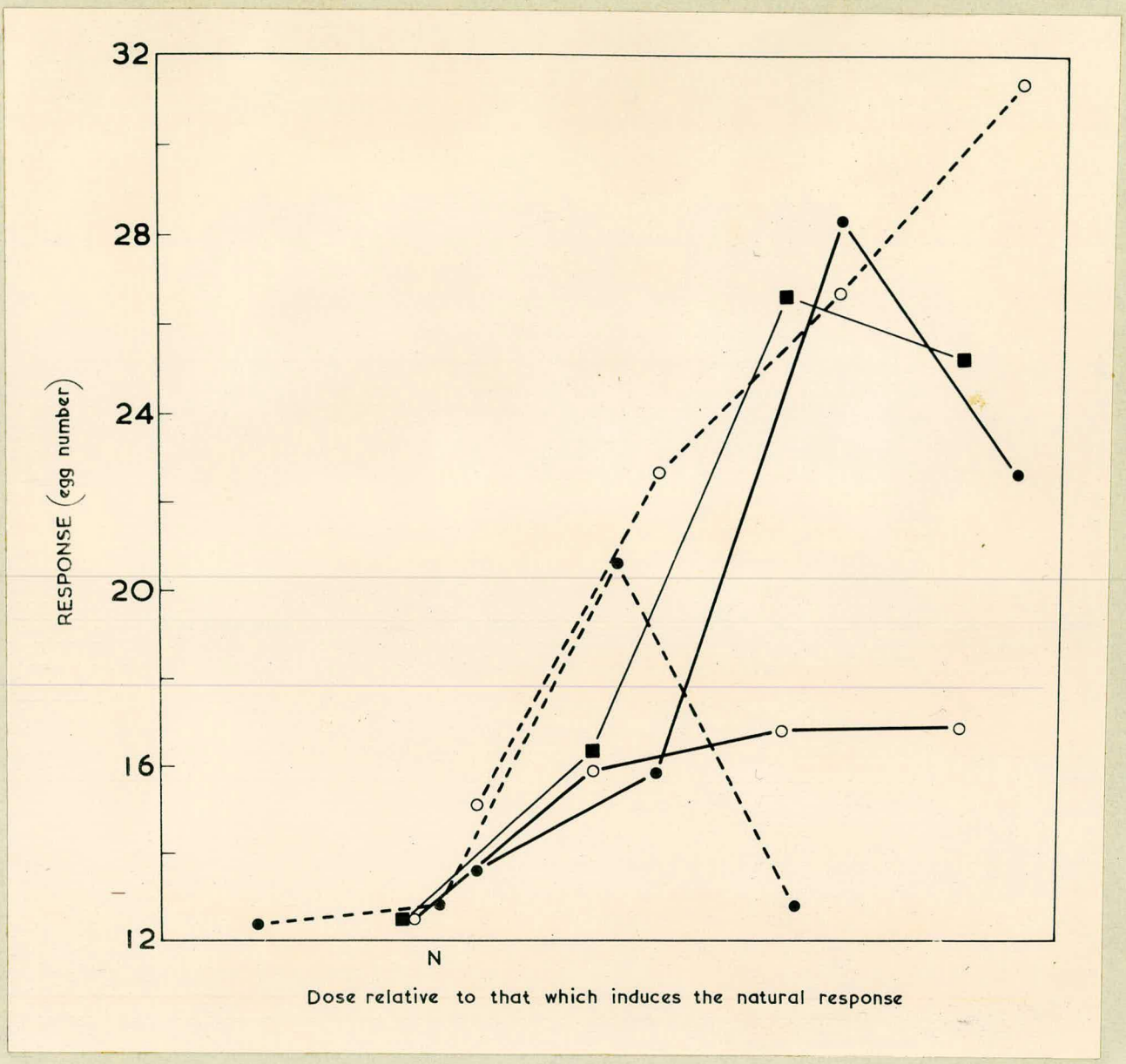

FIG. 9 The dose-response curves shown in Fig. 8 laterally adjusted to pass through the mean natural ovulation rate.

reference to their slope. The gradient of the early part of the curve should be related to the ovarian sensitivity to endogenous hormones, and on this basis the lines can be ranked : LI < LN < C = HN < HI, in order of increasing sensitivity. An examination of the unadjusted curves in Fig.8 indicates that the HI line has the most sensitive ovaries, and that the LN and C lines do not differ from each other, but have more sensitive ovaries than the HN and LI lines.

The different ways of comparing the dose-response curves only disagree with respect to the ranking of the natural lines. It would therefore appear reasonable to conclude that the ovarian sensitivity of the lines selected for induced ovulation rate (i.e. sensitivity) has been changed in the direction of selection. By contrast, selection for natural ovulation rate appears to have had a much smaller effect on ovarian sensitivity, and might have even changed it in the opposite direction to that of selection.

DISCUSSION

The analyses of the selected lines do not, unfortunately, lead to clear and decisive conclusions. They do, however, give an indication of the changes which have taken place in the selected lines, and it is intended to discuss these results before continuing to discuss ovulation rate as a component of litter size and fitness.

Selection for natural ovulation rate has increased and decreased the number of eggs shed relative to the control. The results of the examination of the males, however, suggest that follicle-stimulating activity has increased in the high line, but remained constant in the low line. It would appear therefore that ovarian sensitivity of the low line must have decreased relative to that of the control. However, the information from the nulliparous females indicates that the ovarian sensitivities of the HN, C and LN lines could not be differentiated, and that if anything, the ovarian sensitivity of the LN line was greater than that of the HN line. This apparent contradiction is not as serious as it initially appears, because whereas selection was carried out on post-lactational females, nulliparous females were used in the analysis. Such females differ in age, weight, and the experience of gestation and lactation. The above results can therefore be accommodated if it is assumed that one or more of these factors has resulted in differential changes in ovarian sensitivity taking place. The possibility that selection may have produced such a differential response is supported by the initial comparison of post-lactational and nulliparous females, which showed that the higher ovulation rate of the post-lactational females was largely the result of an increased

ovarian sensitivity. Further, the fact that the differences in natural ovulation rates between these two groups of females could not be accounted for by the increase in weight, together with the conclusion of Roberts (1956) that age has little effect on ovulation rate, indicates that the differential change in ovarian sensitivity is the result of pregnancy and/or lactation. The supposition that part of the difference between the post-lactational ovulation rates develops during lactation and pregnancy is supported by the observation that the difference between the nulliparous ovulation rates of the HN and LN lines is very small. Furthermore, the presence of this difference, despite the possibility that the ovarian sensitivities of the two lines differed in the opposite direction, together with the information from the males, indicates that the differences in follicle-stimulating activity are present without the previous experience of pregnancy or lactation, and consequently supports the conclusion that the differential change is a change in ovarian sensitivity.

It would, therefore, appear safe to conclude that the increase in the number of eggs shed by the HN line is mainly the result of an increase in the level of gonadotrophin activity, and that both FSH and LH activity contribute to this change. By contrast, the decrease in the low line appears to be the result of the failure of the ovarian sensitivity to increase during pregnancy and lactation.

