

THE DEVELOPMENT OF FUNDULUS HETEROCLITUS IN SOLUTIONS OF LITHIUM CHLORID, WITH APPENDIX ON ITS DEVELOPMENT IN FRESH WATER

BY

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WITH NINETEEN FIGURES

The eggs of *Fundulus heteroclitus* are hardy and, therefore, stand experimental treatment most satisfactorily. Since they are capable of development in both salt and fresh water it is possible to eliminate all of the physical effects of hypertonic solutions, for effective solutions of a salt may be applied in fresh water that have a final pressure lower than that of normal sea water. Mathews has stated that the eggs of this fish are peculiarly adapted to treatment with solutions because they appear to be easily penetrated by nearly all sorts of ions, and they are quite insensitive to variations in osmotic pressure of the solutions. Whether this statement is absolutely true or not there can be little doubt of the fact that the ions resulting from LiCl in solution do penetrate the membranes and give an effect that is certainly not due to osmotic pressure, since the same result is obtained with solutions of LiCl in both salt and fresh water, the one being hypertonic while the other is a hypotonic solution. Whether or not the membranes are easily or quickly penetrated is a question better answered by a study of the results.

The fact that lithium salts produce a marked and really specific effect on development was observed first in 1893 by Herbst while studying the eggs of the sea-urchin. The definite type of embryo thus produced was termed by him the lithium larva. Soon after this several investigators studied the action of lithium salts on the development of the frog's egg and found that it induced decided effects, yet they failed to show that a distinct type of embryo

resulted, although Gurwitsch concluded that his radial larva was the specific result of the lithium action. Morgan then in 1902 carried on experiments to test the effects of LiCl on frog's eggs, and again in the following spring he used a number of lithium salts along with other solutions for comparison. He finally concluded that lithium produced in the development of the frog's egg a typical larva just as Herbst had shown for the sea-urchin. Both of these workers claimed for similar reasons that the effect was due to the chemical action of the lithium ion and not to osmotic or pressure effects; hence the specific character of the embryos. Loeb, Mathews and others have subjected the eggs of the fish, *Fundulus heteroclitus*, to solutions of lithium salts, but only to observe the physiological effects of the solutions, ignoring the morphological changes induced.

With these results in view Prof. T. H. Morgan kindly suggested that I study, from a morphological standpoint, the effects of lithium salts on the fish's egg. It is, therefore, a pleasure to avail myself of this opportunity of expressing my indebtedness to Professor Morgan for his kindly advice and criticism throughout the progress of this work. The experiments were conducted during the past summer while occupying a table in the United States Fish Commission Laboratory at Wood's Hole, and I wish to thank the authorities of this laboratory, particularly the director, Dr. F. B. Sumner, for the courtesies extended me while there.

The *Fundulus* egg differs in its mode of development from both that of the sea-urchin and the frog, hence it is of interest to note whether or not an effect such as that produced by lithium is at all comparable in the three cases. Of course it is fully realized that no strict embryological comparison should be made among types of development so different as these, yet one may find little objection to a comparative study of the chemical effects produced in the three widely different forms by one and the same element, lithium. At present I am not in a position to state that the abnormalities which have been induced in the *Fundulus* egg by means of LiCl are specific for lithium, as a number of other salts must first be experimented with. The abnormalities described are not exceptional, but general, occurring in as large a per cent.

of the eggs as could be expected. All of the experiments were repeated frequently—some as often as a dozen times—and always with constant results.

#### METHOD

The following treatment and precautions were used throughout the experiments. It was decided to keep the eggs as nearly as possible under normal conditions by using solutions of the LiCl in ordinary sea water, and to run experiments with fresh and distilled water solutions merely as checks on these. Normal sea water controls were carried in each experiment and as a further check eggs were kept developing in ordinary fresh and distilled water. A series of solutions of LiCl in sea water was prepared in strengths  $\frac{1}{8}$  n,  $\frac{1}{4}$  n,  $\frac{3}{8}$  n,  $\frac{1}{2}$  n,  $\frac{5}{8}$  n,  $\frac{3}{4}$  n,  $\frac{7}{8}$  n, and normal, a normal solution of LiCl being equivalent to about a 4.25 per cent. solution; the eggs were then subjected to these to ascertain the various effects of different strength solutions. It was found that those solutions weaker than  $\frac{5}{8}$  n produced no apparent effect during the early stages of development. In the  $\frac{3}{4}$  n,  $\frac{7}{8}$  n, and normal solutions the eggs showed varying degrees of abnormalities, the ones in the stronger solutions dying after a few hours. A series was then prepared between  $\frac{5}{8}$  n and  $\frac{3}{4}$  n so as to obtain different degrees of abnormal development. These solutions were 2.62 per cent., 2.82 per cent., 3.02 per cent. and 3.22 per cent. LiCl or .62 n, .66 n, .71 n and .76 n. These four strengths were then used in all of the experiments. A fresh water series was also prepared and I found, as Mathews had, that a  $\frac{1}{4}$  n solution was the minimal poisonous dose, that is, the weakest solution preventing the formation of an embryo; it will be noted that this is only about one-half the strength required to affect the eggs when in a sea water solution of LiCl. A  $\frac{7}{10}$  n or 1.48 per cent. LiCl solution in distilled water was resorted to, though its effect was rather severe, being about the same as that produced by a 3.22 per cent. LiCl solution in sea water. Controls were always taken from the same bunch of eggs as those on which the experiments were performed. It is quite noticeable that *Fundulus* eggs withstand solutions many

times stronger than those used to affect the eggs of the sea-urchin and frog.

