ON THE GENETICS OF SUBNORMAL DEVELOPMENT OF THE HEAD (OTOCEPHALY) IN THE GUINEA PIG

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INTRODUCTION

With the solution of the immediate problems of the numerical phenomena of heredity and of the organization of hereditary units in the cell, the center of interest in genetics is tending to shift towards questions of the physiology of gene action. Most of the genetic differences which it has been convenient to study have had to do with rather superficial characters in which the differential activities of the genes probably take place in cells of rather late developmental stages. This has its advantages on the physiological side since in such cases the chain of processes intervening between primary gene action and the observed character difference is likely to be relatively short. On the other hand, it would be of great interest to learn something of the nature of the genetic control of the more basic developmental processes—type of polarity, cleavage, gastrulation and the formation of the embryo. The subnormal development of the head shown by otocephalic monsters seems to involve processes at this level. Such monsters, ranging from types normal except for slight reduction of the mandible or of the premaxilla, to a type in which the head lacks jaws, nose and eyes and the brain terminates in the medulla, have been appearing for years in relatively large numbers in a particular inbred strain[1] of guinea pigs. However poor these may be as material for the study of heredity it has seemed worth while from the standpoint of developmental physiology to learn as much as possible of their genetics. The present paper supplements a previous account (WRIGHT and EATON 1923).

Questions of the genetics of characters (in the broad sense) are conveniently divided into three categories. First is the determination of the ultimate factors, (genetic or environmental) and the evaluation of their relative importance. Second are questions of the time and mode of action of these factors. Third is the task of tracing and explaining the interactions within the organism which intervene between the primary action of the factors and the observed character changes. The present paper is

[1] This strain (family 13) is one of 23 strains maintained by brother-sister mating since 1906 by the Bureau of Animal Industry of the U.S. DEPARTMENT OF AGRICULTURE. The author was in charge of these experiments from 1915 to 1925. Since that time this strain has been maintained at the UNIVERSITY OF CHICAGO.

devoted primarily to the first group of questions but attention will be called to such deductions as can be made immediately with regard to those of the second group. A recent paper (WRIGHT and WAGNER 1934) is devoted primarily to the third group of questions.

GRADES OF DEFECT

Figure 1 gives semi-diagrammatic representations of these grades. "In grade 1, the only obvious defect is more or less reduction of the lower jaw. In grade 2 no mandible can be felt externally. In grade 3 the ears are con-

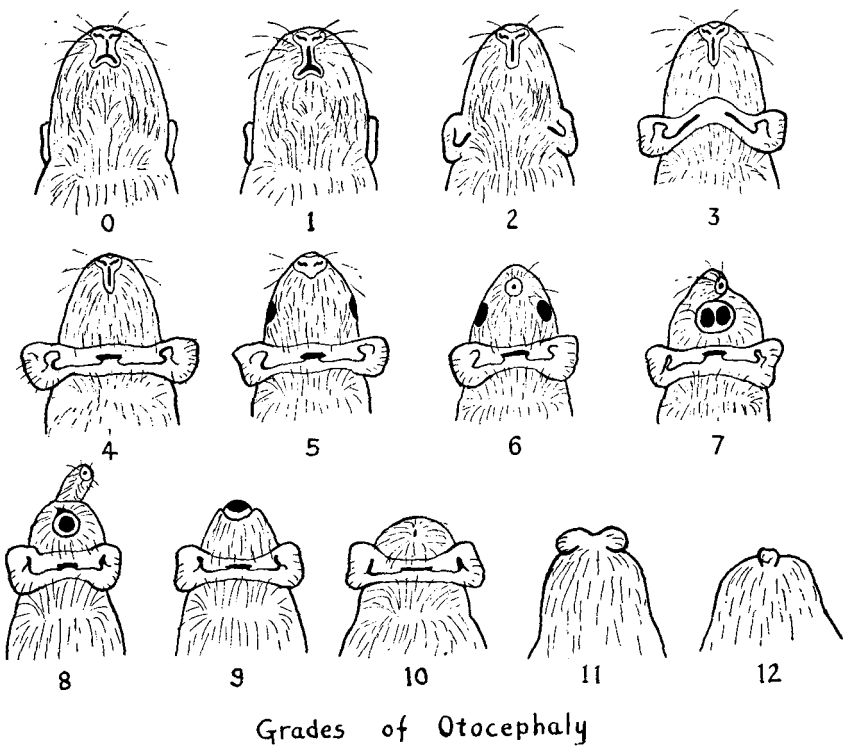

FIGURE 1.—A semidiagrammatic representation of the main series of grades of otocephaly.

nected under the throat by bare skin. In grade 4 there is only a single median ear opening on the throat. In grade 5 the mouth and upper incisors are lost. In grade 6 the nostrils fuse. In grade 7, the eyes are in contact below a narrow nasal proboscis or are more or less fused. This fusion is complete in grade 8. The proboscis is lost in grade 9, the eye in grade 10, and the ear opening in grade 11. Two small ears are the only externally visible organs of the head left. In grade 12, the body rounds off in front of the shoulders, with no sign of a head except a single small median external ear" (WRIGHT and EATON 1923). There have been a few aberrant types. In grade A the principal defect is the shortness of the snout and lack of upper

incisors. Grade 4A combines this defect with those of grade 4. In grades 8A and 9A, the cyclopian eye appears to be on top of the head, since an upper jaw is present below it. The widest departure from the usual series has been in the case of seven monsters, born since 1925 in the stock of the Bureau of Animal Industry, but which Dr. H. C. McPHEE and Mr. O. N. EATON, who have been in charge, have kindly permitted me to discuss here. In these monsters there are not only no jaws (as in grade 5) but no external ears or ear openings and no external eyes in the small orbits. They are called grade 5C.

The lower grades (1 to 5) belong to the type of monster known as agnathia. In the higher grades, agnathia is combined with gross defect of the brain, manifested externally in grades 7 to 9 by cyclopia. The highest grades (10–12) are of the type known as aprosopus. The series from 3 to 12 and including 8A and 9A but not A, 4A and 5C, agrees well with I. G. ST. HILAIRE'S category "otocéphalien," which he distinguished from the closely allied category "cyclocéphalien" in which there is cyclopia without mandibular defect. The term otocephaly will be used as a convenient designation for the whole series.

Animals of grade 1 often live from 4 to 6 days. Two lived longer, 20 and 38 days respectively. On death it was found that the growth of the lower incisors which did not meet the upper incisors, had forced their mouths open. Even the most advanced grades are healthy and nearly normal in size up to the time of birth, following which they die in a few minutes of asphyxiation. One female born since the closing of the tabulation on which the present paper is based lived to an age of 12 months. She differed from normal in having only one (median) lower incisor. Her parents produced two monsters among 12 young; and her sire mated with a sister of her dam produced 1 in 7. This female has had two litters by a half-brother. The 5 young were all normal. Two of the offspring mated with each other produced two monsters to one normal.

OCCURRENCE IN DIFFERENT STRAINS

Otocephalic monsters appear sporadically in many stocks of guinea pigs. In the experiments of the U. S. Bureau of Animal Industry through June 1922 (WRIGHT and EATON 1923), 4 had appeared among 11,000 animals of mixed ancestry. Records were at hand at that time from 24 inbred strains, maintained since 1906 exclusively by brother-sister mating (except for one maintained by parent-offspring mating). Fourteen of these had never produced any monsters of these types (12,000 young). Nine had produced 28 of the monsters in 13,000 young. The one remaining strain (family 13) had produced 50 in 3300, with significant differences among its branches.

These data can now be supplemented by the records of the U. S. Bureau of Animal Industry from July 1922 to December 1925 and of stocks descended from these but bred at the UNIVERSITY OF CHICAGO from 1926 through 1930. The following statements are based on the entire body of data.

Strains with no ancestry of family 13 but which had produced at least one otocephalus yielded a total of 34 in 24,000. Taking into account the considerably larger number of animals from strains which have never produced such monsters, it may be concluded that the ordinary rate of occurrence is less than 0.05 percent. In contrast family 13 has now produced a total of 272 in 6,275 young, or 4.3 percent.

One of the inbred strains which is included above among those which produced no otocephalics during the period of tabulation was family 2. This family has produced some 6,000 young in the stocks of the Bureau of Animal Industry and of the UNIVERSITY OF CHICAGO. As shown later, no otocephalics have appeared in first crosses between it and even the high producing lines of family 13. Yet I am informed by Mr. O. N. EATON who has continued to breed the family in the stocks of the Bureau of Animal Industry, that a group of 160 animals wholly descended from one mating in the 19th generation of brother-sister mating has included seven otocephalics, all of the peculiar earless type described as grade 5C, which has never appeared in any other stock. He informs me that no otocephalics of any sort have appeared in other branches of family 2 in the Bureau stock since 1925, in agreement with the earlier history of the family and with the record of the branches still maintained in Chicago. Mr. EATON informs me that otocephalics have continued to appear freely in the Bureau stock of family 13 since 1925 but these are not included in the present tabulation.

It is clear that inbreeding *per se* does not bring about abnormal development but that special genetic tendencies affecting both frequency and type may occur and be isolated and fixed in particular inbred strains.

The record of family 13 begins with a mating made in 1906 between two animals from the stock of the U. S. Bureau of Animal Industry. The foundation female had previously been mated with another male, but no otocephalics were recorded among 400 inbred descendants. The foundation male was mated with 3 other females. One otocephalus appeared among 2200 inbred descendants. Thus the otocephalic tendency of the original animals does not seem to have been very strong.

