Abnormalities in Frog Embryos Induced by Centrifugation

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Introduction

The centrifuge has long served as a tool in the experimental analysis of development of both vertebrates and invertebrates. In the early days of experimental embryology when attention was largely centered on the external environment, the eggs of many forms, including the frog, were centrifuged to determine the influence of gravity upon development. Subsequently the method was employed to bring about a redistribution of the contents of the newly fertilized but still unsegmented egg to see if any abnormality in cleavage pattern or later development resulted. The names of such pioneers as O. Hertwig and T. H. Morgan are associated with work of this sort on the frog.

To Konopacka ('08) belongs the credit for first investigating the possibility of variation in degree of abnormality, following centrifugation of frog eggs at various cleavage stages. She found that in general the number of abnormal embryos was greater when the eggs were centrifuged during the early cleavages rather than immediately after fertilization. She pictured as examples of such abnormalities numerous embryos with persistent blastopores and some headless monsters.

Konopacka's work was followed by that of McClendon ('09) who, however, was primarily interested in a chemical analysis of the various layers into which the egg contents could be separated by the centrifuge. Mention is made of McClendon only because his studies were the direct stimulus for a later investigation by Jenkinson ('14) which included a detailed description of a considerable number of abnormal tadpoles produced by centrifuging freshly fertilized eggs. These abnormalities consisted primarily of disturbances in varying degrees of anterior structures; the olfactory pits, forebrain and eyes, midbrain, skull, and mouth were either distorted or entirely lacking. In general the middle and posterior portions of the body were normal, though there were some instances of a persistent yolk-plug. There were a few monsters, in addition, resulting from excessive centrifugation, which possessed double tails as well as the usual derangements of anterior structures. These cases are especially interesting in the light of the results to be described in this paper.

Many of the results obtained by Jenkinson were not easily accounted for, coming as they did before the development of our knowledge of inductors and especially of the important role played by the roof of the archenteron in the determination of the neural tube. Jenkinson, in keeping with the times, attempted to explain most of the abnormalities in terms of mechanical difficulties imposed by abnormal distributions of

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yolk and fat. This was correct up to a point, but now we go much further and seek the answer in distortions in the pattern of formative movements and the dislocation of all-important organizing materials. One might, in fact, write a new interpretation based solely on Jenkinson's data to which, except for limited observations (e.g., Beams, King, and Risley, '34; Beams and King, '37), little has been added up to date. A complete redescription seems in order, however, because in the experiments to be reported, the various conditions obtained by him have been duplicated and also supplemented by many new facts.

Material and Methods

Eggs of several species of frogs as well as two species of salamanders were employed in these experiments. Since, however, the eggs of *Rana pipiens* comprised as large a portion of the total material as all the other species together, the results to be described apply solely to this form. It may be noted in passing that the fragmentary results obtained with other species differ in no essential respect from those with *Rana pipiens*.

Eggs at various stages of development were selected for centrifugation and a variety of centrifuge speeds was employed, for the purpose of identifying the most susceptible stage and the most effective speed. Any single experiment consisted in selecting a cluster of 75-100 eggs of a given stage, placing them with gelatinous membranes intact in a 12.5 cm. tube and centrifuging (Sorvall Angle Centrifuge, Type SP) at a selected speed for three minutes. Since the eggs were free to rotate in their membranes, they quickly oriented themselves with the animal hemispheres in the centripetal position as the centrifuge gained speed, and thus it may be assumed that the axis of the effective force was essentially the same for all eggs of any given stage. A total of thirty batches of eggs was treated in this fashion, of which fifteen were of *Rana pipiens*. Ten of the other fifteen groups were distributed between three other species of frogs and two of salamanders. The remaining five batches were also from *Rana pipiens*, but were obtained during the winter months by means of pituitary implants. Because of the low percentage of fertilization, these have not been included with the others. From the same mass that supplied a particular group of eggs for centrifugation a control group was also selected.

After centrifugation all groups were allowed to develop in individual three-inch stender dishes or finger bowls containing pond water which was changed daily. If the eggs continued to develop through the few hours following centrifugation, they could be expected to continue to live and to have a mortality rate no higher than the controls until their stored yolk material was exhausted. Then, because most of them had defective mouths or none at all, they died off very quickly.

A sample of, on the average, twelve eggs, both experimental and control, was fixed in Smith's modification of Tellyesnicky's fluid immediately after centrifugation for subsequent gross and miscroscopic study. Additional samples were fixed at one to three day intervals for the next ten days if the experimental larvae lived that long. Those larvae selected

for sectioning were first studied and sketched and either stained in borax-carmine before sectioning or sectioned first and stained with Harris' or Delafield's hematoxylin.

Experimental Results

Table I summarizes the fifteen groups of *Rana pipiens* eggs in terms of stage of development at the time of centrifugation, speed of the centrifuge, and subsequent developmental history. The stage numbers employed are from the table for normal development of *Rana sylvatica* (Pollister and Moore, '37).

