GENETIC CONTROL OF OVULATION RATE AND
EMBRYO SURVIVAL IN MICE.
I. RESPONSE TO SELECTION

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NUMBER of young born per parturition in litter-bearing mammals is determined by two parameters, number of ova shed per estrus and proportion of these represented by normal fetuses at birth. The latter may be less than unity because of fertilization failure, failure to implant, or death of the embryo between implantation and the end of gestation. The losses may be the result of either zygotic or maternal factors or of interactions between the two.

FALCONER (1960, 1963a) has reported that response to selection for large litter size in a line of mice resulted from an increased number of ova shed, whereas a line selected for small litters did not decrease in ovulation rate but rather increased in embryonic mortality. BATEMAN's (1966) results of a similar experiment appear to show the same pattern, though no unselected control was available for comparison. This kind of result led FALCONER (1963a) to suggest that both components are under genetic control, and further that they are genetically independent or at least partially so. Additional evidence for such a conclusion can be found from experiments involving inbred lines and their crosses (see for example BOSHIER 1968, and the review by ROBERTS 1965). On the other hand, the two traits are related phenotypically (BOWMAN and ROBERTS 1958) and because of the nature of the reproductive process in mammals it is unlikely that they are genetically independent throughout their entire range of variability.

Although there is the indirect evidence cited for separate genetic control of the two components of litter size, direct evidence obtained by selecting for the components themselves does not appear to have been reported. The problem is of interest, not only with reference to the genetic correlation between the two, but also from the standpoint of methods of effecting genetic change in litter size. The results mentioned above suggest a high genetic correlation between ovulation rate and litter size. If the heritability of ovulation rate were higher than that for litter size, this might represent a situation where indirect selection would be more effective than direct.

This paper reports the results of an experiment in mice in which selection was practiced, in separate lines, for ovulation rate and for embryo survival, with the objectives of estimating realized heritability of these two parameters and of evaluating the effect of genetic change in them on litter size. Selection for both parameters was carried out in lines superovulated each generation as well as in un-

treated lines. Numerous experiments have been carried out on the effects of exogenous gonadotropins in mice, several of which show differences between inbred strains or selected lines in response (McLaren and Michie 1959; Fowler and Edwards 1960; McLaren 1962; Wilson and Edwards 1963; Gates 1965; Lin and Bailey 1965). Variation in natural ovulation rate presumably is a function of pituitary and ovarian factors; that following injection of gonado-tropins might be expected to be due primarily to differences in ovarian sensi-tivity. Selection for high embryo survival, or more specifically the ability to gestate a large litter, might be carried out more effectively when all females have a surplus of potential embryos, i.e. following superovulation. Consequently, gonadotropin treated lines were included in the experiment. The four selected lines were then each evaluated in the presence and absence of superovulating treatment to obtain information on the degree of interaction between selection and test environments.

MATERIALS AND METHODS

This experiment was carried out concurrently with the selection for litter size reported by Bradford (1968), using the 4-way cross stock and general management procedures described in that paper. Selection procedure in this experiment was as follows:

Each line was initiated by mating 21 first generation 4-way cross males to two 4-way cross females each. To obtain second litters from post-partum matings from both mates, the two females assigned to each male were placed in the mating cage 4 days apart. The second female remained in the mating cage 13 days, and was then transferred to a separate cage (cage 2). The male was transferred to cage 2 24–48 hr after the first female had produced a litter and pre-sumably remated, but before the second female gave birth. By this means a high proportion of both mates throughout the experiment produced second litters about 25 days after the first litter.

All female progeny up to 4 per litter from first litters were mated at approximately 9 weeks of age to non-littermate males from their own line, checked daily for mating plugs, and sacrificed as described below to obtain counts of corpora lutea (CL), implants (I), and normal fetuses (N) where appropriate. Males (21) and females (42) from second litters (18 and 36 after generation 9) were then selected on the basis of an index combining information from full and half sisters according to the method described by Henderson (1963). Since no estimates of heritability of the parameters being selected for were available at the outset of the experiment, a value of .20 was assumed arbitrarily for use in the index calculations. For the same reason it was further assumed that the variance due to maternal effects was zero. That maternal effects can exist in such material has been emphasized by Falconer (1963b and elsewhere). However, maternal effects were probably much less important in this experiment than in Falconer's, because of the later mating age as discussed by Bradford (1968).

The individuals selected from second litters were mated at 9 to 10 weeks of age, mates being assigned at random except for the avoidance of sib mating. This latter restriction was removed at generation 8, matings thereafter being completely at random including, therefore, a limited number of sib matings.

Four lines were selected for 9 or for 10 generations according to the above plan. They were:

Line designation	*Selection criterion*
O	Number of CL, counted at 7–8 days gestation. Implants (I) were also counted, but were not considered in selection.
O-T	Number of CL, as for line O, except that females providing the selec-tion information were superovulated each generation, using 3 Inter-national Units each of Pregnant Mare Serum (PMS) and Human Chorionic Gonadotropin (HCG), as described by Bradford (1968).

E   Number of normal fetuses (N) at 16 days gestation, as a ratio to num-
                ber of CL, the ratio weighted by N.

E-T   As for line E, except that females providing the selection information
                were superovulated. Females selected from second litters and mated to
                propagate the line were not treated (also for line O-T).

