EXPERIMENTAL STUDIES AIMING AT THE CONTROL OF DEFECTIVE AND MONSTROUS DEVELOPMENT¹

A SURVEY OF RECORDED MONSTROSITIES WITH SPECIAL ATTENTION TO THE OPHTHALMIC DEFECTS

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TWENTY-NINE FIGURES

INTRODUCTION

The problem of the causes underlying defective development has recently received very exhaustive and illuminating treatment by Mall ('08). Basing his conclusions on his own study of 163 pathological human ova and on recent results of work in experimental embryology, he suggests that the human monsters are—with the exception of the hereditary 'merosomatous' terata—due to injurious influences of atypical environmental factors. He makes the specific suggestion—which seems justified in the light of evidence brought forth by him as well as by clinical data—that the monstrous development of some ova may be due to their inadequate nutrition owing to the imperfect implantation in a diseased uterus. It would seem that this hypothesis may hold good at least for some pathological embryos aborted during the first two months of pregnancy. It is obvious, however, that Mall's interpretation could not be extended to monstrous fetuses of the later months of pregnancy or to monsters after full-term birth; for an ovum suffering from lack

¹ This contribution is based on a paper read by title ("Is defective and monstrous development due to parental metabolic toxemia?") at the meeting of the American Association of Anatomists held at St. Louis, December 29, 30, 1914. *Anat. Rec.*, vol. 9, no. 1, pp. 133-137.

of nutrition could not well be imagined to live on to these later stages of development.

Some environmental factors must be looked for other than faulty implantation of the ovum, to account for the occurrence of such cases. The results of investigations in experimental embryology and teratology by Dareste ('91), Roux ('95), Hertwig ('96, '10), Féré ('94), Morgan ('02, '04) Stockard ('07, '09) and others, who obtained monstrous development of ova which had been subjected to the action of physical or chemical modifications of the environment, suggested to me that the human as well as the other mammalian monsters may be due to the physical or chemical action of some substances in the blood of one of the parents on either one of the sex cells or on the fertilized ovum, respectively.

Stockard ('09) has expressed the unsupported view that cyclopia in man may possibly be due to an excess of magnesium salts in the blood of the mother, and in later publications ('10 b), concluding from his experiments with alcohol solutions, he suggests that the cyclopean defect might perhaps be attributed to alcoholism of either one of the parents. It is rather evident that this latter assumption may be regarded as justified only for a negligibly small percentage of monsters. For such defects are not infrequently found in mammals as well as in other vertebrates where the possibility of alcoholism is entirely eliminated. The solution of this problem appeared to me to be elsewhere. Since the metabolism is the main source to which chemical modification of the body might be traced, I concluded that the toxic substances found in the blood of individuals afflicted with some metabolic disturbances might be the ones which could be made responsible for the origin of monstrous development.

MATERIAL AND METHODS

To test the validity of this hypothesis it would be necessary to produce experimentally in mammals such disturbances of metabolism as diabetes, nephritis, jaundice, etc., to mate thus diseased animals and eventually to study the heredity of such offspring as they may beget. Such experiments, however, are

difficult to perform in the absence of certain facilities, which are not usually accessible to the biologist. On the other hand, the spontaneous occurrence of these diseases in animals is too rare to permit of conclusive breeding experiments. It was necessary, therefore, to confine myself to a preliminary step in the investigation. This consisted in subjecting eggs of an oviparous vertebrate to the action of some toxic substances found in the urine under certain pathological conditions of metabolism.

The eggs of *Fundulus heteroclitus* were chosen, and after being fertilized they were exposed in early (1 to 2, 2 to 4, or 4 to 8, and 8 to 16, cells respectively) cleavage stages to the influence of solutions in sea-water of such substances as urea, butyric acid, lactic acid, acetone, sodium glycocholate, ammonium hydroxide, etc. Only with two of these substances, viz., butyric acid and acetone, have so far definite and positive results been obtained.

In the case of butyric acid, 10 cc. of a $\frac{1}{12}$ to $\frac{1}{14}$ gram molecular solution in 50 cc. of sea-water was found to be approximately the optimal solution, i.e., causing the greatest number of eggs to develop in a defective manner. If fertilized *Fundulus* eggs were left in this solution for fifteen to twenty hours and afterwards transferred to pure sea-water a great number of them gave rise to monsters. Similar results were obtained with solutions of acetone. Here the eggs were exposed in a number of dishes to the action of solutions of 20, 25, 30, 35, 40, 45 and 50 cc. of a gram molecular solution of acetone added to 50 cc. of sea-water. A varying relative number of deformed embryos was found in all dishes, increasing with the strength of the solution up to 40 cc. of acetone and decreasing in solutions still stronger, which caused an increase in the death-rate of the eggs. The length of exposure to the action of acetone was forty-eight hours in most and twenty-four and seventy-two hours respectively in some experiments. It was found that while long exposures increased the mortality of the eggs the difference in effect on the surviving eggs was very slight between exposures of twenty-four and those of forty-eight or even seventy-two hours. This points to the probability that it is mainly the initial effect

of the toxic solution on the ovum that causes it to develop in an atypical manner. The eggs were fixed and preserved after Child's sublimate-acetic-formaline method and were left in 4 per cent formaldehyde until the time of their imbedding. They were imbedded in celloidin-paraffin, chloroform being used as a clearing agent. Sections were cut 6 or 7 μ thick, stained with Delafield's hematoxyline and counterstained with erythrosin.

SURVEY OF THE MORPHOLOGICAL RESULTS

1. Deformities of the sense organs and the mouth

The morphological results obtained in both series of experiments, with butyric acid and acetone, being very much alike, it will suffice to state that the deformities enumerated here were found to be common to both. Great numbers of cyclopean embryos were found in both these series of experiments. I have recorded in my observations the occurrence of transition from two normal eyes in the typical position in the head all the way down through the more or less closely approximated eyes or eyes of a double composition and true cyclopia to complete anophthalmia (figs. 1-7) as described by Stockard ('09) in his experiments with magnesium chloride and alcohol, and by Lewis ('09) in his pricking experiments. Other defects in the development of the eyes such as asymmetric monophthalmia (fig. 8) and microphthalmia (fig. 9) were also found to occur abundantly. In some embryos all that could be detected of the eyes were lenses only or a rudiment of the choroid coat with or without a rudimentary lens. Coloboma, by which is understood a patency of the embryonic fissures of some parts of the eye, was found in some cyclopean as well as in two eyed but otherwise defective embryos.