TABLE I

Group No.	Developmental Stage	Speed of Cent. (Round Figures)	Developmental History
F-19	4-8 cells (Stage 5)	1900 r.p.m.	Continue cleavage. Few gastrulae. Development stops.
F- 5	6-8 cells (Stage 5)	2900 r.p.m.	No development.
F-29	16-20 cells (Stage 6+)	1300 r.p.m.	Normal development.
F- 7	Medium Blastula (Stage 7)	1100 r.p.m.	All embryos normal except one with defective eyes.
F- 6	Medium Blastula (Stage 7)	1800 r.p.m.	Essentially normal. Some distortion due to diffusely distributed yolk.
F- 8	Medium Blastula (Stage 7)	2800 r.p.m.	No development.
F-17	Late Blastula (Stage 9)	2400 r.p.m.	Defective head and yolk mass through blastopore.
F- 1	Initial Blastopore (Stage 10)	2500 r.p.m.	Rudimentary head; double tail.
F- 2	Initial Blastopore (Stage 10)	2800 r.p.m.	No development.
F- 3	Initial Blastopore (Stage 10)	2900 r.p.m.	No development.
F- 9	Crescentic Blastopore (Stage 11)	1100 r.p.m.	Normal development.
F- 4	Crescentic Blastopore (Stage 11)	2500 r.p.m.	Defective head; bud-like secondary tail.
F-10	Medium yolk plug (Stage 12)	1800 r.p.m.	Normal development.
F-14	Medium yolk plug (Stage 12)	2400 r.p.m.	Normal development.
F-30	Late yolk plug (Stage 12)	2400 r.p.m.	Normal development.

An examination of this table reveals that the stage of development most affected by centrifuging as reflected by resulting abnormal larvae is that of the early blastopore, i.e., the stage at which the formative movements of gastrulation are just getting under way. (Cf. group numbers F-1 and F-4.) Although the resulting abnormalities are less pronounced, the stage immediately preceeding blastopore formation likewise appears susceptible (Group F-17). A further interesting fact about these three groups is that modification of development has followed a centrifugal speed of 2400-2500 r.p.m. That this is the most effective speed is shown by groups F-2 and F-3 where the higher speeds of 2800-2900 r.p.m. produced such distortions that further development was inhibited, an group F-9 where normal development followed the very low speed of 1100 r.p.m. The especial susceptibility of the early period of gastrulation is further emphasized by groups F-14 and F-30 where the usually effective speed of 2400 r.p.m. has failed to interfere with normal development of later stages of gastrulation. Earlier cleavage stages will continue development following the minor distortions offered by low speeds (group F-29), but moderate or high speeds (F-19 and F-5) produce displacements of materials that cannot be overcome.

Analysis of the specific abnormalities presented by the F-1, F-4, and F-17 groups, from which a total of approximately seventy-five embryos was obtained, demonstrates that no two embryos are absolute duplicates of each other. They are all featured by pronounced deficiencies and abnormalities of the head region, but each possesses particular peculiarities and variations of its own. The outstanding variations of the common irregularities can best be presented by sample cases. Descriptions of six such cases follow.

Case 1. This is an individual selected from a group of embryos centrifuged at Stage 10 at 2500 r.p.m. and allowed to develop three days thereafter. These embryos attained a length of approximately 6.0 mm. and successfully hatched into free-swimming larvae. All were featured externally by a rounded, foreshortened head, distended, oedamatous abdomen, and a ventral or ventro-lateral supernumerary tail. There was no surface indication of the sense organs, nor were there oral glands or a stomodaeal pit. (Fig. 1a)

The forebrain of the embryo is confined to the limits of eight 15u sections, and at the extreme anterior end the brain wall is thick and irregular. This area is interpreted as a rudimentary telencephalon, although there are no olfactory anlagen present to assist in the identification. The floor of the telencephalon leads immediately into a diencephalon featured by an abnormally thin roof. Immediately beneath the diencephalon and connected with it by a narrow stalk lies a single optic cup (Fig. 2) with a thick retinal layer and an outer layer which is pronouncedly pigmented. There is no indication, however, of a lens rudiment. The epiphysis and hypophysis are entirely lacking.

The midbrain appears to be normal as is the anterior end of the hindbrain (first 8-10 sections). A normal pair of otocysts is associated with this normal portion of the hindbrain. But tracing posteriorly, the hindbrain gradually flattens and divides into two (Fig. 3). One of the

two hindbrains thus formed retains its regular position and is obviously the brain of the embryo proper; the other assumes a left-lateral position. In similar fashion the notochord first flattens into a diffuse mass and then divides so that each brain has a notochord associated with it. Posteriorly the lateral neural tube and notochord continue out into the supernumerary tail.

