

# THE EFFECTS OF LOW TEMPERATURE UPON THE DEVELOPMENT OF FUNDULUS

## A CONTRIBUTION TO THE THEORY OF TERATOGENY

WM. E. KELLICOTT

*From The Marine Biological Laboratory, Woods Hole, and the Biological Laboratory, Goucher College*

There have recently appeared several embryological contributions of importance, based upon the development of the familiar minnow, *Fundulus heteroclitus*. Stockard ('09, '10, '13) and more recently Werber ('15, a) have described a large number of abnormal and monstrous embryos resulting from chemical treatment of the fertilized eggs and have made several suggestions regarding the *modus operandi* of the treatments used and the causes of monstrous development. And in the works of Stockard ('15) and Reagan ('15) advantage is taken of the possibility of causing, also by chemical treatment, certain embryonic abnormalities, in attacking a group of mooted questions in normal embryogeny and histogenesis.

During the summer of 1915, at The Marine Biological Laboratory, I was able to carry out a rather extended series of experiments on the production of monstrosities in *Fundulus* by a method which involved no direct chemical stimulation and which thus permits me to test certain previously suggested causes of abnormal development in the species studied. The method is so simple and so markedly effective that it seems desirable to make a brief report upon it and to point out its bearings upon the general theories of teratogeny, although at this time I shall not attempt to give an extended analysis either of the precise results obtained or of the exact mechanism of the disturbance of normal development.

In a word the method consists simply in subjecting the fertilized ova or young embryos to the temperatures of the ordinary household refrigerator, namely, 8–13°C. for a few hours or days.

In connection with the work on Teleosts in the Embryology Course at The Marine Biological Laboratory, we have for several seasons made a practice of placing the eggs, at various stages of development, in the refrigerator in order to slow their development and thus secure certain stages at hours convenient for study. It was noticed that a slight reduction in temperature merely slowed development without leading to any serious disturbance in morphogenesis, while a few hours at a temperature below 12–14°C. were followed, upon returning the eggs to the laboratory, by a considerable mortality and by very frequent abnormality (the eggs of *Tautogolabrus* (syn. *Ctenolabrus*), not *Fundulus*, were then used). In fact abnormalities in nuclear and cytoplasmic arrangements were to be noted while the eggs were still at the lower temperature. It was not until last summer, however, that the more precise examination of the results of this treatment was undertaken. While these experiments were in progress Loeb ('15) reported certain observations on the effects of much lower temperatures upon the development of *Fundulus*. His results will be discussed and compared with my own later in this paper. My apparent neglect to test certain points of disagreement is due merely to the fact that his paper did not come to my attention until some time after the spawning season of *Fundulus* was entirely past, which made such an attempt impossible.

The eggs were inseminated in the usual manner, i.e., dry, with the addition of testis teased in only a few drops of seawater. They were placed in finger bowls with a half-inch or so of water; the bowls were covered to prevent evaporation, and placed on the shelves of the refrigerator. The range of temperatures used was secured merely by using higher or lower shelves, in compartments alongside or below the ice compartment. The water was renewed daily, or in some instances every other day, the fresh water being at the refrigerator temperature. That covering the bowls with glass plates did not

materially affect the oxygenation of the eggs is sufficiently indicated by the normal development of similarly handled controls in the room temperature. The possible effect of darkness in the refrigerator was controlled in only one instance, when no effects were observed.

If the temperatures used be not too low (11–13°C.) the only marked immediate effect seems to be the retardation of normal development, which is resumed at its usual rate when the ova are returned to the ordinary temperatures of the laboratory (20–24°C.). It is a simple matter thus to prolong the age of the early blastula from the normal average of about five hours, to more than two weeks, that is some sixty-five times, and probably it might be prolonged much more. Such blastulas, replaced in the room, may develop and hatch quite normally, or they may form embryos showing marked abnormalities, indicating that underlying their normal appearance earlier there was an actual disturbance of some kind. One can not always say by superficially inspecting the entire blastula, or later stage, whether it is completely normal or not.

Subjection to a lower temperature (8–10°C.) for a few hours or days, is usually followed either by death or by abnormal development, normal development after such treatment being found in only a small percentage of the total treated. It should be noted, however, as a point of some importance, that in every experiment where development continued at all, after removal from the refrigerator, at least a small percentage of the embryos developed normally.