#### RESULTS

When *Fundulus* eggs are placed in LiCl solutions shortly after fertilization, or before the two-cell stage, a very constant modification in the early stages of development will be noticed. The protoplasmic cap or blastoderm becomes unusually prominent, bulging up in an arched fashion that is much more marked than in normal eggs. After reaching about the sixty-four cell stage and later, a clear bubble-like appearance is noted in the living eggs below the disc (Figs. 1 and 2). On sectioning and staining the blastoderms at this stage it is found that the bubble-like appearance is due to the greatly enlarged condition of the segmentation cavity (Fig. 13). The central periblast has been forced from its normal place close below the blastoderm (Fig. 14) and pushed down into the yolk mass at the same time causing the cap to arch more decidedly above on account of the strain thus induced about its periphery. This certainly looks like an osmotic effect but one fails to see how it could be attributed to such a cause when it is remembered that the same condition exists both in the sea water LiCl solution and in the distilled water solution (compare Figs. 1 and 2), the one being hypertonic while the other is hypotonic and thus in the two cases opposite, and not equal, effects would be expected.

*Lithium chlorid* in all cases most obviously delays the development (compare Figs. 1, 2, 3 and 4 with 11; 5 and 8 with 6). In the stronger solutions, eggs as old as forty-three hours, show the blastoderm still as a polar cap and in these older stages it presents a most interesting appearance. On examining such living eggs the blastoderm is found to be greatly raised and in some cases almost pinched away from the yolk. When one looks down on the top of this blastoderm it is seen to suggest very strongly a gastrula of some holoblastic egg such as that of a sea-urchin or starfish, the center appears thin while the peripheral curved surface has a much thicker appearance as if the center showed below it the blastopore and the sides represented the

folded double wall of the gastrula seen in profile. Stages were seen in which this "blastopore" appeared very large as if invagination was just beginning, and other stages showed various degrees of decrease in the size of the blastoporic appearance (Figs. 15 and 16). These eggs finally died, in many cases on account of the blastodermic edges becoming approximated, thus pinching the cap entirely away from the yolk. On studying sections of these peculiar forms it was found that as the segmentation cavity became abnormally large, thus pushing the blastodermic roof up into a more decided arch, the edges or periphery of the blastoderm were brought closer together at the same time becoming thicker and showing more cell layers than were seen in the crown or top of the disc (Figs. 15 and 16). The periblast seems more loosely connected with the blastoderm than is usually the case; thus it is readily understood how the latter may finally pinch away from the yolk mass—compare Figs. 13, 15 and 16. These figures also make clear the manner in which the blastopore-like image in the living egg seems to decrease in size—as if a blastopore was closing; this is merely the visual effect produced by the decreasing circumference of the blastodermic periphery as it becomes puckered together like the mouth of a sac. No dividing cells were found in these sections of the blastoderms, and when it is recalled how abundant such cells usually are in sections of fish blastoderms at this stage we are struck with the inhibition or decrease in division rate caused by the LiCl, and hence the slow rate of growth. Normally a forty-hour egg would have the young fish well formed. No indication of invagination was shown about the edges of these blastoderms, but it may be possible that if eggs in this condition were transferred from the strong solutions back to sea water that the slight recovery thus induced might cause them to continue development and perhaps to slightly invaginate as this appears to be the easiest direction of growth. Lack of material during the latter part of the season prevented the trial of such an experiment.

Many eggs die when the blastoderm becomes confined to the polar region as is indicated above, but in rare cases they survive and develop to the extent that the blastoderm thins out and

## EXPLANATION OF FIGURES.

All were drawn from camera sketches of the living eggs except figures 5, 6, 8 and 9.

Fig. 1. Egg from a normal  $\frac{1}{10}$  LiCl distilled water solution when twenty-two hours and twenty minutes old. *sg*, Segmentation cavity; *od*, oil drops.

Fig. 2. When twenty hours old in 3.22 per cent. LiCl in sea water.

Fig. 3. Twenty-three hours old in 2.82 per cent. LiCl in sea water. *gr*, Germ ring; *es*, embryonic shield.

Fig. 4. Twenty-two and one-half hours old in 2.82 per cent. LiCl sea water.

Fig. 5. Nine days old, taken from 3.02 per cent. LiCl when twenty-eight hours old and placed in pure sea water. *h*, Head; *t*, tail; *f*, fin.

Fig. 6. Control embryo nine days old. *ht*, Heart.

Fig. 7. In 2.82 per cent. LiCl fifty-four hours old.