The entire pharynx is abnormal. Anterior to the tip of the notochord it exists only as a solid protrusion of endoderm abutting upon the caudal face of a mass of prechordal mesoderm. (This term "prechordal

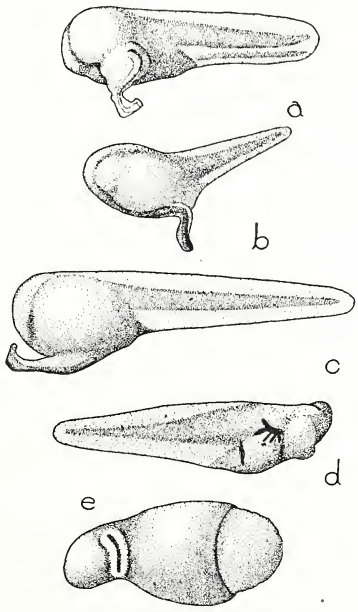
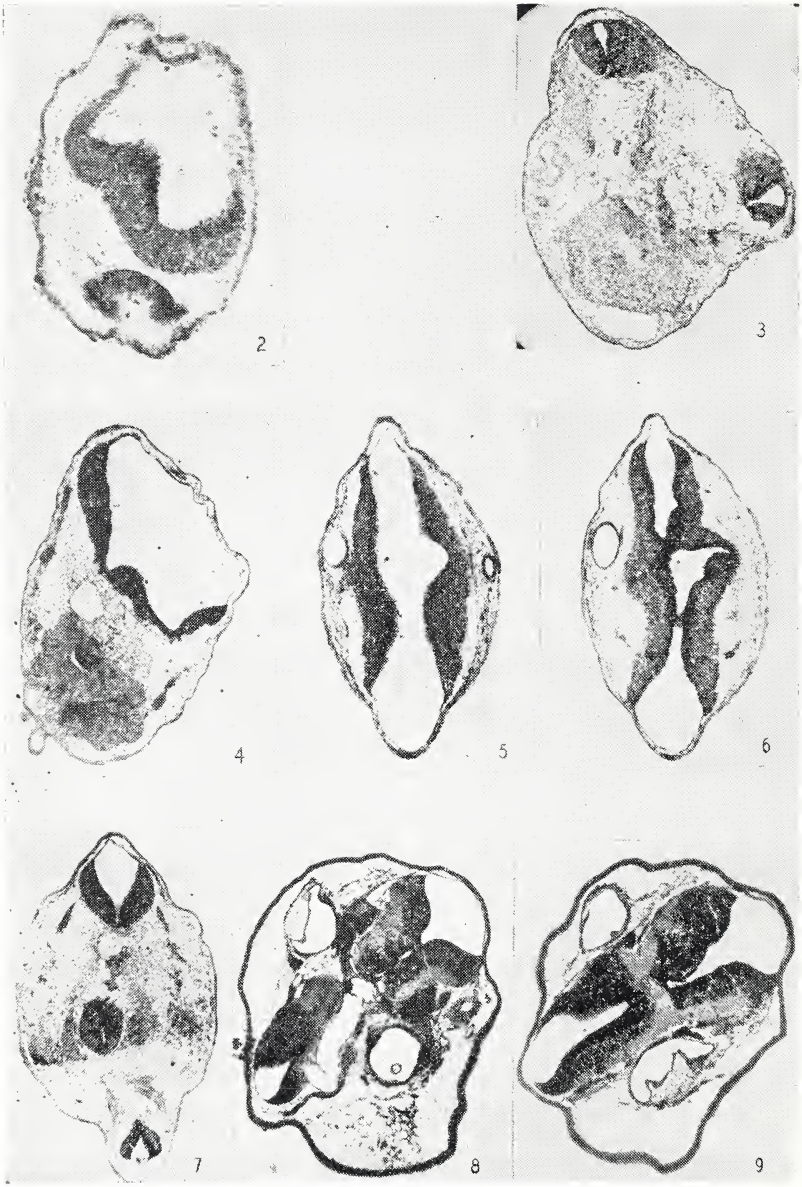


Fig. 1.

mesoderm" is being used in the general sense to include both mandibular and prechordal plate mesoderm. Cf. Adelmann '32.) From the level of the tip of the notochord back to the level of division of the hindbrain the pharynx is roughly circular and its lumen alternately open and occluded (Fig. 4). No distinguishable pouches are present.

The pharyngeal region also features very abnormal visceral and mesodermal elements. Anterior to the notochord the prechordal mesoderm exists only as an undifferentiated mass extending forward to the level of the diencephalon and occupying a strictly median-ventral position. There is no bilateral differentiation of this material at all. Posteriorly there are irregular mesenchymal condensations on each side of the pharynx which probably represent arches two, three, and four, though the absence of definitive pharyngeal pouches makes this interpretation uncertain. Rudimentary external gill filaments are present on the right side only (Fig. 4).

PLATE I



Explanation of Figures

Fig. 2. Section through anterior end of Case 1 showing cyclopean eye devoid of lens.

Fig. 3. Case 1 at level of heart, picturing secondary embryo and irregular notochordal mass on left side.