Stockard ('09) has also described a peculiar change in the form and position of the mouth of the cyclopean embryo. The mouth in such embryos has the appearance of a snout, a proboscis-like structure, and is pushed down below the cyclopean eye. This displacement into the ventrolateral position Stock-

Figs. 1-29 Sketches of monstrous *Fundulus* embryos.

Fig. 1 Normal *Fundulus* embryo, twelve days old, to show the position of the eyes; *h.*, heart.

Fig. 2 Synophthalmia bilentica, from $\frac{1}{16}$ gram molecular butyric acid, eighteen days old.

Fig. 3 Synophthalmia bilentica, from $\frac{1}{12}$ gram molecular butyric acid, twenty-eight days old, with 'proboscis'—mouth, *m.*

Fig. 4 Synophthalmia bilentica, from $\frac{1}{12}$ gram molecular butyric acid, twenty-eight days old.

Fig. 5 Synophthalmia unilentica, from $\frac{1}{12}$ gram molecular butyric acid, twenty-four days old.

Fig. 6 Cyclopean embryo, showing one unusually large median eye with fused olfactory pits, *o.p.*, and proboscis-like mouth, *m.* From $\frac{1}{12}$ gram molecular butyric acid, eighteen days old.

Fig. 7 Anophthalmic embryo with club-tail and distended ear vesicles, from acetone solution (40 cc. gram molec. sol. to 50 cc. sea-water), thirteen days old.

Fig. 8 Asymmetrically monophthalmic embryo, with greatly distended ear vesicles, *e.v.*, one pectoral fin only and club-tail. From acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water), thirty-two days old.

ard attributes to the circumstance that the cyclopean eye being frontally located has caused the mouth to move downward. This interpretation, however, seems to be insufficient in view of the fact that I have observed in my experiments this occurrence not only in cyclopean monsters (fig. 6) but also in some cases of synophthalmia (fig. 3), asymmetric monophthalmia (fig. 8) and in many cases of dorsal microphthalmia (fig. 9). In some asymmetrically monophthalmic embryos (fig. 29) the mouth, while being apparently normal in shape, occupied the position in which normally the lacking eye would have to be located.²

Abnormalities of the olfactory pits were also almost invariably found to occur in embryos exhibiting various degrees of median 'cyclopia' (fig. 6) as well as in asymmetrically monophthalmic embryos (fig. 20). They usually corresponded to the anomalies of the eyes of a given embryo, that is, were either blended into one median pit or exhibited various degrees of approximation or fusion respectively in the cyclopean embryos. In the asymmetrically monophthalmic embryos where the mouth had taken the position of the missing eye, the nasal pit of the side possessing the eye was usually found to be in the normal position, while the pit belonging to the side lacking the eye has sometimes been found to be located posterior to the mouth. This unilateral ectopia of the nasal pit in the monophthalmic embryo is probably secondary to the ectopia of its mouth. These changes in shape and position of the mouth as well as of the olfactory pits are apparently due to processes of regulation after an blastolytic destruction of a certain area at the anterior end of the early embryo's body.

In a great many embryos the auditory vesicles reached enormous size, which on microscopic examination seemed to be due

² In a previous note ("The influence of products of pathologic metabolism of the developing teleost ovum," Biol. Bull., vol. 28, no. 1, pp. 51-57), it was stated (p. 54) that in some cases of asymmetric monophthalmia an open orbit was found on the side lacking the eye. This error was made owing to the transparency of the living specimens. It was the mouth in the exact position of the eye that was mistaken for an 'open orbit.' The error was found when the drawings of the living embryos were compared with the fixed specimens.

Fig. 9 Microphthalmic embryo (small eyes dorsally located), with distended ear vesicles, *e.v.*, From $\frac{1}{16}$ butyric acid, twenty-nine days old.

Fig. 10 Greatly malformed cyclopean embryo with dorsally located eye, rudimentary pectoral fins and club-tail, from $\frac{1}{2}$ gram molecular solution butyric acid, thirteen days old.

Fig. 11 Greatly malformed embryo from acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water) with one rudimentary lateral eye, without fins, club-tail; *p.c.*, distended pericardial vesicle.

Fig. 12 Extremely malformed anophthalmic embryo from acetone solution (20 cc. gram. molec. sol. to 50 cc. sea-water), fourteen days old.

Fig. 13 Greatly deformed anophthalmic embryo, with head partly constricted off from the rest of the body. From acetone solution (30 cc. gram molec. sol. to 50 cc. sea-water), sixteen days old.

Fig. 14 Extremely malformed, oedematous embryo, with one rudimentary lateral eye, distended ear vesicles, *e.v.*, with club-tail and without pectoral fins. From $\frac{1}{2}$ gram molecular butyric acid, fourteen days old.

Fig. 15 Amorphous embryo from acetone solution (40 cc. gram molec. sol. to 50 cc. sea-water), sixteen days old.

Fig. 16 Egg with amorphous tissue fragments on yolk-sac, from acetone solution (30 cc. gram molec. sol. to 50 cc. sea-water), thirteen days old.

Fig. 17 Meroplastic embryo with rudimentary eyes, from acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water), twelve days old.

to an oedematous distension (figs. 7, 8, 9, 14). In some monophthalmic embryos which had hatched a similar observation was made to the one recorded by Stockard ('09) in his experiments. These embryos "swam in circles, often whirling around with great rapidity, much as Japanese waltzing mice do. Others swam in irregular spirals and only progressed in a straight direction with difficulty." In most of them I observed that when forced to swim in a straight forward direction they would immediately drop to the bottom of the dish, while they were able to swim for some distance along the wall of the finger-bowl in which they were kept. Stockard attributes this functional anomaly to "a defective muscular arrangement, the animal's body being slightly bent or twisted so that it is unable to straighten perfectly." From my own observations and Stockard's ('10 a) microscopic findings I am rather inclined to think that in these embryos—at least in my own experiments, if not also in those of Stockard's—the locomotor anomaly is due to defects in the semi-circular canals.