The branching lines of descent from this pair, reaching in some cases to the 32nd generation of brother-sister mating, are shown in figure 2. It is convenient to apportion these lines to 18 substrains, each starting from a single mating. The otocephalics are represented by black dots in the figure.

Inspection shows that the incidence of abnormality has been far from uniform. The first case appeared in generation 2 and no others came until generation 8. Three of the substrains which began in the early generations produced no monsters among 199, 196 and 301 young respectively. The 3 other substrains which originated before the 8th generation produced an average of about 1 percent (2 in 151, 4 in 457 and 5 in 629 respectively).

The later representatives of the family fall into two large branches descended from two matings of the 13th generation, with common ancestry

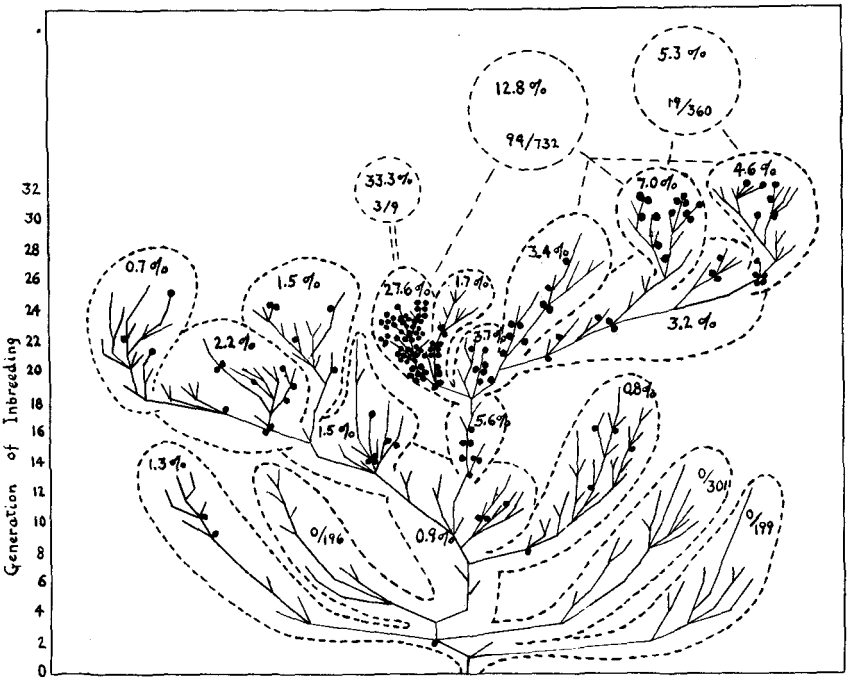

FIGURE 2.—The distribution of otocephalic monsters (black dots) among the branches of family 13, tracing exclusively by brother-sister mating to a single foundation pair. The percentage of monsters in each branch is indicated. The occurrence in three groups descended from crosses within the family are indicated in circles at the top.

4 generations earlier. One of these two branches (descendants of mating 13–13–7) is here divided into 4 substrains ranging from 0.7 percent to 2.2 percent in production of otocephalics (3 in 451, 5 in 325, 6 in 395 and 9 in 408 respectively). These do not differ significantly from each other. Their grand average is 1.5 percent (23 in 1579).

The other large branch (descendants of mating 13-13-1) began at once to produce a distinctly higher percentage of monsters. Excluding the descendants of one mating (13-19-1) to be discussed later, 6 recognized substrains ranged from 3.2 percent to 7.0 percent with no significant differences over a period extending from the 13th to 32nd generation of inbreed-

ing (7 in 124, 7 in 189, 8 in 252, 9 in 264, 13 in 185 and 11 in 240 in order of time). In addition, 19 of the monsters appeared among 360 animals derived wholly from later generations of this group but not from brother-sister matings. These were offspring or descendants of males treated with lead (COLIN 1931). Dr. COLIN'S analysis of this and other data led him to the conclusion that the lead treatment had no demonstrable genetic effect in any respect. Dr. STRANDSKOV (1932) has made a similar study of the effects of X-rays, using the same branch of family 13 as material. While he found a definite reduction in the size of litters produced by X-rayed males, probably indicative of dominant lethal mutations, he concludes that "in no case does the percentage of otocephalics in the progeny of X-rayed males or in their descendants differ significantly from those of contemporary controls." These data are not included in the tabulations of the present paper. It is obvious that there is no significant difference between the 5.3 percent monsters from Dr. Colin's data and the average of 4.4 percent from the 6 substrains referred to above. Combining, we have a group of 1614 animals starting with the offspring of one pair in the 13th generation but ranging to the 32nd generation of brother-sister mating among which 74 otocephalic monsters (4.6 percent) are distributed with remarkable randomness. It is to be noted that the record of this branch as a whole differs significantly ($5.2 \times$standard error) from the record of the descendants of mating 13-13-7, raised and recorded contemporaneously. Its record differs even more from the early history of the family.

We have so far excluded the record of the most highly producing subbranch of the above high producing branch. Figure 2 brings out the unprecedented incidence of abnormality beginning with a mating (13-19-1) of the 19th generation. This line had produced 17 in 79 (21.5 percent) at the time of the previous paper. Before it became extinct as an inbred strain, through dearth of viable females, it had produced 67 in 355 or 18.9 percent. The detailed record is given in figure 3 and from this it may be seen that there was marked and significant heterogeneity. The original mating produced 2 in 20. One daughter mating produced 7 in 31 and descendants for 4 more generations continued to produce large numbers (44 in 129). The other daughter mating from 13–19–1 produced 2 in 17 and in the next generation two matings produced 6 in 19 and 1 in 3 respectively, clearly demonstrating that the high tendency was present. Yet in the following generation only 2 appeared among 28 young and from this time no more appeared although 99 normals were born. The indication is that the abrupt increase in tendency toward abnormality appeared in one of the pair 13–19–1, in a heterozygous condition, due presumably to a mutation, was transmitted in some lines and lost in others. It may be noted that there is probably heterogeneity even among the matings which

produced monsters. Thus the difference between 2 in 20 and 11 in 26 is 2.4 times its standard error. Among the 9 matings which produced at least 15 young, of which at least 1 was an otocephalus, there is only 1 chance in 20 that the percentages could vary as much as they did by chance (χ^2 test).

The above data from the high producing line may be supplemented by 9 young of which 3 were otocephalic, from matings other than brother-sister, but within the strain. Including these but excluding the substrain in which the high tendency was lost (mating 13-22-5 and descendants) 65 monsters were produced in 234 young or 27.8 percent.

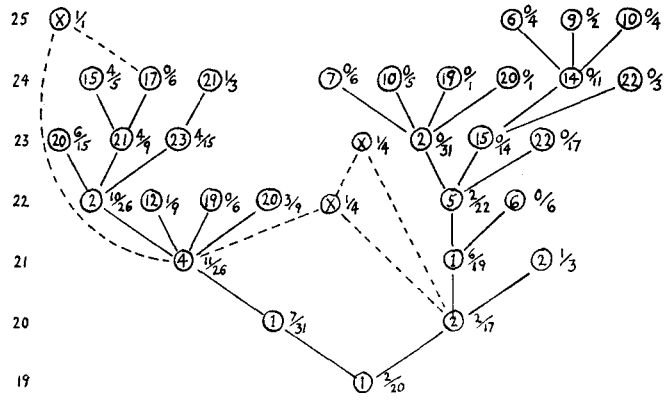

FIGURE 3.—The pure-bred descendants of mating 13-19-1. The number of otocephalic monsters and the total number of young from each mating are indicated in fractional form. All matings were between brother and sister except the three marked X whose connections with their parental matings are shown by broken lines.

A number of animals of this group were mated with animals from the large related group (descended from 13-13-1) averaging 4.6 percent. The total record from such crosses and descendants was 94 in 732, or 12.8 percent, significantly higher than 4.6 percent, and clearly showing that the high tendency was transmitted at least in some cases.

DEDUCTIONS FROM FAMILY HISTORY

Before analyzing further the records of this high producing branch, it will be well to consider what deductions can be made from the rest of the family. The first suggestion is perhaps that the monsters represent Mendelian segregants. The condition, being lethal, cannot of course be a simple dominant. The ratio of normals to monsters in those matings which produced at least one, show that it cannot be a simple recessive. The ratio should be less than 3:1 in data in which only those matings are tabulated which produced at least one recessive. Actually, the 34 monsters recorded from the family, excluding the descendants of 13-13-1, came from 28

matings which produced a total of 485 young. The ratio of normals to monsters is 13.3:1 among matings averaging 17.3 young. Similarly the 74 otocephalics among the descendants of 13-13-1 (excluding those of 13-19-1) were produced by 57 different matings among a total of 812 young. The ratio of normals to monsters is here 10:1 from matings which produced an average of 14.3 young. The 96 matings of this group which produced no monsters had a total of 802 young or an average of only 8.3 per mating, suggesting that their failure to produce monsters was largely if not wholly a matter of chance.

The hypothesis of segregation of one factor being eliminated, it might be urged that the monsters depend on the assemblage of a particular combination of two or more genes, each present in the stock in a heterozygous state. This would be plausible enough if the matings had been made at random within a large stock, but it is ruled out by consideration of the statistical effects of the brother-sister mating actually practiced. Brother-sister mating tends automatically toward the establishment of homozygosis. The number of heterozygous factors in each line should be reduced about 19 percent per generation (in the absence of selection for the heterozygous state). Most lines should soon become homozygous in some combination of the genes present and this combination must of necessity be one yielding fairly normal development. A few lines might become fixed in all but one of the factors for otocephaly and continue to carry this one in a heterozygous condition for several generations. During this period, these matings would produce about 25 percent monsters, but this, as noted, is contrary to the observations. Matings producing monsters in lower frequencies than 25 percent due to heterozygosis in 2 or more factors might be present in the early history but should give way to the 1 factor ratio or to complete fixation of normality. The incidence of monsters through the 32 generations of brother-sister mating is utterly at variance with this expectation.