Miscellaneous structural details that might be mentioned include a normal gut posterior to the pharynx, a normal appearing heart, and a pronephros—all belonging to the embryo proper. The lateral secondary embryo has in addition to the notochord and neural tube only a complement of mesodermal somites.

To summarize, we have a cyclopian embryo accompanied by the deficiencies usually associated with such a condition; namely, reduction of the forebrain and suppression of its bilaterality, a similar suppression of the prechordal mesoderm, absence of the stomodaeum, and abnormalities of the pharyngeal region. It is extremely interesting that this embryo conforms generally and in many of its particulars to those cases of complete cyclopia in Amblystoma described by Adelmann ('34). It also offers support to Adelmann's interpretation of cyclopia in terms of suppression of bilateral development of the prechordal mesoderm. In this instance, however, the "suppression" is probably that of absence of the material rather than its failure to develop properly. It is likely, that is, that the potential "head organizer" has been dislocated by the action of centrifugal force. At one and the same time head mesoderm has largely been removed from its normal position, thus bringing about head abnormalities, and has been transferred to a new location where it has induced the formation of a secondary embryo. The exact nature of such a translocation is a problem in itself to be discussed after further data are brought forward in the cases to follow. Before leaving this case, however, there is one additional point deserving attention, namely, that the single, median optic cup has no lens.

As is well known, the extent to which lens formation is dependent upon the optic cup varies widely among the amphibia, ranging from absolute dependence in the case of forms such as *Rana fusca* to complete independence in *Rana esculenta*. *Rana pipiens*, interestingly enough, is one amphibian in which the optic cup lens relationship has not been analyzed. It would seem, however, in the light of the lens free condition existing in this cyclopian embryo that *Rana pipiens* belongs in the same category with *Rana esculenta*, otherwise the induction of a lens by the single cup would have come about. If this is true, then the potential lens ectoderm must be included with those other materials believed to have been moved during centrifugation. This point will be discussed further elsewhere.

Fig. 4. Flattened hindbrain, semisolid pharynx underlain by diffuse visceral mesoderm, and rudimentary gill filaments of Case 1.

Fig. 5. Section through elongated rhombencephalon of Case 2; note asymmetrically sized otocysts.

Fig. 6. Beginning of subdivision of brain, Case 2.

Fig. 7. Case 2. Rhombencephalon in three parts. Top, primary brain; bottom, secondary brain; middle, proximal portion of forward extending, blindly ending brain pouch.

Fig. 8. Section through Case 3, slightly posterior to figure 9, showing hindbrain separated into two, elongated notochord also dividing, and secondary otocysts. Primary brain, top; secondary brain, bottom.

Fig. 9. Beginning of division of hindbrain, Case 3. Secondary otocysts in this section.

Case 2. This embryo is one of the same group from which Case 1 was selected. It is similar in many respects to the former, but offers certain points of fundamental difference. Externally it is comparable to the previous embryo except that the supernumerary tail has a strictly ventral rather than ventro-lateral origin. Internally, however, it exhibits far greater deficiencies of the head.

The eighteenth section from the anterior end (Fig. 5) reveals an elongated rhombencephalon flanked by a pair of otocysts, the one on the left being considerably smaller than that on the right. Anterior to this level the brain is merely a thin-walled sac surrounded by scattered mesenchyme. There is no mesencephalon, prosencephalon, hypophysis, or epiphysis; there are no eyes, nasal placodes, or oral glands. Prechordal mesoderm is entirely lacking, nor is there an anterior foregut or stomodaeum.

A few sections caudad the ventrally elongated brain is roughly divided into three parts (Fig. 6). These subdivisions, connected at this level, soon separate (Fig. 7) as distinct branches of the neural tube, each underlain by a portion of the notochord. The uppermost section of the tube is that of the embryo proper and the lower one represents that part of the tube which, accompanied by the notochord, extends into the ventral supernumerary tail. The intermediate section deserves special attention because it projects backward as a blindly ending pouch lying ventral to the most anteriorly existing part of the pharynx. The latter is distorted and flattened and gives off a pair of vaguely defined and unidentified pouches. This neural diverticulum is also surrounded by an unorganized array of mesenchymal condensations representing the pharyngeal arches. The setup is very similar to a case described by Adelmann ('34) in Amblystoma in which a portion of the hypothalamus was found pushed "back into the anterior portion of the pharynx for a considerable distance, producing an intussusception of its walls" (op. cit., p. 231). In this instance, however, the diverticulum is accompanied by a notochord and except for its most proximal portion has a thin roof; thus it must be interpretated as a projection from the floor of the hindbrain rather than as an abortive forebrain. Unquestionably, then, we have an embryo entirely devoid of a forebrain and midbrain and all related and associated structures.

Other structures to be noted are the heart in the correct position relative to the primary embryo, though subnormal in size, and the pronephros possessed by both the primary and secondary embryo. This last is in contrast to the first case in which the secondary embryo did not possess a pronephros.