Corpora lutea were counted under a dissecting microscope at 10–15× magnification on females anesthetized with pentobarbitol. Counting was done under anesthesia since in our experience the CL are more easily seen in the live animal. The bursa was removed and the ovary grasped by forceps in the region of the hilum which tended to keep the ovary and CL well vascularized. Implants were counted at 7 or 8 days gestation (lines O and O-T); these were typically quite uniform in size at this stage, and the few which did not appear normal were not recorded separately. In lines E and E-T, the number of implants was estimated as the sum of the number of moles, abnormal and normal fetuses, the last being so classified on the basis of both size and vascularity. As will be seen, the number of normal fetuses as estimated at approximately 16 days appeared to provide a very close estimate of litter size at birth. In cases where no mating plug was recorded, stage of gestation was estimated from size of the fetuses. Since there were always a number of pregnancies of known age, this estimation could be done quite accurately.

Counts for all items were recorded separately for right and left sides, but all data reported represent the sum of both sides.

All counting was done by one of two persons, with occasional checking between the two for consistency of procedure. Implants and normal fetuses can be counted with a high degree of accuracy. BRAMBELL (1948) discussed potential sources of error in estimating number of ova shed from counts of corpora lutea. These include trapped ova, deep imbedding and fusing of the CL, and occasionally an apparent difficulty in distinguishing between functional corpora and those from a previous estrus. Size, color and consistency were all used as means of detecting the latter in the present study, and it is believed that the counts at least for untreated females were quite accurate. Some counting errors undoubtedly occurred in superovulated mice, although considering that the counting was done by experienced persons and with the mean and range involved (of the order of 33 and 15–60, respectively) the percentage error in actual counts of CL was probably small.

Generation interval with the procedure followed was 4 months. Lines O and O-T were kept synchronous, as were E and E-T, but the two pairs of lines were 60 days out of phase, to spread work load and space requirements. The unselected control line used in these experiments, line C described by BRADFORD (1968), had a generation interval of 3 months, and consequently once annually produced litters contemporary with each of the sib-selected lines considered here.

Two experiments comparing the performance, in the presence and absence of gonadotropin treatment, of females of the two pairs of selected lines were carried out. The first of these involved generation 8 females from lines E and E-T, and the second, generation 9 females from lines O and O-T. In each case the females used were those from litters in which 4 or more females were available. A random sample within each such litter was assigned to treated and untreated experimental groups. A random sample of untreated control line (C) females was also included in the first experiment.

The numbers of CL and losses at the two stages were also recorded for a sample of females from generation 15 of a line selected for litter size (line S1, BRADFORD, 1968) to permit comparison of these parameters on that line with those of lines O and E.

Regressions of generation mean on generation number were calculated for several parameters for the four lines O, E, O-T and E-T. All the regressions were based on data beginning with generation 2. This was done because (1) the generation 1 means may have been biased upwards by heterosis, the dams being original 4-way cross females, (2) older females were used in lines E and E-T in generation 1, (3) the PMS dosage used for line O-T was less than 3 I.U. in generation 1.

RESULTS

Mean values for the various parameters measured are presented by generation

for the different lines in Tables 1–4 and graphed in Figures 1–4. The means in Tables 1 and 2 are based on females actually pregnant, including an occasional individual in which all fetuses were resorbing at the time of examination. Conception rates were very high in untreated females, 90 to over 95% after one week, and much lower, 45–70%, in gonadotropin treated females.

Selection for ovulation rate in untreated females: Line O responded to selection for increased number of CL at the rate of 0.26 per generation, based on the linear regression of generation mean on generation number (Figure 1). This represents a realized heritability of slightly less than .10. The increase in number of CL was closely parallelled by an increase in number of embryos implanted ($b = .27$), although the secondary selection differentials measured for the latter (Table 5) were less than 40% as large as the selection differentials for number of CL.

Litter size of this line, on the other hand, did not deviate significantly from that of an unselected control after 10 generations of selection. The regression of mean litter size on generation number was non-significantly positive for first litters, and non-significantly negative for second litters (Table 3). There was an apparent increase in post-implantation embryonic mortality to the extent that litter size remained essentially constant in spite of the increase in number of embryos implanted. Direct evidence for this post-implantation loss was provided by generation 11 animals, which were sacrificed in late rather than early pregnancy (Table 1).

TABLE 1

Mean numbers of corpora lutea (CL), implants (I) and normal fetuses (N) in untreated lines of mice selected for ovulation rate (line O) and embryo survival (line E)

| Generation* | Line O | | | | Line E | | | | | |
| | Number of ♀♀ | Mean number | | | Number of ♀♀ | Mean number | | | N/CL | (N/CL)N |
		CL	I	N		CL	I	N		
1	129	10.1	8.3	. .	155	10.4	9.3	8.3	.81	7.0
2	112	9.5	8.6	. .	106	10.5	9.3	8.3	.81	7.1
3	113	10.6	8.8	. .	144	10.7	9.6	9.0	.86	7.9
4	127	10.4	9.1	. .	142	9.0	8.5	8.0	.88	7.2
5	116	10.0	8.7	. .	121	9.3	8.9	8.3	.89	7.6
6	119	12.9	9.4	. .	113	10.0	9.6	9.0	.91	8.3
7	121	11.0	9.8	. .	141	9.8	9.5	8.8	.90	8.2
8	122	12.1	10.2	. .	98	10.8	9.8	8.9	.81	7.6
9	99	11.9	10.4	. .	121	11.7	11.0	10.1	.87	8.9
10	74	13.1	11.3	. .	114	11.8	11.2	10.2	.87	9.3
11	107	11.0	10.2	8.0	106	11.8	11.3	10.6	.90	9.6
s†		2.6	1.9			1.9	1.8	1.9		
$b \pm s_b$‡		.26±	.27±			.23±	.27±	.25±	.0036±	.25±
		.11	.04			.09	.06	.06	.0038	.05

* Selections initiated among generation 1 animals.
† Mean intra-generation standard deviation.
‡ Regression of generation mean on generation number, beginning with generation 2.

