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Cell number in early embryos from strains of mice selected for large and small body size

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SUMMARY

Embryos were recovered 3½ days *post coitum* from females of three replicate large- and small-selected Q-strain lines, together with their unselected control lines. Selection had been carried out by D. S. Falconer, resulting in large and small lines which differed two-fold in adult body weight. Females of the large lines yielded significantly more embryos than those of the other lines. Embryo cell number showed significant heterogeneity among replicates, but was similar in large, small and unselected lines. The data are not consistent with the hypothesis that the divergence in adult body weight is due to a uniform difference in rate of cell division throughout development.

Little is yet known of the physiological basis of genetic variation in mammalian growth. In the rabbit, differences between large and small breeds may be already apparent before implantation. Castle & Gregory (1929) found that, 168 h after mating, large-breed embryos were convincingly greater in diameter than those of a small breed and, 48 h after mating, contained an average of 22 cells as against 14 cells for the small breed. The difference in cell number was of doubtful significance. In a later study of the same breeds (Gregory & Castle, 1931), a difference in cell number in the same direction was found at the 8–12-cell stage (40 h after mating), but not at the 4-cell stage (32 h after mating).

In an analogous study, we have compared cell number in early embryos of mice selected by D. S. Falconer for large and small 6-week body weight. Selection (see *Mouse News Letter* **41**, 22–23; 1969) was started in 1964 from the random-bred Q strain, and had been proceeding for 12–15 generations at the time the embryos were examined. Three replicate lines (A, D, E) were included, each consisting of a large-selected, a small-selected and an unselected control line. There was a two-fold difference in adult body size between the large and small lines.

Embryos were flushed from the uteri of pregnant females 3½ days *post coitum*, counted, classified into morulae or blastocysts according to whether cavitation had occurred and placed in 0·25 % sodium citrate for 5 min. After fixation in acetocarmine for 24 h, they were squashed, stained with 0·5 % basic fuchsin and the nuclei counted using phase microscopy. The results are presented in Table 1.

The number of embryos recovered per female showed significant heterogeneity between small, control and large lines, though not between replicates (Table 2). The heterogeneity arose mainly because, in each replicate, the large females yielded more embryos than did either of the other two lines.

In contrast, the number of cells per embryo (Table 3) showed significant heterogeneity between replicates, with line D embryos consistently less advanced than those of lines

Table 1. *Number and stage of development of eggs recovered 3½ days p.c. from Q females selected for large and small body size and unselected (control)*

Replicate	Small	Control	Large
A			
No. of females	6	7	5
Mean. no eggs	8·7 (40)*	8·1 (45)	12·6 (44)
% blastocysts	84·6	93·0	86·4
Mean cell no.	48·3	47·8	40·3
D			
No. of females	6	6	6
Mean no. eggs	5·0 (21)	8·5 (37)	10·0 (48)
% blastocysts	96·7	74·5	75·0
Mean cell no.	41·6	34·8	34·0
E			
No. of females	7	6	10
Mean no. eggs	8·6 (50)	7·7 (35)	10·1 (78)
% blastocysts	78·3	71·7	92·1
Mean cell no.	43·7	35·9	43·1
Replicates pooled			
No. of females	19	19	21
Mean no. eggs	7·5	8·1	10·7
% blastocysts	84·5	80·5	85·9
Mean cell no.	45·0	40·1	39·8

* The total number of eggs used for cell counts is shown in parentheses.

Table 2. *Analysis of variance of the numbers of eggs shed by the 9 groups of mice included in Table 1*

Source of variation	Degrees of freedom	Mean square	F
Between replicates (A, D, E)	2	14·01	1.9
Between treatments (small, control, large)	2	57·87	7·8**
Replicate–treatment interaction	4	13·09	1·8
Within-group	50	7·42	—

** $P < 0{\cdot}01$

Table 3. *Analysis of variance of cell number in eggs from the 9 groups of mice included in Table 1*

Source of variation	Degrees of freedom	Mean square	F
Between replicates (A, D, E)	2	2716	5·5***
Between treatments (small, control, large)	2	1011	2·0
Replicate–treatment interaction	4	535	1·1
Between females	50	494	4·8***
Within females	339	104	—

*** $P < 0{\cdot}001$

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A and E, while no significant differences in cell number were seen between the small, control and large lines. Highly significant heterogeneity between females was found, and differences between lines were therefore assessed relative to the between-female mean square. The percentage of embryos which had reached the blastocyst stage of development was significantly correlated with mean cell number.

Thus there is no indication that the differences in adult size which have been achieved by selection are reflected in any differences in stage of development or cell number in pre-implantation embryos. In so far as the adult size difference involves cell number rather than cell size, what corresponding difference in cell number might one expect to see in embryos at the stage we examined? An adult mouse contains about 10^{12} cells, requiring an average of about 40 rounds of cell division; a twofold size difference represents an average of one extra cell division; if this difference were distributed evenly throughout development, one might expect embryos of the large line to undergo 1·025 cell divisions for each division in the small line. This is equivalent to a difference of only 4 cells at the 40-cell stage. The three replicates, A, D, E, give estimates for the large-minus-small difference in cell number of $-8{\cdot}0 \pm 5{\cdot}8$, $-7{\cdot}6 \pm 4{\cdot}1$ and $-0{\cdot}6 \pm 5{\cdot}1$ respectively (Table 1), yielding a joint estimate of $-5{\cdot}57 \pm 2{\cdot}81$, which differs significantly from the expected difference of $+4$ cells ($P < 0{\cdot}01$). Our data therefore contradict the hypothesis that the large-selected and small-selected lines owe their adult size difference to a uniform, genetically determined difference in rate of cell division.

Such a hypothesis is also inconsistent with the data of Castle & Gregory (1929) and Gregory & Castle (1931). The difference in rate of development which they reported for early rabbit embryos (approximately 1·3 cleavages in the large breed for every 1 cleavage in the small, from the 4-cell stage) would, if it continued throughout ontogeny, result in about a 1000-fold difference in adult body weight, rather than the three- to four-fold difference which they observed. Evidently in that instance a difference in growth rate manifests during early cleavage but not later, while in the case of Falconer's size-selected mouse lines the difference in growth rate is confined to the post-implantational period.

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