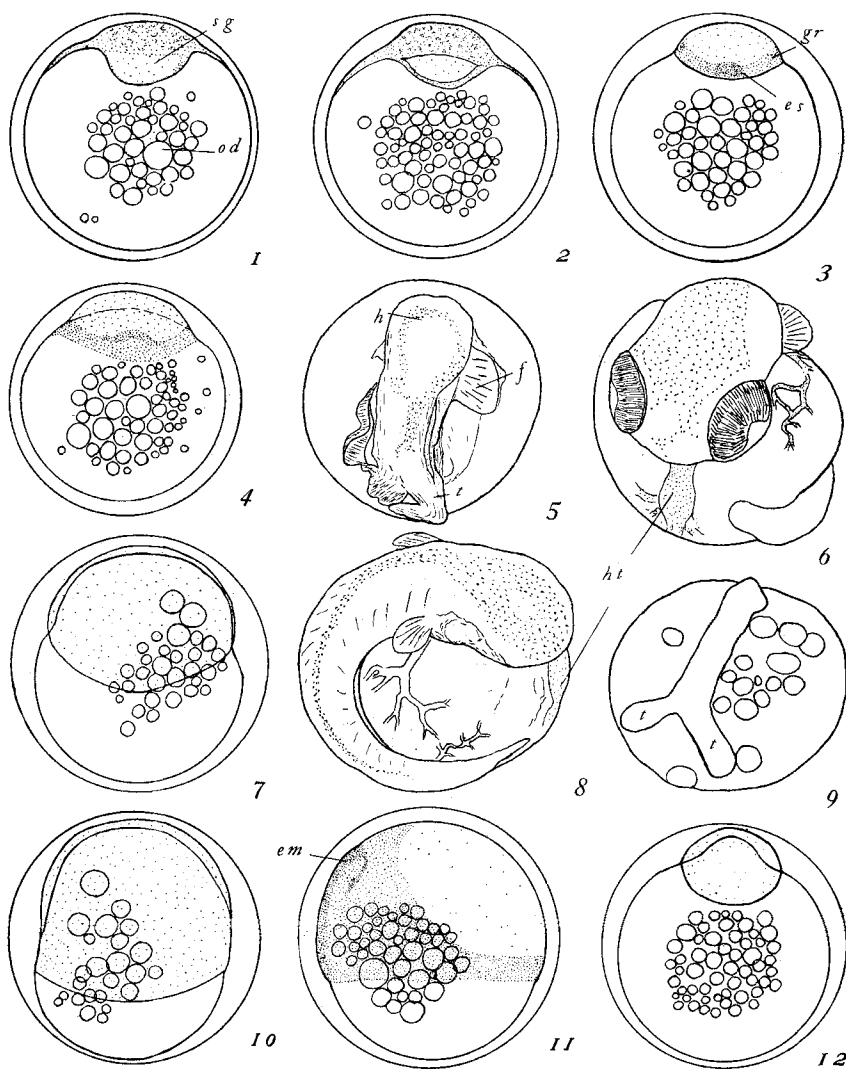
Fig. 8. Embryo with no eyes, nine days old, from 2.82 per cent. LiCl into sea water at thirty-two hours old. *ht*, Heart.

Fig. 9. Cauda bifida normal  $\frac{1}{2}$  LiCl forty-eight hours. *t*, Tail.

Fig. 10. In 2.82 per cent. fifty-four hours old.

Fig. 11. Control twenty-four hours old. *em*, Embryonic thickening.

Fig. 12. In 3.02 per cent. thirty-one and one-half hours.



flattens slightly, and in some cases forms an embryonic shield in this polar position. Fig. 18 shows such a shield which, forming in a cap forty-six hours old, extended no further over the yolk than a normal high segmentation stage would or than the germ area shown in Fig. 1 or 2 does. A longitudinal section of this shield is shown in Fig. 17 and it is seen that the folding and cell arrangements are more or less normal, in this case little or no indication of the germ ring was found beyond the edges of the shield. Fig. 19 shows a section through a blastoderm with the embryonic shield thickening well marked. In Figs. 3 and 4, embryos about twenty-three hours old, the germ area is still high up on the yolk, but the germ ring and embryonic shield have reached a stage of development commonly seen when the germinal area has descended about one-third of the way down the yolk. In these eggs the embryonic area never completely covers the yolk, the blastopore always remains open and through it the yolk is seen to protrude. *These instances show most clearly that a second effect of lithium chlorid is to prevent the down-growth of protoplasm in the egg.*

The embryos that result from the eggs just mentioned are peculiar, being necessarily short as the embryonic material only extends part of the way down the yolk. They are also poorly formed in the head region and the tail seems to dwindle out suddenly as a small pointed end. Figure 5 shows such a monster in position on the yolk when nine days old; a normal control embryo of the same age is shown in Fig. 6. These short embryos were cleared and stained and finally studied in sections. They usually lacked all indications of eyes, either optic vesicles or lens, but the ears were being formed and the trunk organs were present. As is seen in the figure the fins are poorly developed.