TABLE 2

Mean numbers of CL, I and N in superovulated lines of mice selected for ovulation rate (line O-T) and embryo survival (line E-T)

| Generation | Line O-T | | | Line E-T | | | | | |
| | Number of ♀♀ | Mean number | | Number of ♀♀ | Mean number | | | N/CL | (N/CL)N |
		CL	I		CL	I	N		
1	77	21.2*	8.9*	73	37.3	14.2	10.5	.29	3.5
2	75	33.5	14.8	53	33.1	11.3	7.9	.24	2.5
3	74	31.6	12.8	74	29.3	12.6	9.7	.33	3.7
4	53	33.6	13.4	67	25.4	9.2	7.7	.30	2.8
5	53	34.0	14.4	69	27.4	13.8	10.7	.38	4.8
6	50	31.8	12.5	73	30.3	14.7	11.2	.37	4.7
7	62	39.0	15.3	105	29.3	15.6	12.8	.45	6.4
8	65	29.7	13.4	68	29.2	14.8	11.5	.41	5.3
9	45	35.6	14.3	82	26.7	13.9	11.6	.45	5.9
s		11.8	7.5		9.6	6.6	5.1		
b		.23± .46	.03± .17		—.37± .37	.58± .26	.60± .18	.028± .005	.51± .09

* Less than 3 I.U. of PMS used this generation only.

Selection for embryo survival in untreated females: The results for line E are similar to those from line O in that direct response to selection occurred, but differ with respect to change in litter size. Embryo survival in line E, measured as N/CL, increased markedly over the early generations, from .8 to .9, and then varied somewhat but was again .9 at generation 11. Ovulation rate declined abruptly at the third generation and then increased steadily, the net increase being very similar to that in line O. As a result, litter size in this line increased more than 25% above that of the control.

As indicated earlier, selection in line E was based on N/CL, multiplied by N. The intent was to select for increased embryo survival while avoiding selection against number of CL, as would have occurred by selection solely for a ratio of which this was the denominator.

The relative selection pressure applied for the different parameters with this procedure is indicated in Table 5. Since selection was based on an index combining information on varying numbers of full and half sisters, selection differentials cannot be calculated in the usual fashion. The index was calculated using an assumed heritability of .20. After the data were collected, the genetic gain in each of the parameters predicted to result from the selections actually made, and assuming heritability values ranging from .05 to .25 in increments of .05, were computed. It is recognized that all but one of the "selection differentials" calculated for each line are secondary selection differentials. Thus they do not necessarily predict accurately the change in the secondary traits (MAGEE 1965). However, this does provide a means of examining the selection actually applied. Only those values for heritability = .20 are given in the table. As can be seen
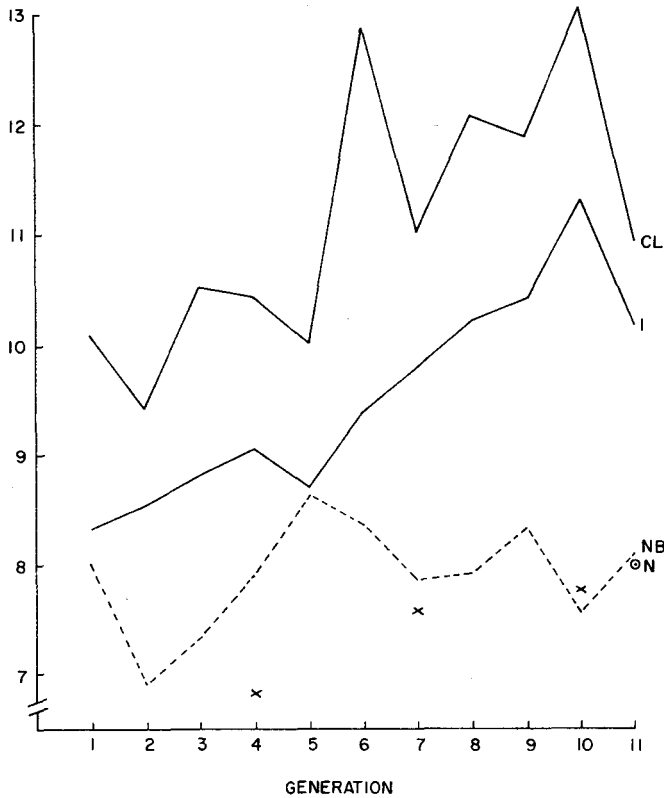
FIGURE 1.—Response to selection for ovulation rate. Line O, untreated.
    CL—Corpora lutea
    I—Implants
    N—Normal fetuses at 16 ± 2 days.
    NB—Number born in first litters (untreated females in all lines).
    X—Number born in first litters of a contemporary unselected control (line C).

from the table, use of a different heritability value (usually lower) in the index would have been somewhat more appropriate in some cases. However, the function of the index was to rank the litters available for selection, and a different heritability value would have resulted in only minor changes in rank.