Posteriorly the embryo is normal except for the secondary tail which is complete with neural tube, notochord, and muscle somites.

Case 3. Case 3 is another representative of eggs centrifuged at Stage 10 at 2500 r.p.m. It was allowed to develop for six days and attained a length of 8.5 mm. Externally (Fig. 1b) it presented the familiar rounded, foreshortened head and ventral supernumerary tail, the latter arising at the anterior trunk level rather than from the head. Oral

glands, stomodaeum, and all external evidence of sense organs were again lacking.

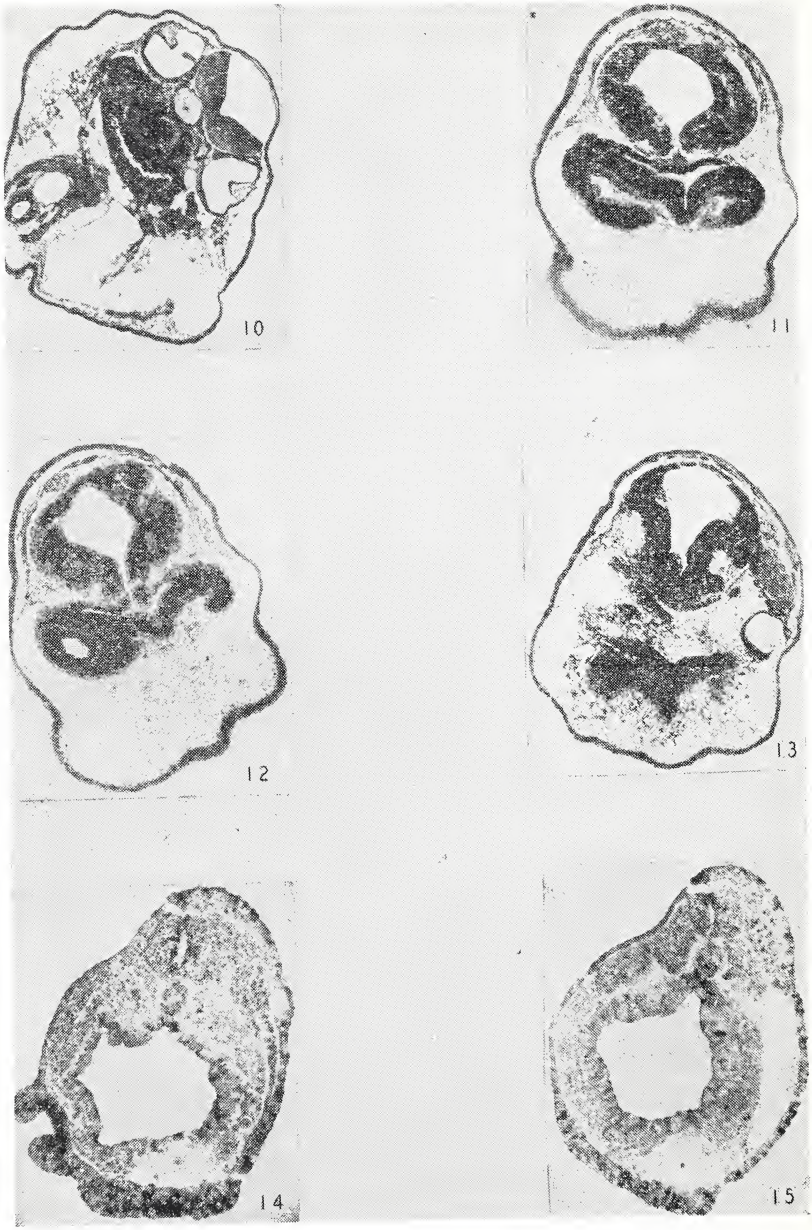
Internally this embryo is very similar to Case 2 in that the forebrain and midbrain are entirely absent. So also are the eyes, hypophysis, and olfactory organs. There are, however, two important differences.

First, the pharyngeal region (Fig. 10) is even more reduced than in the preceding case. The pharynx occurs only as a flattened endodermal mass with an indistinct lumen and without pouches. It is impossible to distinguish definitive visceral arches and there are no external gills.

Secondly, the large hindbrain divides into two (Fig. 8) farther cephalad, and each of these hindbrains is flanked by a pair of otocysts, the secondary pair being somewhat anterior to the primary pair (Figs. 9-10). This is extremely interesting because of the possible role played by the hindbrain in bringing about the formation of otic vesicles. Stone ('31), for example, assigns to the potential medulla an important part in the induction of the otic placodes. If this is true, then the extra pair of otocysts in this embryo can be accounted for on the basis of the extra brain. The occurrence of these selfsame otocysts would thus support Stone's conclusion. It so happens, however, that whereas doubling of the hindbrain is usual for most of the embryos studied, this is the only instance of a corresponding doubling of the otocysts. As a matter of fact, the embryos customarily have a single pair only and in a case yet to be described there are none at all. It seems much more likely that the factors bringing about otocyst formation do not reside solely in the hindbrain, if at all. Harrison ('35) has concluded that the mesoderm adjacent to the prospective ear ectoderm is the primary inductor of the otocyst and this is apparently substantiated by the recent results of Yntema ('39), which show that determination of the ear ectoderm progresses considerably even before that ectoderm is contiguous to the neural folds. The determination of the ear, therefore, apparently occurs sometime during gastrulation, and the explanation of abnormalities of the otocysts in this and other embryos probably is to be found in the translocation of essential mesodermal materials as a result of centrifugation.