When weaker solutions of LiCl were used, such as 2.62 per cent. and 2.82 per cent., the formation of the embryo approached nearer the normal, but even in the 2.62 per cent. no embryo was ever developed in an entirely normal way. In all cases the rate of development was retarded, the heart beat was slower, and the blood was colorless, seeming to lack the hemoglobin constituent. The latter condition must have lessened the respiratory efficiency to quite an extent, a fact which would also contribute to their slow



rate of development. Embryos in these weaker solutions often showed cauda bifida (Fig. 9), and others that were more nearly normal in form had rather irregular and twisted outlines, some of these having the tail end bent at right angles to the body axis. These malformations of the caudal end are no doubt due to the slow descent of the protoplasmic mass over the yolk and in some cases to the entire failure of this mass to completely enclose the yolk. This causes the embryo to be short, crowded into less space, and twisted in appearance.

A most noticeable feature in these living embryos was the apparent absence of eyes (Fig. 8 a nine-day embryo). When one looks at a batch of developing fish eggs after the embryos have formed, a most conspicuous feature is the large black eyes on each egg—the lack of such an appearance is striking in the lithium embryos—and the entire mass of eggs has, therefore, a pale, unhealthy look. Sections of the heads of these larvae show that in many cases no indication whatever of eyes existed, while in some a small deep-seated poorly-formed eye was found, which, on account of its probable paleness, was entirely unnoticeable in the living embryos.

The germinal area in some eggs advanced over the yolk in a very peculiar manner. Figure 10 shows a thin cellular sac that extends more than half way down the yolk, constricting it slightly about the equator and giving the entire egg a rather unusual shape. At the border of the protoplasmic sac little or no indication of germ ring or embryonic shield could be distinguished, though these structures must have existed to some extent since at later stages short malformed embryos would arise appearing in life as if their tail ends were entirely lacking. Figure 7 shows a circular cap which does not extend so far down the yolk as in the last case, but the very strong line of demarcation which often exists between yolk and protoplasm is well shown. Figure 12 shows a rare case in which a small part of the yolk is pulled up into the blastodermic cap as a small polar cone.

The rapidity of action of the LiCl solution, the degree of abnormalities and death rate produced by solutions of different strengths as well as the periods in development at which the solutions seemed

most and least effective may best be treated by referring directly to notes taken while testing these several questions.

As was mentioned before the rapidity or ease with which metallic ions penetrate the membranes of *Fundulus* eggs is uncertain. But the examples cited below seem to show that lithium ions, at any rate, enter with comparative readiness and produce abnormalities which in these experiments, at least, can not be attributed to other causes. Eggs that were fertilized "dry" and then placed in sea water LiCl solutions of 2.82 per cent., 3.02 per cent. and 3.22 per cent. showed characteristic lithium effects after a period of only three hours, while in the eight-cell stage, the blastoderms bulging up abnormally. The control from the same lot of eggs failed to show any such modifications. Again on applying a series of solutions of LiCl in sea water of strengths of 1.59 per cent., 2.12 per cent., 2.65 per cent., 3.18 per cent., 3.71 per cent. and 4.25 per cent. and examining after sixteen hours the eggs in the three weaker solutions presented a normal appearance, while those in the three stronger solutions contained a large per cent. of abnormal forms, all in the strongest solution being very abnormal. The blastoderm was arched and the segmentation cavity appeared as a bubble below. After being in the solutions forty-eight hours the 2.65 per cent. lot contained twenty-six per cent. undeveloped eggs and many abnormally-shaped embryos, some with cauda bifida and irregular outlines. The 1.59 per cent. and 2.12 per cent. lots also showed malformed embryos though not so many as in the 2.65 per cent. lot. Thus it is shown that weak solutions do not affect the development in its early stages though they later cause the production of deformed embryos. In another experiment eggs were subjected to 2.82 per cent. LiCl when twenty-two hours old, and others when twenty-five hours old were put into 3.02 per cent. LiCl. When they were all forty-four hours old, the ones which had been in the stronger solution, though for three hours less, showed more marked modifications than those in the weaker. These facts seem to show that LiCl does enter the egg with sufficient readiness for all experimental purposes.

The degree of effectiveness for the different strength solutions may be illustrated as follows: When forty-six hours old many eggs

in 2.62 per cent. LiCl show a faint line indicating the embryo, and there are also a large per cent. of dead eggs present. In the 2.82 per cent. solution at this time there are still more dead ones; the