The results in Table 5 indicate that the secondary selection differentials for CL in line E were small and included both positive and negative values in the early generations, but then increased, as might be expected, as embryo survival increased. The correlation between CL and N doubled (.35 to .70 approximately) between generations 1 and 5, and then remained near the higher level. The selection differential for CL was negative at generation 3, but not nearly large enough to explain the decline in mean value which occurred in generation 4.

Selection was strongly positive for I, N, N/CL and of course for N^2/CL, the actual selection criterion. It may be noted that the increase in litter size in line
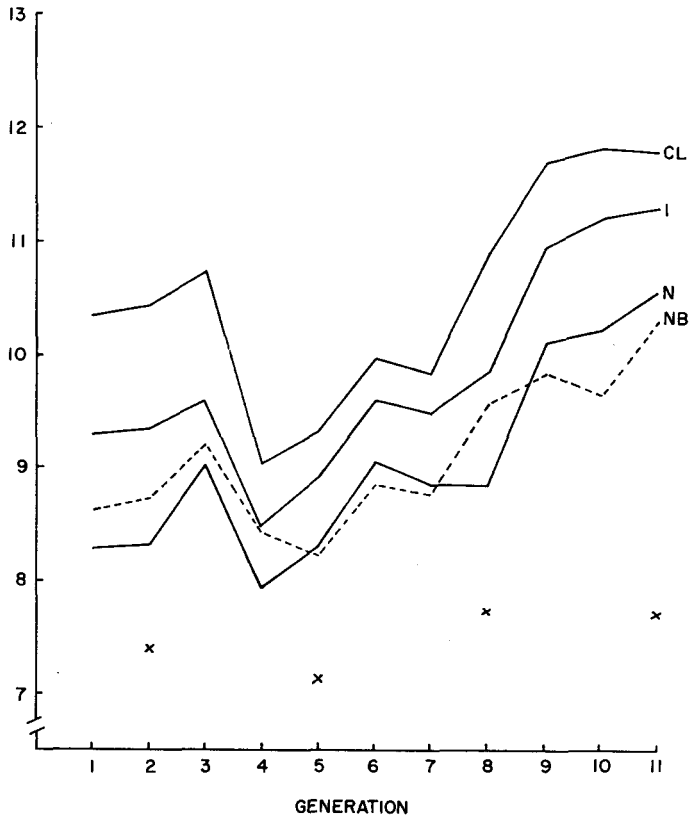
FIGURE 2.—Response to selection for embryo survival. Line E, untreated.

TABLE 3

Total number of young born per litter in first and second litters of lines O and E

	Line O				Line E			
	First		Second		First		Second	
Generation	Number of litters	$\overline{X}$	Number of litters	$\overline{X}$	Number of litters	$\overline{X}$	Number of litters	$\overline{X}$
1	41	8.0	35	9.9	44	8.6*	40	9.5
2	42	7.0	38	9.3	41	8.7	41	9.4
3	42	7.3	42	9.2	40	9.2	40	9.6
4	40	7.8	40	9.0	41	8.5	39	10.2
5	42	8.6	41	9.7	42	8.2	42	10.1
6	41	8.4	39	8.4	40	8.9	38	9.4
7	42	7.9	41	8.6	42	8.8	42	9.7
8	42	7.9	41	9.5	41	9.6	38	11.6
9	41	8.3	40	9.3	36	9.9	36	9.2
10	36	7.6	35	8.6	36	9.7	36	10.6
11	36	8.1	36	7.8	36	10.3	36	10.4
b		.07± .05		—.10± .06		.17± .05		.09± .08

* Females in line E (and E-T, table 4) were 16–18 weeks old at mating in this generation only. In all other cases, mating age was 8–10 weeks.

TABLE 4

Total number of young born per litter in first and second litters of untreated females of lines O-T and E-T

	Line O-T				Line E-T			
	First		Second		First		Second	
Generation	Number of litters	$\overline{X}$	Number of litters	$\overline{X}$	Number of litters	$\overline{X}$	Number of litters	$\overline{X}$
1	41	7.9	35	9.3	41	9.1*	38	9.8
2	42	8.0	37	9.4	42	8.2	41	8.9
3	41	7.8	41	8.7	42	7.6	39	7.8
4	41	8.0	38	9.0	40	8.4	40	9.4
5	40	7.6	37	8.6	42	7.8	41	8.2
6	41	6.8	41	7.6	39	7.7	37	7.9
7	42	6.8	41	8.0	42	8.3	41	9.0
8	41	8.0	41	8.3	42	8.9	42	10.1
9	41	8.0	39	7.6	36	7.9	35	7.8
10	33	7.4	30	7.8	35	9.6	35	9.9
b		—.03 ± .06		—.20± .05		.13± .07		.10± .12

* See footnote (*), Table 3.

E after 10 generations of selection (Table 3) nearly equalled that of a line directly selected for litter size for 14 generations (line S1, Table 7).