The heart and pericardial cavity are present, but very much reduced in size. The pronephros occurs only in the primary embryo, another point of contrast with Case 2, and, as pointed out above, the secondary tail arises from the anterior trunk region. Projecting into the accessory tail for a short distance posterior to its level of origin is a yolk-filled lobe of the intestine. Again, this secondary tail possesses a spinal cord, notochord, and somites.

Case 4. This embryo (Fig. 1c), one of a group fixed eight days after centrifuging, has been selected for brief description because of two very outstanding deficiencies not found in any of the others. First, however, mention may be made of the points of similarity to the first three cases. Once again the forebrain and midbrain regions are lacking along with all the anterior sense organs. No prechordal mesoderm is present and the visceral arches are represented only by a diffuse, shapeless



Explanation of Figures

Fig. 10. Case 3, posterior to figure 8, picturing primary otocysts, and flattened, partly occluded pharynx.

mass of cartilage beneath the greatly reduced and occluded pharynx. There are no pouches or gill filaments. The secondary neural tube arises from the left side of the flattened hindbrain, but gradually moves ventrally and from that position projects out into the secondary tail.

The two special defects are: (1) the heart and pericardial cavity are completely lacking; (2) there are no otocysts, either primary or secondary. For the moment no interpretation of the first is offered and the second has already been briefly considered in conjunction with Case 3.

Case 5. Case 5 has been selected from the F-4 group. The eggs of this group were centrifuged at Stage 11 and allowed to develop thereafter for slightly over three and one-half days. The tadpoles averaged 9 mm. in length at the time of sacrifice.

The embryo to be described has a short, tapering head (Fig. 1d) without oral glands, mouth or external evidence of eyes. External gill filaments are present on the right side only. The abdomen is much less oedamatous than in the previously described cases and there is no supernumerary tail. (Two other members of this group have stubby secondary tails arising from the right side of the trunk.)

The telencephalon is represented only by a cranially projecting mass of neural tissue, which is solid at the extreme anterior end but contains a lumen at about the level of transition with the diencephalon. It is impossible to distinguish cerebral anlagen, and between this abortive telencephalon and the ventrolateral somatic ectoderm only diffusely scattered mesenchymal cells intervene. The diencephalon, though less distorted than the telenchephalon, is decidedly abnormal; it occurs only in primordial tubular form without distinctive thalamic differentiation. The connection of the median-ventral eyes to its ventral floor also contributes to its abnormal character by the addition of a median ventral aperture in its floor. The midbrain and hindbrain appear to be normal.

The eyes in this embryo are represented by two optic cups fused together anteriorly and across the midline, but separate caudally. They bear a striking resemblance in their structure and relations to the diencephalon to the eyes of a 9.5 mm. Amblystoma embryo with partial cyclopia described by Adelmann (op.cit.). The retinal layers of the two cups are continuous across the midline (Fig. 11). Anteriorly the pigment layers are also continuous, but posteriorly (Fig. 12) each joins with the lateral margin of the ventral brain (optic) aperture. Especially interesting is the fact that there is a single lens only, which is associated with the right hand optic cup (Fig. 11). Furthermore, this lens is not one formed in the normal course of events, but is one which has been produced secondarily from the edge of the iris in a manner reminiscent of the known frequent cases of lens regeneration in this manner. This

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Fig. 11. Case 5. Partial cyclopia, with retinal layers continuous across midline. Note small lens attached to edge of iris of right cup.

Fig. 12. Same case of partial cyclopia as above, slightly posterior. Pigment layers continuous with brain wall.

Fig. 13. Single extra otocyst of Case 5. Visceral mesoderm irregular.

Figs. 14-15. Sections through trunk, Case 6. Assymetrical spinal cord and muscle somites.

would lend further support to the point made earlier that in *Rana pipiens* the lens normally is a self-differentiating structure whose materials have, by the nature of the experimental conditions, been transferred or at least suppressed.

There is a single extra otocyst on the left side and at a level far anterior to the normal position. Its most anterior margin appears in a section at about the level of transition between the diencephalon and mesencephalon containing a diminishing fragment of the left optic cup. As Fig. 13 shows, it is well developed and is not only located abnormally far cephalad, but is also ventral to the usual otocyst position. There is no corresponding organ on the opposite side. Farther posterior and normal in both position and structure lie the regular otocysts of the embryo. The occurrence of this single abnormally located otocyst is best explained in terms of the dislocation of a mesodermal inductor (Cf. Harrison '35) because it is difficult to see how the hindbrain could play any role in the formation of an organ that occurs far anterior to that level.