2. General defects; 'meroplastic embryos'

Besides the already referred to deformities of the sense organs there were found in both butyric acid and acetone solutions great numbers of embryos which developed in a much more defective manner, the malformation involving practically the entire bodies. Thus curiously deformed dwarfs with vestigial eyes or blind, or slender, elongate, greatly malformed embryos (fig. 11) often with waistlike constrictions (figs. 12 and 13) were of not infrequent occurrence. A considerable number of eggs were recorded with amorphous embryos (fig. 15) or only amorphous fragments of tissue (fig. 16) on the yolk-sac. The amorphous embryos would correspond in homology to those found in man and described by Mall (l.c.) and others. On microscopic examination of several amorphous embryos it was found that the nervous system, the notochord and the viscera while having developed, are highly abnormal and rudimentary in structure. The sections present pictures similar to those of

Fig. 18 Egg with 'solitary eye,' *s.e.*, and three very small clastolytic tissue fragments, *t.f.*, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), twelve days old.

Fig. 19 Asymmetrically monophthalmic embryo, with club-tail, without pectoral fins. On the yolk-sac at a distance from the embryo is seen an 'isolated eye,' *i.e.* From acetone solution (40 cc. gram molec. sol. to 50 cc. sea-water), 12 days old; *p.c.*, pericardial vesicle, *y.s.*, yolk-sac.

Fig. 20 Asymmetrically monophthalmic embryo with 'proboscis'—mouth, without pectoral fins. From $\frac{1}{2}$ gram molecular butyric acid, twenty-eight days old.

Fig. 21 Duplicity anterior; both components are anophthalmic. From acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water), sixteen days old.

sectioned amorphous fetuses of man. The amorphous fragments may be compared to the 'nodular forms' of Mall which he found in some aborted human ova.

By far the most numerous were found to be eggs in which only a part of the body (fig. 17) had developed—'meroplastic embryos' (Roux, l.c.). In these the hind parts of the bodies were missing to a greater or lesser extent, while the anterior parts were often extremely deformed, their shape being much distorted and only more or less rudimentary eyes being present. Many of them in

which an anterior half of the body had remained would belong to the class designated by Roux (l.c.) as 'hemiembriones anteriores,' which he and subsequently other investigators found to develop from the frog's egg if one of its first two blastomeres was punctured with a hot needle.

The other cases of meroplastic development concern embryos in which either more than the anterior half of the body had developed, or less. They range all the way from those in which hardly much more than the tail was missing to those in which only a very small anterior part of the head (usually with one or sometimes with two vestigial eyes) was present.

3. Independent development of an eye from a fragment of the medullary plate

By far the most curious and most significant of all the meroplastic ova recorded in these experiments were some in which all that was left of the embryo was a fragment of brain tissue with a solitary eye. The fragment of brain tissue was in some cases somewhat larger than the solitary eye to which it has given rise, while in others it was smaller. In one of these eggs (fig. 18) in which the solitary eye was rather defective—the choroid fissure being patent—several small amorphous fragments of tissue could be observed at different points of the yolk-sac at a considerable distance from each other. Since I have observed such fragments of tissue on many other eggs in which either a defective embryo or nothing else besides the amorphous fragments had developed, I am inclined to think that such cases give us a clue as to what processes may be involved in bringing about the effects recorded in this work.

The 'solitary eyes' when observed in the living egg had the typical appearance of eyes and no doubt could be felt that the interpretation of these sporadic cases was correct. However, as no other similar case was known, it seemed rather improbable that a small fragment of the medullary plate would be able to go on developing independently so far as to give rise to such a complex organ as the eye. The possibility suggested itself that the 'solitary eye' of such an egg may be connected with an

embryo which had sunken in the yolk-sac leaving the eye on the surface. The main objection to this would, of course, be that such an egg would die in a very short time, for the embryo could not possibly receive a sufficient oxygen supply; and the death of such a sunken embryo would soon cause autolysis of the ovum. More convincing proof seemed to be furnished by the fact that the yolk-sacs of the eggs, owing to the method of fixation, were translucent, while the embryo had turned white and opaque. If an embryo had sunken into the yolk-sac, it could thus easily have been detected. But, in spite of very careful examination of the ova with solitary eyes, not a trace of an embryo could be seen within their yolk-sacs. Moreover, in order to establish this fact of the independent development of the eye on the firm basis of unmistakable evidence, I sectioned one of these eggs. I have purposely chosen the egg in which besides the solitary eye several (some three or four) very small fragments of tissue could be observed on the yolk-sac, thinking that the latter ones might possibly offer a basis for the interpretation of the morphogenesis of the solitary eye.

The microscopic examination (fig. 22) of the sectioned egg proved that my interpretation of the case as that of a 'solitary eye' was perfectly correct. In the sections it can be plainly seen that the blastoderm had overgrown the entire yolk-sac just as this takes place in normal development. But no embryo can be seen in the yolk of any of the sections. On the yolk-sac besides the solitary eye there can be seen on some sections the fragments of tissue mentioned above, one of which makes the impression of a defective spinal cord and when followed out in successive sections is seen to give rise to the eye in question. Some blood vessels have also developed in a very abnormal fashion and no heart is present. Of the other amorphous tissue fragments one proved to be another eye, although very rudimentary in structure. The nervous elements of this second eye are obviously greatly inhibited in development, neither a choroid nor an iris are present, but the cornea and the lens have developed to a degree permitting of safe identification. The distance between the position on the yolk-sac of this eye

rudiment and that of the well developed solitary eye being as much as 262μ it seems evident that a blastolytic fragmentation of the early embryonic material and a subsequent shifting of the fragments to distant parts of the yolk-sac's surface has occurred.

Two more eggs with solitary eyes were cut into sections and a microscopic examination again confirmed the correctness of their being interpreted as such.

Several eggs were also found with asymmetrically monophthalmic and otherwise malformed embryos in which a completely isolated tissue fragment with a well developed eye could be seen on the yolk-sac at a distance from the embryo which made its connection with the same appear impossible. One of these eggs was sectioned and the microscopic examination revealed the fact that the isolated eye was in no connection whatsoever with the embryo.