Initially the examination of the inducedlines appears to lead to a much clearer picture - the response to 4 i.u. PMS has increased and decreased relative to the control, and, furthermore, this change is shown by the nulliparous females. Unfortunately, however, in spite of these observed differences in ovarian sensitivity, and the indication from the males that the high line had a higher level of gonadotrophin activity than

the low or control lines, the nulliparous females of the two lines had virtually the same ovulation rates. This makes the results very difficult to interpret, and unfortunately does not contribute to an understanding of the relationship between natural and induced ovulation. Furthermore, it is difficult to construct a physiological relationship between ovarian sensitivity and body weight, unless it is simply that larger mice have larger ovaries, and therefore are more able to respond to a particular level of gonadotrophin activity. This hypothesis is difficult to verify as ovarian weight is affected by the number of maturing follicles and/or corpora lutea present. The presence of a relationship between body weight and ovarian sensitivity is, however, supported by the fact that the difference in body weight between the natural lines increases at a time when differential changes in ovarian sensitivity have been postulated to take place.

This evidence, together with the initial observation of a positive phenotype correlation between body weight and the response to a standard dose of PMS, gives an even stronger indication of the relationship between body weight and ovarian sensitivity when it is realised that the larger mice are shedding more eggs in response to lower concentrations of hormones.

Unfortunately the inconclusive nature of some of the results on which the above discussion is based makes it very difficult to be categorical about the postulated causes of the observed changes in ovulation rate, or about the relative susceptibility of the components of ovulation rate to change by selection. However, the evidence for the presence of a genetic correlation between body weight and ovarian sensitivity is sufficient to show that the concept of a relationship between body weight and gonadotrophin

production, mediated *via* the pituitary, and independent of ovarian sensitivity outlined by McLaren (1962) is inadequate to account for the correlation between ovulation rate and body weight.

The small correlated changes in nulliparous ovulation rates have not only contributed to the inconclusive nature of the results of the examination of the susceptibility of the various components of ovulation rate to change by selection, but also mean that comparisons between the two estimates of the heritability of natural ovulation rate are not strictly valid. However, it is interesting to note that the two estimates are of the same order, and that both experiments lead to the conclusion that there is a genetic correlation between ovulation rate and body weight, which increases with age.

By contrast to body weight, there is no evidence of a correlated response in litter size. The comparison of the post-lactational and nulliparous responses of the lines indicates that this is largely the result of a low genetic correlation between the ovulation rates of the two types of females. Such a low correlation might be expected between the induced post-lactational and natural nulliparous responses, but one would have expected that selection for natural post-lactational ovulation rate would have changed the natural nulliparous response. It would be nice to be able to speculate that this is the result of selection for a high post-lactational response favouring large body weights which, because of the maternal effect on body weight, would in turn have favoured small maternal litters, and *vice versa*; but as selection was carried out within litters this is not possible.

Now, how do these results help us to understand the contribution

of ovulation rate to litter size and fitness? If the litter sizes of the lines had remained constant in spite of correlated responses in the nulliparous ovulation rates, it would have been possible to conclude that ovulation rate was not a limiting factor to litter size. However, the fact that ovulation rate has both increased and decreased under artificial selection indicates that it has not been subjected to intense natural selection pressures, and consequently has not been limiting fitness. This conclusion is in agreement with the prediction of Falconer (1960b) that ovulation rate would be expected to have an intermediate optimum. The results of selection for litter size by Falconer (1960b and 1963) however did not support this postulate, as ovulation rate increased in both the high and low lines relative to the control. This failure of ovulation rate to decline in the low line was ascribed to the variation in ovulation rate being masked by variation in embryonic mortality. An alternative explanation, which is supported by the fact that the high line was found to have the highest proportion of preimplantation losses, and a higher proportion of post-implantation losses than the control, is that there is a positive genetic correlation between ovulation rate and embryonic mortality. Under these circumstances, and if the genetic variation in embryonic mortality was greater than that in ovulation rate, selection for low litter size would have been expected to produce the increased embryonic mortality and ovulation rate which was observed.

Unfortunately, the results presented here do not provide sufficient information about the relationships between ovulation rate, litter size and embryonic mortality for any conclusions to be made regarding these interpretations of the results of selection for litter

size. However, even though most of the decrease in the natural ovulation rate of the LN line has been ascribed to the failure of ovarian sensitivity to increase during lactation and pregnancy, Falconer's unidirectional response of ovulation rate would not have been anticipated from the present results.

SUMMARY

Ovulation rate in the mouse was studied both genetically and physiologically. The genetical investigation took the form of a half-sib analysis and a selection programme. The latter involved the selection of post-lactational females for high and low natural ovulation rate, and high and low induced ovulation rate, together with the maintenance of an unselected control line. These lines were then examined physiologically, in an attempt to assess the effects of selection in terms of the level of activity of pituitary gonadotrophins and the sensitivity of the ovaries to these hormones.

The half-sib analysis gave an estimate of 24% for the heritability of natural ovulation rate. The selection programme indicated that the heritabilities of natural and induced ovulation rates were 33% and 15% respectively. Both experiments indicated the presence of a positive genetic correlation between both natural and induced ovulation rate and body weight which increased with age. There were no correlated changes in the litter sizes of the lines.

The physiological analysis of the lines consisted of a comparison of the number of eggs shed by nulliparous females in response to various doses of PMS with the number shed at natural oestrus. In addition, the testis and seminal vesicle weights of the males were examined. The results indicated that the increase in the natural high line was largely the result of an increased level of pituitary gonadotrophin activity, whilst the decrease in the low line was ascribed to a failure of the ovarian sensitivity to increase during pregnancy and lactation. Selection for induced ovulation rate appeared to have

changed the ovarian sensitivity in the direction of selection, and to have increased the level of gonadotrophin activity in the high line. The analysis of females was unfortunately complicated by an apparently low genetic correlation between the post-lactational and natural nulliparous responses. It was suggested that this could be one reason why there have not been any correlated changes in the litter sizes of the lines.

From the increase and decrease in natural ovulation rate under selection, it was concluded that ovulation rate is not a limiting component of fitness.

During the course of this work, it was necessary to investigate the relationship between natural and induced ovulation, and to study the use of dose-response curves in the examination of ovarian sensitivity to endogenous gonadotrophins. In order to preserve the continuity of the genetical investigation, this study is presented as an appendix. This work indicated the presence of a negative feed-back system which controlled the release of FSH from the pituitary. An examination of the effects of exogenous gonadotrophins on this system indicated that FSH was released from the pituitary during most of the cycle, but reached a peak around oestrus. It was concluded that the ovarian response to doses of gonadotrophins above 2 i.u. PMS, or its equivalent, was the response to the exogenous hormone, rather than the response to an unknown combination of exogenous and endogenous hormones.

APPENDIX

AN INVESTIGATION OF THE RELATIONSHIP BETWEEN NATURAL AND INDUCED OVULATION.

Introduction

In the Introduction to the main body of this thesis, it was stated that the reason for selecting for induced ovulation rate was to apply selection pressure to ovarian sensitivity to endogenous gonadotrophins. It was assumed that this could be done by selecting for the response to a standard dose of PMS, and that the most suitable dose would be the one that would induce the ovulation of approximately the same number of eggs as would be expected to be shed at natural oestrus. However it was realised that the grounds for making this assumption were tentative, and this appendix is the presentation of an investigation of the relationship between induced and natural ovulation rates.

It was decided that the best way to investigate both the validity of the assumptions described above, and the justification for assessing ovarian sensitivity in terms of the response to exogenous gonadotrophins, was to consider the effects of exogenous gonadotrophins on the intrinsic follicle stimulating system. The key to this study was the observation that small doses of PMS appeared to reduce the number of eggs shed. This arose from the results given in Table 2 and Fig. 1 of the thesis, which showed that although the natural response and the response to a zero dose of PMS were similar (as would be expected from the work of Burdick, Watson, Ciampa and Ciampa, 1943), the responses to small doses of PMS were lower than that at natural oestrus.