Selection following superovulation: The lines selected for the same parameters following superovulation, lines O-T and E-T, differ from the corresponding untreated lines in several respects (Table 2). The differences may be accounted for in part, but only in part, by the fact that selection was less intense in the treated lines due to the lower conception rates. The latter of course constitute a form of natural selection, which may have had a significant effect on the results obtained.

Line O-T results provided little evidence of direct response to selection, and the only significant correlated response was opposite in sign to that expected. Numbers of CL, in particular, and of I, tended to vary from generation to generation, but there was no discernible trend over generations in either (Table 2 and Figure 3). Litter size of the selected, untreated sisters also failed to increase (Table 4), and in fact in second litters it declined significantly. Further, the proportion of females becoming pregnant following treatment did not improve over the course of the experiment. These results and the marked increase in individual variability following treatment (see Tables 2, 6) are consistent with those for a line selected for litter size following superovulation (line S-T, BRADFORD 1968).

Line E-T, on the other hand, responded to selection, and in fact showed higher realized heritability values than line E. Mean values for CL, I and N in line E-T were all higher in generation 1 than for several generations following. Beginning with generation 2, CL declined irregularly, though not significantly,

TABLE 5

Genetic change expected to result from the selection actually practiced if heritability = .20*

A. Lines selected for number of CL

Generation of selection	Untreated-line O		Superovulated-line O-T	
	CL	I	CL	I
1	.52	.20	1.44	.73
2	.51	.15	2.02	.93
3	.62	.18	1.98	1.16
4	.41	.37	1.85	.45
5	.51	.50	1.58	.68
6	.85	—.06	1.69	.38
7	.37	.33	2.14	.49
8	.56	.02	2.10	.74
9	.68	.18	1.73	.60
Total	5.03	1.85	16.54	6.17
$9 \times b$†	2.33	2.39	2.09	.32

B. Lines selected for (N/CL)N

Generation	Untreated-line E					Superovulated-line E-T				
	CL	I	N	N/CL	(N/CL)N	CL	I	N	N/CL	(N/CL)N
1	—.07	.15	.38	.041	.58	.62	.48	.67	.014	.36
2	.05	.23	.46	.036	.63	.42	.92	.92	.024	.48
3	—.13	.18	.25	.030	.45	.17	.46	.60	.017	.38
4	.21	.31	.38	.021	.49	1.17	1.21	1.00	.021	.57
5	.25	.32	.41	.018	.50	.35	.80	.74	.024	.54
6	.21	.32	.32	.012	.42	—.39	.06	.29	.014	.25
7	.19	.23	.35	.016	.43	—.13	.33	.59	.023	.67
8	.22	.37	.47	.026	.56	—.68	.30	.54	.030	.61
9	.17	.24	.34	.015	.46	—.07	.54	.64	.026	.62
Total	1.10	2.33	3.37	.215	4.53	1.46	5.10	5.99	.193	4.52
$9 \times b$†	2.05	2.40	2.23	.032	2.23	—3.32	5.25	5.45	.252	4.55

* Both primary and secondary selection differentials treated as primary selection differentials for these calculations.

† $9 \times b$ = linear regression coefficient of generation mean on generation number (from Tables 1 and 2) multiplied by number of generations of selection, as a measure of net response.

whereas I increased from 11+ to about 14, and N from about 8 to 12, both significant. Litter size of the females selected and mated without treatment to continue the line also increased, though much less than N of the treated females. The increase in size of first litters was .13 ± .07 per generation, that for second .10 ± .12 per generation.

The very large pre-implantation losses in all treated females raise some question about the validity of CL counts as estimates of ova produced in superovulated females. The possibility of some anovular follicles becoming luteinized and counted as CL has not been ruled out. However, failure of many eggs from superovulated females to develop normally has been reported by other workers. GATES (1965), for example, reported as low as 52% of eggs cleaved from super-
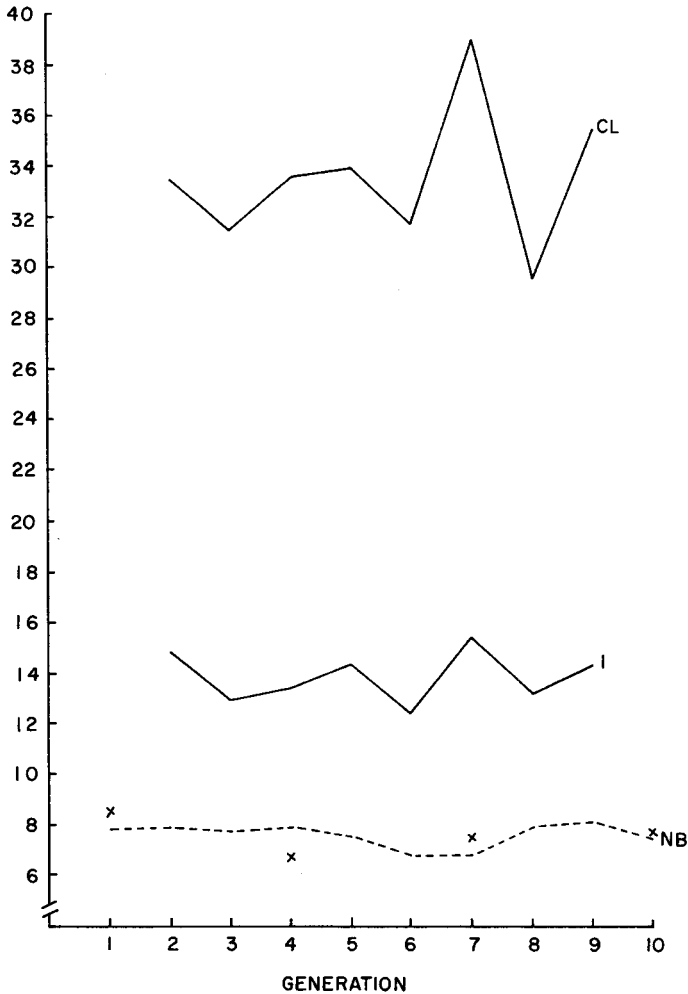
FIGURE 3.—Response to selection for ovulation rate. Line O-T, superovulated.

