Genet. Res., Camb. (1981), **38**, *pp.* 25–46
With 2 text-figures
Printed in Great Britain

The control of body size in mouse chimaeras

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(*Received* 5 *November* 1980 *and in revised form* 17 *December* 1980)

SUMMARY

Aggregation chimaeras were made from embryos of strains of mice selected for large and small body size and of unselected controls. The strains were combined in pairs marked by albino coat colour and by allozyme variants at the *Gpi-1* locus. The proportion of cells derived from each component was scored visually in the coat melanocytes and by electrophoresis in ten other organs or tissues (blood, liver, lung, spleen, spinal cord, brain, pituitary, kidney, adrenal and testis). The object was to find out how body weight is related to cell proportions in the body as a whole and in the separate organs. Individuals varied widely in their mean cell proportions but there were significant differences between organs within individuals. Body weight was linearly related to the mean cell proportions which accounted for most, or possibly all, of the chimaeric variance of body weight. No one of the organs studied could be identified as being solely responsible for growth control, or as having a predominant influence on growth. The weights of some organs were probably influenced to a small extent by their own cell proportions independently of the individual's mean, but the differences of body weight were too great to be accounted for by the summation of localized effects on organs. The mean cell proportion, averaged over individuals, was close to 50%, proving that there was no tendency for cells from the larger component to outgrow those from the smaller. It is concluded that growth control must be systemic, but it was not possible to decide whether the systemic effect comes from some particular organ not studied, or is in some undefined way the consequence of the cell proportions in the body as a whole. There was some evidence, though it was inconclusive, that chimaeras show 'heterosis' for body weight.

1. INTRODUCTION

The aggregation chimaeras to be described in this paper were made by the fusion, or 'aggregation' of two 8-cell embryos derived from different strains. The resultant chimaeric mice contain two populations of cells, one derived from each of the constituent strains that provided the two embryos. Individual chimaeras

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differ widely in the relative proportions of the two cell populations in their bodies. This variation of cell proportions provides an opportunity to study the cellular control of any characteristics by which the two constituent strains differ. Nesbitt (1978) gives a preliminary account of a study of behavioural differences by this means. We made chimaeras of strains differing in body weight with the object of finding out how body weight is related to the cell proportions in the body as a whole and in some of the organs. If there is one particular organ that controls growth rate, we should expect to find body weight to be related to the cell proportions in this organ and not in any other organ. If there is a growth controlling organ or tissue that is derived from a single progenitor cell, we should expect to find a discontinuous distribution of body weight in the chimaeras, some being like one of the constituent strains and some like the other. If the cells of the constituent strains differ in their intrinsic rates of proliferation, we should expect the adult chimaeras to contain a higher proportion of cells from the faster growing strain. Finally, if the growth of individual organs is influenced by their own cells, we should expect to find organ weights to be related to the cellular proportions in that organ. These are the main questions that we set out to answer.

The strains from which the chimaeras were made were differentially marked by variants of the enzyme glucose phosphate isomerase, GPI, at the *Gpi-1* locus, so that the cell proportions in organs and tissues could be estimated by electrophoresis. They were also marked by albino so that the cell proportions in the coat melanocytes could be estimated visually. Some of the chimaeras have been described in two preliminary accounts (Roberts *et al.* 1976; Falconer, Gauld & Roberts, 1978*a*). All the chimaeras marked by the enzyme variant are included in the total of 87 chimaeras to be described here.

2. STOCKS USED AND CHIMAERAS OBTAINED

The mice used were from the Q-strains described by Falconer (1973). There were six replicate lines selected independently for large size, six selected for small size and six unselected controls. Selection was initially continued for 23 generations, after which it was relaxed. At generation 27 most of the lines were found to be polymorphic for the *Gpi-1* locus, and some were segregating for the albino gene, *c* (Garnett & Falconer, 1975). In order to construct stocks suitably marked for making the chimaeras, crosses were made between replicate lines at generations 34, 35, 42 and 43. Pairs of strains differing from each other at both marker loci were constructed from the large lines, from the control lines, and from the small lines, as shown in Table 1. In all three pairs of strains $Gpi\text{-}1^a$ was associated with albino (*c*) and $Gpi\text{-}1^b$ with coloured ($+^c$). After the strains had been made homozygous for their markers they were maintained by random mating. All the chimaeras to be described were made after homozygosis of the markers had been proved. They were made over a period of four years. The mean weights of the constituent strains over this period are given in Table 1.

Having two strains of each size meant that chimaeras from strains of different

sizes could be made reciprocally, and also that chimaeras could be made from strains of the same size. There were thus 9 possible types of chimaera. Of these, 8 are represented in the data, though one type by only 3 animals. Table 2 shows the types of chimaera, their designations and the numbers obtained.

Table 1. *Origins and body weights of the strains used, and markers made homozygous*

Q-lines crossed	Markers	Mean weight (g) males at 6 wks.
LD × LE	*Gpi-1*^{a} *c*	33·4
LB × LF	*Gpi-1*^{b} +	32·7
CA × CB	*Gpi-1*^{a} *c*	25·5
CD × CE	*Gpi-1*^{b} +	21·7
SA × SB	*Gpi-1*^{a} *c*	17·2
SF*	*Gpi-1*^{b} +	16·4

* This strain was not started from a cross, though subsequently a few animals from SE were introduced.

Table 2. *Numbers and types of chimaeras obtained*

				Chimaeras survived and used				
					Overt		Single-component	
Type	Embryos transferred	Chimaeras born	Success rate (%)	Total	♀	♂	Larger	Smaller
L/C	215	6	2·8	5	1	2	0	2
C/L	312	23	7·4	22	5	11	1	5
L/S	346	8	2·3	8	2	4	2	0
S/L	1110	31	2·8	26	4	15	5	2
S/C	122	4	3·3	3	1	2	0	0
L/L	181	8	4·4	6	3	0	3	
C/C	208	8	3·8	8	2	3	3	
S/S	132	9	6·8	9	2	6	1	
					20	43	8	7 9
Totals	2626	97	3·7	87	63		24	

The following terminology will be used. The chimaera types are designated by two letters referring to the sizes of the constituent strains, L for large, C for control and S for small. The first letter always refers to the strain marked by *Gpi-1*^{a} and albino. Thus L/C and C/L, for example, are reciprocal types of L↔C chimaeras, made from two different L-strains and two different C-strains. Chimaeras made from strains of the same size, as L/L, will be referred to as like-size chimaeras. Chimaeras displaying both cell populations in some part of the body will be referred to as overt chimaeras. Animals obtained from aggregated embryos but having only one cell population will be referred to as single-component chimaeras because, though not in fact chimaeric, they have been obtained by the same procedure and treatment. There were no animals that were non-chimaeric in the coat but chimaeric elsewhere in the organs studied.

Altogether there are 63 overt chimaeras for study, of which 47 were from strains differing in size and 16 were like-size chimaeras. The sex ratio among the overt chimaeras is not significantly different from the expected 75% of males (McLaren, 1976). There are 24 single-component chimaeras, which will be used for various comparisons. The proportion of 28% single-component chimaeras is in line with other studies (See Falconer and Avery, 1978, for a discussion of their origin).