Now, in view of the similarity between the natural response and the response to a zero dose of PMS, one would expect the response to

increasing doses of PMS to start at the natural level and gradually rise, or, if the exogenous hormone did not have an effect until it was present in large quantities, to remain at the natural level until the 'threshold' was passed, and then to increase. In view of these expectations, it was felt that the observation of a reduced response was worthy of further investigation.

Part of this work has already been published as a letter to Nature, however, as details of the data were not included, and in the interests of continuity it is proposed to present the work without reference to the paper and simply to include a reprint of the paper at the end of this appendix.

Materials and Methods

The CGT used throughout was 'Pregnyl' (Organon Ltd.), and unless otherwise stated the PMS used was 'Gestyl' (Organon Ltd.).

Mice of the Q strain, described in the body of the thesis, were used throughout.

Unless it is stated to the contrary, ovulation was induced by treatment with PMS at 17.00 hours on day 0, followed by CGT at 12.00 hours on day 2. The ovulation rate was then scored in the manner previously described on day 3.

Results and Discussion

In this investigation, the resons for conducting a particular experiment are often not apparent until the results of the previous experiment have been discussed. It is therefore proposed to discuss the results of a particular experiment when it is completed, and to introduce the new experiment in the light of this discussion.

A. An Examination of the Reduced Response and its Interpretation

(i) Confirmation of the existence of the Reduced Response, and a possible hypothesis to explain it.

In the following experiments, unless otherwise stated, all mice

were 6 to 10 week-old virgins, and in dioestrus at the time of experiment.

The responses to doses of 0, 1/16, 1/8, 1/4, ½, 1, 1½, 2, 2½, 3, 3½, 4 and 6 iu.PMS, each followed by 2 i.u. CGT, were first examined. The results, which are given in Table A1 and Fig.A1a (solid circles), support the supposition that low doses (around 2 i.u.) induce lower responses than very low or high doses, i.e. there appears to be a dip in the early part of the dose-response curve. A similar dip in the early part of the curve was also seen in a small experiment in which a different preparation of PMS ('Equinex' ; Ayerst Ltd.) was used, as is shown by open circles in Fig. A1a and Table A/2.

At this stage it was thought that the presence of LH activity in the PMS could be a complicating factor, and so the experiment was repeated with a preparation (NIH-FSH-S2) of purified FSH. A preliminary trial resulted in a decline from 14.7 to 8.7 eggs when the dose was increased from 0.0 to 0.15 mg., as can be seen in Fig. A/1b and Table A/3a. Similar results were also obtained in a second experiment covering a wider range of doses, as shown by solid circles Fig. A/1b and Table A/3b. In this experiment care was taken not to confound the dose with the volume of the injection by giving all doses in only two volumes.

From these results we may conclude that the dip in the dose response curve is a real phenomenon, the explanation of which could have interesting consequences.

The results may be interpreted as evidence of an interaction between the exogenous hormone administered and the intrinsic follicle stimulating system. The possibility of such an interaction is supported by the work of Szontágh and Uhalric (1964), which indicates the presence of a direct negative feed back system controlling the secretion of pituitary gonadotrophins in the rat, and that the pituitary is the probable site for

TABLE A/1. The Mean Number of Eggs Shed by Nulliparous Females in Response to Various Doses of PMS given at Dioestrus.

Dose of PMS (i.u.)	No. of Mice Treated	No. Ovulated	Mean Response	S.E.
1/16	16	15	12.26	1.65
1/8	16	13	12.92	0.74
1/4	14	13	13.46	0.86
1/2	18	17	11.12	1.43
1	14	13	12.08	0.88
1 1/2	15	14*	11.34	1.02
2	15	15	10.53	0.82
2 1/2	15	15	10.87	0.80
3	15	14	13.14	1.18
3 1/2	14	12	12.42	1.42
4	14	14	13.71	1.31
6	15	13	15.08	1.29
0	15	14	13.43	1.49
N	16	14	15.57	0.35

*1 individual was omitted from this group as it deviated by more than 4 standard deviations from the mean (calculated including this individual).

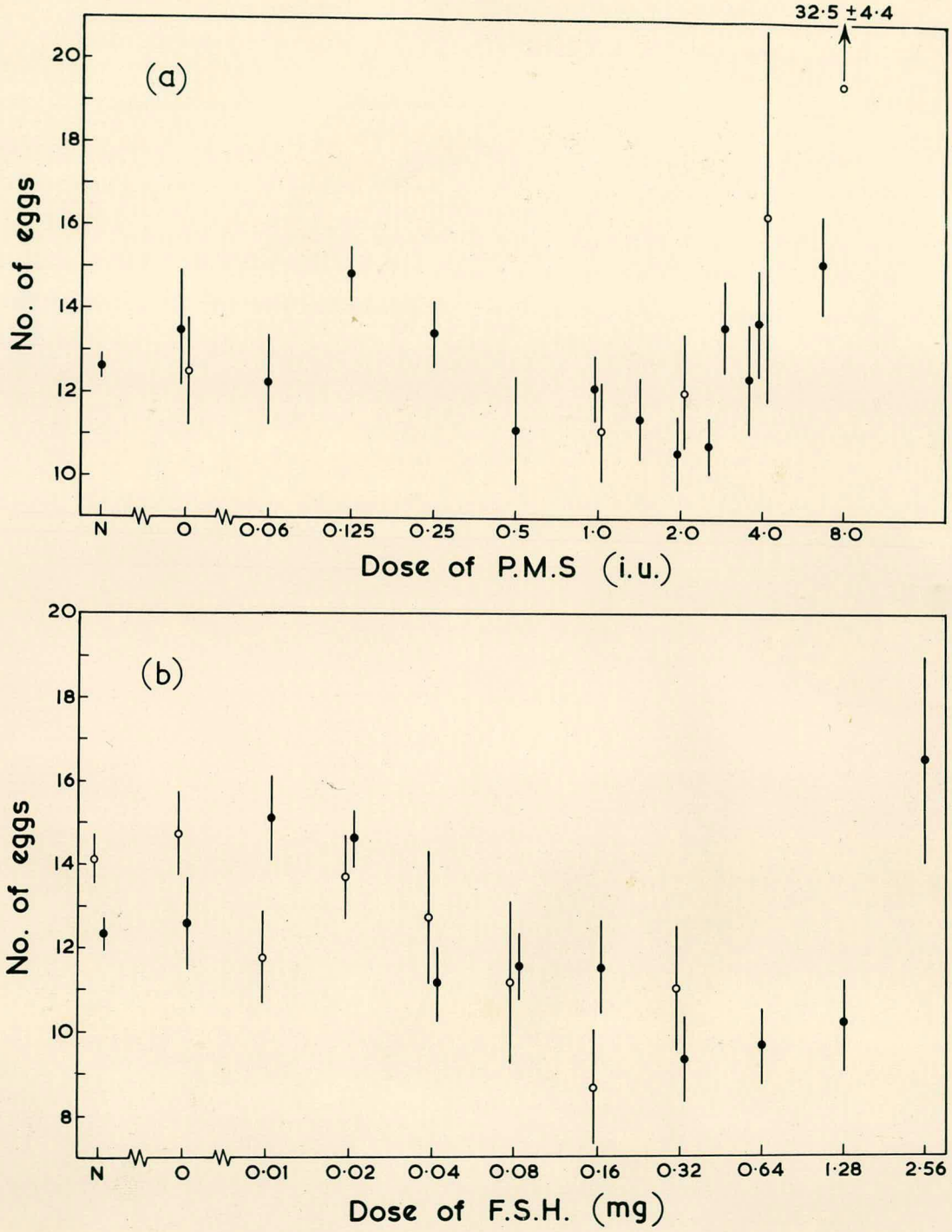
(a)
32·5 ±4·4
No. of eggs
20
18
16
14
12
10
N
O
0·06
0·125
0·25
0·5
1·0
2·0
4·0
8·0
Dose of P.M.S (i.u.)
(b)
No. of eggs
20
18
16
14
12
10
8
N
O
0·01
0·02
0·04
0·08
0·16
0·32
0·64
1·28
2·56
Dose of F.S.H. (mg)

FIG. A/1 The mean number of eggs shed (± S.E.) by nulliparous females in response to treatment at dioestrus with hormones with FSH activity; (a) PMS - 'Gestyl' (solid circles) and 'Equinex' (open circles); (b) purified FSH.