The pharynx in this embryo is very abnormal, though less distorted than in the previously described cases. It terminates blindly only three sections anterior to the rostral end of the notochord and there is consequently no oral cavity. It assumes fairly regular contours from this level posterior, but there is one less pouch on the left than the right and the hyomandibular pouches are missing. The next four pouches on the right are present with the second and third opening into the opercular cavity; on the left the fifth pouch is lacking and only the second opens to the exterior. It may be pointed out in this latter connection that the internal gills and opercular cavity on the left are considerably reduced as compared with the right.

The lack of bilateral differentiation of the prechordal mesoderm is once again a striking feature. The cartilagenous mandibular arch is also absent. The hyoid and branchial arches are present, but better developed and more nearly normal-sized on the right than the left. There is a corresponding lag in the development of the branchial musculature on the left.

All body structures from the post-pharyngeal region caudad are normal.

Case 6. Case 6 has been selected primarily because it is representative of a considerable number of embryos with a persisting posterior yolk mass (Fig. 1e) and certain distortions of the spinal cord. It comes from a group centrifuged at Stage 9 and 2400 r.p.m. and allowed to develop 42 hours thereafter.

The head region is more nearly normal than in any of the previously described embryos. The most conspicuous deficiency is the absence of a stomodaeal pit and, therefore, of the hypophysis. There is only one olfactory placode, which is on the right. The oral glands are assymetrical, the one on the right being essentially normal, but the left one has the form of an elongated slit fused with the right.

The forebrain and midbrain appear normal except for distortions due to diffusely distributed yolk, the former exhibiting primary optic vesicles. The embryo is too young for the lens placodes to have developed. In contrast to the regular optic vesicles, there is a single otic vesicle only, which occurs on the right side and is still attached to its parent ectoderm. The hindbrain is regularly formed.

The spinal cord of this embryo is distinctive by being assymetrical throughout its length. Figure 14 pictures the cord at an anterior level. The right side appears normal, but the left wall is considerably thicker and its cells are continuous with a ventro-lateral wedge of cells having the appearance of a hyper-developed sensory placode or neural crest. It is to be noted, too, that the mesodermal somite on this side is considerably reduced (Figs. 14-15). This is in direct contrast to Holtfreter's ('33) observation that contact with the developing musculature causes the wall of the spinal cord to thicken. Here we have the thicker wall associated with reduced musculature.

The final point to be noted is that this embryo has a persistent blastopore through which a large yolk mass still projects to the exterior.

Conclusion

For purposes of interpretation the more outstanding irregularities described in the above cases may best be brought together under the headings of the particular organs or groups of organs involved.

1. **Pharynx, Mesoderm, and Visceral Arches:** The pharynx in cases 1, 2, 3, and 4 occurs only as a partly solid, partly tubular endodermal mass without distinguishable pouches. In case 5 it is more nearly normal, though considerably distorted, and in case 6 appears normal.

Prechordal mesoderm occurs only in cases 1, 5, and 6, showing no bilateral differentiation at all in the first and fifth and appearing normal in the last. There is no distinguishable prechordal mesoderm at all in the other three, i.e., cases 2, 3, and 4.

The visceral skeleton in the first four cases exists only as unorganized masses of mesenchyme or cartilage. In case 5 the mandibular arch is absent, but the other arches are present, though assymetrical in form. The last embryo is too young for the arches to have formed, but appears normal.

2. **Brain:** The forebrain in all cases has been affected, ranging from its complete elimination in cases 2, 3, and 4 to its essential integrity in case 6, with cases 1 and 5 being somewhat intermediate. The midbrain is likewise absent in cases 2, 3, and 4, but present and normal in the remaining three. Abnormalities of the hindbrain occur in all but the last two cases and consist of a division into two hindbrains, one primary and one secondary, with the latter continuing as a spinal cord into the supernumerary tail.

These brain abnormalities are obviously of two distinct sorts: (1) deficiency or absence and (2) duplication. Either one or both of

two causative factors may have been involved in producing these irregularities. They may be the result of either the literal removal of potential brain materials to new sites and thus to new determinations, or if such materials have not been shifted, the necessary head organizer may have been translocated.

The evidence favors the latter interpretation, for when one compares the degree of development of the brain in the several cases with that of the prechordal mesoderm, an important correlation is immediately obvious. The total absence of the forebrain and midbrain in cases 2, 3, and 4 is found paralleled by a similar total absence of prechordal mesoderm. We find a materially reduced forebrain and normal midbrain in cases 1 and 5 correlated with existing but bilaterally undifferentiated prechordal mesoderm. The normal brain of case 6 goes hand in hand with normal mesoderm. It is very probably true, therefore, that these abnormalities of deficiency are the result of translocation of the head organizer which is identified with the prechordal mesoderm. This material, located as it is in the primitive dorsal blastoporic lip, is ordinarily the first to be invaginated. Since effective centrifugation of the eggs has occurred just as invagination is beginning, it is very likely that these dorsal lip materials have been among those dislocated.