The possibility that heterozygosis might be retained by a system of balanced lethals was discussed in some detail in the previous paper. It was shown to be untenable from the relatively large size of litter characteristic of family 13 and the absence of any increase in litter size following outcrosses with other stocks. Dr. GEORGE HAINES (unpublished work) has found from an examination of 338 female guinea pigs of the stock of the Bureau of Animal Industry that only 8.7 percent of the corpora lutea were not represented by foetuses and only 7.9 percent were represented by dead foetuses. The average number of corpora lutea per female was 2.59. For 55 females of family 13, included in the above, the average number of corpora lutea was 2.93 of which 14.3 percent were not represented by foetuses and 6.8 percent were represented as dead foetuses. Only 11 of these

females of family 13 had been mated with males of their own family but the record of these was substantially the same: an average of 2.63 corpora lutea, 13.8 percent missing foetuses and 6.9 percent dead. Clearly there is no approach to the 50 percent loss of ova required by the hypothesis of balanced lethals. It can be concluded definitely that the monsters are not Mendelian segregants.

As regards the large group descended from 13-13-1 but excluding 13-19-1 and its descendants, the figures give no evidence that the matings which produced monsters differ from those which did not, or indeed that any mating differed from any other in its tendency. The conclusion which is indicated is that a certain combination of genes had become fixed in this group from its beginning (13th generation of inbreeding), the physiological effect of which is such that each individual has about one chance in 22 (4.6 percent) of falling below the threshold of normality in development of the head. In the group descended from 13-13-7, a constant tendency yielding about 1.5 percent seems to have become fixed. Different tendencies were probably present in the earlier branches but the smaller number of monsters (0.5 percent) make the demonstration uncertain. Even this low tendency, it should be recalled, is at least ten times that among guinea pigs in general.

Even though the monsters cannot be genetic segregants, it is possible that they may differ genetically from their normal brothers and sisters on the hypothesis that each one depends on a separate mutation (using the term in its broadest sense). The particular genetic complex fixed in 13-13-1 may be one which includes an unstable gene or which induces irregular behavior of a particular chromosome.

On this hypothesis the monsters would be distributed at random among the litters of the homozygous line. The same sort of distribution would hold if the genetic complex reacts with non-genetic factors of such an accidental nature as to act wholly on individual embryos.

Most environmental factors would act on litter-mates alike. Malnutrition or disease of the mother, the factors invoked by most teratologists, belong in this category. So also does size of litter. Such factors if effective would *tend* to make litters consist either wholly of monsters or wholly of normals. They would at least cause the occurrence of two or more monsters in the same litter more frequently than from mere chance. Any difference between females of the same stock should also have this effect.

OCCURRENCE IN LITTER-MATES

In order to test this important question, all litters of family 13 have been classified by size and number of monsters. Since different branches of the family differ considerably in mean litter size it is necessary to sub-

divide the family into groups of similar incidence of abnormality. The family has been subdivided for this purpose as follows: Group A includes all of the family except the descendants of 13-13-1 and has an average of 1.0 percent of monsters. Group B includes the descendants of 13-13-1 (except those of 13-19-1) and has an average of 4.6 percent. Group C includes the pure-bred descendants of 13-19-1, except for the low line which originated in 13-22-5. The average is 27.8 percent. Group D includes the descendants of 13-22-5 and cross-bred descendants of a few animals from 13-19-1 which gave no evidence of transmitting the high tendency. The average of this rather heterogeneous group is 3.8 percent. It might well have been included with group B had it not seemed desirable to keep the latter group homogeneous by descent. Group E consists of cross-breds from groups C and B which trace to 13-19-1 through one or more of 4 animals known to transmit the high tendency, but only through one parent. These averaged 10.6 percent. Finally group F consists of cross-breds descended through both parents from the above 4 animals. These averaged 22.0 percent. The data are given in table 1.

The distribution of monsters in litters of each size, expected under random sampling, can be calculated as follows. Let q be the chance of abnormal development in the group in question, $1-q$ be the chance of normal development. In litters of 2 the chance that both will be normal is $(1-q)^2$, that one will be a monster and one normal $2q(1-q)$; and finally that both will be monsters is q^2. In litters of 3 the chances of 0, 1, 2 and 3 monsters are simply the appropriate terms in the expansion of $[(1-q)a+qA]^3$ where a stands for normal and A for abnormal. The expectations for larger litters are given by expansion of the appropriate power of the binomial.

Inspection of the table shows that there is rather close agreement between the observed and the calculated results. The data are combined in a final table, in which the expected numbers are the sums of the expectations in the separate groups (which is not the same as expectations based on the grand average frequency of monsters). Excluding singletons, the number of wholly normal litters is slightly in excess of expectation, of litters with 1 monster only slightly in defect, while litters of 2 or more are again slightly in excess.

	LITTERS OF 2 OR MORE		
NUMBER OF OTOCEPHALICS	OBSERVED	EXPECTED	DIFFERENCE
0	1810	1795.6	+14.4
1	173	191.8	−18.8
2 or more	32	27.6	+4.4

These deviations are in the direction expected if there are factors at work which act on litter-mates simultaneously. But they are so slight that

they might well have arisen by chance. The probability (from χ^2) that as great a system of deviations might arise by chance is .10 (one degree of freedom). It may be concluded that factors which act separately on each litter-mate (chance mutation, accidents of implantation, et cetera) are enormously more important than factors which tend to act on litter-mates simultaneously (condition of mother, et cetera).

It should be noted, however, that a minor influence from factors of the latter sort is not ruled out. Neither is it wholly hopeless to look for specific factors. A factor which showed a correlation of r with occurrence of abnormality would be responsible for a correlation of only r^2 between litter-mates. Thus one might readily detect a direct correlation of .10 or .20 between an external factor and occurrence of otocephaly and be unable to detect the resulting correlation of .01 or .04 between litter-mates.

EFFECT OF SIZE OF LITTER

The data given in table 1 can be used for one such test. Size of litter is, to a considerable extent, an indicator of the condition of the dam (WRIGHT 1922). The question whether otocephalics occur equally frequently in all sizes of litter is tested for each group and all combined in the last two columns of table 1. The total number of young in each size of litter is simply multiplied by the proportion of otocephalics in the group in question to get the expected frequency under random distribution. It will be seen that this agrees rather closely with the observed number in most cases. The grand totals, obtained as before by addition of the figures for the groups, show, however, an excess of monsters in litters of 1 and 2 and a deficiency in litters of 4 or more. The probability of obtaining such deviations by random sampling is .01 (combining the data into 3 classes: less than 3, 3, more than 3; the probability is .04 if 5 classes are retained). This is rather definite evidence that conditions indicated by a small litter have a slight excess tendency to induce otocephaly.

In the preceding paper, evidence for environmental effects was looked for in various ways. It was shown that there was no appreciable difference in frequency in first and later litters or in last litters, a factor shown later to be of considerable importance in certain other cases, including polydactyly and white spotting (WRIGHT 1926). The seasonal distribution showed however a slight predominance in winter and early spring when conditions are apt to be poor. A slightly larger percentage of the normal litter-mates of monsters died at birth and between birth and weaning than among normals in other litters from the same matings. There was a corresponding but statistically insignificant difference in birth weight between litter-mates and non-litter-mates. The monsters themselves were about 11 percent lighter than their normal litter-mates. The most significant indica-

TABLE 1

Distribution of litters of family 13 by size, number of otocephalics and family branch.

GROUP	SIZE OF LITTER	OBSERVED NUMBER OF LITTERS					EXPECTED NUMBER			TOTAL LITTERS	TOTAL YOUNG	NUMBER OF OTOS.	
		0 OTO.	1 OTO.	2 OTOS.	3 OTOS.	4 OTOS.	0 OTO.	1 OTO.	2 OR MORE			OBSERVED	EXPECTED
A	1	143	2	—	—	—	143.6	1.4		145	145	2	1.4
	2	364	8	1	—	—	365.8	7.2	0.0	373	746	10	7.2
q=.0097	3	402	9	2	0	—	401.1	11.8	0.1	413	1239	13	12.0
	4	215	8	0	0	0	214.5	8.4	0.1	223	892	8	8.7
	5	78	1	0	0	0	75.2	3.7	0.1	79	395	1	3.8
	6	15	—	0	0	0	14.2	0.8	0.0	15	90	0	0.9
Total		1217	28	3	0	0	1214.4	33.3	0.3	1248	3507	34	34.0
B	1	108	6	—	—	—	108.8	5.2	—	114	114	6	5.2
	2	189	18	2	—	—	190.3	18.3	0.4	209	418	22	19.2
q=.0459	3	185	26	0	1	—	184.2	26.5	1.3	212	636	29	29.2
	4	84	12	1	0	0	80.4	15.5	1.1	97	388	14	17.8
	5	7	2	0	0	0	7.1	1.7	0.2	9	45	2	2.1
	6	1	0	0	0	0	0.8	0.2	0.0	1	6	0	0.3
	7	0	1	0	0	0	0.7	0.3	0.0	1	7	1	0.3
Total		574	65	3	1	0	572.3	67.7	3.0	643	1614	74	74.1
C	1	12	11	—	—	—	16.6	6.4	—	23	23	11	6.4
	2	19	13	5	—	—	19.3	14.8	2.9	37	74	23	20.6
q=.2778	3	9	13	5	0	—	10.2	11.7	5.1	27	81	23	22.5
	4	7	6	1	0	0	3.8	5.9	4.3	14	56	8	15.5
Total		47	43	11	0	0	49.9	38.8	12.3	101	234	65	65.0