TABLE A/2. The Mean Number of Eggs Shed by Nulliparous Females in Response to Various Doses of 'Equinex' - Ayerst PMS.

Dose of PMS (i.u.)	No. treated	No. ovulated	Mean response	S.E.
1	10	10	11.20	1.18
2	11	11	11.73	1.40
4	10	10	16.30	4.73
8	10	10	32.50	4.37
0	13	11	12.91	1.32

TABLE A/3. a and b — The Mean Number of Eggs Shed by Nulliparous Females in Response to FSH at Dioestrus.

Dose of FSH (mg.)	a				b			
	No. treated	No. ovulated	Mean response	S.E.	No. treated	No. ovulated	Mean response	S.E.
0.01	12	11	11.73	1.14	13	11	15.27	1.15
0.02	12	11	13.73	0.98	13	11	14.64	0.82
0.04	11	11	12.70	1.67	12	12	11.08	0.96
0.075	10	10	11.20	1.90				
0.08					13	12	11.58	0.76
0.15	12	11	8.64	1.37				
0.16					12	12	11.67	1.27
0.3	10	10	11.10	1.48				
0.32					12	12	12.24	1.01
0.64					12	11	9.73	0.88
1.28					13	12	10.16	1.07
2.56					14	11	16.45	2.46
0	21	15	14.73	1.02	13	10	12.60	1.37
N	12	10	14.20	0.74	11	11	12.27	0.76

such an interaction. However, the characteristic of a negative feed back system is that it maintains the activity of the system at a constant level, and should not allow the reduction in activity demonstrated in these experiments. The reason for this apparent anomaly becomes clear when we examine the way in which the system maintains the equilibrium. The basic requirement is that the level of activity of the end product should be inversely correlated with its rate of formation. In this case, the circulating FSH must suppress the rate of its release from the pituitary; i,e. the hormone must have two activities - pituitary suppression and follicle stimulation. Now, if we assume that the biochemical bases of these two activities are to some extent different, and that the exogenous hormone is relatively more efficient in its suppression of the pituitary than in its stimulation of the ovary, then the reduction in the resultant level of activity of the follicle stimulating system is bound to follow when the dose is within a certain range. In view of the different nature of these two activities, and the fact that FSH is not a simple compound, the independence of the two activities seems a reasonable supposition. Also, when one takes into account the fact that the exogenous hormone is administered non-physiologically, and that it is not mouse FSH, the assumption of differential activity also becomes acceptable.

If the observed dose-response curves are now interpreted in the light of this hypothesis, it can be seen that each is the sum of two underlying curves, which represent the endogenous and exogenous contributions to the circulating levels of follicle stimulating activity. As the dose increases, the initial decline is the result of the rate of increase in pituitary suppression being greater than the rate of increase in the level of follicle stimulating activity of the exogenous hormone. However, as the suppression reaches its maximum, the situation becomes reversed, and the

number of eggs shed increases in response to increasing doses of exogenous hormone.

From the observations of Edwards et al. (1963), it can be seen that the increased response to low doses of PMS at metoestrus, relative to oestrus and dioestrus, was interpreted as evidence for the release of FSH at metoestrus, the endogenous and exogenous hormones complementing each other. However, the argument presented above leads to the conclusion that the higher the rate of endogenous release, the lower the response to low doses of exogenous hormones. The only way that the response could be as high as the natural response, when the pituitary is actively releasing FSH, is for the level of follicle stimulating activity at the particular stage to have no effect on the number of eggs shed 55 hours later. In view of the fact that Edwards et al. found similar responses to high doses, and that the responses to 4 i.u. at different stages of the cycle (described previously) did not differ, the probability of there being a stage of the cycle at which the level of FSH activity did not affect the number of eggs shed 55 hours later is remote.

In view of the contrasting conclusions from the hypothesis proposed here, and that of Edwards et al., it was decided to examine the response of Q mice to low doses of FSH at oestrus and metoestrus, and, if the greater response at metoestrus was manifest, to try to differentiate between the two hypotheses. Interest in this experiment was further stimulated by the observation in the preliminary trial (Table 1) that the response at metoestrus tended to be greater than at dioestrus, which in turn was greater than at oestrus.

Theoretically the most efficient way to test for the dip is to examine the response to very low doses and to doses which would be expected

to produce the maximum interaction. For this reason, the responses to doses of 0, 0.01, 0.16, 0.32 and 0.64 mg. FSH, followed by 2 i.u. CGT, and the natural response were examined in the hope that the responses at natural oestrus, 0 and 0.01, would give an estimate of the response without interaction, while the responses to 0.16, 0.32 and 0.64 mg. would give an estimate of the interaction. The results shown in Fig. A/2 and Table A/4 are disappointing, in that the responses to 0. and 0.01 mg. FSH differ widely. However, in view of the responses to the other doses, it would appear reasonable to conclude that the dip is greater at oestrus than at metoestrus. If these observations are superimposed on the response at dioestrus, it is evident that the order of response is metoestrus > dioestrus > oestrus, which agrees with the trend shown in the preliminary trial.

The present hypothesis can account for the results of Edwards et al., while that of Edwards cannot account for the decrease in response as the dose is increased. However, let us consider possible additions to the Edwards hypothesis that would enable it to account for the dip. For example, it is possible that the dip is the result of an interaction between the dose of FSH activity used to induce follicle development and the dose of CGT used to induce follicle rupture. This would appear unlikely, as 2 i.u. CGT are adequate to induce the ovulation of the natural number of eggs following very low or high doses of FSH activity. Nevertheless, the possibility was examined on a small scale, and the responses to 0, 1, 2 and 4 i.u. PMS followed by $\frac{1}{2}$, 2 and 6 i.u. CGT are shown in Table A/5. From this it can be seen that $\frac{1}{2}$ i.u. CGT is inadequate to induce the ovulation of the natural number of eggs, and that there is no difference between the responses after 2 and 6 i.u. CGT. Unfortunately there is no evidence of a reduced response

