CYCLOPIA AND RELATED DEFECTS AS A LETHAL MUTATION OF FOWL*

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(With Plates 14–16)

(*Received* 15 *March* 1955)

The weird and bizarre appearance of cyclopean monsters in early times gave rise to legends and myths. Later, when men began to think in terms of more concrete causality, their urge to understand such out-of-the-ordinary events often produced mutual suspicion. Witness the following account of proceedings had at a 'genrll court for New Haven, the 15th of January, 1652': 'The Gouerne^{r} acquainted the Towne that the cause of calling y^{m} together this day is about a monsterous pigg, w^{ch} was brought forth by a sow of John Vincons: it was like a pigg in the body & leges, but wthout haire, the skin being white; the head something like a piggs head, but y^{e} nether chapp, something like the nether chapp of a man, one eare something like a piggs eare, the other like two little teates hanging downe, one great red eye in y^{e} face of it, and from the forehead a peece of skiney flesh hanging downe, hollow like y^{e} member of a man, w^{ch} hath made the Magistrate to whom it was first showne feare that some bestialitie hath bine comitted: it was now showne to y^{e} Towne, and every man was desired to looke vpon it, to see if by the visiage or any other markes there may bee anything discovered that way: but after some time of consideration, no man spake so as to accuse any, wherefore y^{e} Marshall had order to bury it' (Dexter, 1917).

In modern times and perhaps still because, as Ballantyne (1904) put it, cyclopia is such a very monstrous type of monstrosity', descriptive and experimental teratologists have dealt extensively with this type of malformation. The experimental production of cyclopia by various physical and chemical means was reported for chicken embryos, the use of X-rays being especially successful (cf. Ancel, 1950; Wolff, 1948). It remains in doubt, however, if these experimental methods duplicate the manner of origin of spontaneously arising cases of cyclopia.

As far as hereditary transmission is concerned, our knowledge is limited to the extensive studies on otocephalic guinea-pigs (Wright & Eaton, 1923; Wright, 1934; Wright & Wagner, 1934) in which cyclopia occurred as one step in a graded series of malformations. Hence our discovery of familial cyclopia in fowl seemed of more than passing interest. The present report will present a brief description of the gross morphological appearance of these malformed embryos and a discussion of their genetic history. More detailed accounts of structure and development are to follow in another place and at a later date.

* Supported by a grant from the American Cancer Society, on recommendation of the Committee on Growth, National Research Council.

Morphology

The malformations with which we are concerned can be arranged in a series that reaches from relatively minor facial defects to nearly complete absence of the head. Within this series we find types of abnormalities that severally can be distinguished and, in fact, are being used as taxonomic units of teratology, such as arhinencephalia, synophthalmia, cebocephalia, ethmocephalia, cyclopia, otocephaly, microcephaly and acephaly. For classificatory purposes and as a descriptive short-cut, terminologies of this kind may be useful and unavoidable, but from the developmental point of view their significance is limited by the fact that they grade into each other. It will be seen in the present material that what appears to be a succession of small and blending, quantitative steps, probably results from one fundamental aberration which in its process of realization stops at an earlier or later stage according to the presence or absence of numerous modifying factors.

The lowest grade of defect with which we have to deal here is characterized by absence of the premaxillae (Pl. 14, fig. 1). The whole premaxillary bone, including its nasal, palatine and maxillary processes, is lacking. The nasal bone has a blunt anterior surface. It is covered with a narrow margin of horny skin. In some cases a small horny tip projects downward into the oral cavity. In other instances the lateral ramus of the nasals is defective, leaving the nasal orifices without anterior border. The eyes are normal in size and location. Such embryos usually complete development, but fail to break the shell. On occasion, however, chicks of this kind hatched. They always appeared to be entirely blind, indicating that even in these low-grade defects the central nervous system is involved. By 'spoon-feeding' we raised one such chick to the age of about 3 months when it was killed (Pl. 14, fig. 2). Complete absence of the upper beak, with grossly normal size and position of the eyes, occurred as a rare variant.

In succeeding steps, the eyes are transposed forward until they touch each other in the midline (Pl. 14, fig. 3). In addition to lack of the premaxilla, the nasal bone is reduced in size and its shape is abnormal. The next steps in this sequence of increasing abnormality are represented by eyes that have developed from primordia in varying conditions of fusion or, perhaps more correctly, from contiguous and defective primordia which combine to form eyes with a greater or lesser extent of doubling in their constituent structures. These eyes are within one orbit. The coalescent corneas often give the appearance of a horizontal figure ∞. There is a typical proboscis above the orbit, usually with a single nasal opening (Pl. 14, fig. 4; Pl. 15, figs. 5, 6).

In a still more extreme condition one finds a single median eye on the roof of the oral cavity (Pl. 16, figs. 7–9). These truly cyclopean eyes often are of normal size and shape. The proboscis is usually smaller than in the lesser degrees of abnormality and may lack an opening. The lower beak to all appearances may still be entirely normal. The ears are little, if at all, displaced toward the ventral midline. The tongue is curled upward. The forebrain is not separated into hemispheres. The midbrain is frequently herniated, a large fluid-filled vesicle overlying the forebrain. Embryos of this kind usually died between 16 and 18 days of development; some succumbed even earlier; a few were found living in the shell at the end of the incubation period.

Among embryos surviving the thirteenth day of incubation, the most extremely deformed in the present series represent a gradual transition to nearly complete acephaly.

The single median eye is reduced in size; there may only be a pigment spot below the epidermis or no sign of any visual organ (Pl. 16, fig. 10). The proboscis disappears. The mandible is reduced in size or lacking. The ear openings are approximated toward the ventral midline. Finally, there is no visible indication of nose, eyes or mouth; the skull and brain are mere vestiges. Such embryos generally died at about the end of the second week of development.