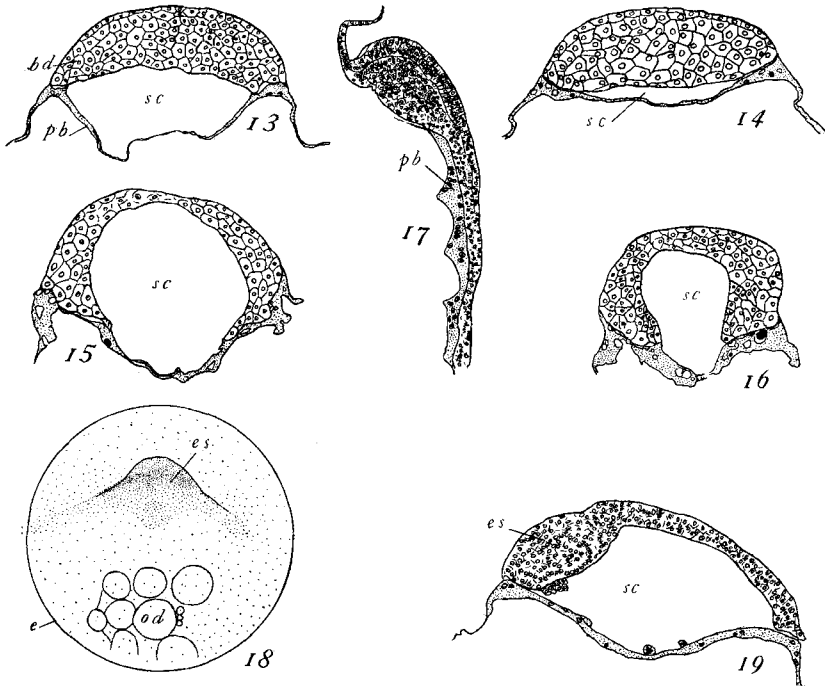


Fig. 13. Seventeen hours old in LiCl normal solution. *bd*, Blastoderm; *pb*, periblast; *sg*, segmentation cavity.

Fig. 14. Control ten hours old. *sc*, Segmentation cavity.

Fig. 15. In 3.02 per cent. LiCl forty-three hours old. *sc*, Segmentation cavity.

Fig. 16. In 3.02 per cent. LiCl forty-three hours old. *sc*, Segmentation cavity.

Fig. 17. Longitudinal section embryonic shield from Fig. 18. *pb*, Periblast.

Fig. 18. Embryonic shield in polar cap forty-six hours old in 3.22 per cent. LiCl. *es*, Embryonic shield; *od*, oil drop; *e*, border of blastoderm.

Fig. 19. Section of polar cap twenty-two hours old in 3.22 per cent. LiCl. *es*, Embryonic shield; *sc*, segmentation cavity.

germ ring has not grown completely over the yolk except in rare cases, but the embryo is formed in the embryonic shield. Among those in 3.02 per cent. none of the blastopores have closed and the

embryos are all short and abnormal, with a still larger number of dead eggs. In the 3.22 per cent. LiCl less than half are still alive; all of the blastopores are unclosed; some of the embryos are fairly well developed in the embryonic shield, while some of the embryonic caps are still at the upper pole with the embryos forming in a thickening, like an embryonic shield, which often lies immediately over the pole of the egg (Fig. 18); others have blastoderms which may finally pinch away from the yolk and die (Figs. 15 and 16).

In another experiment the control embryos are distinctly formed when fifty-four hours old; while in the 2.62 per cent. LiCl the blastopores are only about two-thirds closed and embryos are forming in the embryonic shields. In 2.82 per cent. LiCl blastopores are still wide open; in 3.02 per cent. caps, with slight embryonic shields, are present; and in the 3.22 per cent. LiCl all seem to have stopped developing and many have the blastoderms broken away from the yolk and floating freely within the egg membrane.

When the same eggs are sixty-nine hours old forty-five are alive in the 2.62 per cent. LiCl solution. The embryos are all well formed, though a few have failed to close the blastopore. In 2.82 per cent. LiCl only seven out of thirty-five are still alive, or 20 per cent., and only one of these has a definite embryo formed. In 3.02 per cent. LiCl only five out of fifty-three eggs are living, or 9.5 per cent., and these are all deformed. Of those in the 3.22 per cent. LiCl solution sixteen are dead and one is living, or 6 per cent. alive. In this one the cap is scarcely half way down the yolk.

At ninety-six hours old these eggs are in the following condition: The 2.62 per cent. LiCl lot have thirteen out of fourteen still living, all with well formed embryos though far behind the control in development. The 2.82 per cent. ones have not fared so well; only three out of seven are still alive but these, also, have well formed embryos. The one survivor of the 3.22 per cent. LiCl solution is now dead, the cap has pulled back off of the yolk, which on the day before it had half covered, and is now a dead mass of cells at the upper pole.

The embryos in the 2.62 per cent. LiCl solution when nine days

old are far behind the control in development; the heart beat is only about one-half as fast as normal, and the yolk is badly shrunk so that the entire embryo swings about freely in the egg membrane. The 2.82 per cent. LiCl embryos are still alive, though they also have much shriveled yolks and are otherwise like the ones in the 2.62 per cent. solution.

Eggs of various ages were subjected to the LiCl solutions to ascertain whether or not there were periods in development when they appeared more sensitive than at other times. As a further means of deciding this question, eggs were also removed at intervals from the LiCl solutions and placed in pure sea water. Eggs which were sixteen hours old (early germ-ring stages) were placed in 3.02 per cent. LiCl and when they were forty hours old they had become very abnormal and many were dead. Eggs put into 3.02 per cent. LiCl when seventeen hours old are described thus, when eight days old: The pigment is confined almost altogether to the yolk area and is largely of the black type, little if any of the brown being present; the blood is colorless and the heart beats slowly; the eyes have little or no color and in most cases seem absent, and the yolk has decreased greatly in size. Of the eggs put into 2.82 per cent. LiCl when nineteen hours old almost the entire lot died, the remaining ones resembling those which had been in the same solution since fertilization. If put into 2.62 per cent. LiCl when twenty hours old they appear, when forty-four hours old, similar to those that have spent their entire life in a solution of this strength. Those put into 2.82 per cent. when twenty-two hours old appear at forty-four hours almost normal though a little behind in their development. The extreme sensitivity of the nineteen-hour and twenty-hour stages as thus indicated is noteworthy. Figure 11 shows a normal egg somewhat beyond this age, and it is seen that the germ ring has just turned the equator of the egg. Other eggs were put into 3.02 per cent. LiCl when eighteen, nineteen, twenty, twenty-one and twenty-two hours old, respectively, and kept in this solution for three days. Then they were transferred to pure sea water again. Seventeen days later they were still alive but developing very slowly and seemed far from being ready to hatch while the control