3. METHODS

The method of aggregation followed was that described by Bowman and McLaren (1970). The host females to which the cultured embryos were transferred were mostly from the Control strains, though later females from the CFLP strain (Carworth, Europe) were used. At first, vasectomized males were used to induce pseudopregnancy in the host females; later the females were mated to entire males genetically marked by *Re Re*, and the chimaeras were then reared in litters with the progeny of the mating. The success rates in obtaining live young from aggregated embryos was rather low (Table 2). Dissection of host females that failed to produce litters proved that the losses of chimaeric embryos were almost all pre-implantation. The success rate of the C/L type is significantly higher than in the others. This may have been due to intrinsic properties of the C/L strain combination, but is more likely to have been due to unidentified technical factors because most of these chimaeras were made over a short period of time when no others were made.

The proportion of albino in the dorsal coat pigmentation was scored visually in 5 percent intervals. This was done at 3 weeks, 6 weeks, and when the chimaera was killed. It was also done again later, on the dried skins, for reasons that will be explained later. Most of the chimaeras were killed at 6 weeks of age, but those of one type, the C/L, were kept for breeding tests and were killed at 50 weeks. (The breeding tests conformed to expectation and are not described.) The organs studied are listed later, in Table 6. The carcasses were thoroughly drained of blood before removal of the organs. The proportions of the enzyme markers in the organs were estimated by electrophoresis of serial dilutions (Klebe, 1975), the electrophoresis being done by the method described by Shaw and Prasad (1970). The enzyme extracted from the smallest organs – pituitaries and adrenals – was barely enough for the serial dilutions. Consequently the assays of these organs were obtained from only some of the mice, and the cell proportions are less reliably estimated than those of the other organs. (Ovaries were also assayed but are not included in any of the analyses because too few records were obtained.) The cell proportions were estimated by the serial dilution method as follows.

Chimaeras contain a mixture of the two allozymes, and produce two bands on the gel, hybrid bands being absent from the tissues studied. The two allozymes have approximately the same specific activities (Padua, Bulfield & Peters, 1978), so the relative density of staining of the bands depends on the relative amounts of the two allozymes in the extract, and this in turn depends on the proportions of

the two cell populations in the tissue. Two dilutions of the extract are found which equalize the density of staining of the two bands, so that the amount of one allozyme in one dilution is equal to the amount of the other allozyme in the other dilution. The relative amounts of the allozymes in the original extract are then found from the dilution factor. Equality of staining is most easily judged by making a series of dilutions in equal steps and noting the dilutions at which each band just becomes invisible, i.e. the extinction points. Choice of the concentration of the initial extract and of the dilution factor depend on two things: (*a*) the number of dilutions that can be run in parallel on the same gel, and (*b*) the most extreme cell proportions that it is desired to quantify. The number of dilutions that could be run on the same gel was fifteen, and the most extreme cell proportions were taken to be 5% and 95%. By running artificial mixtures of the allozymes in the proportions 5:95, the initial concentration and the dilution factor were chosen so that the allozyme at 5% was visible in dilution-0 but not in

Table 3. *Scale of cell proportions*

(P%, estimated from the number of dilution steps, n, by which the extinction points differ, with a dilution factor of $r = 0{\cdot}8$).

n	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
P%	50·0	55·6	61·0	66·1	70·9	75·3	79·2	82·7	85·6	88·2	90·3	92·1	93·6	94·8	95·8

dilution-1, while the allozyme at 95% was visible in dilution-14 but not in dilution-15. By choice of an appropriate value of dilution-0 for each organ-extract, it was found that a dilution factor of 4/5 met these requirements, i.e. each dilution had a concentration of 0·8 of the next stronger one. The cell proportions are then obtained as follows. Let r be the dilution factor and n the number of dilution steps by which the extinction points differ. Then the proportion of cells giving the stronger band is $P = 1/(1+r^n)$. The cell proportions are not linearly related to the number of steps by which the extinction points differ. Consequently the method is more sensitive to differences of cell proportions at the extremes than it is near the middle of the range. Table 3 gives the cell proportions corresponding to the step differences. Tests of known mixtures of the allozymes showed that 50:50 mixtures had the same extinction points ($n = 0$), and that the method gave reliable readings for other mixtures. The small differences in activity noted by Padua *et al.* (1978) were therefore not enough to cause detectable error.

There were not enough chimaeras to justify analysing the sexes separately. Therefore for analyses involving body weights, except where otherwise stated, the weights of females were converted to male-equivalents by multiplying them by the factor 1·2, which was previously found to apply to the Q-strains generally (Falconer, 1973). In order to eliminate some of the environmental variation of body weight, adjustments were made for differences in the size of litter in which the chimaeras were born. The male-equivalent weights were adjusted by regression to a standard litter size of 4. The regression coefficient used was $b = -0{\cdot}59$ g per unit of litter size. This regression was estimated from the two control strains since most of the chimaeras were reared by females of these strains. Where mice of the

constituent strains are used for comparisons, their weights are adjusted to the same standard of 4, by the regressions estimated from the strains themselves, which were −0·87 for the two large strains, −0·59 for the controls, and −0·19 for the two small strains. The adjusted weights of the constituent strains contemporaneous with the chimaeras to which they gave rise are given in Table 4.

Table 4. *Mean 6-week weights (g) of the constituent strains for comparisons with the chimaeras*

(The weights are of males contemporaneous with the chimaeras and adjusted by regression to a standard litter size of 4.)

Chimaera type	Component L	Component C	Component S	Difference
L/C	35·35	24·38	—	10·97
C/L	41·32	28·88	—	12·44
L/S	35·96	—	16·32	19·64
S/L	37·98	—	17·30	20·68
S/C	—	23·01	17·79	5·22
	1st	2nd	Mean	
L/L	37·86	41·22	39·54	
C/C	28·70	23·25	25·98	
S/S	17·97	17·97	17·97	

4. RESULTS

(i) *Cell proportions*

There are several questions to be answered about cell proportions in overt chimaeras before body weight is brought into consideration. The main question is whether there is any tendency for cells from the larger constituent strain to outgrow those from the smaller. Other questions concern the distributions of cell proportions, the correlations between organs, and whether there are real differences in proportions between organs of the same individual.

Mean cell proportions. Let P be the proportion of cells from the larger strain ('large' cells for short), in any organ of an individual. The mean of all the organs measured in a particular individual estimates the cell proportion in the body as a whole. This will be referred to as the mean cell proportion and symbolized by $\bar{P}$. The mean cell proportion of an individual, $\bar{P}$, will first be taken as the unweighted mean P of all the organs measured, paired organs being averaged. Table 5 shows the mean value of $\bar{P}$ averaged over the individuals in each chimaera type. None of the means is significantly different from 50%, nor are the chimaera types significantly different from each other. The conclusion is therefore clear that, when averaged over all organs, there is no tendency for one cell population to outgrow the other.