Considerable variation was observed among different lots of eggs, some showing few abnormalities after a treatment that resulted in very few normally hatching larvæ from other lots of eggs. It should also be noted that in one, and in only one, control, did the total proportion of abnormal embryos equal the minimum observed after subjection to a low temperature for more than five hours; and in no other case did the abnormalities in the control even approach the minimum in treated lots.

Eggs placed in the refrigerator within two to five minutes after insemination usually proceed to form a germ-disc which differs

from the normal in being smaller, that is in containing less protoplasm, and in being markedly more convex. This increase in convexity may simply indicate a decrease in surface tension directly due to the lower temperature. If the temperature be not lower than about 12°C. cleavage continues slowly and with little or no apparent abnormality, save that the blastomeres, like the germ-discs, are smaller and unusually convex, and therefore more than normally separated from one another. Stages of eight to sixteen cells may be found twenty-four to thirty-six hours after fertilization.

At a temperature of about 8–10°C. the effects are quite varied. In the first place, in a considerable and widely variable proportion of the eggs no true germ-disc is formed, cleavage rarely occurs, and after a time the protoplasmic parts become wholly vacuolated, giving no sign of living processes, either during continued treatment to cold or after their removal to a higher temperature. Other eggs, however, are not killed by this treatment; a few may form small germ-discs and cleave regularly once or twice during twenty-seven to thirty hours. Or cleavage may become very irregular, blastomeres later showing wide differences in size and extensive divergences from the typical arrangement. Many instances were noted where the germ-disc was very imperfectly formed, the thin protoplasmic cap remaining spread over one-fourth to one-third of the egg, with occasional mounds, cell-like though without cell-walls, scattered irregularly over it. In still other instances there were no true cellular structures whatever present, even after several days, and aggregations of nuclear substance might be seen scattered promiscuously through a cytoplasmic mass of irregular form. Several eggs were noted in which the protoplasm had collected in several distinct and widely separated regions. After several days all these eggs showed at least two kinds of material derived from the cytoplasmic part of the egg; one was dark and granular, quite opaque but still apparently active, the other clear and vacuolated and apparently no longer living.

It should be noted that throughout this paper the appearances described are those given by microscopic examination of total,

unfixed eggs. The results of the detailed study of sections of such eggs, which will perhaps enable me to determine more precisely the effects of the cold, will be reported separately at a later time.

Upon removal to room temperature, after having been some hours or days in the condition just described, many eggs failed to develop, and after some hours longer died without undergoing any apparent structural changes. On the other hand, most of those eggs in which there were present considerable masses of the darker granular material showed some processes of development, exhibiting before their death every degree and form of abnormality, from irregular protoplasmic masses, suggesting cellular structures, to hatched larvæ, well-formed though usually abnormal in some respect. In a very few instances (three were noted) normal larvæ resulted from the development of these dark, irregular, non-cellular masses.

When the eggs were allowed to develop normally for a few minutes (fifteen to thirty) before refrigeration, the general results did not seem to be markedly different, although there are some indications that fewer embryos developed normally.

Two lots of eggs were allowed to develop normally for twenty-two to twenty-three hours after insemination before they were placed in the refrigerator at about 11°C. At this age and at room temperature, the germ-ring is formed and just commencing its extension around the yolk. From a fourth to a third of these were dead after twelve hours in the refrigerator. Most of the remainder, after eleven days in the cold were alive, and upon transference to the room developed, some into normally hatching larvæ, others into larvæ with various defects and abnormalities, some very pronounced. Upon removal after twenty-one days many died and nearly all of the survivors developed very abnormally; but two hatched normally after ten to eleven days in the laboratory, i.e., thirty-two to thirty-three days after fertilization.

One lot of eggs was allowed to develop for forty-three hours before refrigeration at about 10°C. At this age the germ-ring is just closing, the embryo is well established and the optic vesicles

in some instances just forming, in others clearly formed. After eight and one-fourth days at 8–10°C. these were removed to the room; nearly all were alive but development had continued very slowly so that none showed beating hearts (normally the heart begins to pulsate about twenty-four hours after the establishment of the optic vesicles and the closure of the germ-ring). Some were already abnormal at the time of their removal, many others became abnormal as development in the room continued, but about two-thirds hatched normally after eight to eleven days longer.