ovulated adult females of one strain. Such a value would account for most of the pre-implantation loss observed here.

Genotype-environment interaction: Results of the two experiments comparing the performance of the different lines each in the presence and absence of gonadotropin treatment appear in Table 6.

These results are in good agreement, with one possible exception, with the results already presented and predictions from them.

In every case, performance of untreated females of the lines selected without treatment exceeded that of untreated females from treated lines, and vice versa, although the differences did not reach statistical significance, at $\alpha = .05$, in all cases.

The interactions, however, were significant ($P < .05$) in every case, although
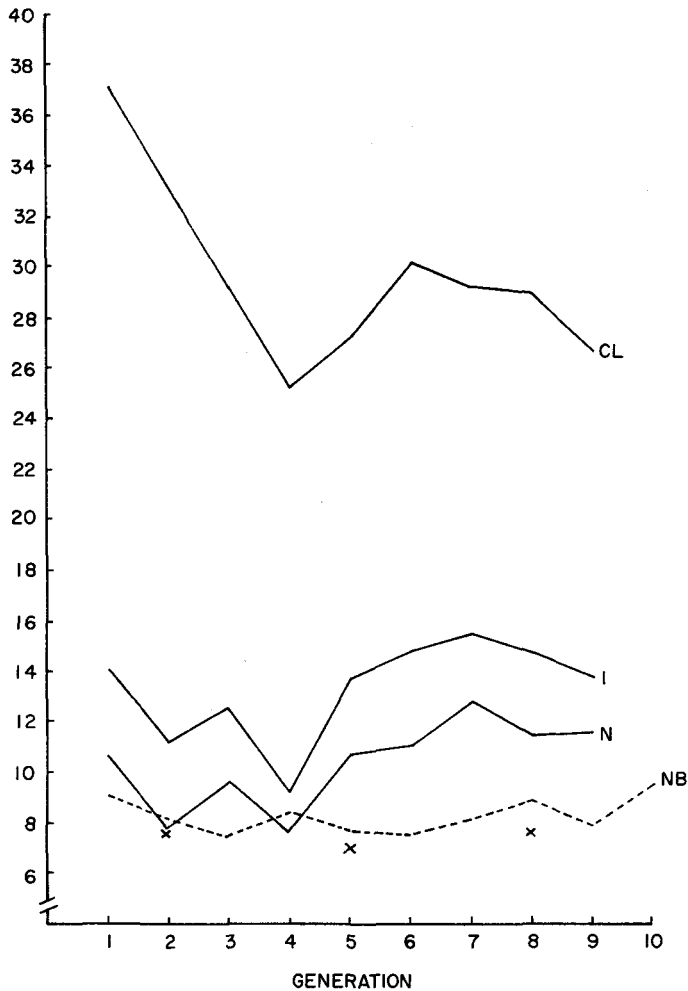
FIGURE 4.—Response to selection for embryo survival. Line E-T, superovulated.

the results of this analysis should probably be interpreted with some caution. It is obvious that the assumption of common variance within all groups is not valid when treated and untreated groups are combined. Furthermore, the coefficient of variation was higher in treated groups, so that a transformation to correct for effects of scale would provide only a partial adjustment.

Untreated females of line O-T, in which litter size of untreated females had already been shown to be no better than and possibly inferior to that of an unselected control (Table 4), had low values of CL and I compared to all other lines. Line E-T, untreated, had a number of CL less, but not significantly so, than line C, consistent with the slight decrease in CL observed under treatment (Table 2). In spite of this lower potential litter size, line E-T females had sufficiently lower losses that they had .79 more normal fetuses at 16 days than did line C females.

TABLE 6

Performance of lines selected in the presence and absence of gonadotropin,
each tested in the presence and absence of treatment

Line	Generation	Number of ♀♀		Untreated Mean			Number of ♀♀	Treated Mean		
				CL	I	N		CL	I	N
Experiment 1										
E	8	67	$\overline{X}$	11.0	10.0	9.0	45	27.3	13.6	10.5
			s	1.8	2.2	2.2		8.8	6.6	4.7
E-T	8	42	$\overline{X}$	9.1	8.6	7.8	37	30.2	15.6	11.7
			s	1.5	1.4	2.0		10.7	7.1	4.7
C	11	25	$\overline{X}$	9.6	8.9	7.0	..	...	...	...
			s	1.4	2.5	2.9				
Difference: E *vs.* E-T				P<.01	P<.01	P<.01		n.s.	n.s.	n.s.
E *vs.* C				P<.01	P<.01	P<.01				
E-T *vs.* C				n.s.	n.s.	n.s.				
Line (E, E-T) by treatment interaction significant (P<.05) for CL, I, N										
Experiment 2										
O	9	44	$\overline{X}$	11.6	10.7	...	26	31.1	13.6	...
			s	2.2	1.5	...		7.3	6.7	...
O-T	9	36	$\overline{X}$	8.8	8.1	...	19	40.0	15.6	...
			s	1.6	2.1	...		11.6	8.8	...
Difference: O *vs.* O-T				P<.01	P<.01				P<.01	n.s.
Line by treatment interaction significant(P<.05) for CL, I.										