In view of these findings no doubt can now be felt that the 'solitary eye' (embryone absente) and the 'isolated eye' (embryone praesente) offer ample evidence of the fact that a very small fragment of the medullary plate may be able to develop independently of the rest of the embryo's anlage far enough to give rise to such a complex organ as the eye. *This is, as far as I am aware, the first case on record of the independent development ('self-differentiation,' Roux) of the eye.*

4. Defects of the brain

The destructive influence of butyric acid and acetone on the developing egg of *Fundulus* manifests itself also in the severe injuries sustained by the central nervous system. In teratophthalmic embryos the brain was in most cases found to be abnormal to a greater or lesser degree. The forebrain may often be unpaired while the mid- and hind-brain of the same embryo are bilateral in symmetry. The hemispheres of one brain may often differ considerably in size and shape as well as in other respects. Thus, e.g., there may be seen a striking developmental inhibition in one hemisphere where most of the

Fig. 22 Photomicrograph of section of egg with 'solitary eye,' (Cf. fig. 18); *y.s.*, yolk-sac, *o.g.*, oil globule space. $\times 80$.

Fig. 23 Photomicrograph of transverse section through the eye region of a normal *Fundulus* embryo, fourteen days old, one day after hatching. $\times 80$.

nervous elements had not proceeded beyond the neuroblast stage, while the other hemisphere may be made up of apparently well developed nerve cells and fibers. It was also often observed both in the brains and the spinal cords of teratophthalmic or otherwise defective embryos that wherever the neuroblasts failed to differentiate into nerve cells a great many large, clear and empty tissue spaces (figs. 26 and 27) were present. The probability suggests itself that these spaces in the living embryos may have been filled with body fluid. For, the heads of some defective embryos when observed in the living or even the preserved specimen were distended to a degree suggesting oedema. As was mentioned before, the same is true also for the ear vesicles. On microscopic examination it can invariably be seen that the usual size of the latter ones is due to (an apparently oedematous) distension of the semi-circular canals. A condition of hydrops is thus produced in the embryo which (since the fish brain has neither lateral ventricles nor a choroid plexus) might perhaps be considered as homologous with the internal hydrocephalus of man. Furthermore, the cranial (figs. 25 and 26) cavity may be unusually large, often containing some fibrin. Both this condition and the intracerebral oedema may often be present in the same embryo. Genetically these dropsical conditions probably are due to an arrest in the development of the blood or lymph vascular system.

5. Defects of the blood vascular system

There is a very wide range of variation in the deformities to which this system is subject. The heart is almost perfect in some embryos in which cyclopia is the only superficially noticeable defect. In more extremely malformed embryos, however, the heart may be only an exceedingly delicate, straight tube in some embryos, while it may be absent altogether in others. It is interesting to note in this connection that while acardia was frequently observed in eggs in which a whole although malformed embryo had developed, a heart, though usually more or less of a rudimentary structure, but functioning, could be

found in some eggs in which only meroplastic embryos or no embryo at all had developed.

While in some embryos the heart can be plainly seen to be connected with the great vessels of the embryo and indirectly with the larger vessels of the extraembryonic area, no such continuity exists in most of the deformed embryos which develop without a complete circulation, or, often without any circulation whatsoever. To J. Loeb ('93) belongs the credit for this remarkable discovery, which is now corroborated by Stockard's ('15) and my own findings. The blood vessels of the yolk-sac may sometimes be present in the form of irregular, dense, apparently continuous networks, or in some cyclopean embryos they may approach in pattern and size the normal blood vessels, while in cases of more extremely malformed embryos only blood islands may be seen scattered in the yolk-sac. Yet blood vessels are usually found in such cases in the embryo itself. It may well be said that only few embryos which develop in butyric acid and acetone solutions, while possessing blood vessels, have a circulation continuous with the extraembryonic area.

This accounts for the interesting fact that very often large lacunae filled with erythrocytes are seen in the bodies of some monstrous embryos as well as in the extraembryonic areas. These lacunae are very striking at the first glance at a living ovum on account of the bright red color of the erythrocytes. Another observation which was first recorded by Stockard ('15) and which I have also frequently made is that a large mesenchyme space, usually in the head region or sometimes in other parts of the body, may frequently contain many leucocytes of apparently the polymorphonuclear variety (fig. 24). The elements of the blood which come from different sources are thus seen to be isolated due to absence of a continuous system of circulation. The bearing of these data on the problems of vasculogenesis and haemogenesis is evident and I expect to discuss it elsewhere.

That these data may also have an important bearing on the genesis of some forms of hydrocephalus has already been pointed out above. It is easy to imagine that even in man a local in-

Fig. 24 Photomicrograph of a transverse section through the eye region of a monstrum synophthalmicum bilenticum (Cf. fig. 2); *l.c.*, leucocytes, *o.c.*, optic chiasma. $\times 100$.

hibition in the development of the blood and lymph vessels in the head region may result in accumulation of fluid in some parts of the head, even in the lateral ventricles of the brain due to lack of adequate drainage. Careful anatomical investigations of some well diagnosed cases of hydrocephalus may possibly bear out the validity of this assumption.

A case described by Smith and Birmingham ('89) may be of interest in this connection. They report a fetus aborted during the fifth month of pregnancy with general oedema due to complete absence of lymphatic vessels. On microscopic examination of the skin and subcutaneous tissue they found in the sections large spaces, "in some parts clear and empty, in others filled

with a colloid material, evidently coagulated lymph." From their appearance and position they concluded that they were dealing with greatly distended lymph spaces, the overdistension being the result of the absence of a lymphatic drainage system.

6. Deformities of the appendages

One of the most frequently found malformations concerns the fins. Both the pectoral and caudal are very often rudimentary (figs. 7, 9 and 10) and diminutive in size. In some deformed embryos the pectoral fins or all fins may be absent altogether (figs. 11, 14, 19 and 20) and the tail in such embryos is club-shaped. I have also often recorded embryos in which only one pectoral fin was present (fig. 8) while only one embryo was found with three pectoral fins. One is reminded by these deformities of the fish's appendages of well known cases in the human being with which they seem homologous. The club-foot in the human fetus and congenital absence of upper limbs in man have often been found. Likewise cases of supernumerary upper limbs have been recorded in man as well as in other mammals.