The embryos with low-grade defects probably are, in the great majority of cases, prevented from hatching because the defectiveness of their beak interferes with their breaking through the membranes and egg shell. In the presence of more extreme abnormalities the whole oral cavity is grossly abnormal or may even be entirely lacking. Death of such embryos may, at least in part, be due to starvation on account of their inability to swallow albumen (Witschi, 1949).

Because of the transgressive nature of expression of the malformations under discussion, it will be impossible to present the results of our breeding experiments in terms of closely circumscribed teratological categories. In what follows, we shall refer by the non-committal term of perocephaly to the whole array of defects, extending from abnormalities which grossly involve only the upper beak to microcephaly. We shall subdivide the data into two groups under the headings of 'rudimentary upper beak' and 'cyclopia'. All embryos and chicks in which the premaxillary bones were absent, but in which the eyes were normal in size and location and in which the nasal openings occupied their normal place, will appear under the classification of 'rudimentary upper beak'. All other malformations, reaching from noticeable synophthalmia to microcephaly, will be included in the 'cyclopia' grouping.

ORIGIN AND INHERITANCE

All embryos with perocephalic malformations, covered by the present report, appeared among the descendants of crosses of our stock of recessive rumpless fowl to three different and unrelated breeds. The recessive rumpless mutation itself (Landauer, 1945) had turned up in the F_2 generations and subsequent progenies of crosses between White Leghorn and Creeper chickens and between White Leghorn and Silver Spangled Hamburg fowl. The breeding history of these crosses made it clear that the genes for recessive rumplessness had been present in the Leghorn flock in a submerged condition, and that they had only come to phenotypic expression after plus modifiers had been removed by outcrossing.

Penetrance and expressivity of recessive rumplessness were low. *Inter se* matings of recessive rumpless birds produced many 'normal overlaps', and complete absence of all tail vertebrae was relatively rare. The majority of birds with an abnormal tail skeleton fell into a series of conditions intermediate between total lack of the tail and its normal development. It was evident that the expression of recessive rumplessness was still under the influence of multiple modifying genes.

In experiments, the principal results of which have been reported elsewhere (Landauer, 1955), we sought to determine if incidence and degree of expression of recessive rumplessness could be raised by transferring the mutation into the genotypes of certain other stocks. Three breeds were used in these crosses, viz. Silver Gray Dorking, Rhode Island Red and Jungle fowl. It will be shown that malformations of the perocephalic type made their appearance in the descendants of each of the three resulting families or of crosses between them.

Crosses with Dorking Stock. In 1948 we made reciprocal crosses between Silver Gray Dorking and recessive rumpless fowl, all of the latter being of the intermediate rumpless kind. No unusual malformations were found in the progenies of these matings. In subsequent years descendants of this cross were bred to each other. The F_2 and F_3 generations included a few embryos with cyclopia and kindred abnormalities. We decided at that time to make systematic observations on the occurrence and possible genetic interrelationship of these malformations.

In 1951 we had five pens of descendants of the original crosses, these being F_4 generations. There were sixty-three females in all. Sixteen of these produced progeny with perocephaly as shown in Table 1. The forty-seven females whose recorded progenies (numbering in size between 1 and 126) did not include embryos with malformations of the type under discussion had a total of 1514 offspring. It is likely, as will appear hereafter, that many of these females were actually carriers of these defects. It is remarkable that all five cocks used in the matings transmitted the malformations. Four of these males were full brothers, the fifth being a half-brother. They had had no sibs with perocephaly.

Table 1. *F_4 progenies of a cross between Silver Gray Dorking and recessive rumpless fowl*

	Survivors of 13th day of incubation	Cyclopia	Rudimentary upper beak	Perocephaly (%)
11 mothers producing progeny with cyclopia or rudimentary upper beak	594	12	4	2·7
5 mothers producing only progeny with rudimentary upper beak	120	0	5	4·2

In 1952 we had four pens with F_5 matings derived from the original Dorking × recessive rumpless crosses. In two of these pens (pens 2 and 3), the females were from matings that had produced offspring with cyclopia as well as rudimentary upper beak; the females in the other two pens (pens 4 and 5) came from those 1951 mothers whose progenies had

Table 2. *F_6 progenies from crosses of Dorking and recessive rumpless fowl. The progenies represent the survivors of the 13th day of incubation*

	No. of mothers producing			Progenies			
Pen	Cyclopia and rudimentary upper beak	Rudimentary upper beak, but not cyclopia	Neither type of defect	No.	Cyclopia	Rudimentary upper beak	Perocephaly (%)
2	3	—	—	116	4	0	3·4
		—	11	103	0	0	0
3	3	—	—	143	3	4	4·9
		2	—	94	0	2	2·1
		—	16	786	0	0	0
4	1	—	—	57	1	0	1·8
		3	—	96	0	4	4·2
		—	15	322	0	0	0
5	2	—	—	138	6	0	4·3
		1	—	33	0	1	2·6
		—	17	982	0	0	0

included embryos with rudimentary upper beak, but none with cyclopia. The males in pens 2, 3 and 5 had been used in the previous year and were known to give perocephalic progeny; the cock in pen 4 had had sibs with rudimentary upper beak, but none with cyclopia. The results of these matings are shown in Table 2.

Among thirty-five daughters of perocephaly-producing parents mated to sons of the same derivation (pens 2 and 3) eight progenies yielded embryos with perocephalic defects; these eight progenies contained a total of 353 survivors of the 13th day of incubation, including seven embryos with cyclopia (2·0%) and six with a rudimentary upper beak (1·7%), the total incidence of perocephaly amounting to 3·7%. Among the twenty females which were daughters of rudimentary upper beak (but not cyclopia) producing matings (pen 5) three had perocephalic abnormalities in their progenies; these three mothers had a total of 176 offspring, six of which had cyclopia (3·4%) and one a rudimentary upper beak (0·6%); the total incidence of perocephalic embryos was 4·0%. Finally, among nineteen females whose sibs had included individuals with rudimentary upper beak (but not cyclopia), when bred to a cock with a similar history (pen 4), four produced progenies containing perocephalic individuals; in a total of 153 offspring one showed cyclopia (0·7%) and three a rudimentary upper beak (2·0%). The incidence of perocephaly in this type of matings amounted to 2·6%. There is no indication that the several types of matings had yielded significantly different results.