GROUP	SIZE OF LITTER	OBSERVED NUMBER OF LITTERS					EXPECTED NUMBERS			TOTAL LITTERS	TOTAL YOUNG	NUMBER OF OTOS.	
		0 OTO.	1 OTO.	2 OTOS.	3 OTOS.	4 OTOS.	0 OTO.	1 OTO.	2 OR MORE			OBSERVED	EXPECTED
D	1	41	0	—	—	—	39.4	1.6	—	41	41	0	1.6
	2	65	5	1	—	—	65.7	5.2	0.1	71	142	7	5.4
q=.0383	3	40	3	1	0	—	39.1	4.7	0.2	44	132	5	5.1
	4	11	3	0	0	0	12.0	1.9	0.1	14	56	3	2.1
	5	3	0	0	0	0	2.5	0.5	0.0	3	15	0	0.6
	6	1	0	0	0	0	0.8	0.2	0.0	1	6	0	0.2
Total		161	11	2	0	0	159.5	14.1	0.4	174	392	15	15.0
E	1	15	6	—	—	—	18.8	2.2		21	21	6	2.2
	2	29	6	2	—	—	29.6	7.0	0.4	37	74	10	7.9
q=.1064	3	28	7	1	0	—	25.7	9.2	1.1	36	108	9	11.5
	4	14	2	0	0	0	10.2	4.9	0.9	16	64	2	6.8
	5	1	1	1	0	0	1.7	1.0	0.3	3	15	3	1.6
Total		87	22	4	0	0	86.0	24.3	2.7	113	282	30	30.0
F	1	17	6	—	—	—	18.0	5.0		23	23	6	5.0
	2	22	9	2	—	—	20.1	11.3	1.6	33	66	13	14.5
q=.2195	3	15	12	3	1	—	14.7	12.4	3.9	31	93	21	20.4
	4	6	8	1	0	1	5.9	6.7	3.4	16	64	14	14.0
Total		60	35	6	1	1	58.7	35.4	8.9	103	246	54	53.9
Total	1	336	31	—	—	—	345.2	21.8		367	367	31	21.8
(A to F)	2	688	59	13	—	—	690.8	63.8	5.4	760	1520	85	74.8
	3	679	70	12	2	—	675.0	76.3	11.7	763	2289	100	100.7
	4	337	39	3	0	1	326.8	43.3	9.9	380	1520	49	64.9
	5	89	4	1	0	0	86.5	6.9	0.6	94	470	6	8.1
	6	17	0	0	0	0	15.8	1.2	0.0	17	102	0	1.4
	7	0	1	0	0	0	0.7	0.3	0.0	1	7	1	0.3
		2146	204	29	2	1	2140.8	213.6	27.6	2382	6275	272	272.0

tion found was in relation to size of litter, the factor considered above with larger numbers. Putting all of these indications together, there can be little doubt that unfavorable conditions tend to increase the chances of this sort of abnormal development, but that the effect is so slight that it produces no appreciable tendency for litter-mates to develop alike. In such a line as 13-13-1 (excluding 13-19-1) each otocephalus is almost wholly the result of a localized chance event, supplementing a genetic tendency common to all members of the group.

GENETIC POSSIBILITIES

Before going into more detailed analysis of the genetic situation, it will be well to review the various genetic possibilities. First is the question whether this basis is Mendelian at all or not. It may conceivably be cytoplasmic as in certain plastid characters of plants, in which case the hereditary tendency would presumably pass largely or wholly down the maternal line.

Second, even though nuclear (Mendelian) it may be the heredity of the mother and not of the young that is effective. The abundant evidence (discussed later) that monsters may be produced by toxic products has led most of those who have conceded any role whatever to heredity to suggest this mechanism (STOCKARD 1909, WERBER 1915, CHIDESTER 1923). It is well known that tendencies toward disturbed metabolism (the diabetic diathesis, gouty diathesis, et cetera) are inherited in man. WERBER has shown that butyric acid and acetone produce monsters, including cyclopia, in large numbers from treated Fundulus eggs. The argument that the frequent production of monsters by a strain may be an indication of hereditary metabolic disturbance affecting the mother is complete, except for direct evidence that it is actually true in some specific case.

A third hypothesis is that the heredity is Mendelian but is that of the egg nucleus before maturation instead of after fertilization. Experiments discussed later have shown that the most effective period for production of cyclopia in fish eggs is before first cleavage. There is no *a priori* reason then why prematuration heredity should not be the effective agent, as it has been demonstrated to be in the well known cases of voltinism and certain serosa colors of the silk worm (TOYAMA 1913), direction of coiling of the shell in Lymnaea (BOYCOTT et al 1930), larval lipochrome colors in Gammarus (SEXTON and PANTIN 1927) and various lethal effects in Drosophila (REDFIELD 1926). Since the heredity of the egg before maturation is identical with that of the mother, this hypothesis is hardly distinguishable on purely genetic grounds from the preceding. The practically random distribution within litters, discussed above, is however more easily accounted for on this hypothesis than on that of a maternal influence.

The fourth hypothesis is that the effective heredity is that of the zygote, with the sperm contributing as much as the ovum. This hypothesis may be divided according to the postulated mode of action of the gene. The time of action may be as early in development as suggested by the critical period for the production of such monsters by environmental agencies or it may be any later period. If there is early action, it means that the multiple visible effects probably trace back to a single common divergence from the normal developmental process. The alternative is separate action of the zygote heredity in the various affected tissues.

Exclusively paternal heredity (possibly of heredity transmitted by the Y chromosome) is a minor possibility as are such complications of zygote heredity as ordinary sex linkage.

The genetic basis may, of course, be a combination of two or more of these possibilities.

TESTS OF PATERNAL HEREDITY

The fourth hypothesis differs from those preceding in that the heredity of the father plays a role. It is thus of first importance to determine whether the father has in fact any influence.

It was noted in the previous paper (WRIGHT and EATON, 1923) that 711 young from matings between females of family 13 and males without ancestry in this family included only 1 otocephalus. During the period of these crosses (1916–1922), the average production of females of family 13, mated with brothers, was 2.7 percent, which yields an expectation of 19 in 711 instead of 1. This was interpreted as indicating that inheritance from the father was an immediate factor. That the mother is also important was shown by reciprocal crosses among which no otocephalus appeared among 373 young.

The above data were collected incidentally in a study of the effect of cross breeding on other characters. It has seemed desirable to make more direct tests of this important question. Accordingly females of the highest producing line (13-19-1) were mated with males from an inbred strain (family 2) which had produced no otocephalics in 3500 young up to 1925. (As noted earlier, one recent branch of this family has produced 7 otocephalics of an aberrant type.) There were no otocephalics among 88 cross-breds. The most significant results are from 5 females which were mated with brothers, either before (♀ 7979) or after the mating with the male from family 2. (Their records are given on the following page.)

The significant difference (3.7 × standard error) in the occurrence of otocephaly among the progeny of these females, depending on the breeding of the male, shows clearly that factors are introduced by the sperm which affect the fate of the developing egg. Matings between males of this line

NUMBER OF TESTED FEMALES	×BROTHER (FAMILY 13)		×♂ OF FAMILY 2	
	OTOS.	TOTAL	OTOS.	TOTAL
7979	6	19	0	2
17796	6	14	0	9
19797	4	9	0	12
19873	3	9	0	3
21171	4	15	0	5
	23	66	0	31

of family 13 with females of family 2 similarly failed to include any otocephalics (95 normal young). One male (4708) produced 7 otocephalics in a mating with a sister (31 young) but none in 14 from a female of family 2. Clearly heredity from the egg is important as well as from the sperm.

Another test of the sperm effect was made by mating two males of the high line (13-19-1, average production 27.8 percent) with females from the late generations of the large group from 13-13-1 (excluding 13-19-1) whose average production was consistently 4.6 percent. Male 24321 (from mating 13-22-20) produced 13 otocephalics among 95 young and male 24517 (from mating 13-22-2) produced 4 in 27. The 4.6 percent monsters expected from these females is raised to 14 percent and 15 percent by mating with these males from a line with a higher tendency. The difference in the case of ♂ 24321 is 4.2 times its standard error. The number from the other male is too small to be of much significance by itself but confirms as far as it goes.

These data, supplemented by that discussed in the next section in connection with sex incidence, demonstrate, it is believed, that the strong hereditary tendency toward otocephaly in the strain of guinea pigs under consideration is neither a matter of toxemia of the mothers of the monsters, nor of cytoplasmic transmission, nor of a conditioning of the egg by its nucleus before maturation and fertilization. It is, on the contrary, a function of the heredity of the egg after fertilization.

RELATION TO SEX

One specific factor of otocephaly, which is Mendelian and zygotic is easily demonstrated, namely, sex. At the time of the preceding paper, the ratio of females to males was 55 to 26 among these monsters, although there was approximate equality in the number of females and males (461 and 447 respectively) among the total progeny of the parental matings. The departure from equality among the monsters is over 3 times its standard error and was interpreted as a fairly certain indication that female sex predisposes toward development of the defect.