The fact that such defects can be produced in the fish by chemical action suggests that in mammals they may be due to like causes.

7. Duplicities

The tendency of butyric acid and acetone to produce twins seems to be only slight for I have observed only a few such cases. I have recorded only one case comparable to the 'Siamese twins' type of the human. In this egg two deformed embryos with a common heart had developed on opposite sides of the egg. Some other cases of twin formation found in these experiments belong to the type known as 'duplicitas anterior' (fig. 21) which Speeman ('04) produced experimentally by tying a ligature around the fissure between the first two blastomeres of the amphibian egg. Several cases of duplicity have also been recorded, the nature of which I expect to ascertain by microscopic examination.

THE MORPHOLOGY OF TERATOPHTHALMIA

After this general survey of the recorded monstrosities I shall now briefly consider the morphology of some such ophthalmic defects as I have so far been able to examine in sections with a view of a more exhaustive treatment of the topic in the complete account of the results of these investigations. Before, however, presenting a few of the cases which I have studied, some terms may be defined, the use of which to me seems to be desirable.

The various degrees of the one-eyed condition which have been recorded by previous writers (Speeman, Stockard, Lewis) as well as by myself, I shall henceforth classify in the following manner:

- I Monophthalmia asymmetrica (s. lateralis)
- II Cyclopia (s. Monophthalmia mediana) $\left\{ \begin{array}{l} \text{(a) perfecta} \\ \text{(b) synophthalmica} \end{array} \right.$
- III Synophthalmia $\left\{ \begin{array}{l} \text{(a) unilentica} \\ \text{(b) bilentica} \end{array} \right.$

The first term is well known because it already has been used by Ahlfeld and adopted by Stockard and so it will need no further comment.

In the case of cyclopia the distinction is made between 'perfect' ('true') cyclopia and 'synophthalmic' cyclopia. The first is to indicate the presence of a single median eye which on microscopic examination is found to be single throughout and nowhere exhibiting evidence of its being composite in character. Under the latter one will be classified such cases where on macroscopic examination a single median eye (with one lens) is found present, while the microscopic examination of sections reveals its composite character. Under 'Synophthalmia' will be classified cases of 'fused' eyes where the composite character of the eye (or eyes) is plainly discernible *in toto*. If such an optic apparatus should possess one lens only it will be designated as 'Synophthalmia unilentica,' while in the presence of two symmetrically placed lenses 'Synophthalmia bilentica' will be used as the descriptive term.

A few illustrative examples will now be given, after which a discussion of the morphogenetic factors of teratophthalmia will be taken up.

Figure 5 represents a case of unilentic synophthalmia. The egg was subjected between the second and third cleavages, —i.e., about $2\frac{1}{2}$ hours after insemination—to the action of 10 cc. of $\frac{1}{12}$ gram molecular solution of butyric acid for twenty hours and then transferred to pure sea-water. Already on superficial examination the composite structure of the eye is easily recognized. It is seen that the approximation of the two eye components is so close as almost to give the appearance of perfect cyclopia. Transverse sections (fig. 26, p. 549) show that the eye is composed of two incomplete optic cups facing each other and enclosing a single lens of about the usual size. The cornea and iris are normally developed and the retinal layer is well differentiated. Two optic nerves are seen to pass out of the eye in a few loose bundles of fibers and, after having formed a chiasma, to enter the opposite sides of the brain.

The incompleteness of the fused optic cups is probably due to the circumstance that at a very early stage of development a large part of the ophthalmoblastic material had been eliminated from development owing to the chemical alteration caused by the butyric acid. The injury sustained by the embryo must apparently have been the severest at the most anterior point of the main body axis, diminishing gradually posteriorwards. The following data seem to substantiate this interpretation. The forebrain is unpaired (fig. 25) and the rest of the brain is when followed in successive sections posteriorwards seen gradually to present more and more distinctly the condition of bilateral symmetry. The midbrain and hindbrain while being bilateral, exhibit, however, a certain other abnormality. The injury here was apparently mainly restricted to the blood and lymph vessels, the earliest anlagen of which seem to have been arrested in their development. This condition can be recognized by the great number of large, clear and empty spaces (fig. 26) in the tissues of the posterior parts of the brain, which in the living embryo have apparently been filled with fluid owing to

the existing imperfection in the circulation. A condition of oedema has thus apparently resulted from lack of drainage. No other abnormalities of these parts of the brain or any other part of the embryo can be seen, which makes it appear very probable that the anterior part of the embryo body is the most sensitive one and thus subject to the highest degree of injury.

In figure 2 is seen an eighteen days old embryo exhibiting the condition of 'synophthalmia bilentica.' This egg had been subjected to the action of 10 cc. of a $\frac{1}{16}$ gram molecular solution of butyric acid in 50 cc. of sea-water for twenty hours, after which time it was transferred to pure sea water. In the specimen *in toto*, the eyes, while being very close together, could not be considered as fused. On microscopic examination, however, it was found that the eyes were fused more posteriorly (fig. 24, p. 544) and that the fusion is the more intimate the more posterior is the section examined. If all sections be examined it can be clearly seen that the highest degree of the injury sustained by the egg due to the treatment is in the most anterior part of the embryo's body, i.e., in the region of the eyes. The abnormalities found in this part of the body besides the fused eye involve also the olfactory pits, which are so closely approximated as to be partially fused, and the forebrain, which is unusually small in size and unpaired. The transverse sections of this embryo are somewhat oblique and the double eye being somewhat asymmetrically located in relation to the chief body axis, in the figure the lens of one eye can be seen to be sectioned about midway while of the other lens the most posterior part is cut. The two lenses, however, can be clearly made out in the figure. The choroid coats which are imperfectly developed and the well differentiated

Fig. 25 Camera lucida drawing of a transverse section anterior to the eye region of a monstrem synophthalmicum unilenticum (Cf. fig. 5), showing unpaired forebrain, *f.b.*, greatly enlarged cranial cavity occupied by some fibrin, *f.* $\times 140$.

