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Cell number in relation to heterosis during embryonic growth of mice

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1. INTRODUCTION

Cell size and cell number are two components of growth which may be affected differently by genetical and environmental factors, as was shown by Robertson (1958, 1960) in *Drosophila*. He found that in the early stages of selection for large and small body size the predominant change is in cell number, while heterosis effect is exhibited mainly through increased cell size. Similar investigations in relation to heterosis of body size in mammals are lacking and the present preliminary experiments were performed to study this problem during the embryonic life of the mouse.

Crossing of inbred strains of mice is often accompanied by the enlargement of body size in F_1 hybrids. However, maternal effects may not permit heterosis to be manifested during embryonic life and in the newborn mice. Of two strains grown in our laboratory, KP and KE, only the females of the KP strain produce heavier young when mated with an alien male. Young born by KE mothers are the same weight, irrespective of whether they are crossbred or inbred. However, in this strain embryonic heterosis may be revealed in competition between the two genotypes. It has been shown that in mixed litters, resulting from heterospermic insemination of KE mothers, F_1 hybrid embryos grow heavier than inbred ones developing in the same uterus (Musiałek, 1966, 1967). Thus the method of heterospermic insemination is useful for studying heterosis during embryonic life.

Counting of nuclei in tissue homogenates was used for estimating cell number. Since the presence of many different tissues and cell types in the whole embryo might obscure the results, it was decided to restrict these studies to one organ only. Liver, whose size correlates very significantly with embryo size, was therefore chosen to investigate the problem of whether cell number or cell size is involved in the heterotic growth of mice.

2. MATERIALS AND METHODS

Two inbred strains of mice: KE—genotype ccaabbPP, and KP—genotype CCaabbpp (Krzanowska, 1965) were used. Virgin females about 10 weeks old, kept previously for 2 weeks on an artificial light regime (dark period between 11 p.m. and 8 a.m.) were artificially inseminated in cyclic oestrus at 11 a.m., using the

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method of Dziuk & Runner (1960). Sperm obtained from vasa deferentia of one KP and two KE males was mixed with 0·2–0·4 ml. of boiled milk and used for insemination of two to four females. The proportion of sperm from the two sources was adjusted to allow for the lower fertilizing capacity of KE males (Krzanowska, 1960, 1964) and to obtain about equal numbers of inbred and crossbred offspring.

On the 13th, 14th, or 15th day of pregnancy (day of insemination was designated 0) pregnant females were killed and all embryos weighed on a torsion balance to the nearest 1 mg. Embryos were inspected for the presence of pigment in the eyes which appears in F_1 hybrids only, both inbred strains having pink eyes (KE because of the gene albino; KP because of pink-eyed dilution). Embryonic livers were gently dissected, weighed on a torsion balance to the nearest 0·1 mg., and transferred into separate tubes containing 0·1 ml. of 1 % citric acid.

A modification of the technique described by Solomon (1957) was used for counting nuclei. After standing for 1 hour the tissue was homogenized with a glass plunger and a further 0·4 ml. of 1 % citric acid was added. The suspension was centrifuged at 2500 r.p.m. for 5 min. and the supernatant was removed. The pellet was broken up with a glass rod and then suspended in 2·0 ml. of a weak solution of toluidine blue (about 0·002 %) in distilled water. This fluid was added gradually with continuous mixing. Two drops of the suspension were placed in the haemocytometer and two big squares (0·1 $mm.^3$ each) were counted to estimate the number of nuclei.

Only those females containing embryos sired by fathers from both strains were used. Of these there were eleven KE and four KP females. Only 14-day-old embryos were investigated in this latter group. After artificial insemination the litter size of KE females was slightly lower than normal (5·3 instead of about 6·6) but was unaffected in KP females (6·5).

3. RESULTS

In all age groups body and liver weights were heavier, and the number of nuclei in the liver larger in F_1 hybrids than in inbred embryos. This holds true both for KE and KP mothers. All differences were significant (Table 1). The relative difference was highest in 13-day-old embryos where the heterotic increment amounted to 30 % for body weight, 83 % for liver weight and 68 % for the number of nuclei in the liver.

For embryos of KE mothers the linear regression of liver weight on body weight was calculated. It was highly significant both in inbred and in F_1 hybrid embryos and the difference in slope between these two groups was non-significant (Fig. 1). The relative liver weight (in mg. per 100 mg. of body weight) increased during the embryonic period studied; it amounted to 3·7 % and 5·2 % on day 13; 6·4 % and 7·2 % on day 14; 8·1 % and 8·2 % on day 15 in inbred and crossbred embryos, respectively.