Table 6 shows the cell proportions, P, in each organ separately, averaged over all individuals. It also shows the variance of P about 50% and the pooled variance within chimaera types. A significant reduction of the within-type variance, when

Table 5. *Mean cell proportions in overt chimaeras*

(The values tabulated are the means of $\bar{P}$%, where $\bar{P}$ is the mean cell proportion of all organs of an individual. The standard errors are derived from the variance of $\bar{P}$ among individuals. For the L/L, C/C and S/S types, $\bar{P}$ is the proportion of cells from the albino, *Gpi-1*^{a} component; for all the other types it is the proportion of cells from the larger component strain. The standard errors are based on the pooled variance within chimaera-types.)

Chimaera type	number of individuals	Mean of $\bar{P}$, %, ± S.E.
L/C	3	28·6 ± 12·1
C/L	16	41·2 ± 5·2
L/S	6	53·2 ± 8·6
S/L	19	49·0 ± 4·8
S/C	3	45·5 ± 12·1
L/L	3	37·1 ± 12·1
C/C	5	52·6 ± 9·4
S/S	8	61·0 ± 7·4

(Note: The standard errors of the C/L and S/L types given in Falconer, Gauld & Roberts (1978*a*) were inappropriate, and the conclusion that C/L differed significantly from 50% was wrong.)

Table 6. *Means, variances about* 50% *, and within type variances of the proportions of* Gpi-1^a *in all overt chimaeras*

	All overt chimaeras ignoring type				
		Variance about 50%		Within type	
Organ	Mean ± S.E.	D.F.	Variance	D.F.	Variance
Coat	45·9 ± 3·4	63	715	55	655
Blood	46·4 ± 3·9	63	939	55	857
Liver	50·9 ± 3·1	62	581	54	572
Lung	49·7 ± 3·0	63	559	55	561
Spleen	47·1 ± 3·0	61	559	53	491
Sp. Cord	44·9 ± 3·0	47	448	39	440
Brain	47·9 ± 2·1	63	272	55	265
Pituitary	46·4 ± 3·5	50	627	42	444
Kidney	48·1 ± 2·8	63	503	55	487
Adrenal	49·8 ± 3·5	46	552	39	509
Testis	51·3 ± 4·0	43	684	36	712
Mean $\bar{P}$	47·5 ± 2·7	63	452	55	441

compared with the variance about 50%, would suggest that there were differences between type means, or between the overall mean and 50%. In fact the reduction is significant only in the pituitary ($P < 0{\cdot}01$). Here the differences between pituitary means are largely attributable to the configuration of missing pituitary values which causes the means of two types, L/L and S/S, to take extreme values. We regard this as a chance effect and proceed under the assumption that for each organ and each chimaera type the cell proportion is distributed about a mean of 50%.

Distribution of cell proportions. Several series of chimaeras have shown that the

cell proportions in the coats have a more or less uniform distribution, all proportions in overt chimaeras being about equally frequent (Falconer & Avery, 1978). In this respect chimaeras differ from X-inactivation mosaics, which are much less variable in cell proportions in the coat. Falconer & Avery (1978) showed how a uniform distribution could arise from the sampling of cells to form the primary ectoderm in embryos of chimaeras. We have examined the distributions of cell proportions in the organs studied here, to see if they also show uniform distributions. In χ^2 goodness of fit tests, using 8 class intervals of width 12·5 %, there were significant ($P < 0{\cdot}05$) deviations from uniformity in six organs, namely liver, lung, spleen, spinal cord, brain and kidney. Moreover Table 6 shows that only the blood has a variance which exceeds the theoretical value of 833 for a uniformly distributed percentage. We conclude that the cell proportions in most organs have a distribution which is more concentrated around 50 % than the uniform distribution. The theory of Falconer & Avery (1978) therefore cannot be right in all details. To account for the distributions found here, however, it is only necessary to suppose that some cell mixing occurs before the separation of the primary ectoderm from the primary endoderm. Note that the variances in Table 6 do differ appreciably between organs, and that the brain, surprisingly, has smaller variance than the mean $\bar{P}$. We shall return to this point later in this section.

Correlations of cell proportions. The cell proportions in the organs of individuals are highly correlated. Table 7 gives the simple correlations of all organ-pairs in all the overt chimaeras irrespective of type. The correlations range from 0·37 to 0·89, and the average is 0·73. There are no obvious differences among the organs in the mean level of their correlations with other organs, except the testis which is on average clearly less highly correlated than other organs. The fact that blood does not show a higher than average correlation with other organs shows that contamination by blood has probably not introduced any serious error.

The left- and right-hand members of the paired organs are much more highly correlated than are different organs, all the correlations being over 0·9. These correlations are shown in the diagonal of Table 7. The coat was treated as a 'paired organ' in the following way. As mentioned under Methods, the coats were rescored from the dried skins. As far as possible the scoring was based on the dorsal part of the coat in order to correspond with the scoring of the live animal. The skins of all the overt chimaeras were examined in turn four times, scoring first the whole dorsal coat, second the left half, third the right half, and finally the whole coat again. The repeated whole-coat scores will be used in the next section. The left-side and right-side scores are used as a paired organ.

A question of interest is whether organs on the same side of the body are more highly correlated than organs on different sides. This possibility was tested by comparing ipsi-lateral and contra-lateral correlations among the paired organs. Ipsi-lateral correlations were calculated from, for example, left kidney with left adrenal and right kidney with right adrenal, these two correlations being then averaged. Contra-lateral correlations were the average of left kidney with right adrenal and right kidney with left adrenal. There were 6 such inter-organ correla-

tions among the 4 paired organs. The ipsi-lateral correlations were very slightly, but non-significantly, greater than the contra-lateral; the mean difference being $0{\cdot}005 \pm 0{\cdot}005$. There seems, therefore, to be little or no tendency for the cell proportions to differ on the two sides of the body as a whole.

Components of variation in organ cell proportions. The large positive correlations between the cell proportions of all pairs of organs indicate that the main component of variation between individuals is a variable which represents the individuals' mean cell proportions. The unweighted mean, $\bar{P}$, could be used to represent this

Table 7. *Simple correlations between pairs of organs in respect of cell proportions*

(On the diagonal, correlations between left and right sides of paired organs. The mean at the foot of the table is the unweighted mean of the 10 correlations of each organ, excluding left-right correlations. (n) is the number of individuals with records of the organ.)