There is always the possibility, of course, that it is not the head organizer alone which has been moved, but also the associated potential brain material. Evidence will be presented in another connection that points to this likelihood as well. The alternative possibility, i.e., that the organizer materials have been left intact and the brain ectoderm has merely been removed from their sphere of influence, seems unlikely because of the occurrence of secondary embryos. That is, the presumed dislocation of at least a portion of the head organizer has not only resulted in a suppression of the forebrain and midbrain, but apparently is also responsible for the induction of a secondary embryo represented by the double hindbrain and spinal cord and supernumerary tail. It will be recalled that the secondary neural tube invariably has a notochord associated with it, which suggests that a duplication of the organizing archenteric roof has occurred. That this secondary inductor may very well be the dislocated head organizer is also suggested by observations (e.g., Spemann, '31) on the longitudinal polarity of the organizer according to which the head organizer is capable of inducing cephalic structures at trunk level.

3. **Sense Organs, Hypophysis, and Oral Glands:** Certain interpretations of conditions relating to the sense organs have already been presented in conjunction with individual cases, but some supplementary generalizations may be added.

A total absence of eyes in cases 2, 3, and 4, like the absence of the forebrain and midbrain may be the combined result of dislocation of both the medullary ectoderm and inducing substrate. If only one has been so affected, then unquestionably it is the latter.

As for the cases of complete and partial cyclopia, there is little to be added to what has already been said. The facts thoroughly support

Adelmann's interpretation of cyclopia in terms of suppression of the prechordal mesoderm. The one point of difference, namely, the absence of lenses (except for the case of unquestionable regeneration of the lens from the iris), has been discussed elsewhere. An actual translocation of lens forming materials seems to be the best answer.

The same answer must also be given for the absence of oral suckers, olfactory organs, stomodaeum, and hypophysis. As for the otocysts, however, it is undoubtedly a matter of disturbance of associated mesoderm. This aspect of the problem has already been discussed under case 3.

4. **Heart and Pronephros:** Two little is known of the manner of original determination of the bilateral heart rudiments for the results of these experiments to be completely understandable. The suppression in size, and in one case total absence of the heart, may only tentatively be accounted for on a basis of a shift of material by centrifugation.

Only in case 2 does the secondary embryo possess a pronephros. That its occurrence is undoubtedly an aspect of the general phenomenon of induction of a secondary embryo is suggested by the fact of its having been observed by many investigators. Whether a pronephros occurs in a secondarily induced embryo or not is known to depend upon the level in the host at which the inductor is working (Holtfreter, '33).

5. **Translocation of Presumptive Organ Regions:** Centrifugation obviously causes the contents of the germ to be rearranged—not all parts to the same degree, however, for the described results indicate that those materials destined to produce the anterior structures of the embryo have been most affected. Reference to Vogt's maps of the presumptive organ regions of an Anuran reveal that it is those pharyngeal and mesodermal materials that are normally first invaginated plus the ectodermal materials within the limits of the future head that are most affected. That these substances have been shifted is unquestionable, but just how and exactly where they have been moved is not so clear. One might speculate with a fair degree of accuracy on the path followed and the final position assumed by various areas under the influence of centrifugal force exerting itself parallel to the egg axis, but any conclusions derived would necessarily be tentative only and supported solely by indirect evidence. It seems best, therefore, to defer such conclusions until more direct evidence is available. This, it is hoped, will come from experiments combining the techniques of localized vital staining and regulation of the axis of centrifugal force by orientation of eggs in gelatin blocks. These results will be submitted in a later communication.

Summary

1. Eggs of the frog, *Rana pipiens*, were centrifuged at various stages of development and at various speeds. The developmental stage most affected by centrifuging, as reflected by resulting abnormal larvae, was that of the early blastopore. The most effective centrifugal speed was 2400-2500 r.p.m.

2. Numerous embryos with accessory tails and under-developed or defective heads were obtained.

3. The accessory tails possessed a spinal cord, notochord and muscle somites.

4. The head abnormalities included: (a) total or partial absence of the forebrain and midbrain and doubling of the hindbrain; (b) cases of total absence of the eyes, complete cyclopia, and partial cyclopia, the latter two featured by the absence of lenses except for one instance of lens regeneration from the iris; (c) absence of the oral suckers, olfactory organs, stomodaeum, and hypophysis; (d) variable duplications of the otocysts uncorrelated with doubling of the hindbrain, and one case of their complete elimination; (e) suppression of development of the prechordal mesoderm; (f) suppression and distortion of the pharynx and visceral arches; (g) one case of total absence of the heart and its reduced size in the other cases; and (h) one instance of an accessory pronephros associated with the secondary embryo.

5. The abnormalities have been interpreted in terms of a presumed shift of the "head organizer" by the action of centrifugal force.

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