During 1953 our matings from the Dorking-recessive rumpless stock were limited to testing sixty-three daughters of parents which, during the previous year, had yielded neither cyclopia nor rudimentary upper beak. These sixty-three hens were mated to cocks which with other females had produced the perocephalic malformations. In forty-nine of these matings sibs ranging from two to seventy-one in number ($M = 30$) contained no embryos with perocephaly in a total of 1481 survivors of the 13th day of incubation. The fourteen other females produced five cyclopic individuals (1·8%) and eleven with rudimentary upper beak (3·9%) out of a total of 281 embryos and chicks, the total incidence of perocephaly amounting to 5·7%.

Crosses involving Rhode Island Red stock. In 1950 we bred a Rhode Island Red hen (whose mother had been of the sporadic rumpless type) to a cock of our recessive rumpless stock. Twenty-four F_1 daughters from this cross were mated to a male from the Dorking-recessive rumpless line who was known to transmit perocephaly. Ten of the resulting sibships (numbering from 52 to 144) contained among a total of 968 individuals no embryos with perocephaly. The remaining fourteen sibships (34–127 in size) included in a total of 1305 survivors of the 13th day of incubation nineteen embryos with cyclopia (1·5%) and six with a rudimentary upper beak (0·5%). These perocephalic embryos, therefore, were produced by females of Rhode Island Red and recessive rumpless ancestry bred to males with Dorking and recessive rumpless progenitors.

Crosses involving Jungle fowl stock. In 1948 we made reciprocal crosses between Red Jungle fowl and birds from the recessive rumpless stock. F_2 and F_3 generations from these crosses were raised in 1949 and 1950 without yielding perocephalic embryos. In 1951 we bred twelve F_3 females from this Jungle-recessive rumpless stock to cocks of the Dorking-recessive rumpless line, known to transmit the perocephalic malformations. Three of these females produced progenies with a total of 185 survivors of the 13th day of incubation, including two cyclopic embryos (1·1%) and one with a rudimentary upper beak (0·5%). The remaining nine females had 534 offspring in progenies of between thirty-eight and eighty-four diagnosed embryos, none of which had perocephalic defects.

Genetic origin of the malformations. Thus there were three types of matings in which embryos with perocephalic malformations made their initial appearance. The first of these is represented by the F_2 and later generations of crosses between Silver Gray Dorking

and recessive rumpless fowl. The second and third types of matings consisted of females from Rhode Island Red × recessive rumpless or Red Jungle × recessive rumpless ancestry bred to males of the Dorking × recessive rumpless family. The only ancestry which all three kinds of matings have in common is that derived from the recessive rumpless stock. It may, therefore, be safely concluded that the principal genetic ingredients which provide the basis for the development of perocephaly were present in our stock of recessive rumpless fowl.

It was pointed out earlier that the recessive rumpless mutation was recovered in two independent outcrosses of White Leghorn fowl. There are reasons to believe that the perocephalic malformations also trace back to the same White Leghorn stock. The few instances of 'sporadic' perocephaly which we have found during the past 30 years in the course of the routine examination of embryos that had died in late stages of development have all been from our White Leghorn stock. The frequency of occurrence of sporadic cases of cyclopia and allied defects was probably in the neighbourhood of one in 10,000, or less. Secondly, a few additional instances of perocephaly were observed following the treatment of embryos in early stages with various teratogenic substances, and these again were all from White Leghorn stock. In the latter case it must be admitted, however, that much larger numbers of White Leghorn eggs than of eggs of other breeds were used in the experiments. In any event, it seems certain that a low-grade disposition for the formation of perocephaly-like abnormalities was present in the White Leghorn stock. This disposition presumably made its appearance as a hereditary trait following a history of extensive outcrossing.

Additional interfamily crosses. The perocephaly-producing families which have been discussed so far were brought together in subsequent crosses. We shall limit ourselves here to a discussion of the data obtained during 1954. The birds involved in these matings were either of Dorking-recessive rumpless by Rhode Island Red-recessive rumpless or of

Table 3. *Results of crosses between families*

The mothers either were known to produce perocephaly ('tested') or were daughters of tested parents. Within these two main groups are listed first the sibships which produced high- as well as low-grade perocephaly; next sibships containing cyclopean, but not rudimentary upper beak embryos; next sibships with rudimentary upper beak, but not cyclopean embryos; and finally sibships not containing embryos with either type of defect.

Mothers	No.	Size of examined sibships	Survivors of 13th day	Cyclopia	Rudimentary upper beak	Perocephaly (%)
Tested hens	6	23–68	268	27	14	15·3
	1	51	51	3	0	5·9
	1	41	41	0	2	4·9
	2	16 and 19	35	0	0	0
Total	10	—	395	30	16	11·6
Pullet daughters of tested parents	15	5–85	782	80	43	15·7
	5	13–57	149	6	0	4·0
	1	17	17	0	1	5·9
	1	20	20	0	0	0
Total	22		968	86	44	13·4

Dorking-recessive rumpless by Jungle-recessive rumpless origin. Since no differences were found between these crosses, the data are presented together in Table 3. There were ten hens whose progenies in the previous year had contained perocephalic embryos and twenty-two pullets whose mothers had produced embryos of this type.

In comparison with the original three families, the incidence of malformations was much increased in these crosses. In the progenies of the ten previously tested hens 7·6 % of the survivors of the 13th day of incubation were cyclopic and 4·1 % had a rudimentary upper beak. In the progenies of the twenty-two daughters of tested parents 8·9 % cyclopic embryos and 4·5 % with rudimentary upper beak were found among 968 survivors of the 13th day. This represents at least a doubling of the incidence observed in the Dorking-recessive rumpless line which among the three original types of matings had produced the highest incidence of these defects.