Organ	(n)		1	2	3	4	5	6	7	8	9	10	11
Coat	(63)	1	0·92	—	—	—	—	—	—	—	—	—	—
Blood	(63)	2	0·70	—	—	—	—	—	—	—	—	—	—
Liver	(62)	3	0·63	0·66	—	—	—	—	—	—	—	—	—
Lung	(63)	4	0·70	0·85	0·81	—	—	—	—	—	—	—	—
Spleen	(61)	5	0·70	0·86	0·72	0·83	—	—	—	—	—	—	—
Sp. Cord	(47)	6	0·77	0·81	0·74	0·88	0·77	—	—	—	—	—	—
Brain	(63)	7	0·74	0·74	0·73	0·80	0·76	0·85	—	—	—	—	—
Pituit.	(50)	8	0·68	0·80	0·68	0·79	0·82	0·82	0·80	—	—	—	—
Kidney	(63)	9	0·77	0·80	0·82	0·89	0·80	0·87	0·84	0·79	0·92	—	—
Adrenal	(46)	10	0·71	0·76	0·70	0·74	0·78	0·70	0·80	0·85	0·81	0·93	—
Testis	(43)	11	0·54	0·45	0·59	0·52	0·47	0·37	0·58	0·62	0·63	0·72	0·97
Mean			0·69	0·74	0·71	0·77	0·75	0·76	0·76	0·76	0·80	0·76	0·55

variable. However, the variances of $P-\bar{P}$, given in the first column of Table 8, differ substantially between organs and suggest that an average is better estimated iteratively by the weighted mean, $\bar{P}_w$, with weights inversely proportional to $\mathrm{var}(P-\bar{P}_w)$. These variances, $\mathrm{var}(P-\bar{P}_w)$, are given in the second column of Table 8. The values of the weighted mean, $\bar{P}_w$, are very similar to those of the unweighted mean, $\bar{P}$. The mean of $\bar{P}_w$ is $47{\cdot}7 \pm 2{\cdot}6$, the correlation between $\bar{P}_w$ and $\bar{P}$ is 0·994 and the variance of $\bar{P}_w - \bar{P}$ is 5·75.

It would be natural to represent an organ cell proportion P as the sum of two components, the individual's mean, $\bar{P}_w$, and the organ deviation $P-\bar{P}_w$. This representation would be particularly useful if the two components, $\bar{P}_w$ and $P-\bar{P}_w$, were independent. That such independence cannot be assumed is shown by the correlations between $P-\bar{P}_w$ and $\bar{P}_w$ given in the third column of Table 8. Two of the correlations are clearly significant, blood with $+0{\cdot}41$ and brain with $-0{\cdot}63$. The meaning of these correlations can be stated as follows. If an individual has its mean cell proportion above 50 % then the blood tends to be above the mean and the brain below; conversely an individual with mean below 50 % tends to have its blood below the mean and its brain above. Or, in other words, over all individuals blood tends to deviate more from 50 % than the mean does, while brain deviates less. In consequence the blood has a higher variance than the mean and the

brain has a lower variance, as was noted earlier in this section, and shown in Table 6. The relationship between deviation and mean must obviously be non-linear because the deviation $(P-\bar{P}_w)$ must be zero at three points, when $\bar{P}_w$ is 0, 50 and 100%.

Table 8. *Estimates of unexplained variation and the parameters of models relating cell proportions of individual organs to their average*

				Logistic regression of P on $\bar{P}_w$	
Organ	Var $(P-\bar{P})$	Var $(P-\bar{P}_w)$	Correlation of $P-\bar{P}_w$ with $\bar{P}_w$	$\beta \pm$ S.E.	Residual variance
Coat	221	245	0·06	1·05 ± 0·12	246
Blood	225	247	0·41	1·55 ± 0·14	203
Liver	163	160	0·03	1·00 ± 0·10	162
Lung	83	65	0·25	1·13 ± 0·06	63
Spleen	119	121	0·00	1·00 ± 0·09	123
Sp. Cord	91	67	−0·27	0·90 ± 0·06	64
Brain	96	82	−0·63	0·66 ± 0·04	51
Pituitary	122	143	0·20	1·18 ± 0·11	141
Kidney	75	55	0·09	1·05 ± 0·06	55
Adrenal	109	140	0·16	1·13 ± 0·12	139
Testis	342	404	−0·06	0·92 ± 0·19	413

A simple empirical model, which satisfies these constraints and gives rise to the observed correlations, is one that relates $E(P)$ to $\bar{P}_w$ linearly on the logistic scale. If $E(P)$ is the conditional expectation of P given $\bar{P}_w$ the model is:

$$\log \frac{E(P)}{100-E(P)} = \beta \log \frac{\bar{P}_w}{100-\bar{P}_w}.$$

Here the coefficients β vary between organs. A value of β less than unity corresponds to a negative correlation between $P-\bar{P}_w$ and $\bar{P}_w$, a value of β greater than unity corresponds to a positive correlation. Estimates of the β, and the residual variances of the $P-E(P)$, are given in the final two columns of Table 8. We shall not consider this model further except to note that an equally good empirical fit is obtained by the simpler linear regression

$$E(P)-50 = \beta(\bar{P}_w-50)$$

This has implications for the later study of the dependence of body weight on the components $\bar{P}_w$ and $P-\bar{P}_w$. Models of development that could give rise to the observed correlations of $P-\bar{P}_w$ with $\bar{P}_w$ will be considered in the Discussion.

Comparison of residual organ cell proportion variation with assay error. There would be no point in studying the deviations $P-\bar{P}_w$ further unless they represented real organ differences within mice rather than just assay error. To confirm that the $P-\bar{P}_w$ represent real organ effects we need to compare their variances with the error-variance due to errors of estimation of cell proportions. The enzyme-assays were not replicated, but there are nevertheless three ways by which their

error variance can be estimated. These estimates are all biased upwards, so that the significance of differences between organs will be under-estimated. The sources of the estimates are the following. (1) The spinal cord was divided into three roughly equal parts and each part was assayed separately but on the same day. The bias comes from differences in cell proportions between the three parts. (2) The blood was assayed on two occasions – at 6 and 50 weeks in the C/L chimaeras and at 3 and 6 weeks in all the others. The bias comes from changes in cell proportions with time. (3) Paired organs – kidney, testis, adrenal – were assayed separately. The bias comes from real differences between left and right organs.

The cell proportions in the coat need not have the same error variance as the other organs because they were measured differently. For the error variance of the coat scores we have three estimates:

(1) Measurements on the live animals repeated at 3 weeks and 6 weeks,

(2) Two repeated measurements on skins, as described earlier, and

(3) The left and right sides of skins.

Table 9. *Estimates of the error variance of percentage cell proportions*, P

	D.F.	Variance
Enzyme assays		
1. Spinal cord	94	4·77
2. Blood	61	49·23
3. Kidneys	60	36·08
Testes	42	18·15
Adrenals	38	37·84
Coat score		
1. 3–6 weeks	63	0·79
2. Whole skin	62	8·68
3. Half skins	62	45·97

Table 9 gives the various estimates of error variance in enzyme proportions and in coat scores. The estimates are all substantially smaller than the corresponding organ variances in Table 8, except in the case of the kidney where the difference is smaller but is significant ($P < 0{\cdot}05$). For these organs at least we may conclude that the $P - \bar{P}_w$ do measure real organ differences.

(ii) *Weight and cell proportions*

In this section we shall deal first with the relation between body weight and mean cell proportions, and then with its relationship with the cell proportions in separate organs.

Mean cell proportion. Fig. 1 shows the relationship between 6-week body weight and weighted mean cell proportion, $\bar{P}_w$, in the two chimaera types with the largest numbers. Body weights are male-equivalents adjusted for litter size. The mean weights of the contemporary constituent strains, from Table 4, are shown by arrows in the margins. It is very obvious that the weights are strongly influenced by the mean cell proportions. The calculated linear regressions of weight on $\bar{P}_w$

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are shown with the 95 % confidence limits of predicted mean weights. The various parameters estimated from the regression analyses are given in Table 10.