The data to the present time are given in table 2 in some detail and are summarized below. The usual approximate equality of number of fe-

males and males among all of the young has continued. In the last two groups, there were 52.5 percent males and 49.5 percent males respectively among the total young of determined sexes.

Group	♂	♀	?
Sporadics with no ancestry from family 13	13	21	0
Cross-breds ¼ to ¾ family 13	3	10	1
A 13-0-2 excluding 13-13-1	15	19	0
B, D 13-13-1 excluding 13-19-1 except low branches	31	57	1
C, E, F 13-19-1 and crosses, excluding low branches	45	96	8
	107	203	10

Considering the total, it appears that females have about twice as great a tendency to develop in the abnormal direction as males unless there is prenatal death and absorption of at least half of the male monsters. The difference between the sexes is unquestionably significant, the deviation from equality being about 5.5 times its standard error.

With respect to the separate groups, it is noteworthy that there is an excess of females in every case. The deviations from equality are statistically significant in the last two groups, making it certain that sex was a factor among all descendants of mating 13-13-1. Whether it was a factor from the beginning of the history of family 13 and among the sporadic cases outside of this family is less certain, although the actual excess of females makes this the presumption.

Female monsters of this general type are in excess not only in guinea pigs but in other animals. BALLANTYNE states on the authority of TIEDEMANN that both in man and in lower animals (for example, the pig) cyclopia is more common in the female than in the male. We shall return to this point later.

MENDELIAN UNITS

The analysis up to this point indicates that the differences in the otocephalic tendencies of different strains rest on a Mendelian basis. It is of interest to determine whether this consists of a large number of genes with individually small effects or a small number with large effects. The most hopeful point of attack is in the origin and history of the highest producing strain, that descended from mating 13-19-1.

It will be recalled that this high tendency appeared abruptly in the 19th generation of brother-sister mating and 6 generations after the origin of a group (13-13-1) which has given every indication of fixation of tendency in all of its subdivisions (except 13-19-1 itself) over a period of 15 years. It has been pointed out that such fixation, through attainment of homozygosis in some combination of genes, is an almost inevitable theoretical consequence of the close inbreeding practiced. Thus the very origin of the group 13-19-1 points strongly toward a mutation, the effect of which was to increase greatly the chance of abnormal development.

Having arisen, the high tendency was transmitted to at least the seventh generation in some lines but in other lines was soon wholly lost. Reference has already been made to the substrain descended from mating 13-22-5 with a record of only 2 in 121 following 3 or 4 generations of transmission of the high tendency. Two other animals from the high line when tested gave no indication of transmission of the excess tendency. One of these was female 30942 (from mating 13-24-21). Mated with a male descended from 13-22-5 she produced no monsters in 11 young. The other was a male (No. 175 from 13-23-20) which, mated with one of the daughters of the above female produced 1 otocephalus among 9 young, and mated back to two of his own daughters from this mating produced only 1 in 15. The animals of these matings were interbred or in some cases outcrossed to the large group with a record of 4.6 percent. Altogether 204 animals were produced which traced to 13-19-1 through ♀ 30942 and also through ♂ 175 (except for the 11 offspring of 30942 referred to above). These included only 10 otocephalics, or 4.9 percent, a figure which does not differ appreciably from the 4.6 percent present before the postulated mutation arose.

In marked contrast with the records of the above animals were those of four others, descended from 13-19-1. It has already been noted that ♂ 24321 (from 13-22-20) and ♂ 24517 (from 13-22-2) produced 13 otocephalics in 95, and 4 in 27 respectively. The high percentage (13.9 percent) in the first generation not only shows transmission of the high tendency but at least its partial dominance. The fact that 9 of the above monsters were males shows that the high tendency is transmissable from father to son and hence is not sex-linked (assuming males to be XY). Eight daughters backcrossed to the relatively low producing group (4.6 percent) produced 10 monsters in 82 (or 12.2 percent).

Matings were also made with two other males from the high producing line (♂ 24518 and ♂ 23652, which like ♂ 24517 came from mating 13-22-2). One of these (♂24518) had produced 4 in 15 mated with a sister, and the other, ♂ 23652, had produced 6 in 15 in such a mating (matings 13-23-23 and 13-23-20 respectively). The crossbred stock derived from the above 4 males and animals from the low producing stock, was carried for 4 generations. There were 30 otocephalics among 282 or 10.6 percent among those which traced to 13-19-1 through only one parent, while there were 54 among 246 (22.0 percent) which traced to 13-19-1 through both parents. Some matings in the last generation produced large numbers of otocephalics (for example, 7 in 12, 6 in 10, 5 in 17, 4 in 13, 4 in 6) while others of similar ancestry produced only one or none in fairly large numbers. There was continuity of high production traceable along particular lines. All of the data in short are in harmony with the hypothesis of a more or less completely dominant factor for high production which never became

fixed and was therefore lost in some lines, although retained to the end in others.

The exact extent to which this factor increased the percentage of otocephalics is an interesting question. Let Ot_2 represent this factor and its allele ot_2 be that present in the stock with an average of 4.6 percent. Males 24321 and 24517 may either have been Ot_2/Ot_2 or Ot_2/ot_2. If homozygous, all of their offspring from the outcross to the lower lines would be Ot_2/ot_2 and the percentage of monsters (13.9 percent) indicates the chance of abnormal development in a heterozygote. On the more probable supposition that males 24321 and 24517 were heterozygous, only half of their crossbred offspring would be Ot_2/ot_2 and half ot_2/ot_2. Since the incidence of defect in the latter would be 4.6 percent, that in the former must be 23.2 percent in order to give the observed 13.9 percent. The record of the next outcrossed generation suggests the heterogeneity expected with the second hypothesis but the numbers are inadequate. The total production 10 in 82 or 12.8 percent is in sufficient harmony with the expectation, 9.2 percent, of either hypothesis. The incidence in heterozygotes thus may be as low as 14 percent but is more probably in the neighborhood of 23 percent.

On any basis it is certain that dominance is not complete. The inbred high producing line produced 27.8 percent and it has been shown that recessives ot_2/ot_2 were segregating out even from the highest producing matings. Male 175, lacking the high tendency, came from a mating which produced 6 in 15 and back of this for 2 generations of brother-sister mating were records of 10 in 26, and 11 in 26. Female 30942 came from a mating which produced 1 in 3, this from one which produced 4 in 9 and back of this the same two high producing matings as above, 10 in 26 and 11 in 26. In order to account for the high record of these and other matings, it is necessary to suppose that most if not all of the homozygotes, Ot_2Ot_2, at least among the females, developed abnormally. The most probable interpretation is that matings producing between 10 percent and 20 percent abnormals were of the type $Ot_2/ot_2 \times ot_2/ot_2$ while most of those producing higher percentages were $Ot_2/ot_2 \times Ot_2/ot_2$ with perhaps a few $Ot_2/Ot_2 \times Ot_2/ot_2$.

The genetic basis of the differences among the low producing lines of family 13, and between these and other families cannot be settled conclusively. As has been noted, however, there were indications of a segregation of tendencies in the early generations, with fixation of a tendency to produce about 1.5 percent in one line from about the 13th generation (13-13-7) and of about 4.6 percent in another, also from the 13th generation (13-13-1). One might postulate a major factor for otocephaly, introduced by one of the original pair. The later history could then be accounted for as one of loss in some lines, retention and fixation in others. The differ-

ence in the percentage of incidence in 13-13-7 and 13-13-1 can be due to the fixation of different systems of minor modifiers. There is however, no such definite evidence for a major factor ot_1, as there is for the mutation Ot_2 discussed previously.

Reference has been made earlier to crosses between the high line of family 13 and a family (2) which had produced no otocephalics among 3500 young. These crosses were carried to F_3 with the results indicated below.

FEMALE×MALE	F_1		F_2		F_3	
	OTOS.	TOTAL	OTOS.	TOTAL	OTOS.	TOTAL
(13)×(2)	0	88	1	220	2	52
(2)×(13)	0	95	1	109	2	44
	0	183	2	329	4	96
	0.0 percent		0.6 percent		4.2 percent	

The appearance of otocephalics in F_2 and of as many as 4.2 percent in F_3 after disappearance in F_1 is strongly indicative of Mendelian segregation and is compatible with a single recessive major factor (ot_1) as the cause of the difference between the families. The secondary factor Ot_2 may not have been transmitted by all of the animals of family 13 used and if it were transmitted it might well produce no effect except in segregants homozygous for the primary factor.

OCCURRENCE OF HIGH GRADES

The monsters are classified by grade of abnormality, sex and strain in table 2. The majority in all strains are in grades 1 to 5 characterized by reduction in the jaws and associated parts of the skull and musculature, but with no apparent defect of the brain. Grades 6 to 12 have a similar reduction of the jaws but, in addition, defects of the brain, nose and usually eyes. In 9 cases largely from the early records, the monsters were neither preserved nor described with sufficient completeness to make the grade certain. These were probably of grades 2, 3 or 4, but may have been of grade 5 or 6. A considerable number were described sufficiently to assign to grades 3 or 4, the commonest types, but could not be allocated to one of these. The great majority have been graded from the preserved specimens.

The most interesting genetic question is the relation of the high grades with brain defect (6 to 12) to the commoner types with normal brains. In general, strains with a higher percentage of low grade monsters, also show a higher percentage of high grades. The frequency of high grades in

TABLE 2

Distribution of otocephalics by strain, grade and sex.