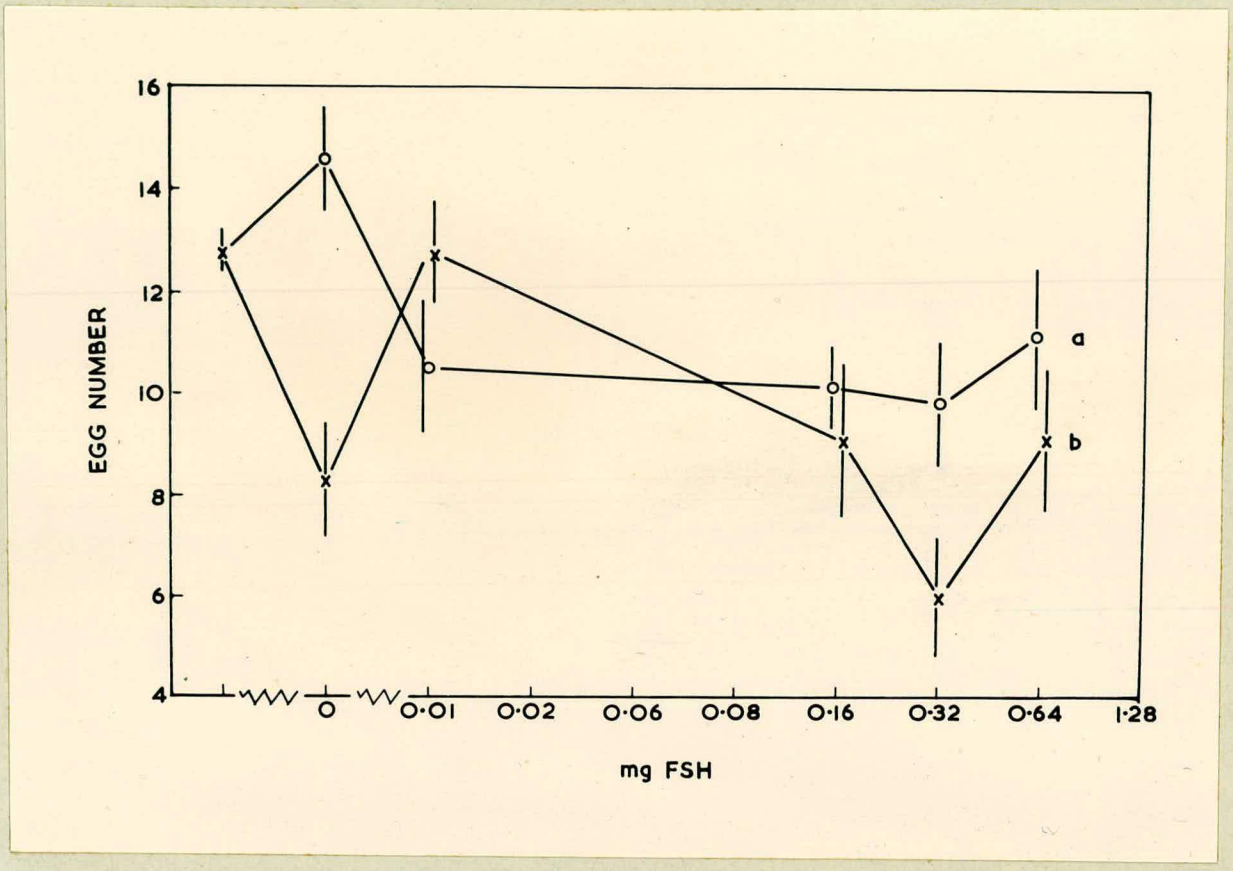

FIG. A/2 The mean number of eggs shed (± S.E.) by nulliparous females in response to treatment with purified FSH at (a) metoestrus, and (b) oestrus.

TABLE A/4. The Mean Number of Eggs Shed by Nulliparous Females in Response to FSH at Oestrus and Metoestrus.

Dose of FSH (mg.)	Met.				Oes.			
	No. treated	No. ovulated	Mean	S.E.	No. treated	No. ovulated	Mean	S.E.
0.01	14	14	10.50	1.29	17	15	12.80	1.00
0.16	15	14	10.07	0.84	14	12	9.08	1.55
0.32	15	14	9.93	1.15	14	12	6.00	1.23
0.64	14	11	11.18	1.45	17	13	9.15	1.39
0	16	14	14.57	1.00	15	13	8.23	1.11
N					13	13	12.77	0.43

TABLE A/5. The Mean Number of Eggs Shed by Nulliparous Females in Response to Various Doses of PMS at Dioestrus followed by Various Doses of CGT.

Within each cell the no. of mice ovulating is given above the no. treated, the mean response is then given together with its standard error.

Dose of CGT i.u. \ Dose of PMS i.u.	0	1	2	4
$\frac{1}{2}$	$\frac{3}{10}$ 11.67 ± 0.34	$\frac{8}{12}$ 8.88 ± 1.08	$\frac{3}{11}$ 10.67 ± 2.60	$\frac{1}{11}$ 13.00
2	$\frac{8}{13}$ 11.62 ± 0.68	$\frac{11}{12}$ 11.82 ± 0.82	$\frac{10}{11}$ 12.70 ± 1.60	$\frac{10}{11}$ 19.00 ± 2.00
8	$\frac{10}{12}$ 12.60 ± 1.16	$\frac{9}{12}$ 12.67 ± 0.75	$\frac{12}{12}$ 11.42 ± 0.97	$\frac{11}{11}$ 18.82 ± 2.37

to 1 or 2 i.u. PMS before 2 i.u. CGT, but, in view of the presence of a dip in all the other trials, and the comparatively few doses given here, it was felt that this was not a serious problem. These results, and the previous argument, appear to rule out the possibility of a PMS - CGT interaction, and consequently it is necessary to devise an experiment to differentiate between the two hypotheses.

(ii) The effect of small doses of PMS on the number of eggs shed at natural oestrus.

According to Edwards, one would expect the injection of a small dose of gonadotrophin into normally cycling mice to increase the number of eggs shed at natural oestrus, but according to the hypothesis outlined above a reduced response would be anticipated. A trial was therefore conducted in which groups of nulliparous females were injected at dioestrus with 0, ¼, ½, 1, 2 and 4 i.u. PMS. The females were then paired with males on the day of injection and their ovulation rate scored at the following oestrus. The results, given in Table A/6, show that there is no significant different between the responses following the different doses, but it is encouraging to note that the trend, if any, is for the response to decline as the dose increases. The fact that there is not an increased response after 4 i.u. could be related to the fact that only 6 out of 11 females treated with 4 i.u. had tubal eggs present on the morning of mating - indicating that 4 i.u. causes a sufficiently large interaction for the synchronisation of psychic and physiological oestrus to be disturbed. In an attempt to make these observations more strictly comparable with those of previous experiments, those mice which had copulated on the third morning after injection were examined separately. Their means and standard errors, shown in Table A/6, reflect the tendency for the response to be reduced

TABLE A/6. The Mean Number of Eggs Shed by Nulliparous Females at Natural Oestrus following a Single Dose of PMS at Dioestrus.

	Total				After 3 days		
Dose of PMS (i.u.)	No. treated	No. ovulated	Response	S.E.	No. ovulated	Response	S.E.
0	15	13	13.46	0.75	7	13.57	0.92
$\frac{1}{4}$	13	9	13.11	0.61	2	13.00	0.00
$\frac{1}{2}$	11	11	13.09	1.00	2	14.50	3.50
1	12	12	12.50	1.38	2	13.00	0.00
2	13	12	12.83	0.73	8	11.65	0.71
4	11	6	12.50	1.17	1	7.00	

following 1 - 2 i.u. PMS.