In order to combine chimaeras of all types into a single analysis, weights were scaled to a standard difference between the constituent strains. For each chimaera a 'relative weight', w, was calculated as follows,

$$w = \frac{W - S}{L - S}$$

where W is the actual weight, and L and S are the mean weights of the larger and smaller constituent strains respectively. Where cell proportions are expressed as

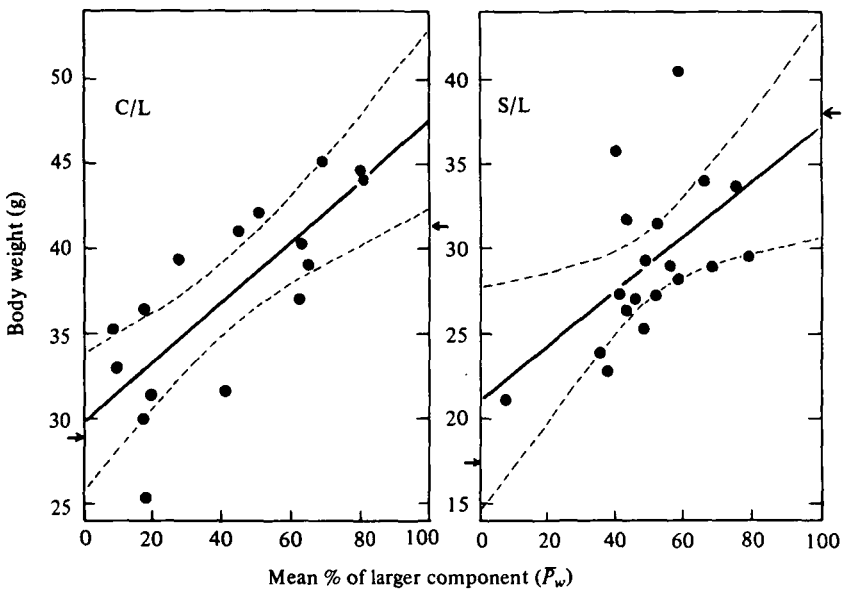

Fig. 1. Body weight at 6 weeks in relation to weighted mean proportion, $\bar{P}_w$, of cells from the larger component. Each point represents one individual. The arrows at the margins show the mean weights of the contemporaneous constituent strains. The lines are the fitted linear regressions of weight on $\bar{P}_w$, with 95 % confidence limits of predicted mean weight.

Table 10. *Regression analysis of body weight* (W) *on mean cell proportion,* $\bar{P}_w$ *in percentage units, for C/L and S/L chimaera types*

	C/L	S/L
Number of mice	16	19
Correlation, W with $\bar{P}_w$	0·77	0·55
Strain difference, D	12·44 g	20·68 g
Regression, W on $\bar{P}_w$	0·177 ± 0·039	0·161 ± 0·058
Intercept at $\bar{P}_w = 0$	29·8 ± 1·9	21·0 ± 3·1
Intercept at $\bar{P}_w = 100$	47·6 ± 2·5	37·1 ± 3·1
Variance of $\bar{P}_w$	639·9	260·3
Total variance of W	33·89	22·05
Residual variance of W	14·65	16·29
σ_z^2 (Table 12)	10·36	10·36
Chimaeric variance not accounted for by $\bar{P}_w$	4·29	5·93

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percentages, relative weights are also expressed as percentages, which means that the $L-S$ difference is standardized to 100. We assumed that the unexplained variation in weight W about any regression on organ cell proportions has a constant variance σ_w^2 over all chimaera types. Thus the variance of the relative weight w is $\sigma_w^2 (L-S)^{-2}$ which varies between chimaera types. All regressions involving w as the dependent variable were therefore estimated by weighted least squares using weights $(L-S)^2$.

The relationship in all chimaeras between relative weight w and mean cell proportion $\bar{P}_w$ is shown in Fig. 2. Estimates of linear regressions within chimaera

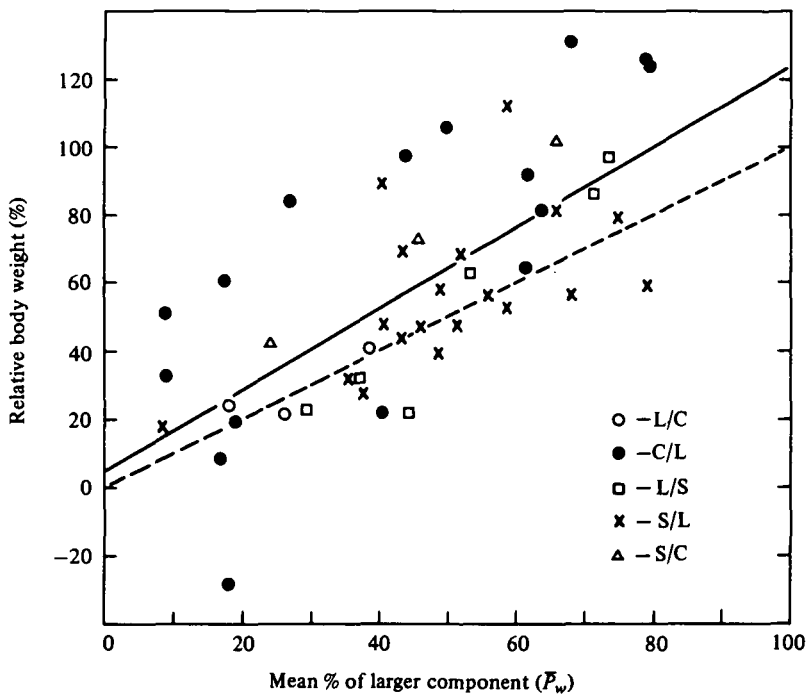

Fig. 2. Relative body weight in relation to weighted mean cell proportion, $\bar{P}_w$, both in percentage units. The continuous line is the fitted linear regression; the broken line joins the means of the constituent strains. Each point represents one individual.

Table 11. *Linear regressions of relative body weight* w *on mean cell proportion* $\bar{P}_w$, *in percentage units, for all five chimaera types*

Chimaera type	Intercept at $\bar{P}_w$ = 50% ± S.E.	Slope	Residual variance of Weight σ_w^2
L/C	48·8 ± 55·5	0·89 ± 2·32	
C/L	79·1 ± 7·8	1·43 ± 0·30	
L/S	50·9 ± 7·7	1·75 ± 0·47	13·55
S/L	56·9 ± 4·1	0·78 ± 0·26	(pooled within types)
S/C	79·6 ± 42·4	1·41 ± 2·37	
Combined	59·6 ± 3·3	1·08 ± 0·18	14·59

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types, and of the overall regression ignoring chimaera type, are given in Table 11. Inspection of both Fig. 2, and intercept estimates in Table 11, suggest that the regression in the C/L chimaera type has a higher elevation than in other chimaera types. However, the F statistics for differences between slopes, and for differences between intercepts assuming a common slope were both non-significant. If there are any differences in elevation they probably resulted from errors in the strain means from which the relative weights were calculated.