ones had hatched from three to four days previous. Thus it seems that LiCl leaves a lasting effect on these embryos and that they are unable to recover, though under normal conditions, for as long as seventeen days after.

Eggs sixty-five hours old with well-formed embryos were placed in 3.02 per cent. LiCl solution; twenty-eight hours later on examining the living eggs no variations from the normal could be detected; fifty-four hours after being in the solution no change was observed, though when nine days old they were behind the control in their development at least two days.

To test the time of greatest sensitiveness in another manner, eggs were taken from the LiCl solutions after being in them only thirty minutes, while in the two-cell stage. The LiCl in this case left no trace of any effect that could be detected in their later development. Again eggs taken from 3.02 per cent. LiCl after six hours treatment were put into sea water, their age being then seven and three-quarter hours, they appeared normal when twenty and twenty-four hours old, but when forty-four hours old they had fallen behind the control in their development. Eggs were taken from 2.82 per cent. LiCl when fifteen and one-half hours old, having been in the solution fourteen hours and were placed in sea water, twenty-four hours later the eggs showed no improvement over those still in the solution. When examined after being out of the solution for sixteen days they still showed little if any recovery. In another case eggs were placed in sea water after being in 2.82 per cent. LiCl for eighteen and one-half hours and others in a 3.02 per cent. solution seventeen and three-quarter hours. When forty-four hours old they were still abnormal but far ahead of those remaining in the lithium solutions. After being in 3.02 per cent. LiCl for twenty-six and three-quarter hours eggs were placed in sea water where they failed to recover, though their death rate was materially lowered; after two days 89 per cent. still were living while of those left in the solution only 9.5 per cent. remained alive. This lowering of the death rate was noted in many instances where morphological recovery from the lithium effect seemed lacking. It appears as though the eggs gain in power of resistance, or rather resistance is no longer required

after being transferred to the sea water and hence though they may not recover in one sense, they are better able to survive than they would be under the depressing effects of the lithium.

During one experiment a very strange result was noted: The entire series of LiCl solutions failed to give any visible effect for more than the first day of development, but later the characteristic effects were manifested and those eggs that were removed from the solutions and placed in sea water during the day of apparent non-effectiveness later showed the effects of the LiCl on their subsequent development in the sea water, although no effect was observable when they were removed from the lithium solutions.

The above experiments were all repeated several times and the particular instances cited merely serve as examples to illustrate the general results obtained. From such responses to the LiCl treatment one may be justified in drawing the following conclusions.

#### CONCLUSIONS

*The rapidity with which lithium chlorid in solution affects the egg varies directly as the strength of the solution, eggs being very slow to manifest the effects when placed in dilute solutions, while a response was obtained on one occasion within three hours in rather strong solutions.*

*The degree of abnormality and the death rate also vary directly as the strength of the lithium chlorid solutions, striking abnormalities being found in 3.22 per cent. solutions while similar malformations in a less marked degree with a much lower death rate were noted in the 2.62 per cent. solutions.*

*The lithium chlorid solution affects the development of the embryo at any stage in which the eggs may be placed in it. But the extent of the abnormalities seem to vary inversely with the age at which the eggs are subjected to the solution, showing merely a lessened rate of development with other minor effects if placed in the solution during late stages.*

*When eggs are once affected by the lithium chlorid solution—which is found to be the case if they remain in it as long as six hours—*

*they are unable to completely recover from this effect even though they be placed in pure sea water during the remaining period of their development.*

#### REVIEW OF LITERATURE

Herbst, in '92, '93 and '96, conducted extensive experiments to test the action of a large number of salts on the developing eggs of the sea-urchin. He found that of a number of salts used, those of the metal lithium produced the most decided results, and further that with the different salts of lithium the results were the same. Thus the conclusion was reached that the action of these salts was not due to their several acid radicals but to their common lithium ion. Herbst, therefore, stated that the abnormalities induced were typical lithium effects, not being obtained by the use of salts of other metals. He used the term "lithium larva" to designate the peculiar monster that resulted. The effect of lithium on the sea-urchin's egg seemed chiefly to cause "exogastrulation," that is, the endoderm instead of becoming infolded by invagination became really inverted and formed a sac connected with the ectoderm or outer gastrula wall by a short stalk, thus producing a three-part larva, two sacs, the gastrula wall and the primitive gut, joined by a short tube or stalk. From the end opposite the stalk the primitive gut sac in many instances formed another small sac representing, as Herbst claimed, the vasoperitoneal pouch. The ectoderm sac at times became smaller and occasionally finally disappeared leaving the entire blastula wall endodermal. The larva recovered slightly so as to form the calcium framework when removed from the lithium solutions; the amount of recovery depending upon the length of time it had spent in the solution. This briefly is the main action as found by Herbst in three genera of sea-urchins treated with, in all, thirteen salts of lithium. The features of especial interest to us are the marked inversion of the layers and the resulting enlarged embryonic cavities.