Fig. 26 Photomicrograph of a transverse section through the eye region of the same embryo as in figure 25, showing the two components of the synophthalmic eye, facing one another, with one lens, *l.* Many tissue spaces, *t.s.*, are seen in the brain. The cranial cavity in the region of the optic lobes, *o.l.*, is greatly distended, *o.c.*, optic chiasma. $\times 160$.

retinal layers are seen to be perfectly coalesced ventrally, while dorsally they arch inwards to leave an opening for the optic nerves. The latter come as separate bundles of fibers from each one of the eye components and fuse into one trunk just at the point of emerging from the double eye. This common optic nerve trunk is in other sections seen to enter only one hemisphere of the brain. The cavity between the sclera and the brain has widened out and is on both sides near the head integument occupied by large leucocytes of apparently the polymorphonuclear variety. These leucocytes are seen in all sections of the embryo densely filling spaces between the tissues or they may also be found more scattered in the mesenchyme. The blood vessels of this embryo as well as of its extraembryonic area are rather scarce, and the discontinuity of the latter ones being very striking, the suggestion is at hand that the circulation of the embryo was imperfect. This would account for the accumulation of white blood corpuscles in the mesenchyme as well as for the numerous apparently oedematous interstices which they often filled. Summarizing the abnormalities found in this embryo, it may well be said that the degree of injury sustained by it, being highest in the immediate region of the eyes, diminishes along the main body axis posteriorwards, the mid-brain and hindbrain, excepting the anomalies due to an inhibited circulation, being apparently normal in all other respects.

Of a much higher degree is the injury sustained by the case of perfect cyclopia with a supernumerary lens, cross sections of which are represented in figures 27 and 28. The egg had been subjected in the eight-cell stage to the action of 35 cc. of a gram molecular solution of acetone in 50 cc. of sea-water for forty-eight hours. The embryo when killed was twenty-seven days old. A single median eye is seen in a transverse section (fig. 27) which is smaller in size than a normal eye, the lens only being disproportionately large. All other structures of the eye, viz., cornea, iris, choroid coat and retina are present, the latter having differentiated in a defective manner. At a somewhat more posterior level of the brain, the following view is presented (fig. 28). Laterally from the eye on one side is seen a large well

Fig. 27 Photomicrograph of a transverse section through the eye region of a twenty-seven days old cyclopean embryo, one day after hatching, showing a small median, imperfectly differentiated, eye with a disproportionately large lens. The forebrain is very abnormal, and tissue spaces, *t.s.*, point to oedema. From acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water). $\times 160$.

Fig. 28 Photomicrograph of a transverse section of the same embryo as in figure 27, at a more posterior level, showing supernumerary lens, *l.*, on one side and optic anlagen on the other side of the cyclopean eye. $\times 160$.

differentiated lens surrounded by an epithelial capsule and anteriorly and laterally by a cornea, which is found to be in continuation with the cornea of the cyclopean eye. On the other side of the eye between its lateral border and the head integument is to be seen a large patch of cells with deeply stained nuclei and a little higher upwards on the same side (o. a.) and bordering the integument two more such patches of tissue can be seen in close approximation. On careful examination it was found that these three patches of cells represent fragmentary optic anlagen. The one of these optic anlagen which borders the eye has even differentiated into a small retinal layer which is in about the same stage of differentiation as the same structure of the cyclopean eye. There is only one optic nerve present which in more posterior sections can be seen to enter as a trunk one of the hemispheres. The brain is much deformed and more so anteriorly, where its symmetry is obscured, than posteriorly, where its bilateral symmetry can be recognized without difficulty. Many large clear tissue spaces can be seen in the brain in almost all sections which in the living apparently represented persistent early embryonic vascular anlagen and were filled with body fluid owing to lack of drainage caused by an imperfect circulation.

This embryo I regard as one instance of the perfect cyclopean condition, for only one complete eye has developed. Such indications as are present of the other optic anlage give us a clue to the genesis of the single-eyed condition in this case. Although the injury sustained by the embryo is not localized and is of a high degree, it is highest in the most anterior portion of the body, diminishing gradually posteriorwards along the main body axis. The anterior portion of the very early embryonic anlage has apparently undergone great destructive alterations of a blastolytic nature, owing to which the ophthalmoblastic material of one side was fragmented and dispersed while the corresponding material of the other side has been injured less severely and has given rise to an imperfectly developed median eye. The material which would in the normal embryo eventually be represented by the interocular area seems to have, owing

to some regulatory processes, moved lateralwards, where it has given rise to an independent lens. To the same regulatory process is it probably due that the ophthalmoblastic material of the less injured side has been shifted medianwards to give rise to the cyclopean eye.

The case of 'asymmetric monophthalmia' which I shall now describe will, I believe, also point to unmistakable evidence that in teratophthalmic embryos the sustained injury is usually the severest at the anterior end of the body.

The egg had been subjected for forty-eight hours to treatment with acetone, 35 cc. of a gram molecular solution of which were added to 50 cc. of sea-water. The embryo was killed one day after hatching when it was sixteen days old. On examination *in toto* there was seen to be present only one eye in the usual lateral position while the mouth occupied exactly the position of the missing eye. In transverse sections (fig. 29) it is seen that the one eye present is well developed and apparently normal in structure. No indication of another eye or optic anlage can

Fig. 29 Photomicrograph of a transverse section through the eye region of a monstrum monophthalmicum asymmetricum, from acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water), sixteen days old, one day after hatching. The mouth, *m.*, occupies the position of the missing eye. $\times 120$.

be found anywhere in the sections and only one optic nerve is seen in the more posterior sections to pass out of the retina and terminate in the optic lobe of the opposite hemisphere. The brain is symmetric as far as its bilaterality is concerned, but is asymmetric in regard to the position occupied by the two hemispheres in relation to the main body axis. As can be seen in the figure, the hemisphere of the side where the eye has developed, is in its normal position while little can yet be seen of the other hemisphere, which has been shifted posteriorwards and comes to view in more posterior sections. The same posteriorward displacement has also affected the olfactory pit and the semi-circular canals of the side lacking the eye. There are no other abnormalities to be recorded for this embryo. The abnormalities found, however, justify the conclusion that the injury sustained by the early embryo was chiefly a unilateral one at the anterior end of the body. Owing to this injury the ophthalmoblastic material of one side has suffered complete destruction. On the same side, owing to subsequent processes of regulation, the mouth has arisen in what was to be the position of the eye and a posteriorward displacement of the brain hemisphere of the injured side has taken place. The sustained injury, while being lateral from the median axis of the body, was severest at its most anterior end where it has eliminated the material for an entire organ.