The conclusions that can be drawn from the regression coefficients are rather limited. The regression lines must pass through the weight of the larger strain at $\bar{P}_w = 100\,\%$ and of the smaller strain at $\bar{P}_w = 0\,\%$. The expected linear regression of weight on cell proportion in percentage units is therefore D/100, where D is the difference in weight between the constituent strains; and the expected linear regression of relative weight on $\bar{P}_w$ is 1. The slope of the fitted overall regression agrees well with this expectation, but the intercept at $\bar{P}_w = 50\,\%$ is significantly higher than the expected value of 50. There is no evidence from a graphical study of the residuals that the regression is non-linear. We can therefore conclude that the body weight is linearly dependent on the cell proportion in some organ or organs.

The variance of weight and its partitioning by the regression analysis is more informative than the regression coefficients themselves. The regression on $\bar{P}_w$ partitions the variance as

$$\text{total variance} = (\text{variance due to } \bar{P}_w) + (\text{residual variance}).$$

An alternative partition is

$$\text{total variance} = (\text{'chimaeric variance'}) + (\text{'non-chimaeric' variance})$$

where 'chimaeric variance' is associated with differences in cell proportions and 'non-chimaeric' variance is the remainder not associated with cell proportions. The non-chimaeric variance is mainly environmental, but it contains also a component due to genetic differences within the constituent strains because individual chimaeras made from the same two strains will not have exactly the same genotypic values of their component embryos. This is non-chimaeric variance because it is not associated with differences of cell proportions. The two components of the non-chimaeric variance, however, do not need to be distinguished in what follows. The two partitions of total variance are not identical because the variance due to $\bar{P}_w$ forms part, but not necessarily all, of the chimaeric variance. There may be components of chimaeric variance not attributable to $\bar{P}_w$. In particular there may be components of chimaeric variance attributable to regressions on the deviations $P - \bar{P}_w$ of organs which influence body weight. We wish to know whether such components can exist, for it is only by identifying them that we shall be able to identify organs which influence body weight. By comparing

$$\begin{aligned}\text{total variance} = {} & (\text{chimaeric variance due to } \bar{P}_w) \\ & + (\text{chimaeric variance not due to } \bar{P}_w) \\ & + (\text{non-chimaeric variance})\end{aligned}$$

with the regression partition we deduce that

$$\text{chimaeric variance not due to } \bar{P}_w = (\text{residual variance}) - (\text{non-chimaeric variance}).$$

The possible existence of chimaeric variance attributable not to regression on $\bar{P}_w$, but to regression on the $P - \bar{P}_w$, can therefore be inferred from comparison of estimates of residual and non-chimaeric variances.

Table 12. *Non-chimaeric variance of body weight, estimated from like-size and single-component chimaeras*

Genotype	D.F.	Variance, g^2
Large	12	12·99
Control	13	8·82
Small	9	9·09
Pooled	34	10·36

Table 13. *Regressions of relative body weight,* W *on proportion of 'large' cells in each organ. For each organ* W *is regressed in turn on* P *alone, on* $\bar{P}_w$ *alone, on both* $\bar{P}_w$ *and* P.

Organ	Number of individuals	Linear regression w on $P \pm$ S.E.	Residual variances of W about regression on		
			P alone	$\bar{P}_w$ alone	both $\bar{P}_w$ and P
Coat	47	0·78 ± 0·15	16·10	14·59	14·30
Blood	47	0·68 ± 0·13	16·11	14·59	14·80
Liver	46	0·63 ± 0·17	18·74	13·94	13·98
Lung	47	0·85 ± 0·16	16·40	14·59	14·87
Spleen	45	0·80 ± 0·18	17·85	14·10	14·34
Sp. Cord	39	0·99 ± 0·18	13·93	13·07	13·36
Brain	47	1·22 ± 0·26	17·79	14·59	14·87
Pituitary	38	0·77 ± 0·18	17·47	13·94	14·21
Kidney	47	0·95 ± 0·16	14·77	14·59	14·73
Adrenal	33	0·64 ± 0·17	14·51	11·29	11·64
Testis	34	0·36 ± 0·18	21·56	14·77	14·80

An independent estimate of the non-chimaeric variance comes from single-component chimaeras and from overt chimaeras of like-sized strains. These chimaeras contain cells of a single size-genotype, that is to say the origins of their cells are either all Large, or all Control or all Small. Their variance represents all of the non-chimaeric variance, including any variance due to genetic differences within the constituent strains. Table 12 gives the estimates of the non-chimaeric variance derived from each of the three size-genotypes. The estimates do not differ significantly by Bartlett's test, so they are pooled to give a joint estimate of 10·36 g^2. The residual variance about the regression on $\bar{P}_w$ was estimated as 14·65 and 16·29 respectively in the C/L and S/L chimaera types (Table 10), and as 14·59 over all chimaera types (Table 11). None of these estimates differ significantly from the estimated non-chimaeric variance. Thus although there may be components of chimaeric variance not accounted for by $\bar{P}_w$, whose sum is best

estimated as $14{\cdot}59 - 10{\cdot}36 = 4{\cdot}2$, there is no significant evidence that such components exist.

Separate Organ Cell Proportions. It has been established that each organ cell proportion, P, may be expressed as the sum of two components $\bar{P}_w$, which is common to all organs, and the organ deviation $P - \bar{P}_w$ which depends on the organ but is approximately linearly dependent on $\bar{P}_w$ in some organs. The fact that the body weight is linearly dependent on $\bar{P}_w$ establishes a dependence on the cell proportions in one or more organs. To associate this dependence with a particular organ the dependence of body weight on the deviation $P - \bar{P}_w$, additional to the dependence on $\bar{P}_w$, must be established. An additional dependence of body weight on $P - \bar{P}_w$ would be indicated either by P being a better predictor of body weight than $\bar{P}_w$, or by a partial regression of body weight on P, eliminating $\bar{P}_w$.

Table 13 gives the statistics derived from simple regressions of relative body weight on P alone and on $\bar{P}_w$ alone, and from the multiple regression on $\bar{P}_w$ and P together. The main interest lies in the residual variances about these regressions: the lower the variance, the better is the predictor of body weight. The results are easily summarized: (1) for each organ, the organ itself (P) is a poorer predictor than the mean ($\bar{P}_w$). (2) For each organ, the addition of $\bar{P}_w$ to P gives a better prediction than P alone, and the decrease of residual variance is significant ($P < 0{\cdot}05$) for all organs except spinal cord and kidney. (3) For each organ, the addition of P to $\bar{P}_w$ does not give a significantly better prediction than $\bar{P}_w$ alone. In fact the residual variances are increased for all organs except the coat for which, however, the decrease is not significant.

Two conclusions can be drawn from these results. First, from (2), no one of these organs, with the possible exception of the spinal cord and kidney, can be solely responsible for determining body weight. Second, from (3), no one of these organs can be identified as playing a predominant role in determining body weight. The question of whether there may be some other organ that is the principal growth-controlling organ will be considered in the Discussion.