The data of Table 3 suggest, furthermore, that *all* offspring of known producers of perocephalic progeny in turn have in their progenies individuals with these defects, provided that numerically adequate tests are made. The ten hens in the 1954 matings all had in the previous year produced some offspring with cyclopia as well as rudimentary upper beak. Yet, during 1954 one of these hens had three cyclopean offspring, but none with a rudimentary upper beak; another hen had two embryos with rudimentary upper beak, but none with cyclopia among her offspring; and two of the hens produced small progenies without defects. The twenty-two daughters of tested parents showed a similar distribution. Only one of them had no defective offspring in a small progeny, and most of the progenies of the six pullets which among their offspring had either cyclopic embryos but none with rudimentary upper beak or the reverse distribution were also quite limited in number. It is presumably significant that the nine largest of the thirty-two sibships all included embryos with both types of defect.

Seasonal variations in incidence. Our early observations on perocephaly suggested that the incidence of these malformations may show seasonal fluctuations. An analysis was, therefore, made for the data of each of the years 1951–4. The number of weekly settings of eggs during the hatching season was as nearly as possible divided into equal halves and incidence of the malformations determined for the first and second part of the season. The first part of the experimental season was in each year from the middle of February to the middle of April, the second part from the latter part of April to the middle of June. The results are shown in Table 4.

Table 4. *Incidence of perocephaly for all relevant matings during* 1951–4 *according to season*

Year	Part of season	Size of progenies	Embryos with perocephaly	Significance
1951	First	1236	15	$\chi^2 = 6{\cdot}094$
	Second	1115	29	$P = 0{\cdot}014$
1952	First	939	12	$\chi^2 = 18{\cdot}382$
	Second	1146	52	$P < 0{\cdot}001$
1953	First	842	58	$\chi^2 = 0{\cdot}358$
	Second	921	57	$P = 0{\cdot}563$
1954	First	610	34	$\chi^2 = 49{\cdot}757$
	Second	753	138	$P < 0{\cdot}001$

It can be seen that in three of the four years the incidence of perocephalic malformations rose sharply during late spring, with highly significant differences in each instance. During 1953 the differences were not significant. Calculation of the combined probabilities for the four seasons gave a χ^2 with $P < 0{\cdot}01$.

A search was then made for the causes of these seasonal fluctuations in incidence of

perocephalic malformations. These efforts have not been successful thus far. They will be briefly discussed in what follows. Among factors of the internal environment *maternal age* might play a role. A chance to test this was provided in one of our 1954 pens which was composed of eight hens and five pullets. The data are shown in Table 5. It is evident that age of the laying mothers made no difference in occurrence of the seasonal trend. Another possible factor influencing incidence of perocephaly might be *parity*, i.e. the position of particular eggs with reference to the preceding number of eggs laid by the individual mother. This question was studied by calculating for pens 1 and 2 of 1954 the regression of the number of eggs produced (x) from the beginning of the laying year to the end of the experimental season on relative frequency of perocephaly during the second half of the season (y). For pen 1 the coefficient $b_{xy} = +0{\cdot}155$ and for pen 2 $b_{xy} = -0{\cdot}101$. Clearly, there is no relation between preceding egg production and the chances of an embryo to bear the perocephalic defects.

Table 5. *Incidence of perocephalic malformations according to season for yearling and 2-year-old hens (Pen 1, 1954)*

Age of mother	Part of season	Size of progenies	Embryos with perocephaly	Significance
1 year	First	112	7	$\chi^2 = 8{\cdot}179$
	Second	147	27	$P < 0{\cdot}01$
2 years	First	165	11	$\chi^2 = 10{\cdot}240$
	Second	195	35	$P < 0{\cdot}01$

Among external factors, *nutrition* is unlikely to have had an influence on trends in the incidence of perocephaly since our breeding birds are kept in pens without runs (the 2-year-old hens having been off range for about 18 months) and are given the same ration at all times. It is equally improbable that conditions of *incubation* were responsible for the observed changes in incidence of perocephaly: temperature variations were slight and not influenced by the season; changes in relative humidity, though somewhat under the influence of seasonal trends, do not seem sufficiently pronounced or consistent to account for the existing facts.

Another possible source of variation in external conditions is the *air temperatures during the day eggs were laid*. Eggs are taken once a day (in the afternoon) from our breeding pens to an air-conditioned storage room. There is no doubt that the developmental processes that have gotten under way at the time of laying are brought to a stop more promptly in early spring than later on. Hence it can be taken for granted that the eggs laid and incubated during May and June were at a somewhat more advanced stage at the beginning of incubation than those produced during late winter or early spring. In experiments with Creeper fowl, it was found that such differences accounted for differential survival of lethal homozygotes (Landauer, 1944).

If the seasonal trend in incidence of perocephalic malformations is brought about by temporal changes in the developmental stage reached prior to incubation, it might be possible to obtain supporting evidence in one of two different ways. First, and assuming that the temperatures of the external environment during late spring favour the survival of perocephalic embryos that die earlier in the season, corresponding differences in the opposite direction could be expected to obtain in early embryo mortality. Secondly, on the assumption that high air temperatures on the day of laying and a consequently more

advanced condition of the blastodisk prior to incubation are detrimental to embryos that would otherwise have developed into normal overlaps, it might be possible to duplicate these conditions experimentally by pre-incubating eggs at relatively low temperatures before exposing them to the normal incubation routine.