By dividing the weight of the liver by the number of nuclei in this organ, mean cell size was calculated (Table 1). This tends to be larger in F_1 hybrid than in inbred embryos on the 13th day of pregnancy, but smaller on the 15th, although the level

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Table 1. *Body and liver weight and the number of nuclei in the liver of inbred and F_1 hybrid embryos developing after heterospermic inseminations*

Character investigated	Day of pregnancy	Inbred embryos, mean ± s.d.	F_1 hybrid embryos, mean ± s.d.
		In KE mothers:	
Body weight (mg.)	13	(8) 86·5 ± 18·63	(8) 113·0 ± 18·60*
	14	(14) 158·4 ± 24·58	(9) 182·2 ± 17·26*
	15	(11) 257·4 ± 45·68	(8) 312·3 ± 26·81†
Liver weight (mg.)	13	(8) 3·21 ± 1·65	(8) 5·88 ± 1·92†
	14	(14) 10·18 ± 2·78	(9) 13·08 ± 2·36*
	15	(11) 20·72 ± 4·43	(8) 25·68 ± 2·67*
Number of nuclei in the liver (millions)	13	(8) 2·24 ± 1·10	(8) 3·77 ± 1·08*
	14	(14) 6·07 ± 1·63	(9) 7·76 ± 1·76*
	15	(11) 13·14 ± 2·79	(8) 17·94 ± 1·86†
Cell weight (mμg.)‡	13	(8) 1·45 ± 0·23	(8) 1·54 ± 0·27
	14	(14) 1·67 ± 0·16	(9) 1·71 ± 0·16
	15	(11) 1·59 ± 0·22	(8) 1·45 ± 0·14
		In KP mothers:	
Body weight (mg.)	14	(14) 168·9 ± 16·15	(12) 186·5 ± 11·18†
Liver weight (mg.)	14	(14) 12·81 ± 1·99	(12) 15·26 ± 1·43†
Number of nuclei in the liver (millions)	14	(14) 7·25 ± 1·02	(12) 8·95 ± 1·45†
Cell weight (mμg.)‡	14	(14) 1·75 ± 0·24	(12) 1·73 ± 0·23

Numbers of embryos examined are given in brackets.

* Difference between F_1 hybrid and inbred significant at 5% level.
† Difference between F_1 hybrid and inbred significant at 1% level.
‡ Cell weight (mμg.) = $\frac{\text{liver weight (mg.)}}{\text{number of nuclei (millions)}}$.

of significance was not reached in either case when the same age groups were compared.

Cell size changed with age, being the largest in 14-day-old embryos. The difference between day 13 and 14 is significant for inbred embryos, while that between day 14 and 15 is significant for F_1 hybrids. These calculations show that the relation between liver weight and the number of nuclei in the liver is rather curvilinear, which complicates direct comparison of the lines of regression for inbred and cross-bred embryos. However, Fig. 2 shows that for the same liver weight the number of nuclei and consequently cell size is very similar in both genotypes. The results indicate that heterosis is exhibited through a different growth rate, while both in F_1 hybrids and in inbred embryos growth proceeds along the same curve relating cell number to organ weight.

4. DISCUSSION

When inbred and F_1 hybrids are developing in the same uterus, heterotic effects are increased: F_1 hybrids grow bigger at the cost of inbred embryos which are smaller in heterospermic litters than in normal ones (Musiałek, 1966). Thus heterospermic

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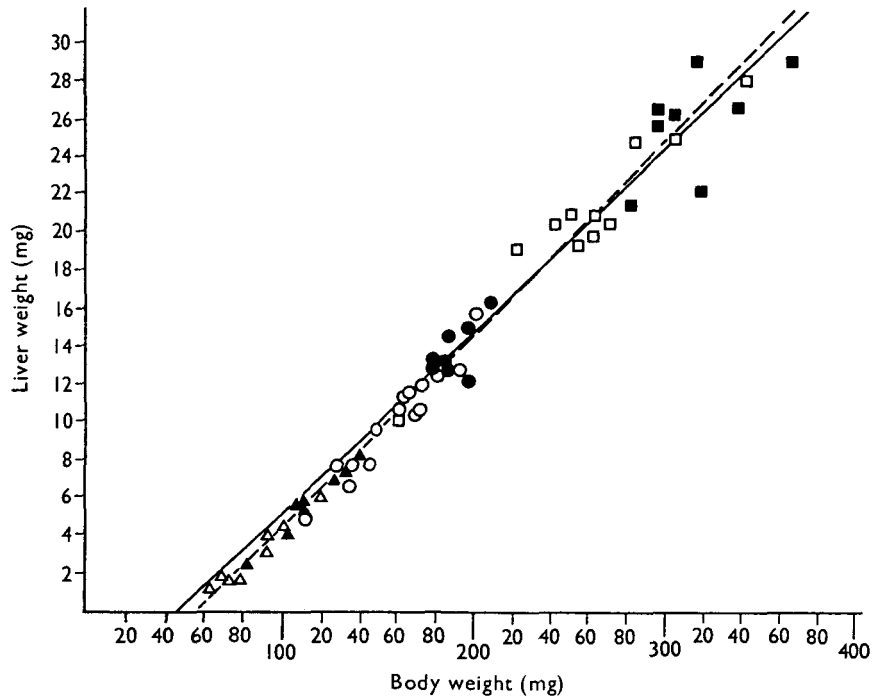