(iii) *Organ weights*

Is the weight of an organ influenced in any degree by the cells that it contains, independently of the cells in the rest of the body? For example, if a liver contains a higher proportion of 'large' cells than the rest of the body, will it be relatively larger in consequence? To answer this question we calculated the partial regression of organ weight on cell proportions in the organ with mean cell proportions held constant. The organ weights were first adjusted to a standard body weight. For this purpose weights were transformed to logs because log organ weights had previously been found to be linearly related to log body weights (Falconer, Gauld & Roberts, 1978*b*). For each organ, regressions of log organ weight on log body weight were calculated from all the chimaeras of all types. These regressions were then used to adjust the organ weights of overt chimaeras to a standard body weight. The kidneys of females were adjusted to male equivalents since the relative weights of female and male kidneys had been found to differ (Falconer *et al.*

1978*b*). The regressions of these adjusted log organ weights were then calculated from all overt chimaeras of unlike-sized strains combined, the regression being the partial regression on the proportion of cells from the larger component in the organ, with the mean cell proportion, $\bar{P}$, held constant. (The unweighted mean was used for these calculations.) The results for eight organs are given in Table 14. None of the regressions is significantly different from zero, though the liver and kidney are close to significance (P ~ 0·06). Seven of the eight regressions are positive, which by a sign-test has a probability of P = 0·07. The organs do not differ significantly in their regressions. The regression pooled within organs is not significantly different from zero (P ~ 0·2). The pituitary and adrenal might be excluded on the grounds that their small size makes their records unreliable. Omitting them the pooled regression is still not quite significant (P = 0·065).

Table 14. *Partial regressions of* log_{10} *organ-weight (adjusted to standard body weight) on proportion of 'large' cells,* P, *in the organ, with mean cell proportion,* $\bar{P}$ *held constant*

Organ	D.F.	Regression ± S.E.	*t*	% effect†	Cell mass diff. %‡
Spleen	42	0·238 ± 0·241	0·99	73	24
Kidney	43	0·198 ± 0·103	1·93	58	44
Brain	44	0·132 ± 0·091	1·45	36	—
Liver	43	0·111 ± 0·057	1·95	29	57
Testis	31	0·067 ± 0·178	0·38	17	—
Lung	44	0·060 ± 0·093	0·64	15	17
Adrenal	29	0·057 ± 0·330	0·17	14	—
Pituitary	35	−0·234 ± 0·301	0·78	−42	—
Pooled (1)*	325	0·076 ± 0·061	1·24	19	
Pooled (2)*	257	0·105 ± 0·057	1·84	27	

* Pooled within organs, (1) all organs, (2) with adrenal and pituitary excluded.

† 100 (antilog of regression − 1). See text for explanation.

‡ Percentage difference, L − S, in cell size, from Falconer, Gauld & Roberts (1978*b*).

Though not conclusive, the evidence does point fairly strongly to a local influence of cell proportions on the size of some organs. A localized effect on organ size is made more credible by the fact that the shapes of vertebrae are influenced by their cellular composition (Moore & Mintz, 1972). If the effect on organ weight is real, could its summation over all organs account for the effect on body weight as a whole? The ratio of body weights of the constituent strains was, on average, about L/S = 1·82, so that the larger was about 82% heavier than the smaller. The comparable percentage differences produced by the localized effects on organs are shown in the column headed '% effect' in Table 14. The meaning of these '% effects' on organs is this. If two individuals have the same $\bar{P}$ and one had, say, a liver with all 'small' cells while the other had its liver with all 'large' cells, then the latter liver would be 29% heavier than the former. So, if all organs had the same localized effect as the liver, and there were no effect of a growth-controlling organ, a mouse with all 'large' cells in its body would be 29% heavier

than one with all 'small' cells. This is much less than the actual difference of 82 %. The localized effects on the liver and the lung are significantly less than 82 %, and so are the two pooled effects. It is therefore very unlikely that the overall body weight is determined simply by the summation of localized effects on organ weights.

The localized effects of cellular genotype, if real, could be the consequence of differences of cell size. The cell size (mass of organ per nucleus) in four of these organs was studied by Falconer, Gauld and Roberts (1978*b*). In all four organs the cells of the Large strain were larger than those of the Small at 6 weeks of age. The percentage difference is given in the right-hand column of Table 14. The correspondence for each organ is not close, but the averages of the four organs are not very different, 44 % for the localized effect in the chimaeras and 36 % for the cell size effect. The correspondence may be no more than a coincidence. We point it out to show that the localized effect of the cellular genotype on organ weight may be mediated through its effect on cell size.

(iv) *Chimaeric heterosis*

Chimaeras are generally acknowledged to be relatively large and healthy individuals. This raises the question of whether they benefit, in a manner analogous to heterosis, from the mixture of cell populations of different origin. We shall therefore examine the evidence for 'heterosis' for body weight in our material. First, however, we must know whether the strains used to make the chimaeras show heterosis in the ordinary genetic sense when crossed. Crosses were therefore made and it is sufficient to say that they showed on average about 12 % heterosis for weight at 6 weeks, heterosis being defined as the difference between the F_1 and mid-parent means.

There are two independent ways in which heterosis in the chimaeras can be looked for: both are suggestive but unfortunately inconclusive. The first way is by consideration of the elevation of the regression line in Fig. 2. If there were no heterosis the points would be equally distributed above and below the broken line joining relative weights of 0 and 100. It is obvious that there are more points above than below. The observed regression does not differ from the broken line in slope but it does so in elevation. The predicted mean relative weight at $\bar{P}_w = 50$ is $59{\cdot}6 \pm 3{\cdot}3$, which is significantly greater than the value of 50 % expected with no heterosis ($t_{45} = 2{\cdot}91$; $P < 0{\cdot}01$). This therefore looks like convincing evidence for chimaeric heterosis. The significance test, however, takes no account of the errors in estimating the mean weights of the constituent strains, and it is not possible to arrive at a reliable figure for this error. It is noticeable that most of the evidence for heterosis in Fig. 2 comes from one chimaera type, the C/L (see Table 11). We therefore think that the evidence from Fig. 2 cannot be regarded as proving the existence of chimaeric heterosis. There is, moreover, a possible reason for the evidence from the C/L chimaeras being spurious. The weights analysed were those at 6 weeks but the cell proportions of these chimaeras were determined at 50 weeks, and the mean of $\bar{P}$ was well below 50 % (Table 5). If there were a progressive reduction of the proportion of 'large' cells with increasing

age, the body weights at 6 weeks would be above their expectations based on the cell proportions at 50 weeks, giving the appearance of heterosis.