In testing the first hypothesis, we scrutinized the data for the years 1953 and 1954 with reference to mortality during the first 13 days of incubation. These two seasons were chosen because no seasonal trend in incidence of perocephaly was found in one of the two years (1953), whereas in the other year (1954) the trend was highly significant (see Table 4). Means of embryo mortality during the first 13 days and for the first and second half of the seasons of each of the two years were found to be as follows: 1953, first half of season 21·7%, second half 20·0%; 1954, first half of season 16·8%, second half 15·4%. In neither of the two years was the difference significant. In view of the relatively low over-all incidence of perocephaly this negative result cannot be considered decisive. Closer inspection of the data showed, however, non-linear relationships. If, for instance, the 1954 data are used to express, for each sibship, 1–13-day mortality during the second half of the season in percentage of mortality during the whole experimental period, a highly significant heterogeneity is found between individual mothers ($\chi^2 = 231{\cdot}97$, d.f. 25, $P < 0{\cdot}001$). If the mortality data are partitioned according to whether the sib incidence of perocephaly during the whole breeding season was low (not more than 16%) or high (above 16%), it was found that in the group with low incidence early mortality was $11{\cdot}23 \pm 1{\cdot}89$%, whereas in the high-incidence group it amounted to $20{\cdot}10 \pm 4{\cdot}21$%. The difference between the two groups amounts to $8{\cdot}83 \pm 4{\cdot}61$%. The evidence, therefore, points against the conclusion that high incidence of perocephaly was accompanied or brought about by reduced early mortality; there is, in fact, a suggestion that early mortality was higher in the group with the greater incidence of perocephaly. It is true that among the sibships with low incidence of perocephaly early embryo mortality was lower during the second part of the season as compared with the first part, and the differences may be significant ($\chi^2 = 4{\cdot}334$, $P = 0{\cdot}03$), but no such changes took place in the sibships with a high incidence of perocephaly. The suggestion provided by these data, viz. that a rise in incidence of perocephaly tends to occur *pari passu* with increased early embryo mortality is reinforced by data which will be discussed below.

Experiments in which the second hypothesis was tested by pre-incubating eggs at lower than normal temperature, followed by cooling and subsequent normal incubation, gave entirely negative results, i.e. the incidence of perocephaly was not raised. We are thus left without explanation of the factors which account for the seasonal variations in incidence of perocephaly. Experiments are now under way inquiring into the possible influence of changes in length of daylight and of oxygen tension in our incubators on incidence of perocephaly.

Sex. During 1953 and 1954 we determined the sex of the perocephalic embryos. The following data were found:

	Rudimentary upper beak			Cyclopia		Total		
Year	♂	♀	Sex?	♂	♀	♂	♀	Sex?
1953	39	36	—	27	16	66	52	—
1954	54	52	7	30	29	84	81	7
Totals	93	88	7	57	45	150	133	7

There is no suggestion of an abnormal sex distribution.

Genetic variance. Our material shows clearly that there is a graded series of malformations which by imperceptible steps leads from a rudimentary condition of the upper beak (associated with blindness) to approximation of the two eyes toward the frontal midline, to the presence of two eyes within one orbit, to typical cyclopia, and in the most extreme conditions to otocephaly and microcephaly.

It is of interest to compare various matings in regard to the total incidence of these defects and also with reference to the relative frequencies of different forms of expression. The incidence of all types of perocephalic abnormalities was analysed for our two 1954 pen matings. There were fifteen females in pen 1, including two which had small progenies that were free of defective embryos. In pen 2 we had seventeen pullets, all full or half-sisters, and one of these had twenty offspring, all of which were normal. Within both pens the sibs showed highly significant heterogeneity with reference to the incidence of perocephalic malformations. For the fifteen sibs of pen 1 $\chi^2 = 84{\cdot}91$, and for the seventeen sibs of pen 2 $\chi^2 = 99{\cdot}72$, with a probability in each case of $< 0{\cdot}001$. Omission from calculation of the few sibs containing no perocephalic embryos had little effect on the results (the χ^2 being 80·52 and 94·45, respectively, with $P < 0{\cdot}001$ in each case). It appears, therefore, that the groups of females in our two pens were heterogeneous in regard to the conditions which permit perocephaly to come to expression.

A similar result emerged when the data from the eighteen pen matings, which between 1951 and 1954 contained our perocephaly-producing birds, were analysed with reference to the proportion of the more extreme kinds of abnormalities (i.e. exclusive of rudimentary upper beak) in the total of embryos with perocephalic malformations. Significant heterogeneity was found to exist between these pens, with $\chi^2 = 32{\cdot}80$ and P between 0·01 and 0·02. There was no indication, however, of a constant relationship between the incidence of the more extreme and of the less pronounced forms of perocephaly. The relative frequency of the more grave defects (otocephaly, cyclopia, synophthalmia) and the percentage incidence of the less severe forms of abnormality (rudimentary upper beak) showed for the 1951–4 pen matings a correlation coefficient of $+0{\cdot}103 \pm 0{\cdot}216$, i.e. entire absence of a mutual relationship. This was to be expected if the modifying genes governing 'expressivity' had been distributed by chance following the original outcrosses. The detailed data are shown in Table 6.

Data of particular interest were secured when the same thirteen females were mated to half-brothers in 1952 and to a distantly related cock in 1953. The results are given in Table 7. As compared with the half-brother-sister matings of 1952, the data for the much less closely related matings of 1953 show the following significant changes:

Embryo mortality during the first 13 days	$+10{\cdot}3 \pm 3{\cdot}24\%$
Number of chicks hatched	$-30{\cdot}4 \pm 5{\cdot}58\%$
Incidence of perocephaly among survivors of 13th day	$+5{\cdot}7 \pm 2{\cdot}22\%$

The coefficients of variation for the two years were as follows:

	1952	1953
Mortality 1–13 days	60·9	69·2
Hatch	12·4	36·5
Perocephaly	49·7	91·6

It will be remembered that the perocephalic defects first made their appearance in the F_2 and subsequent generations of outcrosses between our recessive rumpless stock and

unrelated breeds of fowl. The data of Table 7 demonstrate that a higher degree of outbreeding, i.e. more pronounced dilution or diversification of protective modifiers, led to an increased incidence of perocephaly and to reduced vitality. A similar increase in the incidence of perocephalic monsters, and presumably for the same reasons, was found when the original three perocephaly-producing lines were crossed with each other (data of Tables 2 and 3). The heightened variability in outcrosses is a concomitant of the same forces