The cases described above and many more similar ones which have been studied led me to accept in the main the 'fusion theory' of cyclopia which has in recent years been advocated by Lewis ('09) and Speemann ('12) and to reject as untenable Huschke's ('32) view of the early single anlage of the eye which Stockard has quite recently ('13) adopted in his morphogenetic analysis of cyclopia.

Stockard, who was the first to produce experimental cyclopia in fish by chemical agents, has in his earlier work ('09) suggested that the developmental defect is due to the specific anaesthetic action of the chemicals (magnesium chloride, chloroform, ether, etc.) which he used. He thought that the giving off of the eye anlagen by the brain was inhibited often in an unequal manner

on both sides, thus giving rise by fusion of the inhibited anlagen to various transition stages between two normal eyes and cyclopia and anophthalmia. Recent investigations of McClendon ('12), however, who obtained similar results with a great variety of non-anesthetic substances, have caused Stockard ('13) to abandon his anesthetic theory of teratophthalmia. He now believes that the eye anlage in the medullary plate is single and median in position, that in normal development this single anlage eventually divides into two portions which move lateralwards, where they develop into the optic vesicles. If the embryo is subjected to the action of toxic chemicals this separation of the original single median optic anlage into two, may, he concludes, be inhibited to a greater or lesser degree and thus various degrees of the cyclopean defect may result. He even submitted the theory of the single optic anlage to an experimental test which, he believes, answered the query in the affirmative. It is unfortunate that Stockard's statements are not substantiated by better evidence than that which he brings forth, since he has failed to support his statements by illustrations of specimens *in toto* and in sections of the material which he regarded as permitting of such important conclusions. *Until this evidence is furnished, however, the theory of the single median optic anlage in the medullary plate would hardly seem to be acceptable.* On these grounds, we cannot agree with Stockard's conclusion on the morphogenesis of cyclopean defects.

The 'fusion theory' of cyclopia is, I believe, justified in the main, and has recently been ably supported by Speemann (l. c.), Mall (l. c.) and W. H. Lewis (l. c.). These authors assume a coalescence or fusion of two originally separate optic anlagen. Just how this fusion comes about may still be a matter of discussion. I think that Speemann's and Lewis' suggestions are very illuminating just on this point. Both these authors have produced all those conditions of one-eyedness which Stockard has obtained in his investigations, and which I have recorded in my experiments, a description of some of which is given above.

Both Speemann and Lewis have produced the teratophthalmic condition by mechanical injury of a small area at the ante-

rior end of the early embryo; and Lewis holds that the collapsing of the wound surfaces effected the approximation of the two optic anlagen. The degree of this approximation would depend upon the size of the fragment of interocular tissue which Speemann eliminated by constriction and Lewis by pricking. This, they conclude, would account for the various degrees of the cyclopean condition. Even asymmetric monophthalmia can in this way be accounted for, as it is easy to imagine that in the experiment the injury inflicted to the embryo may sometimes be somewhat lateral from the embryo's main body axis and that a complete eye anlage may thus be destroyed. The teratophthalmic condition would then, as Speemann points out, have to be regarded as a 'defect' (evidently meaning a mechanical defect) rather than a developmental inhibition.

My own conclusions are very similar to those arrived at by Speemann and by Lewis. I have, however, found it necessary to modify the fusion theory of cyclopia to conform to Stockard's and my own findings. The explanation offered by these authors is open to the criticism made by Stockard, namely, that "cyclopean eyes are rarely in size and extent equal to the sum of the two normal eyes combined." It is obvious that this objection is justified and that conclusions based on results obtained from mechanical experiments cannot be extended to cover the results of the chemical experiment or to account fully for the occurrence of the cyclopean defect in nature under unfavorable environmental conditions.

The following modification of the coalescence theory of cyclopia meets the objection raised by Stockard, covers the results of the chemical experiment and thus, I believe, may be extended to the morphogenesis of various cyclopean defects in nature, where the causal factor is undoubtedly a chemical one.

When the fertilized egg is subjected to the action of toxic substances it will sustain an injury, which—depending upon the stage of development, the substance used and the strength of its solution—may be a general one, i.e., involving the whole or a large part of the embryo's body, or a locally restricted one. The results of Stockard's and McClendon's work, as well as some

results of my own experiments, point to a blastolytic injury of a restricted area at the anterior end of the early embryo's body in the case of teratophthalmia. Child's³ ('09, '12) important discovery of the 'axial gradients', according to which the anterior end of a flat-worm's body is the most sensitive one to the action of injurious substances would seem well to justify this assumption. In the early vertebrate embryo, before the organs have been differentiated, probably very similar physiological conditions obtain, and thus we may well assume that its anterior end is the point of least resistance. When the egg is acted upon by a toxic substance, a restricted area at the anterior end of the embryo's median body axis becomes so altered chemically as to be eliminated from further development or it may go on developing to a certain point beyond which it is chemically unable to proceed. This restricted area at the anterior end of the body axis is the region between the future optic anlagen or even the region of those anlagen. The size of the injured area at the anterior end is probably subject to considerable variation, and thus it may comprise the material which would normally correspond to the future interocular area and cause an approximation of the potential optic anlagen or it may extend even over the latter ones, thus eliminating parts of them, while the uninjured parts would coalesce after approximation and form any one of the various degrees of the synophthalmic condition. Or, finally, the injured area may comprise the whole of one potential optic anlage and little or no material of the future interocular area, thus causing the embryo to develop into a cyclopean monster if the uninjured optic anlage is shifted medianwards, or into an asymmetrically monophthalmic monster, if no such change in the position of the uninjured or less injured ophthalmoblastic material takes place.