GRADE	NO ANCESTRY OF (13)		CROSSES INVOLVING (13)			(13) EXCL. 13-13-1		13-13-1 EXCL. 13-19-1			13-19-1 AND CROSSES LOW SEGREGATION		13-13-1 1 PARENT TRACES TO HIGH 13-19-1			13-13-1 BOTH PARENTS TRACE TO HIGH 13-19-1			13-19-1 HIGH LINE			ALL LINES			
	♂	♀	♂	♀	?	♂	♀	♂	♀	?	♂	♀	♂	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	TOTAL
A				1												1				1		1	2		3
1	2					2	1	1	1		1	2	1	3			3		2	1	1	9	11	1	21
2	1	5	1	1		1	3	5	11			1	3	2		1	2		1	5		13	30		43
3		1		2	1	2	1	4	8		1	2	4	4	1	3	6		2	6		16	30	2	48
3, 4	2	4		1		3	4	2	5					1					3	7	1	10	22	1	33
4		3	1	4		3	6	8	17	1	3	2	4	6		8	21	1	8	17	3	35	76	5	116
5		1		1		1		1	2								3					2	7		9
6	1	1					1	2	1					1		1	2			1		4	7		11
7	1		1			1		1														4			4
8	1	2																		1	1	1	3	1	5
9		1				2			1			1							1			3	3		6
10	1	1					2		3		2								1			4	6		10
11																				1			1		1
12																			1			1			1
Misc.	4	2					1										2					4	5		9
A-5	5	14	2	10	1	12	15	21	44	1	5	7	12	16	1	13	35	1	16	37	5	86	178	9	273
6–12	4	5	1			3	3	3	5		2	1		1		1	2		3	3	1	17	20	1	38
Otos.	13	21	3	10	1	15	19	24	49	1	7	8	12	17	1	14	39	1	19	40	6	107	203	10	320
Young	(24,000)		(2500)			(3507)		832	744	38	195	183	137	139	6	114	126	6	111	109	14				
Percent			0.2	0.8		0.9	1.1	2.9	6.6		3.6	4.4	8.8	12.2		12.3	31.0		17.1	36.7					

	OTOS. AS PERCENTAGE OF ALL YOUNG		OTOS. OF GRADES 6–12 AS PERCENTAGE OF TOTAL OTOS.
	1–5	6–12	6–12
No ancestry of family 13	0.10	0.04	26
Crosses of family 13	0.52	0.04	7
13-0-2 (excl. 13-13-1)	0.80	0.17	18
13-13-1 (excl. 13-19-1 except low branches)	3.9	0.55	12
13-19-1 and crosses (excl. low branches)	18.1	1.44	7

13-19-1 is probably significantly greater (2.3 SE) than the frequency in 13-13-1, indicating that the mutation Ot_2, responsible for a greatly increased percentage of otocephalics, increases the frequency of high grades as well as of low grades.

One might expect, indeed, that any factor which increased the frequency of otocephalics would increase disproportionately the chance for the extreme types to appear. The opposite, however, appears to be the case. Among the sporadic cases outside of family 13, *at least* 26 percent were of high grade. At the opposite extreme is line 13-19-1 and its crosses with a total of 19.6 percent otocephalics but only 7.4 percent of the otocephalics of high grade. The difference is significant (3.2 SE). Contrasting the 7.4 percent with the 14 percent of high grades in the rest of family 13, the difference approaches significance (1.7 SE).

Females are about twice as likely to be otocephalic as males. Yet only 3 more high grade otocephalics have appeared among females than among males (20 *vs.* 17). Only one out of 10 female monsters is high grade while one out of 6 is high grade among males. The difference is not statistically significant (1.5 SE) but at least it indicates that there is no tendency toward excessive degree of abnormality among females. Thus, both in respect to strain and sex, the grade of abnormality tends to decrease as the total frequency increases.

It is interesting in this connection that sporadic otocephaly and cyclopia as described in man and lower animals is very likely to be found associated with other abnormalities, for example, in double monsters, and in single monsters with anencephaly, cephalocele, harelip, spina bifida, exomphalus, *situs inversus*, club hand or foot, polydactyly, et cetera. There has been no such tendency in family 13 apart from occasional hydrocephalus in high grades and microphthalmia in grades 5 and 6 which are probably secondary consequences. It appears that *external* factors which tend to cause otocephaly, also tend to cause other abnormalities but that the *genetic* basis in family 13 is specifically related to otocephaly.

The tendency toward specificity of type of otocephaly in particular genetic stocks is especially well illustrated by the unusual character of all of the otocephalics produced in family 2. In these 7 monsters, all called

grade 5C, there was complete absence of the inner ears although these are the most persistent organs of the head in the otocephalics of family 13 and other strains.

THE GENETICS OF ALLIED ABNORMALITIES

Where not experimentally induced, otocephaly and allied defects have usually been reported as occurring as isolated cases which have not suggested heredity as a factor. The occasional reports of two or more in a sibship have been explicable on the basis of either heredity or environmental factors according to the predilections of the author. The most closely similar abnormality to be studied genetically seems to be a "lethal head abnormality" described by LITTLE and BAGG (1924). In this the face was usually shortened, the mandible short or absent, the mouth sometimes lacking. One or both of the eyes might be rudimentary. Breeding data were interpreted as indicating a simple Mendelian recessive lethal. This is in contrast with the situation found in the guinea pigs in which more than one gene is clearly involved and each gene (including one dominant one) affects merely the *percentage* of occurrence.

It is possible, however, that the difference is more apparent than real. In LITTLE and BAGG's data there is a significant deficiency of recessives from parents known to be heterozygous. This may be due, as the authors note, either to the devouring of stillborn young by the mother or to failure of the abnormality to be manifested in all recessives. The latter hypothesis approximates to some extent that required in the guinea pig case. It may be said that the data are consistent with the hypothesis of a single main recessive semi-lethal and indeed that this hypothesis is the most probable one but it is not clear that a more complicated situation is ruled out.

WRIEDT and MOHR (1928), and MOHR (1929) have investigated a lethal abnormality in cattle (*Acroteriasis congenita*) which has points of similarity with otocephaly. The ears are small and approximated, the mandible rudimentary, the palatines lacking, the maxillae defective and the premaxillae bent downwards. These defects suggest an otocephalus of grade 5 but the regular association of these with gross defect of all of the legs (extremities lacking, anchylosis of scapula and humerus, et cetera) make comparison doubtful. They find that this trait has been appearing frequently in herds of Holstein Friesian cattle of Norway. In nearly every case it was possible to trace both parents to a particular imported bull, only a few generations back. The frequencies were in harmony with the interpretation as a simple recessive lethal and the uniformity in degree of abnormality favors this interpretation.

FELDMAN (1932) has reported briefly on a non-lethal defect of the mouse which may possibly be related to the otocephaly of guinea pigs of family

2 (grade 5C). In these mice, there is more or less reduction of the external ear. In extreme cases the middle and inner ears and other parts of the head, especially the eyes, may be affected. He states that this trait is recessive, complicated by 20 percent overlap with normal.

There are probably many more traits whose genetics is like that of the otocephalic guinea pigs than is usually realized. In the guinea pig itself, polydactyly (WRIGHT 1926, 1931) depends on a very similar situation except for the absence of lethal effects. In certain respects this is also true of white spotting (WRIGHT 1928). A well known case in Drosophila is that of reduplicated legs (HOGE 1912) in which the major gene determines merely a certain percentage of abnormals at ordinary temperatures. According to MORGAN, BRIDGES and STURTEVANT (1925), there are many such cases in Drosophila but, being inconvenient for genetic work, they are largely discarded. The outsider is thus likely to obtain an exaggerated impression of the frequency with which genes determine clear-cut, absolute effects. In birds and mammals, cases involving multiple factors and thresholds may easily simulate simple Mendelian heredity throughout experiments as extensive as it is considered practicable to make and an erroneously simple interpretation is thus likely to be assigned. In these slowly reproducing forms, the question as to what constitutes a critical demonstration of a gene is one that deserves more attention than has been paid to it.

THEORIES OF CAUSATION

In the preceding discussion we have attempted to analyze the factors involved in the occurrence of a certain category of monster in certain strains of guinea pigs. It is desirable now to take definite cognizance of the fact that we are dealing with one of the most characteristic types of abnormal development in vertebrates (and perhaps of animals in general) with regard to the causation of which there is an extensive literature. This literature is concerned almost wholly with environmental rather than hereditary factors and with ones of a much more tangible nature than the chance factors, not common to litter-mates, found to determine practically all that is not hereditary in the guinea pigs of family 13. The task is therefore not one of cataloging similar results but of attempting to bring apparently dissimilar results under a common viewpoint.

Agnathia and cyclopia, separate or combined, have doubtless been known from the earliest times as types of monsters occurring in man and the domestic animals. BALLANTYNE points out that many of the minor deities of ancient Greece had teratological characters and that the most striking element in the appearance of POLYPHEMUS may well have been suggested by a human cyclops.

We may pass rapidly over the theories of ancient times, according to

which monsters were looked upon as the result of the play of the Gods, "sports," as signs of divine power or anger or as portents. The oldest known publication on the subject seems to be a brick found in ASHURBANIPAL'S library in Nineveh which gives in cuneiform the prognostication appropriate to each of a remarkable list of monsters (BALLANTYNE, GOULD and PYLE). Somewhat allied was the belief in the influence of heavenly bodies which seemed a rational explanation of "moon calves" as long as astrology flourished. A common mediaeval belief, that monsters are of diabolic paternity was closely related to belief that they are hybrids. Finally may be mentioned the ancient and still prevalent belief that monsters are the result of "maternal impressions."