Table 6. *Records for* 1951–4 *pen matings, showing incidence of cyclopia and rudimentary upper beak among the survivors of the* 13*th day of incubation. Data for females with any perocephalic progeny. Incidence of perocephaly in percentage of survivors; incidence of cyclopia in percentage of perocephaly*

Year	Pen	Survivors 13th	Cyclopia	Rudimentary upper beak	Perocephaly (%)	Cyclopia among all perocephalic (%)
1951	4	350	6	4	2·9	60·0
	5	673	9	3	1·8	75·0
	6	796	10	5	1·9	66·7
	7	386	4	2	1·6	66·7
	8	260	4	2	2·3	66·7
1952	2	117	4	0	3·4	100·0
	3	238	3	6	3·8	33·3
	4	154	1	3	2·6	25·0
	5	179	6	1	3·9	85·7
	6	565	5	9	2·5	35·7
	7	743	10	10	2·7	50·0
	8	100	2	3	5·0	66·7
1953	1	192	4	4	4·2	50·0
	2	122	1	3	3·3	25·0
	3	193	5	3	4·1	62·5
	4	211	6	2	3·8	75·0
	5	180	1	4	2·8	20·0
	6	113	3	1	3·5	75·0
	7	755	51	29	10·6	63·8
1954	1	619	52	28	12·9	65·0
	2	689	61	31	13·4	66·3
Total		7635	248	153	5·3	61·8

Table 7. *Records of thirteen females bred to half-brothers in* 1952 *and to a distantly related cock in* 1953

	1952				1953			
Mothers	Fertile eggs	Dead 1–13 days	Hatched	Perocephaly	Fertile eggs	Dead 1–13 days	Hatched	Perocephaly
672	48	4	39	1	50	3	36	0
696	58	2	51	3	52	11	14	2
729	99	0	90	1	103	22	45	7
1213	30	3	22	1	56	14	27	5
1221	74	7	56	1	61	6	32	3
1228	50	4	38	2	57	13	18	3
1246	63	6	51	1	71	4	55	4
1278	40	2	21	1	27	10	10	0
1298	61	6	44	2	56	18	10	7
1313	76	2	66	1	80	2	59	0
1319	87	1	79	1	79	2	45	18
1321	60	1	45	3	58	10	26	4
1336	60	4	47	2	52	3	23	2
Mean %		5·8±0·98	78·6±2·70	2·9±0·40		16·1±3·09	48·2±4·88	8·6±2·18

Discussion

In the series of malformations that has been discussed, the most common stages are a rudimentary condition of the upper beak (absence of the premaxillary bones), synophthalmia and cyclopia. The more extreme defects, varying from cyclopia anophthalmica to microcephaly, are relatively rare among survivors of the 13th day of incubation, but it should be noted that with an increased incidence of perocephalic defects embryonic mortality became high during early stages of development. The possibility remains open, therefore, that our data do not include the most extreme abnormalities.

The synophthalmic and cyclopean monsters of our material, grossly at any rate, bear a close morphological resemblance to corresponding malformations which have been described throughout the vertebrate phylum from fishes to man. In humans, cyclopia is often, though by no means invariably, associated with other defects, among which polydactylism, lack of the adrenal glands, horseshoe kidney and atresia ani are particularly common. No such associated abnormalities were found in our material.

There are, strictly speaking, no reports dealing with the inheritance of cyclopia as a unit trait. The literature of human pathology contains some case reports suggestive of the hereditary transmission of cyclopia and allied defects. In one of these case histories (Klopstock, 1921) a first-cousin marriage produced sibs one of which was cyclopean and the other was a cebocephalus, with the two eyes in a common orbital cavity. Van Duyse (1898) reported on a sib from unrelated parents in which three consecutive abortions (at three months of pregnancy) were followed by two children with cleft palate (the first lived for 5 days, the second died at birth), a normal child, and then cyclopic twin foetuses. Another twin birth that is of interest was recorded by Ellis (1866). The mother in question had, as primipara and with her first husband, produced a child with cleft palate and imperforate anus. The next delivery, with her second husband, resulted in twin foetuses, of which one was described as an ethmocephalus, the other as a rhinocephalus.

The studies of Wright and his associates on hereditary otocephaly in guinea-pigs command particular interest in relation to our material. The two series of abnormalities converge from seemingly unrelated low-grade conditions toward closely similar defects in their most extreme expression. In chicken embryos the series of perocephalic defects begins with lack of the premaxillary bones as the principal external symptom; in guinea-pig foetuses the corresponding initial stages exhibit a reduction of the mandible. With the appearance of synophthalmia and cyclopia in the abnormal guinea-pigs (Wright's grades 7 and 8) the similarities with the chicken material become manifest, although even in these stages the ears are little, if at all, involved in chicken embryos, whereas the guinea-pigs are completely otocephalic. In their most pronounced expression the monsters of both kinds of animals lack all signs of eyes, nose, mouth and forebrain.

The genetic and environmental forces which in embryos of guinea-pigs and chickens conspire to produce these malformations of the head are in part similar, in part quite dissimilar in the two groups of animals. As far as otocephaly of guinea-pigs is concerned, Wright (Wright & Eaton, 1923; Wright, 1934) arrived at the following conclusions:

(1) In the foundation stock otocephaly occurred as a rare ($<0{\cdot}05\%$) sporadic malformation.

(2) Some, though not all, inbred families, derived from the original stock, gave a somewhat higher incidence of otocephaly.

(3) One inbred family in particular showed in some of its branches sudden increases in the frequency with which otocephaly occurred. One such jump raised the incidence from 1 to 5%, another from 5 to about 27%.