Fig. 1. Regression of liver weight on body weight in inbred (dashed line, open symbols) and F_1 hybrid (solid line, black symbols) mice. Triangles—13-day-old; circles—14-day-old; squares—15-day-old embryos. The calculated regression coefficients are $0{\cdot}102 \pm 0{\cdot}0022$ for inbred embryos and $0{\cdot}098 \pm 0{\cdot}0037$ for hybrid embryos.

litters create an opportunity to investigate embryonic heterosis in uniform environmental conditions provided by the same mother. In the present experiments the difference between crossbred and inbred embryos on day 13 of pregnancy was equal to about 40% of the difference between inbred embryos on day 14 and on day 13. Thus 13-day-old F_1 hybrids were nearly of the same size as 13·4-day-old inbred embryos. This relation is also true for liver weight and for the number of nuclei in the liver, indicating that cell number is the responsible factor. When the same liver weights are considered, number of nuclei seems to be very similar in inbred and hybrid embryos (Fig. 2). Moreover, no significant differences in cell size were found between genotypes (Table 1) when the same age groups were compared.

Liver was chosen as the object of the present experiments for technical reasons, because it is easy to dissect in early embryos and its size correlates with body size. It might be argued that it is not the proper organ for such studies because of the possible presence of polynucleate and polyploid cells. However, during embryonic life such cells are extremely rare or absent both in mice (review of literature given by Wilson & Leduc, 1948) and in the rat (Dvořák, 1963). When counting the nuclei it was noticed that nuclear size varied considerably but the differences may be fully explained by the fact that in such mitotically active tissue there were many very

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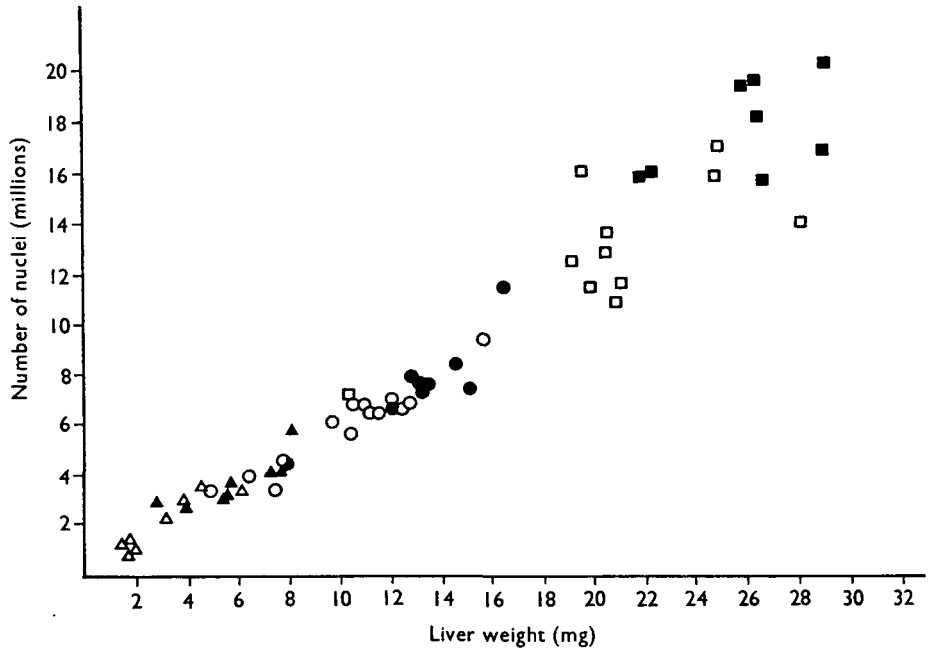

Fig. 2. Number of nuclei in the liver in relation to liver weight in inbred (open symbols) and F_1 hybrid (black symbols) mice. Triangles—13-day-old; circles—14-day-old; squares—15-day-old embryos.

young cells containing smaller nuclei. No difference in this respect was found between crossbred and inbred embryos.

The conclusion seems to be justified that the heterotic effect in mouse embryos is due to more rapid division of cells in early pregnancy. Unfortunately, the method used did not permit embryos earlier than 13 days old to be studied. On the other hand it is known that in homospermic litters there is no effect of heterosis in 96-hour blastocysts (Krzanowska, 1964, and unpublished experiments). This refers even to KP mothers where in such litters F_1 hybrids are significantly larger as newborns Musiałek, 1966). Thus it is probable that heterosis first appears at the time of, or after, implantation.

The present results refer only to embryonic stages when the main factor of growth is increase in cell number (Winick & Noble, 1965). It is possible that in later stages, after birth, when enlargement of cell size begins to play the main role in the growth processes, the mechanism of heterosis may be also changed. This will be the subject of further studies.

SUMMARY

Body and liver weight and the number of nuclei in the liver were significantly higher in F_1 hybrid than in inbred embryos developing in the same uterus after artificial heterospermic insemination. The size of 13-day-old F_1 hybrids corresponds

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to the size of 13·4-day inbred embryos. No significant differences in cell size were found between F_1 hybrid and inbred embryos of the same age. It is concluded that in 13–15-day embryos heterosis takes place through increase in cell number and not cell size.

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