Gurwitsch, in '95 and '96, treated developing frog's eggs with LiCl and other salts, finding that sodium and lithium salts were most effective. His lithium solutions were very weak (0.3 per



cent., 0.4 per cent. and 0.5 per cent.), only about one-half the strength required to affect the *Fundulus* egg when applied in fresh water, and about one-third or one-fourth the concentration necessary to give a response when used in sea water. Frog's eggs under the influence of LiCl showed a retardation in the segmentation of the white area, in rare cases this area failing entirely to divide. Thus they became marked into upper and lower hemispheres and the cells at the sides of the floor of the segmentation cavity seemed to push in tending to fill this cavity. At this period Gurwitsch claimed that his embryos exhibited a radial symmetry but Morgan contradicts this and holds that they are really bilateral although the bilaterality may be less evident than that shown in the normal egg. Gurwitsch also finds that the downgrowth of the substance constituting the upper hemisphere is lacking, as indicated above, and this is without doubt a marked characteristic of the action of LiCl on the fish's egg. This also gives in the two cases an abnormally high position to the developing embryo and may, along with the failure of the blastopore to close, cause the malformed caudal areas of many individuals. Abnormalities in the position of the blastopore and brain, particularly of the former, were found to be the most decided effects of the LiCl solutions.

Madame Rondeau-Luzeau conducted an extensive series of experiments to test more especially the action of several chlorids on development. She found that a double action exists, namely, a physical action due to "hypertonicity" of the solutions and a chemical action depending upon the kind of salt employed. Only in the case of LiCl did the chemical action predominate when weak solutions were tested. In my work the fact that solutions in both fresh and sea water were used seems to preclude, in this case, all chances for the physical or hypertonic effect and leaves the chemical effect alone responsible for the abnormal development observed. Except in the case of LiCl Mm. Rondeau-Luzeau found that death was much oftener due to the osmotic pressure of the solutions than to chemical poisoning. The teratological action of the chlorids appeared to be due to their chemical action in the case of eggs treated after fertilization. The effects of the solutions

were also found to be more marked at some stages than at others, particularly so at the time of closure of the blastopore and of the formation of the medullary folds. LiCl was found to exert a more powerful chemical action than any other salt tried. The minimal amount of this salt that will effect the development of the frog's egg may be represented by an osmotic pressure of 169 cm. (0.3 to 0.4 per cent.); while for KCl, it is 405 cm. (1.2 per cent.); for NaCl, 459 cm. (1.9 per cent.); for MgCl<sub>2</sub>, 485 cm. (1.3 per cent.); and for CaCl<sub>2</sub>, 484 cm. (1.5 per cent.).

Morgan, in '02 and '03, subjected frog's eggs at various stages in their development to 0.4 per cent., 0.5 per cent., 0.6 per cent., 0.7 per cent. and 0.8 per cent. solutions of LiCl. Most of these solutions would be too weak, even though prepared in fresh water, to affect *Fundulus* eggs. Morgan found that in the frog's egg LiCl did produce a specific effect which he attributed to the chemical action of the lithium ion. Delayed development was noted as being a most obvious condition and this is equally true of the fish's egg. Eggs in two-cell and four-cell stages were more affected than those in later segmentation, and those beginning to gastrulate were least affected. Stronger solutions were necessary to give an equal effect when applied in later stages. Similar facts are also indicated in my notes above. But it was found that the *Fundulus* eggs placed in the solutions during cleavage and at the beginning of gastrulation up to the sixteenth or seventeenth hours were equally affected without regard to the stages at which they were subjected to the action of the salt. Another very noticeable effect of this salt was to prevent the downgrowth of protoplasm from the upper hemisphere of the frog's egg, as Gurwitsch also found. This makes gastrulation difficult, so that embryos in the stronger solutions do not pass beyond this stage. The absence of the downgrowth of the protoplasm in the fish's egg has been emphasized above and it will be remembered that in the stronger solutions the blastoderm folded into a ball upon the upper pole and finally pinched itself away from the yolk and died. One type of the frog embryo showed a complete inversion of the layers, the black area folding down into the yolk cells. The complete accomplishment of such a feat by the fish's egg could hardly be imagined,

but it seems possible that it may attempt a similar process, when it is recalled to what extent the periblast area may be forced down into the yolk when the segmentation cavity becomes unusually enlarged as it sometimes does. In the frog the black area becomes more or less spherical and sinks into or is engulfed by the yolk-cells of the white area. The frog eggs often form a sharp line between the black and white hemispheres, usually above the equator. This line, Morgan states, corresponds to the inturned edge of a circular blastopore whose position at or above the equator of the egg is explained by the lack of downward movement of the material of the upper hemisphere. The segmentation cavity of the upper cells was obliterated. This is a very different state of affairs from that seen in the fish where the segmentation cavity becomes so enormously exaggerated. Many short and folded embryos were found in later stages, some also showed cauda bifida; short embryos and forked tails are also common among the late effects of LiCl on the fish embryo.