The one apparent exception noted is the significant difference in CL between treated line O and line O-T females (31.1 *vs.* 40.5). The mean for all females in line O-T in that generation was 35.6. The only difference between the subsample involved here and the total group was that the former came from litters in which more females were raised. None of the other evidence from this line suggests a positive genetic correlation between litter size (untreated) of the dam and response to hormone treatment, and it is suggested that the observed difference is most probably due to chance.

Effects of selection for litter size on ovulation rate and embryo survival: Ovulation rates and pre- and post-implantation losses of the lines selected for the two components and for litter size are compared directly with those of the unselected control in Table 7. The line O and E data are those from generation 11 shown in Table 1 plus some additional from an experiment being reported in more detail in a separate paper (BRADFORD and NOTT, 1969). The comparison of particular interest here is that between the line selected for litter size (S1) and those selected for components.

Line S1 had an increased ovulation rate sufficient to account for nearly all of the increased litter size observed in this line, consistent with the result reported by FALCONER (1963a). Pre- and post-implantation losses were similar to those

TABLE 7

Ovulation rate and prenatal losses in an unselected line (C) and in lines selected for ovulation rate (O), embryo survival (E), or litter size (S1)

Line	Generation	Total number of ♀♀	Mean					
			CL	I	CL—I*	N	I—N†	N/CL
C	14, 15	62	9.8	8.9	0.9	8.0	0.9	.81
O	11	142	11.1	10.0	1.1	8.2	1.8	.73
E	11	142	11.4	10.9	0.5	10.2	0.7	.90
S1	15	56	13.0	11.7	1.3	10.8	0.9	.83

* Pre-implantation losses.
† Post-implantation losses.

in the unselected control, as was therefore the proportion of CL represented by normal fetuses.

DISCUSSION

The experimental evidence available when this experiment was initiated suggested that the components of litter size, ovulation rate and embryo survival, were genetically independent, and that it was probable that the genes affecting embryo mortality were recessive and present at low frequency (FALCONER 1963a). This suggests that of the two, ovulation rate should respond more readily to selection. Further, since increased ovulation rate could account for the observed response to selection for litter size (FALCONER 1963a; BATEMAN 1966, and confirmed here, line S1), it might be expected that selecting for ovulation rate would be an effective means of increasing litter size. The results presented here are in agreement with these expectations only to the extent that both components were changed by selection.

The results from line O indicate that number of ova produced per estrus can be increased fairly readily by selection, and furthermore that the proportion of these which are fertilized and implanted is fully maintained without any direct selection pressure for number of implants. Apparently it does not "cost" the animal much to implant a larger than usual number of embryos. On the other hand, the results suggest that the population tends to maintain a relatively constant mean litter size unless direct selection for larger litters is applied.

Line E results indicate that pre- and post-implantation losses can be reduced by selection, and that a relatively low level of losses can be sustained with an increasing ovulation rate. Although the heritability of embryo survival, whether measured as N/CL, or as (N/CL)N, the actual criterion of selection used, was in fact quite low (Table 5), the effect of the selection was to reduce total losses from about 20% to about 10%, the latter being a surprisingly low value. Measurement of this heritability and interpretation of the relationships among the parameters would have been more straightforward had the pattern of the first three generations, i.e. little selection for CL, been maintained. However, as already pointed out, decreasing the losses automatically increased the correla-

tion between N and CL, thus increasing the selection pressure for the latter. From a biological standpoint, of course, the maintenance of low embryonic and fetal losses with an increasing ovulation rate which actually resulted, is perhaps of equal interest to a possible further reduction (below .1) in total losses. Line E-T provides some information on a high embryo survival line in which ovulation was not increased. The one sample of this line evaluated without treatment (Table 6) indicated lower losses than in the control, but no lower proportionately than in the E. This does not of course indicate what might have been achieved by selection simultaneously for lower losses and constant ovulation rate.

The possible advantages hypothesized for selecting for performance following superovulation were obviously not realized in this experiment. The results for line O-T are essentially completely negative. Ovulation rate failed to increase measurably in either treated or untreated descendants of animals selected for response to treatment, nor did any of the other components under consideration change, at least in a positive direction. The decrease in size of second litters of untreated females (Table 4) and possibly in ovulation and implantation rates without treatment (Table 6) could possibly be explained on the basis of a slight decrease in body weight of this line, a result suggested though not established conclusively for all treated lines in the experiment. This in turn could have resulted from use of a constant dosage of gonadotropic hormones, dilution effect resulting in less response in larger females. The phenotypic correlation between body weight and ovulatory response to gonadotropin was, however, essentially zero when tested in two different samples.