The second way of looking for heterosis is more direct. It involves the comparison of the weights of overt chimaeras with those of single-component chimaeras. The overt chimaeras are those of types L/L, C/C and S/S. Here the constituent strains are of similar size and the variation of cell proportions does not have much effect on weight. The single component chimaeras are from all types; so that, for example, single-component individuals with 100% of cells from one or other of the Large strains are compared with overt chimaeras of the type L/L. Similarly, single-component chimaeras with C cells are compared with C/C overts; and single-component S with S/S. The results are given in Table 15. In all three size-types the overt chimaeras are heavier than the single-component chimaeras, but

Table 15. *Comparisons of the weights of overt chimaeras of like-sized strains with those of single-component chimaeras*

	Size		
Overt	Large	Control	Small
Source	L/L	C/C	S/S
Number	3(2)	5	8
Mean weight (g)	39·47 (35·70)	26·99	24·78
Single-component			
Source	L/S, S/L, C/L, L/L	L/C, C/L, C/C	S/L, S/S
Number	11	10	3
Mean weight (g)	34·44	26·35	21·20

not by much. To assess the significance of the difference, the data were subjected to a two-way analysis of variance, treating overt *vs.* single-component as a fixed factor, weights being first transformed to logs. This gave an F-ratio of 5·7 for 1 and 34 d.f., with P = 0·02. There is, however, a difficulty in accepting this as conclusive evidence of heterosis. One overt L/L individual had an exceptionally high weight of 47·0 g. The mean with this individual omitted is shown in parentheses in Table 15. The analysis of variance with this individual omitted gave $F = 3·2$, P = 0·08, which is not significant. So the evidence for heterosis rests heavily on a single individual and cannot be accepted with confidence.

To summarize: the two independent lines of evidence both suggest that chimaeras show 'heterosis' for body weight, but both comparisons suffer defects which make the conclusion not completely convincing.

5. DISCUSSION

The results have shown clearly that none of the organs studied plays a predominant role in controlling growth. With the possible exception of the pituitary, this is not really surprising. Nevertheless, all appear to play some part in controlling growth because body weight is linearly dependent on the mean cell

proportions in these eleven organs. Can one conclude from this that growth is controlled by the cellular genotype throughout the body; or is there some other organ, not studied, that controls growth? Unfortunately this question cannot be answered conclusively. Evidence for the existence of such an organ would come from chimaeric variance not accounted for by the mean of the organs studied. The estimate of this variance, 4·2 g^2, was not significantly different from zero. All that can be said, therefore, is that we have no compelling evidence for the existence of a growth-controlling organ other than those studied. The absence of a growth-controlling organ is suggested by a study of embryonic growth (Gauld, 1980). From 11 days of gestation till birth embryos of the large strains were found to be heavier than embryos of the small strains, and the differences could not all be attributed to maternal effects. At 11 days, organogenesis has barely started and no organ has completed its differentiation. The embryonic difference in weight can therefore hardly be attributed to any specific organ or tissue. The difference of embryonic weights, after subtracting the estimated maternal effect, amounted to about 11 percent. The much larger difference developed postnatally could be due to a different growth-controlling mechanism.

The mean cell proportions, over all chimaeras made from strains of different sizes, was close to 50%. From this we drew the conclusion that there was no differential cell proliferation: cells from the larger component did not tend to outgrow those from the smaller. One must, however, ask what cell proportions would be expected if the cells proliferated at the rates characteristic of their strains of origin; would it be detectably different from 50%? Mice of the large strains have more cells than those of the small strains at the same age of 6 weeks. The cells of the large strains must therefore proliferate faster than those of the small. Falconer, Gauld & Roberts (1978*b*) give estimates of the total cell numbers in four organs (lung, liver, spleen and kidney) of Large, Control and Small strains. Let N_L and N_S be the cell numbers in an organ of the larger and smaller strains used to make a chimaera. Suppose that the organ in a chimaera starts with 50% of 'large' cells and that the cells subsequently proliferate at their own intrinsic rate. The adult organ will then contain $\frac{1}{2}(N_L + N_S)$ cells. Provided the mean initial proportion is 50 percent, the mean proportion of 'large' cells in the adult organs will then be $N_L/(N_L + N_S)$. Taking the values of N_L, N_S and N_C (for controls) from Table 3 of Falconer, Gauld & Roberts (1978*b*) allows us to calculate the expected cell proportions, $E(\bar{P})$, for the chimaeras of each type, assuming that the cell numbers in the organs studied here are the same on average as the mean of the four organs for which N is known. The expected cell proportions are then as follows:

Chimaera types	L/S, S/L	L/C, C/L	S/C
$E(\bar{P})$(%)	61·8	54·3	57·5

The observed values of $\bar{P}$ (Table 5) differ significantly from these expectations in both C/L and S/L ($t = 2{\cdot}5$) and in the weighted mean of all unlike-size chimaeras ($t = 4{\cdot}2$). We can therefore conclude that the rate of cell proliferation is not cell-specific in the chimaeras.

There are three levels at which the control of growth might be exercised: (1) systemic, all organs being subject to the same control; if there is a single growth controlling organ, it would have to operate in this way, (2) at the level of organs, each organ having its growth determined by its own cellular composition, and (3) at the cellular level, the rate of proliferation being cell-specific. The last of these possibilities is disproved by the consideration of mean cell proportions in the previous paragraph. The second possibility was disproved as the main way by which growth is controlled. It was shown that the weights of some organs are probably influenced by their own cellular composition, but this effect was not nearly enough to account for the differences in body weight associated with mean cell proportions. We are therefore left with the conclusion that growth control must be mainly systemic. But whether the control originates from a particular organ, not among those studied, or is in some undefined way dependent on the overall cellular composition of the body cannot be inferred from the present results.

One aspect of the cell proportions in the organs remains to be discussed, and that is the puzzling correlation, found for some organs, between $P-\bar{P}_w$ and $\bar{P}_w$; i.e. between the deviation of the organ from the mean of the individual and the mean itself. In particular, this correlation was negative for the brain and positive for the blood. The following two developmental models, though not very plausible, may be suggested as ways by which these correlations could arise. Both require the supposition that there is a tendency for the cell proportions to change during development and to change, moreover, in the direction of one or other extreme. Such a change might result from the majority cell-type inhibiting the proliferation of the minority type. In one model the change toward the extremes takes place in the undifferentiated tissues, from which the organs become differentiated sequentially. After differentiation the organs do not change further in their cell proportions. Thus the first-formed organs will have their cell proportions closer to the initial value and the later-formed organs will have them further toward the extremes. This would generate a negative correlation of $P-\bar{P}_w$ with $\bar{P}_w$ in the first-formed organs and a positive correlation in the later-formed ones. In the second model, which is perhaps somewhat less implausible, the change toward the extremes takes place in all organs during the whole course of their development, and the organs do not need to differentiate sequentially. Organs with little cell replacement would change least and would have a negative correlation of $P-\bar{P}_w$ with $\bar{P}_w$; organs with much cell replacement would change most and have a positive correlation. The correlations observed for the brain and blood fit with this expectation. The spinal cord, which would be expected to be like the brain, has also a negative correlation though a smaller one. If this second model were right, one might expect to find changes of cell proportions during the life of the individual, and this can be tested from the blood which was assayed at two ages. The expectation from the model is that the variance would increase with age. The blood was assayed at 6 and 50 weeks in the C/L chimaeras and at 4 and 6 weeks in the others. The variance increased in the C/L, but not significantly ($P \sim 0{\cdot}2$) and it decreased non-significantly in the others, so this test gives no support for this

model. We must therefore leave these correlations as an unexplained feature of the results.

We are indebted to Dr Patricia Bowman who made some of the chimaeras.

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