After considering these investigations upon the effects of lithium one is strongly inclined to conclude that the salts of this metal do exert a specific influence on developing eggs. But I am not now in the position to state that these effects on the *Fundulus* eggs are really specific for lithium, though they have been so repeatedly and constantly obtained that one is led to believe that they are at least characteristic of lithium action on eggs of this form. Other chemical or physical stimuli might possibly produce similar abnormalities; this could be definitely stated only after the study has been continued with a large number of salts of various metals.

#### SUMMARY

1. Lithium chlorid delays development to a most obvious degree.
2. Eggs seem equally affected by the solution when placed in it at any time during cleavage stages; at other times the effects vary. They seem most sensitive about the period when the germ-ring circles the equator; though they are always affected to a greater or less degree; the rate of development is slower, the em-

bryo presents a pale appearance, since the blood lacks color and the pigment spots are fewer than normal.

3. After having remained for as long a time as six hours in LiCl solutions of sufficient strengths to cause abnormalities the eggs are incapable of complete recovery when placed in pure sea water during the remainder of their development.

4. In LiCl solutions the blastoderm is usually prevented from growing downward over the yolk, it therefore bulges up as a cap on the upper pole of the egg. This cap in the stronger solutions constricts its border, thus folding its periphery, and finally pinches itself away from the yolk and dies.

5. The segmentation cavity is enormously enlarged since the central periblast pushes down unusually far into the yolk mass while the blastoderm bulges up giving the cavity a more arched roof.

6. In many eggs the blastoderm never completely encloses the yolk; thus the blastopore remains open and short, peculiarly formed, and often cauda bifida embryos result.

7. In the late embryos the heart beats slowly, the eyes often fail to develop, the blood is colorless and, therefore, appears to lack hemoglobin. These characters taken with the inability to recover from the lithium effect, seems to prove without doubt that such an effect is due to chemical, and not to physical, causes. The fact that similar abnormalities are induced by LiCl solutions prepared with sea water and fresh water, therefore, giving both hypertonic and hypotonic solutions show further its chemical rather than its physical, action.

#### APPENDIX

##### *Notes on the Development of Fundulus heteroclitus in Fresh Water*

At Cold Spring Harbor, Long Island, in the summer of 1904, I collected material for comparing the development of the eggs of *Fundulus heteroclitus* in fresh water with their development in sea water, which is their normal medium. I wished also to ascertain whether or not those embryos hatched in fresh water would

show a higher degree of adaptability for living in this medium than the normally hatched fish. The fresh water at this place, as indicated by later experiments, evidently contained some substance that affected the embryos very strangely, as almost without exception the late embryo assumed a peculiarly twisted position upon the yolk the tail bending up in a circular fashion and striking the body about half way from the head. Almost all of these eggs died before hatching and those embryos that lived to hatch were unable to straighten their bodies and died within a few hours. The control embryos began hatching three and one-half days before these fresh water ones.

During the past summer at Woods Hole this experiment was repeated with the extra precaution of running a distilled water control. Here the results were entirely different in regard to the form of the embryo. Those fish that hatched in the fresh and distilled water exhibited perfectly normal shapes having also occupied the usual position on the yolk. But again the fresh water embryos were late in hatching, being in the earliest case two days behind the control and some were five days late in coming out.

The fresh and distilled water embryos gave an interesting mortality record for different periods of their development. During the first ten to twelve days these eggs were about as hardy as those in the sea water, though from this time until hatching began they died at a rate of over 5 per cent. per day. In one case where there were two hundred and twenty-five eggs when hatching began, thirty-nine, or  $17\frac{2}{3}$  per cent. died that night and only six individuals were hatched. During the next twenty-four hours forty-three, or  $23\frac{1}{3}$  per cent. died, leaving only one hundred and forty-three still alive. The following day, or three days after hatching had begun, only eighteen were alive,  $87\frac{1}{3}$  per cent. having died that night, five dying after hatching. None lived in fresh water longer than ten hours after hatching out. From this it is seen that the fish become peculiarly sensitive to the unusual medium shortly before the hatching time and only a few survive to break through the egg membrane.

The fish that did hatch in the fresh water were certainly no better fitted to live in this medium than those hatched in sea water.

When embryos that had hatched in fresh water were transferred directly to either one-half sea and one-half fresh water, or to pure sea water, they began at once to show quicker movements, and always lived normally in these media.

Thus it is seen that although the eggs of *Fundulus heteroclitus* will develop in an apparently normal fashion in fresh water, so far as form is concerned, they are slower in hatching. The eggs die in large numbers during the hatching period, and those that do hatch are unable to survive unless transferred to sea water. At this period of late development they probably die from the same cause that kills the mature fish if they are put into fresh water.

Zoölogical Laboratory, Columbia University,  
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