Now, when this experiment was being planned it was realised that an increase or decrease in the response may enable the two hypotheses to be differentiated, but if the response did not change significantly it could mean that the endocrine balance was restored before natural ovulation took place. To overcome this problem, similar groups of mice were simultaneously treated with the same doses of PMS as those described above, but the dose was given twice a day (at 9.00 and 21.00 hrs approx.) for three days before pairing, and the treatment continued until a plug was found. If no plug was found after a total of 13 days' treatment, the treatment was suspended. The females were then either left to ovulate naturally, or treated with 2 i.u. CGT on the 14th day and scored on the 15th. It was hoped that under this treatment there would be a relatively constant level of exogenous hormone present, and that ovulation, if it occurred, would be the result of endogenous changes superimposed upon this regime. From the results given in Table A/7 and Fig.A/3, it is evident that such a PMS regime has a more marked effect upon ovulation than has a single injection. Again, 4 i.u. seemed to upset the intrinsic system to such an extent that none of the plugged mice had tubal eggs present. Although it is difficult to compare the effects of PMS on the number of eggs shed at natural oestrus with the effects of PMS or FSH followed by CGT, it is interesting to note that a twice daily dose of $\frac{1}{2}$-1 i.u. PMS appears to correspond to a single dose of 1 - 2 i.u. PMS or 0.2 - 0.6 mg. FSH when followed by CGT. To continue the comparison, it is evident from Fig.A/1 that treatment with FSH causes a greater reduction in the level of activity of the follicle stimulating system than does treatment with PMS, which suggests that it might be possible to effect an even greater

TABLE A/7. The Mean Number of Eggs Shed by Nulliparous Females at Natural Oestrus during and after prolonged PMS Treatment.

Dose of PMS (i.u.)	No. treated	During Treatment				After Treatment				After Treatment + CGT			
		No. plugged	No. ovulated	Mean	S.E.	No. plugged	No. ovulated	Mean	S.E.	No. treated CGT	No. ovulated	Mean	S.E.
0	12	12	12	13.33	0.47	0	0	-	-	0	0	-	-
$\frac{1}{4}$	13	13	6	12.16	0.91	0	0	-	-	0	0	-	-
$\frac{1}{2}$	14	11	5	9.80	0.97	2	2	10.00	6.00	1	1	3.00	
1	13	6	4	9.50	2.46	5	4	12.25	0.63	2	2	5.00	0.00
2	16	7	6	13.67	4.16	6	6	11.83	0.31	3	2	6.00	2.00
4	13	6	0	-	-	5	5	11.40	0.68	2	0	-	-

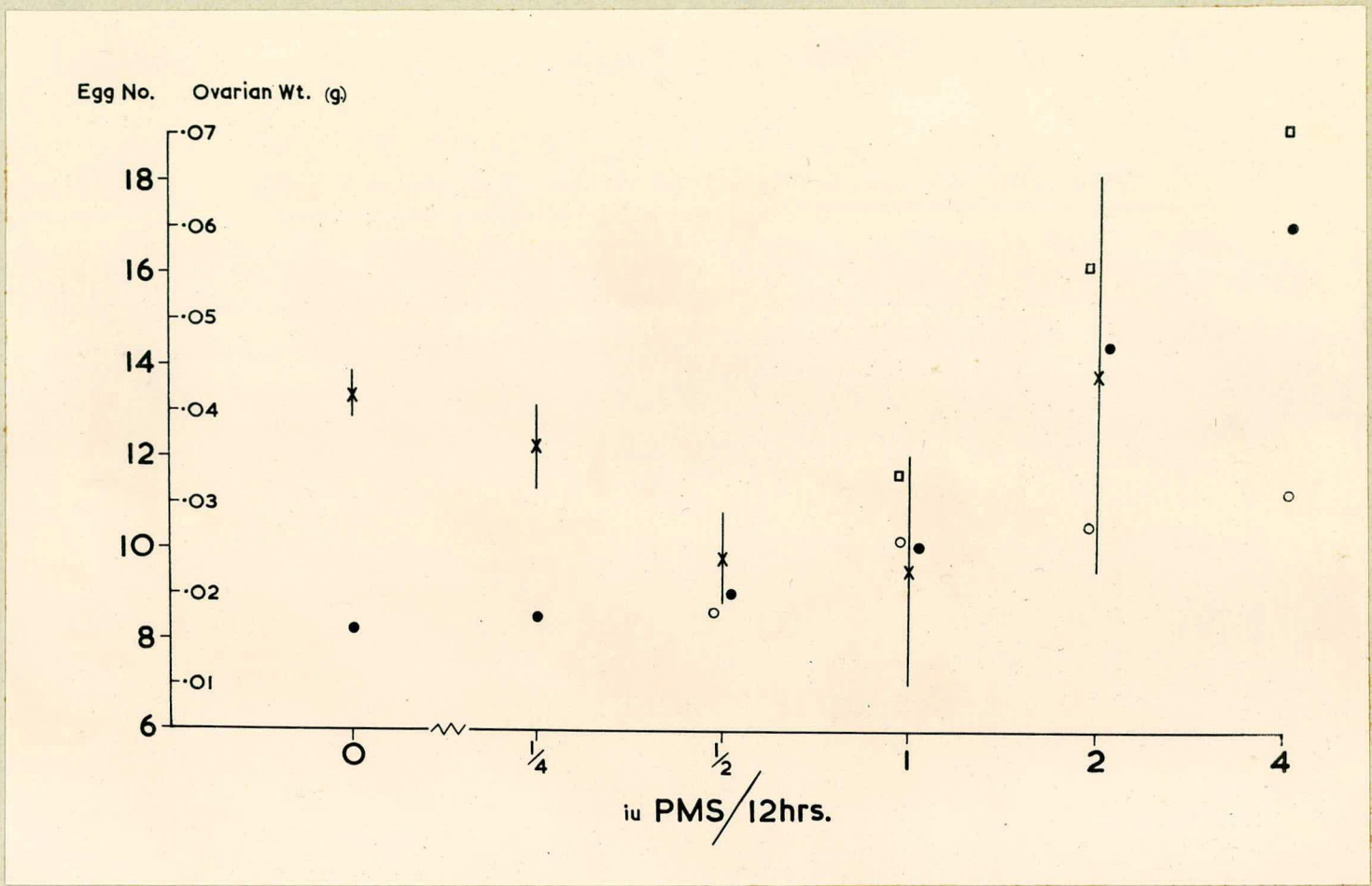

FIG. A/3 The effects of continuous PMS administration to nulliparous females on: (a) The mean number of eggs shed (± S.E.) at natural oestrus during the treatment (crosses), (b) The mean total ovarian weight at natural oestrus during the treatment (solid circles), (c) The mean total ovarian weight at natural oestrus after the treatment (open circles), and (d) The mean total ovarian weight after the treatment plus 2 i.u. CGT (squares).

reduction in the number of eggs shed at natural oestrus by the use of FSH, than by the use of PMS.

This trial therefore supports the theory that the dip in the original dose-response curves is the result of an interaction between the exogenous hormone and the intrinsic follicle stimulating system.

(iii) Discussion.

We are now in a position to be able to discuss the results presented above in the light of the proposed theory, and to see what conclusions it leads to. The presence of a dip at three stages of the cycle suggests that FSH is continually released. This conclusion is supported indirectly by the fact that a series of PMS injections has a greater effect upon the number of eggs shed at natural oestrus than a single injection, which indicates that the ovary is susceptible to FSH for a longer period of time than is covered by the effects of a single dose of PMS. However, before discussing this further, it is necessary to consider the units of time used. Only one set of treatments is started on a particular day; that is, all mice considered suitable for treatment on a particular day are treated at the same time on that day. Thus, the shortest unit of time in measuring a stage in a cycle, which itself only lasts for 4-5 days is 1 day. As a result of this, and the fact that changes from one stage of the cycle to another are gradual rather than sudden events, it is impossible to obtain a group of mice which are all at precisely the same stage of the cycle. Any event therefore which lasts for less than a day will be masked by occurrences on either side of it, and consequently we can only say that the evidence presented leads to the conclusion that FSH is secreted for most of the cycle.