One of these theories (that of hybridism) turns out to be related to a real factor in certain cases, but there is little connection between the mediaeval superstitution and recent experimental verification with fishes.

Among ancient writers who attempted to treat the matter in a scientific spirit, one may find the germs of theories of both hereditary and environmental determination. EMPEDOCLES and DEMOCRITUS, according to BALLANTYNE, held that heredity was wholly paternal and that monsters are due to seminal aberrations, of one sort or another. ARISTOTLE extended this idea to products of both parents. There was little development of the hereditary theory of monsters until recent times. Heredity of minor defects such as harelip and polydactyly has long been recognized. Many authors of the early 19th century (for example, MECKEL) vaguely suggested germinal defect as a cause of the non-viable monsters, but heredity could hardly be taken seriously before the idea of a hereditary mechanism had reached definiteness.

The idea that abnormal development may be a result of mechanical injury and especially of pressure goes back to HIPPOCRATES and ARISTOTLE, and has continued to the present time as one requiring careful consideration in each case. DARESTE's interpretation of the monsters of birds and mammals (including cyclopia) as largely resulting from the pressure of an arrested amnion was a form of this theory which was widely accepted in the latter half of the 19th century. Here, however, pressure is not the ultimate external factor. It is generally accepted today that mechanical forces are important secondary (internal) factors in normal or disturbed morphogenesis (though not in general in the way postulated by DARESTE) but that they are of negligible importance as *ultimate* causes of monstrosity.

During the 18th century, the theory of foetal disease came into prominence under the leadership of MORGAGNI. Apart from direct destruction of tissues, disease was believed to be a factor by causing accumulation of fluid with consequent injury from internal pressure (applied to cyclopia

and otocephaly by BÉCLARD in 1816–17 and DUGÈS in 1827) or by causing amniotic adhesions to the embryo (held by E. G. ST. HILAIRE and many later authors to be the principal source of monstrosity) or by causing malnutrition or intoxication.

WILLIAM HARVEY in 1651, noted that harelip and allied abnormalities could be accounted for by arrest of development at an embryonic stage, a view extended by later embryologists such as HALLER, WOLFF and MECKEL. This theory however is again not one of *ultimate* causes. Arrested development may be the consequence of pressure, or of malnutrition, or of intoxication, or of heredity.

The frequent occurrence of abnormalities among artificially incubated chicks was doubtless recognized as far back as the practice of this art in ancient Egypt. E. G. ST. HILAIRE (in the early 19th century) seems to have been the first to make deliberate experiments. He studied the effect of shaking eggs, pricking, covering with wax or varnish, et cetera, and produced a variety of monstrous types including high grade otocephaly. Environmental disturbance was thus proved to be a real factor. These experiments were continued by I. G. ST. HILAIRE and many others. The most comprehensive series of researches was that of C. DARESTE, published in a long series of papers summarized in 1892. DARESTE found that all effective methods (shaking, varnishing the shell, abnormal temperatures) produced the same series of monsters, frequently including cyclopia and otocephaly, if applied early in incubation. The particular theory which he invoked in the case of cyclopia, arrest of the amnion, was shown to be false by RABAUD on studying sections of cyclopic chicks produced by DARESTE's method. RABAUD found that the anterior medullary plate developed abnormally from the first in cases of cyclopia.

The list of agents which could produce cyclopia and other monstrosities in the chick was greatly extended by FÉRÉ (see BALLANTYNE 1924) who not only confirmed DARESTE'S results but was successful with many others, including penetrating vapors (from turpentine, ammonia, ether, chloroform, et cetera), solutions injected into the albumen of the egg (salt, glucose, many alcohols, acetone, blood, cocaine, morphine, atropine, et cetera). He found no specificity in the production of particular monsters by particular agents and (like RABAUD) no constant relation to abnormality of the amnion. He attributed the effects to malnutrition and poisoning.

While not based on experimental evidence, the conclusions reached by recent students of human monsters are of interest. BALLANTYNE (1904) after a judicial survey of previous theories, leaned toward a combination of the views of DARESTE and FÉRÉ, namely that disturbances of nutrition and toxic effects are primary and that these are especially effective in teratogeny through arrest of the amnion. With respect to cyclopia and

agnathia he noted the frequency of association with other defects as evidence that all have a common basis in pathology of the embryo.

MALL (1908, 1917) made a study of an exceptionally large number of pathological human embryos. The frequent association of monstrosity with a pathological condition of the membranes or of the embryo as a whole (*fetus compressus*, et cetera) led him to express most emphatically the conclusion that in all cases monsters are produced by external influences among which he gave first place to disturbed nutrition (usually due, he believed, to faulty implantation) and second place to injurious substances. Like many others he drew a sharp line between monsters (including cyclopia) in whose causation he believed heredity played no part whatever, and hereditary structural anomalies, such as polydactyly. WILDER (1908) drew a similar line, but because of their symmetry (Cosmobia) was inclined to put cyclopia on the genetic side.

SCHWALBE and JOSEPHY (1913) also impressed by the symmetry of cyclopia and otocephaly rejected the crude mechanical factors such as amniotic pressure. They considered the natural causes as not at all clear. While recognizing that cyclopia can be produced experimentally by external factors they held it still an open question whether an inner factor might not be the usual cause.

We turn now to experiments with lower vertebrates in which the amnion cannot be a factor.

The hypothesis that local injury might produce such monsters was supported by BORN (1897) who demonstrated that frogs with a cyclopian eye could sometimes be produced by splitting the anterior end of the medullary plate. Later LEWIS (1909) produced cyclopia and allied types in Fundulus by pricking the anterior end of the embryonic shield. Less direct in the relation between the injury and the appearance of a cyclopic eye was the result of an experiment by SPEMANN (1904) in which by ligating the egg of Triton (2-cell stage) he obtained two-headed monsters, one head of which was frequently cyclopic (occasionally both). SPEMANN interpreted the cyclopic eye as the result of fusion, following defect of the material between the eye rudiments, a conclusion with which LEWIS concurred. More recent defect and transplantation experiments by ADELMANN (1929, 1930) indicate, however, that there is equal potency with respect to eye-forming capacity in a band across the anterior medullary plate and that the explanation of cyclopia cannot be so simple as mere loss of rudiments of predetermined parts between the eyes.

With regard to agnathia, STONE (1922) has demonstrated that deficiencies in the branchial skeleton can be induced in Amblystoma larvae by removal of portions of the neural crest in medullary plate stages.

STOCKARD (1907, 1909) obtained such an extraordinarily large propor-

tion of cyclopians (50 percent in favorable cases) by treatment of Fundulus eggs (undivided or cleavage) with magnesium salts as to suggest a specific developmental effect. He suggested that cyclopia in mammals might be caused by excess of magnesium in the maternal blood. Later however (1910) he found that alcohol and other anaesthetics were as effective as magnesium salts or more so and suggested that the effect was one of this class of substances on the developing central nervous system. His theory was one of inhibition and arrest of development (of the central nervous system) instead of partial destruction followed by fusion of lateral primordia.

CHILD and MCKIE (1911) interpreted STOCKARD'S results as due to a general inhibition of developmental rate affecting most seriously the anterior medullary plate as the most active and sensitive region rather than as a region reacting to the specific substances used.

MCCLENDON (1912) showed, in fact, that a much wider range of substances than anaesthetics was effective in Fundulus (for example, KCl, NaOH, cane sugar, caffeine) but reached the conclusion that the osmotic condition was the significant one.

WERBER (1915), with the mammalian situation in mind, studied the effect of a number of products of abnormal metabolism on Fundulus eggs. He found that acetone and butyric acid produced cyclopia and many other monsters in large numbers. He held it likely that mammalian cyclopia is due to toxemia resulting from such conditions of the mother as diabetes, jaundice and nephritis and that if heredity is concerned at all, it is maternal heredity of such conditions. As to the immediate mode of action, his theory was one of destruction of sensitive primordia by the toxic agent ("differential blastolysis") and in this respect was similar to the defect theory of SPEMANN and LEWIS rather than to the inhibitory theories of STOCKARD and of CHILD.

KELLICOTT (1916) induced the usual variety of monsters, including cyclopia, merely by subjecting the eggs to low temperatures. KELLICOTT criticized the theory of disturbed nutrition of MALL, the differential blastolysis of WERBER and developmental arrest of STOCKARD as inapplicable to effects produced before cleavage or in early cleavage stages of an egg with indeterminate cleavage. He suggested instead the disarrangement of nuclear or cytoplasmic constituents or both in the egg. As regards the nucleus, this may be considered as similar to the more recent hypothesis of induced mutation or chromosome aberration. In this connection the results of BARDEEN (1907) and of BALDWIN (1919) on the effects of fertilization of toad eggs with X-rayed sperm are of interest since from present knowledge this agent seems to be the one most likely to produce effects of the kind considered by KELLICOTT. These authors obtained abnormal de-

velopment of a particular type, characterized by stunting of all parts. The eyes were especially effected but microphthalmia, not cyclopia, was produced. The picture was one of permanent injury affecting all cells and thus very different from the normal-bodied otocephalics found in guinea pigs.

Monsters of the cyclopic and more extreme types were induced in amphibia, (most successfully in frogs, *Rana fusca*) by LEPLAT (1919). He tried a variety of agents (NaCl, KCl, LiCl, $BaCl_2$, $MgCl_2$ ethyl alcohol, chloroform and chloral hydrate) at various stages of development from first cleavage to closure of the blastopore. He was most successful with LiCl, ethyl alcohol and chloral hydrate. Treatment was most effective during the period of the horseshoe-shaped blastopore. This is a decidedly later period than indicated by most other experiments.