(4) The genetic factors were zygotic. The inbred families presumably had become homozygous for genetic factors providing the basis for a tendency toward otocephalic development, with at least one major mutant step responsible for increased incidence.

(5) There was some indication of strain differentiation with reference to particular peculiarities of otocephaly.

(6) With increasing frequency of otocephaly incidence of the extreme types tended to decrease.

(7) The incidence was about twice as high in females as in males.

(8) Unfavourable environmental conditions (winter) had a slight tendency to raise the incidence of otocephaly.

(9) There appeared to be little, if any, increased foetal death rate in otocephaly-producing matings.

Our own data may be discussed briefly in the light of these observations on and interpretations of the guinea-pig material.

(1) The one ancestral stock common to all perocephaly-producing families was a line of recessive rumpless chickens which in turn had been derived from outcrosses of White Leghorn fowl. These White Leghorns had on rare occasions (probably 0·01% or less) produced sporadic cases of cyclopia and allied defects.

(2) As the recessive rumpless mutation had made its initial appearance in the F_2 generations of crosses between White Leghorn × Creeper and White Leghorn × Silver Spangled Hamburg fowl, so the perocephalic malformations were first encountered among the descendants of crosses between recessive rumpless fowl and unrelated stock.

(3) Following crosses between recessive rumpless and Dorking fowl, the F_2 and subsequent generations contained 2–3% perocephalic progeny among the survivors of the 13th day of incubation. About the same incidence of these malformations was found when F_2 females from crosses of recessive rumpless × Rhode Island Red fowl or recessive rumpless × Jungle fowl were mated to males (F_2 or later) from the recessive rumpless × Dorking fowl stock. When two of the three lines were brought together the incidence of perocephalic defects rose to about 4–5%. When the genotypes of all three sources of origin were combined, there was a further rise in frequency of perocephalic embryos to about 13%.

(4) All the results of our breeding experiments indicate that the genetic factors for perocephaly were present in the foundation stock, that by outcrossing plus modifiers were removed or made heterozygous, and that by the continued admixture of unrelated genes the factors for perocephaly became increasingly released from their cryptic existence. As long as the incidence of perocephaly was low, no relationship could be discerned between the size of sibs and the presence or absence in them of perocephalic individuals (Table 2). But after the incidence of monsters had once risen to a more appreciable level, only small progenies failed to include them (Table 3). It is equally notable that of twenty-one cocks from perocephaly-producing matings which were tested between 1949 and 1954 every single one transmitted the defects. Taking into account the data on incidence of the abnormalities (viz. the impossibility of accounting for them on the basis of segregation), the only explanation which covers all the facts appears to be the assumption that the

perocephaly-producing parents were actually homozygous for the genetic factors producing these malformations, and that the incidence of perocephaly in their progenies depended solely on what proportion of zygotes produced by them had fewer than the number (and kinds) of protective modifiers required for normal development.

Much additional evidence is in harmony with this conclusion, and some of it should here be pointed out. As the frequency of perocephalic defects increased in our material, the proportions of high-grade defects (synophthalmia, cyclopia, microcephaly) and of relatively minor abnormalities (rudimentary upper beak) remained constant among the survivors of the 13th day of incubation. This is contrary to what would be expected on the assumption of increasing homozygosity for a multifactorial complex with graded expression, but is readily understood on the basis of gradual and chance elimination of protective modifiers. At the same time, early embryo mortality increased *pari passu* with rising incidence of perocephaly among the survivors, presumably as another effect of the dilution or elimination of plus modifiers.

(5) It was shown that highly significant heterogeneity existed within groups of females, bred to the same male, in regard to the relative incidence of cyclopia and rudimentary upper beak. In conjunction with the fact that the proportions of different grades of perocephaly remained approximately constant over the years, these observations may be taken as evidence for the random distribution (elimination) of modifiers. There is little doubt that separate lines, producing varying perocephalic phenotypes, could be established by selection.

(6) It has already been pointed out that the frequencies of different grades of perocephaly tended to remain stable during the period of our breeding tests.

(7) In contradistinction to otocephaly in guinea-pigs and cyclopia in other mammals (including man) the two sexes of chicken embryos showed no difference in incidence (or severity) of the perocephalic malformations.

(8) In three out of four years the incidence of perocephaly showed a significant and very pronounced seasonal trend, incidence increasing from early spring toward summer. The proportions of different grades of perocephalic defects were not affected by this trend. The trend appeared also to be independent of the over-all incidence of perocephaly during a particular year or in a specific type of mating.

(9) There was no excessive early embryo mortality as long as the incidence of perocephaly remained low (2–3 % of the survivors of the 13th day), but a perceptible rise in the frequency of perocephalic monsters was accompanied by excessive early mortality.

In Wright's guinea-pig material the genetic tendencies for production of otocephaly were isolated and fixed in particular inbred strains, and the incidence of animals bearing these malformations was enhanced by the occurrence of one or more mutations in certain branches of one inbred family. In our material, on the contrary, the genetic factors for perocephaly were unmasked by outcrossing, and incidence of the defects rose as a consequence of the continued dilution of the original genotype in crosses of different perocephaly-producing strains.

It is evident, however, that even in those of our matings which yielded the highest incidence of perocephaly, multiple modifiers were still at work in producing the variance of expressivity and probably also in determining the level of penetrance. It is quite conceivable, though, of course, beyond the possibility of proof, that the initial step in the history of the perocephalic malformations was a single-gene substitution (or other muta-

tional change) with detrimental heterozygous effect, and that this was followed by a long history in the accumulation of plus modifiers. It may be surmised that these modifiers have no 'specific' adaptive relation to perocephaly, but in a more general way are an integral part of the complex system of genetic units contributing to and reinforcing normal development. In this connexion, it is of some interest to point out that the recessive rumpless condition (i.e. the stock to which the perocephalic malformations could be traced) was from its first emergence associated with reduced adult body size. This relatively small body size has persisted in our perocephaly-producing families. The elimination of genes affecting body size may well have been the first step in the process of unmasking the hereditary basis of perocephaly.