³ Child offers an interesting interpretation for this high degree of sensitivity. According to him the rate of the metabolic reactions seems to be the highest at the anterior end of the planarian's body, decreasing gradually along the main body axis in the direction away from the head. This theoretical postulate of the metabolic 'axial gradient' was substantiated by the rate at which various points along the body axis have given off CO₂, the measurements having been made by Tashiro with his own biometer-method.

Here too, the size of the chemically injured area may vary and thus in some instances a small fragment of the injured potential anlage may survive and develop into a diminutive whole, but somewhat rudimentary, eye, or into an eye fragment with a well differentiated retinal layer and choroid coat.

However, it is necessary to assume that the injury which results in teratophthalmia is sustained at a very early stage of development. At that time the volume of the area which receives the injury is relatively small and such minute parts of the embryo as may represent the potential double anlagen of the eyes, are, since growth proceeds in a cubic proportion, relatively much nearer each other than they would be at a somewhat later stage, e.g., in the embryonic shield. This would account for the fact that the cyclopean eye may be very small in size, much smaller even than the normal eye. For, at this stage, the eliminated area may contain much more material than of one potential eye. The remaining ophthalmoblastic material may undergo some regulatory process to form eventually a single eye of corresponding size or a double, fused eye if enough of the ophthalmoblastic material of both sides survives, or finally if the injury sustained by that material be so extensive as to comprise all of it, the condition of anophthalmia may be the result. This conception of the morphogenesis of teratophthalmia, while recognizing the coalescence theory of cyclopia as justified, also meets the objection which Stockard raised to Speemann's and Lewis' generalizations, namely, that the cyclopean eye is often much smaller than the sum of two normal eyes combined. At the same time, it makes it appear unnecessary to resort to a theory whose probability is so questionable as that of the "single median optic anlage in the medullary plate" (Stockard).

* * *

The mechanism involved in the action of butyric acid and acetone in bringing about the great variety of recorded effects will have to be taken up as one of the further steps of this investigation. At the present time, I can only state that there seems to occur in the eggs when subjected to the action of these solutions

an elimination of material of the blastomeres or of the germ-disc and probably also of the yolk-sac. This elimination of material may be due either to the precipitating or solvent effect respectively of the chemicals which were used in these experiments. Since many eggs were found with living but extremely malformed embryos, the yolk-sacs of which were ruptured allowing some yolk to escape, the suggestion is at hand that another factor—some force like a temporarily increased internal osmotic pressure—may by its action have brought about the fragmentation of the germ-substance. On examining many eggs in which only amorphous tissue fragments can be found and the eggs with 'solitary eyes' (embryone absente) or eggs with 'isolated eyes' (embryone praesente) one gains the impression that either the blastomeres or the germ-disc had been blastolytically fragmented owing probably to both physical and chemical factors. These tissue fragments sometimes appear at such great distances from each other, or even from a malformed embryo, that there seems to be no doubt left that they have moved out of their original position along the axis of the embryo. Having examined many thousands of these pathological ova I believe that I possess enough evidence to justify the assumption of blastolytic processes of a physical or chemical nature, such as fragmentation due to a temporarily increased osmotic pressure or to chemical alteration of some parts due to the solvent or precipitating effect respectively of the chemicals used in these experiments. Mall speaks (l. c.) of a similar process which he assumes for some pathological human ova, and which he terms 'dissociation of cells.' The effects of these apparently blastolytic processes vary enormously. For whatever parts survive them, may go on developing into a whole defective, or a meroplasmic, embryo, or even into an isolated organ.

Just what brings about this great range of variation in the noted effects can at present hardly be more than conjectured. It would seem probable that the stage in which the egg is subjected to the action of the toxic solution may play an important part in that respect. For as Conklin ('12) has shown ". . . stages during kinesis are more susceptible to modification than stages

during interkinesis. Almost all persistent alterations of structure occur during cell division, few of those which occur during the resting period are permanent." However, this and similar other inquiries must be left to future investigation.

CONCLUSIONS AND SUMMARY

1. Starting from the assumption that monstrous development is due to parental metabolic toxemia, experiments were performed in which *Fundulus* eggs were subjected to the action of some substances occurring in the blood or urine respectively of man during metabolic disturbances.

2. With two of these substances, i.e., butyric acid and acetone, positive results have been obtained, a great variety of monsters having been produced which are analogous or homologous respectively to human and other mammalian monsters. The monstrosities concern the eyes (cyclopia, synophthalmia, monophthalmia asymmetrica and anophthalmia), the ear vesicles, the olfactory pits, the mouth, the central nervous system, the heart and blood vessels, the fins (unpaired fin, absence of pectoral fins or all fins, club-tail, etc.) and body form.

3. A condition of hydrops was found in many embryos, due apparently to blood vascular abnormalities which might be considered as homologous to some forms of hydrocephalus in man.

4. In many eggs parts of the embryonic material have suffered destruction, while the remaining parts have developed into anterior hemiembryos or other meroplastic embryos.

5. Eggs have been found in which one eye has developed from a small fragment of the medullary plate independently of an embryo (the 'solitary eye,' the 'isolated eye').

6. Regarding the mechanism of action of butyric acid and acetone the conclusion has been reached and some evidence offered that the various monstrosities are brought about by a process of blastolytic fragmentation due to some factors not yet ascertained.

7. Regarding the formation of the various degrees of the 'cyclopean' defect it is concluded that the fusion theory of Speeman

and Lewis is justified in the main. An additional assumption is made, namely, that the blastolytic process which eliminates parts of the potential interocular or ophthalmoblastic material takes place at a very early stage of development (i.e., before the formation of the embryonic shield).

8. The results obtained tend to justify the assumption that monstrous development may be due to metabolic toxæmia.

The experimental part of this work has been carried out at the Marine Biological Laboratory at Woods Hole, Mass., during the summer of 1914. It is a pleasure to me to acknowledge my indebtedness to the director, Dr. F. R. Lillie, for the facilities granted. I am also under great obligation to Professors Conklin and McClure and to Dr. E. N. Harvey, whom I have often taken occasion to consult on some points of the work. For many courtesies I am indebted to Professor Dahlgren and Mr. W. E. Hoy.

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