Further information regarding the release of FSH could be obtained from bioassays of the pituitaries of mice at different stages of the cycle, but the difficulty of obtaining synchronous groups of mice, and the small size of the mouse pituitary makes this difficult. Furthermore, extrapolations from pituitary to circulating FSH levels are only tentative. In spite of this last problem, it is interesting to examine the results of Robertson and Hutchinson (1962), and Rakha and Robertson (1965), who overcame the former difficulties by using sheep and cattle respectively, both of which have long cycles and large pituitaries. In both cases a drop in pituitary gonadotrophin levels around oestrus is demonstrated, which is in accordance with the conclusions reached here.

B) An Examination of the Number of Eggs shed in Response to CGT at Different Stages of the Cycle, and under Different Exogenous Gonadotrophin Treatments.

The probable presence of FSH throughout the oestrus cycle suggests that it would be interesting to look at both the population of Graafian Follicles at different stages of the cycle, and the effect of PMS on the population of follicles. At the time of scoring the females which had been subjected to continuous PMS treatment, their ovaries were weighed and the results represented in Fig. A/3 show that, although there is a reduction in the number of eggs shed, there is a continuous increase in ovarian weight with increasing dose of PMS. Now, increases in ovarian weight are usually regarded as a reflection of the number and size of follicles present. If this is so, why are fewer eggs shed? This could be the result of an interaction between exogenous PMS and endogenous LH, but the increased number of eggs shed on a regime of 2 i.u. PMS conflicts with this argument. The two arguments can be accommodated if it is assumed that as the endogenous

LH activity decreases, the proportion of follicles which rupture decreases, but that, as the dose of PMS increases, the total number of mature follicles increases, the former effect being initially larger than the latter. However, the observation that mice which had been treated with 4 i.u. PMS for 13 days did not respond to 2 i.u. CGT, and that in all cases it was only after several days with PMS, and after a considerable decrease in ovarian weight, that ovulation occurred, suggests that LH was not the limiting factor. It would appear therefore that the increase in weight was not the result of an increase in the number of mature follicles, but that the actual number of follicles was reduced. Unfortunately, without further information it is impossible to discuss the problem further, but the disruption of the cycle by treatment with gonadotrophins certainly supports the concept of a direct gonadotrophin - pituitary feed back system.

It has already been observed that CGT alone will induce ovulation, and that it will do so after PMS blanks at different stages of the cycle. Now, it would appear reasonable to assume that the number of eggs ovulated at a particular stage of the cycle is a reflection of the number of mature Graafian Follicles present at that stage of the cycle, which in turn reflects the level of FSH activity. Consequently, by examining the response to CGT, inferences can be drawn regarding the presence of FSH activity. For this reason it was decided to study the effects of CGT alone at different stages of the cycle, and to extract from data (part of which has already been presented) the effects of CGT following a PMS blank at different stages of the cycle. The latter is shown in Table A/8. If these results are combined, it would appear that the relative responses to CGT following a blank at various stages of the cycle are in the order: dioestrus = metoestrus > oestrus. If the response to CGT at a given stage of the cycle is now examined - see

TABLE A/8. The Mean Number of Eggs Shed by Nulliparous Females in Response to 2 i.u. CGT Following a 'blank' at Different Stages of the Cycle.

	Metoestrus				Dioestrus				Oestrus			
	No. treated	No. ovulated	Mean	S.E.	No. treated	No. ovulated	Mean	S.E.	No. treated	No. ovulated	Mean	S.E.
'Trial'												
A	16	14	14.57	1.00	13	8	11.62	0.68	15	13	8.23	1.11
B	10	10	14.20	0.70	21	15	14.73	1.02	11	11	12.09	2.18

Table A/9 - it is evident that the responses can be ranked in the order: proestrus > dioestrus ⩾ oestrus > metoestrus.

While the differences within the two sets of data are insignificant, it is interesting to compare the trends within them. The effect of CGT following a blank would be expected to correspond to the effect of CGT alone at a stage 2 days after the stage at which the blank was given. On this basis, the best fit between the two sets of data is obtained if it is assumed that the length of the cycle is five days, but even with the more usually accepted four-day cycle, the agreement is reasonable. In view of the difficulty of precise timing, and variation in the length of the cycle, it was felt that these discrepancies were not serious. The lack of precision in timing could also result in there being a large range of stages over which the response is similar, and account for the ovulation of a normal complement of eggs immediately after a set had been ovulated naturally. In order to show that this was not the case, the responses after 1 - 6 days of treatment with 2 i.u. CGT were examined. The results, shown in Table A/10, suggest that there is a relatively constant supply of mature follicles and that the observations on cycling mice are not an artefact of the timing procedure. The results of continuous treatment and the treatment of cycling mice therefore agree, and, on the grounds of the initial premises, suggest that FSH activity is present throughout the cycle.

If we now consider the comparison of the effects of continuous versus a single dose of PMS, the similarity between the effect of a single dose of 2 i.u. and a continuous treatment of 1 i.u. provides evidence against a very low rate of gonadotrophin catabolism, and hence, for there to be a continuous presence of FSH activity, there must be an almost continuous release of FSH. The results of the response to CGT alone are therefore in accordance with predictions from previous arguments.

TABLE A/9 The Mean Number of Eggs Shed by Nulliparous Females in Response to 2 i.u. CGT at Different Stages of the Cycle.

	No. treated	No. ovulated	Mean
Proestrus	10	6	13.83
Oestrus	16	12	10.33
Metoestrus	14	12	10.08
Dioestrus	14	13	11.08

Analysis of Variance

Source of Variation	d.f.	SS	M.S.	V.R.
Between stages	3	64.4	21.46	0.88 N.S.
Within stages	39	948.6	24.32	
Total	42			

TABLE A/10. The Mean Number of Eggs Shed by Nulliparous Females in Response to Successive Doses of 2 i.u. CGT.

No. of successive doses	No. of mice treated	No. ovulated	Mean Response	S.E.
1	13	9	13.33	1.32
2	11	11	18.00	1.38
3	10	9	14.11	2.10
4	11	10	10.00	1.23
5	11	10	11.20	1.80
6	10	10	9.70	0.94

Conclusions

Although agreement within the above evidence is not perfect, it is sufficiently good to allow us to reach the conclusion that the injection of low doses of hormones with FSH activity can reduce the level of activity of the intrinsic FSH system, and that this property is not specific to a single preparation. Also, we can conclude that the secretion of FSH by the mouse pituitary continues for most of the cycle, reaching a peak around oestrus - not metoestrus as suggested by Edwards *et al*.

Now, how does this affect the interpretation of dose-response curves, in terms of their ability to estimate the sensitivity of the ovary to gonadotrophins? It would appear that the response to low doses is a function of both the dose and the stage of the cycle in which it is administered, but that above certain levels (in this case about 2 i.u. PMS or 1 mg. FSH) the dose becomes the important factor, and consequently the response is now an estimate of the sensitivity of the ovary to this dose. The interpretation of dose-response curves, therefore, is not affected, but the results do indicate that ia may be more efficient to divide the dose of FSH activity, and hence administer it in a way more closely resembling the situation *in vivo*. It can also be concluded that unless the dose is subdivided the best measure of ovarian sensitivity to endogenous gonadotrophins is the response to a dose of FSH activity which induces the ovulation of the same number of eggs as would be expected to be shed naturally; that is, the assumptions that have been made were justified.