It is well recognized that the fund of equilibrating genes varies in its composition between individuals of a species. This variance presumably accounts for the increase in variability often encountered in the presence of mutant genes. I believe that failure to recognize this has often led to a misunderstanding of the actual situation. In discussing the genetical control of stability in development Mather (1953), for instance, says: 'The expression of, for example, the gene "eyeless" in *Drosophila* is much more variable both between individuals and between the two sides of a single individual than is that of its normal allelomorph. The genotype appears to have been adjusted by natural selection to give a relatively uniform development of eye size in the normal fly, but the stability vanishes once the course of development is changed by the introduction of the mutant gene.' In our view, the stability vanishes because, even in inbred stocks, individuals vary in their assets of genes with quantitative effects ('modifiers') one of which was probably the 'normal allele'. In our view, the perocephalic conditions became manifest when a sufficient number of such 'minor' genes had been eliminated or had become ineffective by heterozygosity.

The situation revealed by our present studies on cyclopia and allied defects is by no means unique. It has already been pointed out that we had encountered a closely similar situation in the history of the recessive rumpless mutation. We have also reported earlier that multiple recessive genes are commonly found in stocks of domestic fowl which in concert will completely prevent the phenotypic expression of dominant rumplessness, the 'short upper beak' lethal, and still other mutations. It is readily understandable that recessive genes of this type should have become widely distributed.

In his discussion of obligate heterozygosity as a competitively advantageous condition for the maintenance of equilibrating processes in development, Lerner (1954), in a comparison between *Drosophila* and poultry, comes to the conclusion that 'in the species which are more economical in their reproductive behaviour the margin for elimination of homozygotes may not be adequate to enforce heterozygosity at more than a limited number of loci'. Perhaps it was precisely this situation which made the development of a different type of protection against detrimental mutations a matter of life or extinction and which led, in poultry at any rate, to the accumulation and wide distribution of genes which in proper combination will reduce or entirely overcome the phenotypic expression of such adverse mutations.

SUMMARY

Cyclopia and related malformations have been observed as a lethal condition of fowl. The more important facts and conclusions may be summarized as follows:

1. The morphological expression varied from a condition in which the upper beak was

rudimentary to microcephaly. The whole gamut of defects is referred to as perocephaly. This is subdivided into two groups, viz. 'rudimentary upper beak', comprising the low-grade defects, and 'cyclopia', under which heading we include malformations ranging from synophthalmia to microcephaly. In the rudimentary upper-beak condition the premaxillary bones are lacking. On rare occasions such chicks hatched; they were always blind. Synophthalmia and cyclopia are associated with a proboscis above the median orbit. In more extreme conditions only a median pigment spot was present, there was no oral opening and the ears were closer to the ventral midline than normally (otocephaly). The most extreme cases were microcephalic without any indications of eyes, mouth or ears.

2. Perocephalic malformations made their first appearance in F_2 and later generations of crosses between recessive rumpless fowl and representatives of the following three breeds: Silver Gray Dorking, Rhode Island Red and Jungle fowl. In sibships which included any embryos with these malformations, the incidence was about 2–3 %. When families with a different breeding history were intercrossed, the incidence of perocephaly rose to about 5 %, and upon combining the genotypes of all sources of origin the frequency became about 13 %. The proportions of rudimentary upper beak and cyclopic embryos remained nearly constant during these changes in total incidence of perocephaly. After the 13 % level of incidence had been reached, all but the smallest sibships contained perocephalic embryos. Significant heterogeneity of incidence continued, however, to exist between the individual sibships of pen matings.

3. Twenty-one sons of perocephaly-producing parents were tested in our experiments. All transmitted the malformations.

4. A rising incidence of perocephaly was associated with increased early embryo mortality.

5. The sex ratio appeared to be normal among perocephalic embryos of all grades.

6. A scrutiny of the data obtained during four years showed in three of them a highly significant seasonal trend, the incidence of perocephaly rising from early toward late spring. The causes of this trend remain uncertain.

7. The results of our breeding tests led to the following interpretation. The genetic factors responsible for the occurrence of perocephaly (possibly a single recessive gene substitution) were present in the stock of recessive rumpless fowl. Except for extremely rare 'sporadic' occurrences, the mutation was completely suppressed by modifying genes. The mutation came to light after outcrosses to unrelated stocks and the incidence rose with continued dilution of protective modifiers. It is evident that even in later generations many embryos which were homozygous for perocephaly developed normally.

8. It is suggested that, in fowl at least, the accumulation of recessive modifying genes with individually small effects, but which in concert can completely suppress the action of deleterious mutants, represents a common and important mechanism of equilibration and genetic self-defence.

I am grateful to Dr W. F. Hollander for calling my attention to the incident recorded in New Haven Town Records.

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EXPLANATION OF PLATES

PLATE 14

Fig. 1. Head of embryo, living at 22 days, with rudimentary upper beak.

Fig. 2. A chicken with rudimentary upper beak, raised to the age of three months by artificial feeding. The animal was blind.

Fig. 3. Head of 20-day chicken embryo with rudimentary upper beak; eyes transposed forward (synophthalmia). Down removed.

Fig. 4. Head of embryo, living at 22 days, eyes contiguous and in one orbit, proboscis.

PLATE 15

Figs. 5, 6. Ventral aspect of head of two embryos with synophthalmia.

PLATE 16

Fig. 7. Head of 18-day embryo with cyclopia and proboscis.

Fig. 8. Head of 20-day embryo with cyclopia and proboscis. Tongue pushed upward.

Fig. 9. Head of 17-day embryo with cyclopia and proboscis. Lower beak reduced in size.

Fig. 10. Ventral aspect of head of embryo living at 22 days. Whole head reduced in size. Rudiment of beak. No nasal or oral openings. A pigment spot in ventral midline. Ears transposed toward ventral midline.