Failure of line O-T to respond could be due to many factors, among them improper balance of FSH and LH activities of the treatment administered, and interaction between the exogenous hormones and those produced by the animal's own pituitary. There is, of course, also the possibility that ovarian factors are unimportant relative to pituitary gonadotropin output as a source of variation in number of ova produced per estrus, although McLAREN (1962) has presented evidence that increased ovarian sensitivity was the factor primarily responsible for the increased ovulation rate resulting from selection for litter size in untreated mice. This kind of evidence, and that from differences among inbred stocks in response to gonadotropins cited earlier, both suggest genetic differences in ovarian response to gonadotropin treatment. The results of CHAPMAN (1946) and KYLE and CHAPMAN (1953) with respect to ovarian weight response in immature rats provide evidence not only for genetic variation but for a substantial additive component in the response to gonadotropin treatment. The results obtained here, however, indicate that a negligible fraction of the variation in ovulatory response of adult female mice to gonadotropin treatment is additively genetic.

Line E-T, in contrast to line O-T and to the line selected for litter size following treatment reported earlier (line S-T, BRADFORD 1968), did respond to selection, giving a realized heritability of .20 for the measure selected for. Apparent embryo survival nearly doubled over the nine generations of selection. A slightly decreased number of CL contributed in part to this. It is possible, though unlikely,

that increased accuracy in counting CL as the experiment progressed could account for part of the latter. The increases in proportion of implants represented by normal fetuses and in absolute number of the latter (Table 2) are of course not subject to this possible bias.

In spite of the response to selection under treatment in this line and the evidence that some of this improvement is expressed in the absence of treatment (Tables 4, 6), it is clear that this is not a more effective means of selecting for embryo survival or litter size than selection in the absence of treatment. The pattern of results in Table 6 in fact provides strong support for the view that selection for improvement in a particular situation will tend to be most effective when carried out in that situation.

Direct comparison of the efficacy of indirect selection (for components of litter size) and direct selection for litter size is precluded by the fact that selection for components was based on information from full and half sibs, whereas selection for litter size was based on information from the dam. In general, however, the results of this experiment provide little evidence of an advantage for indirect as compared to direct selection. As just noted, selection following gonadotropin treatment was less effective in improving performance in the absence of treatment than selection among untreated animals, and vice versa. Selection for corpora lutea improved this parameter, but not litter size. Selection for components of litter size among females mated for the first time resulted in more increase (or less decrease) in size of first than of second litters in every case (Tables 3 and 4).

The one exception to this pattern is the increase in ovulation rate in lines E and S1 compared to that in line O. The overall increase in number of CL was similar in lines E and O, although direct selection for CL was practiced only in the latter, and in line S1, selected only for number born, the increase in CL was greater than in either of the component lines. Thus, selection for litter size increased ovulation rate but not embryo survival, whereas selection for ovulation rate did not increase litter size but selection for embryo survival did. Such asymmetrical correlated responses appear paradoxical, but BOHREN, HILL and ROBERTSON (1966) have developed genetic models which suggest that such results are in fact to be expected commonly.

With respect to independence of the components, the failure of litter size to increase in line O, in which ovulation rate increased, implies a negative correlation between ovulation rate and embryo survival. On the other hand, the results for line E, in which the selection procedure led to simultaneous emphasis on the two components, do not indicate this negative correlation. Further investigations of both genetic and physiological aspects of these relationships appear warranted; the stocks developed in this experiment should be useful for such studies.

Note, added in proof: Since this manuscript was submitted, a paper on the effects of selection for ovulation rate has been published (R. B. LAND and D. S. FALCONER, Genetic studies of ovulation rate in the mouse. Genet. Res. **13**: 25–46, Feb. 1969). These authors' results show the same general pattern as those reported here with respect to response to selection for high natural ovulation rate and the lack of correlated response in litter size, but differ with respect to both direct and

correlated response to selection for induced ovulation rate. The latter difference could be due to one or more of several differences between the two experiments, including the method of estimating ovulation rate, age at treatment, dosage, and strain of mice.

SUMMARY

Mice were selected for ovulation rate, estimated by counting corpora lutea (CL), and for embryo survival, the ratio of normal fetuses in late pregnancy (N) to CL. The lines were designated O and E respectively. Paired lines selected for the same criteria were superovulated with PMS and HCG (lines O-T and E-T). To avoid selection for decreased ovulation rate in lines E and E-T, the ratio N/CL was weighted by N. This resulted in little selection pressure on CL in line E-T or early generations of line E, but positive emphasis on CL in line E as embryo survival improved.—Line E-T showed direct response to selection, and some of the improvement in embryo survival was expressed in the absence of treatment. Line O-T did not show direct response, and performance of untreated individuals of this line decreased. Interaction between selection history and test environment was significant for all parameters measured when the four component selection lines were evaluated in the presence and absence of treatment.—Direct response to selection occurred in lines O and E, but litter size increased only in line E. A line (S1) selected successfully for large litters had increased in ovulation rate but not in embryo survival relative to an unselected control (C). Values for CL, I (implants), N and N/CL, respectively, at the end of the experiment were as follows: line C—9.8, 8.9, 8.0, .81; line O—11.1, 10.0, 8.2, .73; line E—11.4, 10.9, 10.2, .90; line S1—13.0, 11.7, 10.8, .83. Thus, selection for each component directly improved that component, but only the response in embryo survival was accompanied by an increase in litter size. Selection for litter size resulted in a marked increase in ovulation rate but the reciprocal relationship was not found.

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