A further conclusion that can be drawn is that there is no need to inject mice at a particular stage of the cycle when looking at the response to doses above that which is required to induce the ovulation of the natural number of eggs.

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Ovarian Response of Mice to Low Doses of Hormones with Follicle-stimulating Activity

An examination of the response of mice to low doses of pregnant mare's serum (PMS) led to the surprising result that, when the dose was reduced below a certain level, the number of eggs ovulated increased.

The primary object of the work was to find the dose of PMS required to induce the ovulation of a number of eggs approximately equal to that shed at natural oestrus (the natural number). The experiments were carried out on a random-bred strain of mice (*Q* strain). Ovulation was induced by injecting PMS ('Gestyl'; Organon Ltd.) followed by chorionic gonadotrophin (CGT) ('Pregnyl'; Organon Ltd.) 43 h later, as described by Fowler and Edwards[1], the eggs being counted 21–24 h after the second injection. Between 10 and 15 mice were used to test each dose.

It was realized that the response might be affected by three factors: (1) the dose of PMS; (2) the dose of CGT; (3) the physiological state of the mouse at the time of treatment. Of these, (1) is the variable to be examined. Factor (2) has been shown by Fowler and Edwards[2] to be relatively unimportant. They found that a dose of 2 i.u. was adequate in treatments designed to induce the ovulation of more than the natural number of eggs. This was confirmed in *Q*-strain mice, and a dose of 2 i.u. was consequently chosen. To reduce variation due to factor (3), all females were 7–9 weeks old, virgins, and in dioestrus at the time of the first injection.

Preliminary studies suggested that the natural number of eggs was shed in response to approximately 4 i.u. of PMS. The interesting point, however, was that low doses appeared to induce the ovulation of fewer eggs than would be shed naturally. Burdick *et al.*[3] found that luteinizing hormone (LH) alone, in doses between 1 and 100 i.u., induced the ovulation of a 'natural complement of eggs'. It appeared therefore that the ovarian response to low doses of PMS might be less than the response to CGT alone, without any PMS. In order to test this possibility further, the response to doses between 0 (that is, water alone) and 6 i.u. PMS was examined. The results, which are shown by solid circles in Fig. 1 *a*, support the supposition that low doses produce less response than very low, or high doses; that is, there appears to be a dip in the early part of the dose-response curve. A similar dip in the dose-response curve was also seen in a small experiment, in

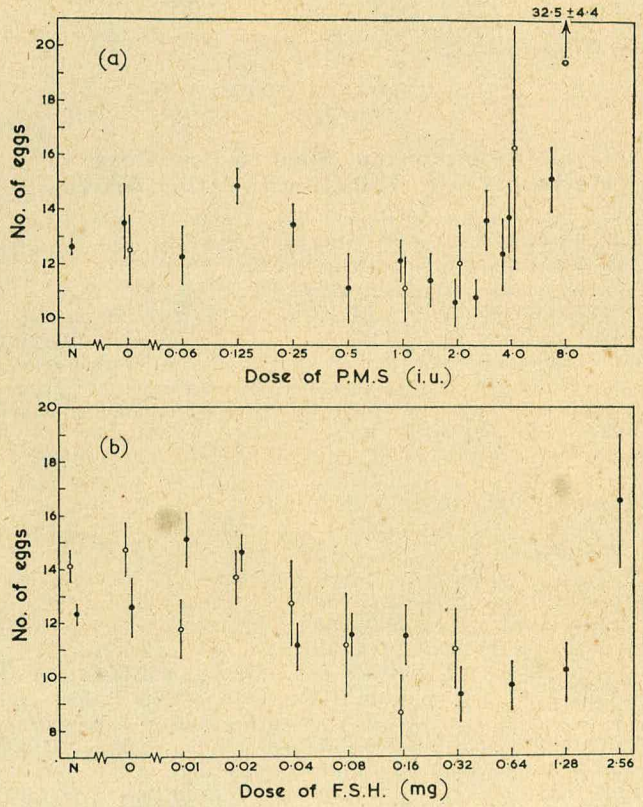

Fig. 1. Ovarian responses to different doses of hormones with FSH activity. Each point is the mean of between 10 and 15 mice, and the vertical lines extend to ± one standard error. *a:* PMS; solid circles, 'Organon'; open circles, 'Equinex'. — *b:* purified FSH—*NIH–FSH–S2*

which a different preparation of PMS ('Equinex'; Ayerst, Ltd.) was used, as shown by open circles in Fig. 1 *a*.

At this stage it was thought that the presence of LH activity in the PMS could be a complicating factor, and so the experiment was repeated with a preparation (*NIH–FSH–S2*) of purified follicle stimulating hormone (FSH). A preliminary trial resulted in a decline from 14·7 to 8·7 eggs when the dose was increased from 0 to 0·15 mg, as shown in Fig. 1 *b* (open circles); and similar results were obtained in a second experiment covering a wider range of doses, as shown by the solid circles in Fig. 1 *b*. The results show that the dip in the dose-response curve is a real phenomenon, the explanation of which could have interesting consequences.

These results may be interpreted as being evidence of an interaction between the exogenous hormone administered by the injection, and the endogenous follicle stimulating system. The possibility of such an interaction is supported by the work of Szontágh and Uhalric[4], which indicates the presence of a direct negative feed-back system controlling the secretion of pituitary gonadotrophins in the rat; furthermore, their work suggests that the pituitary is the most likely site for this interaction. However, the characteristic of a negative feed-back system is that it maintains the activity of the system at a constant level, and should not allow the reduction in the level of activity demonstrated in these experiments. The reason for this apparent anomaly becomes clear when we examine the way in which the system maintains the equilibrium. The basic requirement is that the level of end-product is inversely correlated with the rate of its formation; in this case the circulating FSH must suppress the rate of its release from the pituitary, that is, the hormone must have two activities, pituitary suppression and follicle stimulation. Now, if we assume that the biochemical bases of these two requirements are to some extent different, and that the exogenous hormone is relatively more active in suppressing the pituitary than stimulating the ovary, then the reduction in the resultant level of follicle-stimulating activity is bound to follow when the dose is within a certain range. In view of the difference between the two activities, and the fact that FSH is not a simple molecule, the independence of the two activities seems a reasonable supposition. Also, when one takes into account the fact that the exogenous hormone is not mouse FSH, and that it is administered non-physiologically, the assumption of its differential potency becomes even more readily acceptable.

If we now interpret the observed dose-response curves in the light of this hypothesis, we can see that each is the sum of two underlying curves, which represent the endogenous and exogenous contributions to the circulating level of follicle-stimulating activity. As the dose increases, the initial decline in ovarian response is the result of the rate of increase in pituitary suppression being greater than the rate of increase in the level of follicle-stimulating activity of the exogenous hormone. However, as the suppression reaches its maximum, the situation becomes reversed, and the number of eggs shed increases in response to increasing doses of exogenous hormone.

The evidence presented leads to the conclusion that the injection of low doses of hormones with FSH activity can reduce the level of activity of the endogenous follicle-stimulating system, and that this property is not specific to a single preparation.

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