Meanwhile, CHILD (1911) had shown that abnormalities of as similar a nature as possible to cyclopia of vertebrates could be induced in regenerating fragments of Planaria by means of agents which depress metabolism. A series of forms was produced characterized by approximation of the eyes, fusion of the eyes, absence of eyes or in extreme cases absence of the whole head. The center of defect appeared to be in the nervous system. Pieces of a given length showed higher frequencies of abnormality at successively posterior levels of a zooid, while at a given level, reduction in size of a piece increased the frequency of abnormality.

CHILD interpreted his results on the basis of his gradient hypothesis. According to this, protoplasm is a kind of material which tends to become polarized with respect to rate of metabolism in relation to the prevailing environmental stimuli, much as it is a kind of material which acquires a marked surface-interior differentiation under surface tension. While stimulation spreads from regions of high metabolism to adjacent low regions irrespective of cell boundaries, a high region tends to maintain dominance over adjacent regions approaching (but not equalling) it in level. The consequence is the development of a regular gradient even in assemblages of cells initially heterogeneous in metabolic rate. Such a pattern tends to become stabilized by material differentiation consequent on differences in metabolic rate. In the later elaboration of pattern, this material organization becomes more important than protoplasmic polarity, although the latter tendency continues to be manifested within limited regions of relatively homogeneous character. Such gradients have been demonstrated in many simple organisms including vertebrate embryos (HYMAN 1921). In general, the anterior end of the embryo develops at the high pole of the primary pattern. Experiments indicate that high levels are more immediately susceptible to injurious agents than low levels, although more capable of acclimation during, and recovery after, continued exposure to slightly depressing influences. Cyclopia and allied types are ex-

plained as the type of monster resulting from injury to the susceptible anterior pole of the primary gradient pattern.

This theory is essentially not one of the nature of the ultimate factors of abnormal development but one of the nature of protoplasm. It becomes readily explicable under it that the same type of abnormal development should result from an environmental agency or from a gene complex either of which brings about a general depression of life activities with differential effects on parts of the organism in relation to the gradient pattern.

NEWMAN in a series of papers between 1908 and 1917 showed that the same types of monsters could be produced by making wide crosses (in teleosts) as were produced by environmental agencies. He found a close correlation between rate of development and the degree of normality of the embryos. He found that the monsters which appeared could be classified in two groups, forms with inhibited apical parts (cyclopia, microphthalmia, defective heart) and forms with inhibited basal parts (relatively large head, stunted trunk and tail, in extreme cases isolated eyes or hearts, explicable as due to differential acclimation or recovery.

STOCKARD (1921) confirmed the production of cyclopia, as well as of other types of monsters including twins, by low temperatures. His general conclusions in this paper are in essential agreement with those of CHILD, but emphasize especially the significance of critical moment. After noting (1) that all types of monsters, double and single, may be caused by the *same treatment* and (2) that one type (as cyclopia) may be produced by a great number of different treatments and (3) that all effective treatments are ones which tend to lower developmental rate he concludes (4) that the type of monster depends on the particular developmental moment or moments during which developmental rate is reduced. Thus he finds that the critical moment for twinning comes first followed shortly by those for cyclopia, brain vesicles, the branchial arches and the inner ear.

These principles are in accord with CHILD'S theory except for some qualification of the fourth. Different types of abnormality are to be expected on any basis from treatment after the organs concerned have been formed, as compared with treatment before; and apart from this, the regions of most active metabolism at one stage are not necessarily the same as those of another stage. Under CHILD'S theory, however, radically different types of monsters (due respectively to differential inhibition and differential acclimation) are to be expected from treatment at the same developmental moment, depending on the severity of the treatment or the physiological state of the animals.

On reviewing the accounts of the various experiments on production of monsters, one gets the impression that the critical moments are not as definite as implied by the above theories. Results obtained by HINRICHS

(1925) on treating Fundulus eggs with ultraviolet are especially significant since only a few minutes of treatment were necessary but in general they agree with those of earlier workers, including STOCKARD, as far as comparison is possible. The earlier the treatment the greater the number of monsters. Thus 3 minutes of treatment produced 100 percent monsters if within 10 minutes after fertilization, but only 25 percent at 75 minutes, and only about 5 percent at 3 1/2 hours or at 21 hours (when the axis was in process of formation). The monsters induced by treatment in the one cell stage (first 2 1/2 hours) included a great variety of types: twins and anterior and posterior duplicities, defective hearts, cyclopia, defects of branchial arches (otocephaly), dwarfs, forms with stunted tails, et cetera. Twins and anterior duplicities could not be produced by later treatment but all of the other types could be produced by treatment during cleavage although with decreasing frequency. With treatment after the axis had begun to form, eye defects took the form of microphthalmia rather than cyclopia.

It is to be noted that the effective period for cyclopia is before the appearance of morphological rudiments of eyes, whether the agent be ultraviolet, chemicals or low temperature. It apparently extends, however, from the unsegmented egg to a period shortly after formation of the medullary plate.

These results point to a similarly early time of action of the genes in the guinea pigs discussed here rather than to separate action on the primordia of the various organs.

The results in the inbred line of guinea pigs are, however, in marked contrast to those of experimental treatments in the uniformity of monstrosity produced (apart from mere variation in degree). This probably means that the gene effects are relatively direct and precise in their moments of action, while the response to the experimental agents varies greatly in its promptness, dependent on the physiological state of the organism.

It is interesting in this connection, to return to consideration of the excess of females among otocephalics. As already noted, a similar excess has been noted among cyclopians of man and other animals. It is stated (HIRST and PIERSOL, SCHWALBE) that human double monsters (Dicephalus, Pygopagus) are two or three times as likely to be female as male. In both natural and experimentally produced two-headed monsters, one head is likely to be cyclopian or otocephalic. It may be noted here that two double monsters have occurred in our stocks of guinea pigs. Both were of the cruciate type, two bodies, belly to belly, united anteriorly; in one case with two heads, the other with one head (but four ears) at right angles to the body axes. In the two-headed case one face was normal, the other

otocephalic of grade 6 (Syncephalus thoracopagus janiceps). The tendency toward association of otocephaly, cyclopia and duplicity point toward a close relationship in causation. As noted above, all of these types are produced only by early experimental treatment. The excess of females in all of them suggest that the X chromosome is effective as a primary sex determining factor perhaps through an effect on rate of metabolism near the beginning of development, although differential absorption at a later period is a possible alternative.

CONCLUSIONS AND SUMMARY

Otocephaly is a well known type of monstrosity in vertebrates. In low grades, the defect is restricted to the jaws (agnathia). In higher grades, extreme jaw defect is associated with defect of the brain and associated parts, cyclopia being a common manifestation. In the highest grades, jaws, nose, eyes and all of the brain in front of the medulla are lacking (aprosopus).

Otocephaly is very rare in most strains of guinea pigs (less than 0.05 percent). A particular inbred strain produced 1 percent in its early generations. A branch which arose in the 13th generation of brother-sister mating has produced about 5 percent in all but one of its sub-branches for 15 years. The exceptional branch has produced about 27 percent but tends continually to relapse to 5 percent.

The usual theory has been that this sort of monster is produced in mammals by maternal toxemia and that any tendency to heredity is maternal heredity. Experiments show that this is not the case here. The pertinent heredity is that of the monster itself, equally derived from both parents.

There is evidence of segregation of different tendencies among branches of the family in the early generations. The 5 percent branch must be considered as homozygous for a certain gene complex. It is shown that within this branch the monsters cannot be due to ordinary Mendelian segregation and that there are no balanced lethal factors. Neither can the monsters be due to any important extent to environmental factors of such a sort as to act on litter-mates alike. The randomness of occurrence within litters of each size, indicates that each monster is due to a highly localized chance event, supplementing a genetic tendency common to all members of the group. There is, however, a slight excess in small litters, indicative of some effect of unfavorable conditions.

The evidence indicates that the abrupt change from 5 percent to 27 percent occurrence in one sub-branch in the 19th generation was due to a dominant mutation. This has segregated as a unit in later generations and in outcrosses to the 5 percent branch.

Sex is a factor, there being twice as many females as males among the monsters.

There is evidence of genetic differences in the type of defect. Only 7 percent of the otocephalics of the high producing line show brain and eye defect, while over 26 percent of the sporadic otocephalics of other families were of these extreme types. All 7 of the otocephalics of one inbred strain showed an earless condition not found in any other strain.

It is pointed out that these monsters are similar to ones produced by a great variety of environmental agents (mechanical, chemical, ultra-violet, cold, et cetera) in lower vertebrates. More or less closely allied types have been produced by treatment as early as the uncleaved egg but apparently cannot be produced by treatment later than an early medullary plate stage. It is suggested that the genetic factors in this case act by bringing about (directly or indirectly) a general depression in vital activity at a particular critical moment, with permanent effects on the development at the anterior end of the embryonic axis as the most active and hence most susceptible region at the time of action.

It is noted that in the case of the guinea pigs studied the genetic factors play a less decisive role with respect to the fate of individuals than reported for several other somewhat similar types of abnormality. The genetic complex determines merely the percentage incidence in a strain, while chance differences of such a nature as to be practically as great between litter-mates, as between non-litter-mates of the same genetic constitution, are decisive with respect to individuals.

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