It is interesting to compare certain of these observations with those of Loeb ('15) who found that treatment with a temperature somewhat lower than any I used, namely 7°C., was followed by no abnormalities in development. At that temperature Loeb found that "the newly fertilized eggs can live for weeks . . . without being injured" and that they "developed very slowly but no abnormal embryos were observed, although some of the eggs were kept at a temperature of 7°C. for four weeks" (p. 62). Loeb also used temperatures much lower than any with which I worked and found that even at 0–2°C., if the treatment were not prolonged more than four to seven and one-half hours, and if stages from the time of insemination to about four cells were used, only 20–30 per cent became abnormal upon transference to the room; longer treatment increased the mortality considerably, none surviving forty-eight hours at this temperature. If the eggs were first allowed to develop fifteen hours at a normal temperature (stage given as about one hundred and twenty-eight cells, although at the usual laboratory temperature of 22°C. this stage is reached about five hours after insemination) Loeb found that after two days at 0–2°C. normal development was still possible, although he does not state that abnormal development did not also occur in some individuals. But he found that if the embryo is once formed before treatment, it survives weeks of subjection to 0–2°C. "without any injury." "As soon as it is put back to room temperature it continues to develop" (p. 59).

I am entirely at a loss to interpret the difference between Loeb's observations and my own on this point. It is just possible, referring to the latter instances mentioned above, that at a temperature of only 0–2°C. the living processes of the embryo are so completely stopped that when development is resumed at a higher temperature no disturbing effects are to be seen; in other words not even abnormal development may proceed at so low a temperature. But this possibility seems largely negatived by the observation that extremely harmful results follow similar treatment of uncleaved or four cell stages. And no such interpretation seems possible for his results at 7°C., which should be more nearly comparable with my own at 8–10°C. I observed no instance of normal development occurring after eggs had been three weeks at 8–10°C., although several lots of eggs were tested and among the same lots normal development sometimes followed briefer treatment; and abnormalities were very common after treatment for only twenty-four hours, in some lots even for only five and one-half hours. Two lots of eggs, treated in just the same manner as the others, showed no abnormalities upon development after twelve hours at 8–10°C. It seems quite unlikely, however, that all of Loeb's material could have been so unusually resistant, in view of the rarity with which such lots came under my observation. I may add in passing that Werber ('15, b) notes that he too was able to secure abnormal embryos by treatment with temperatures much higher than those used by Loeb, but he gives no details either of his experiments or results.

It would not be profitable to attempt to describe in detail the abnormalities observed, nor even to enumerate all of them. In general it may be said that the embryos resulting from this treatment showed every degree of abnormality, from irregular masses of protoplasm, alive though exhibiting few of the characteristics of organisms, up to completely normal hatched larvæ. Among several hundred embryos examined there was found almost every conceivable kind of disturbance. Every characteristic that could be observed externally with the lower powers of the microscope showed some degree of abnormality in

some embryo. Certain organs, such as those of the circulatory system—the heart, vessels and blood-cells—and the eyes, were especially subject to abnormality, others such as the ears and covering ectoderm were rarely affected; yet *no structure was found which was not affected in some degree in some embryo.*

By way of general support for this statement I may mention just a few of the observed abnormalities, without mentioning the details of the treatment in individual instances.

In those cases where no definite embryo might be said to have formed, such conditions as the following were noted: formless, non-cellular but 'living' protoplasmic masses; protoplasmic masses with varying degrees of cellular structure; irregular protoplasmic masses (probably cellular) showing suggestions of organs, such as brain fragments, lenses, portions of optic cups, groups of somites, masses of erythrocytes, rhythmically contractile cells arranged either as flat sheets or as tubular 'hearts,' scattered pigment cells of the usual types, endothelial cells over the surface of the yolk, fragments of notochordal tissue.

Among those cases where a more or less complete embryo was formed (connected with the preceding condition by various gradations) a few of the abnormalities noted were the following: two separate and complete embryos on a single yolk; absence of head; absence of tail; large malformed head; short stumpy tail; sinuous body and tail; short deep body and tail; malformed pectoral fins; localized ectodermal proliferations; some regions of the brain not closed dorsally; absence or hypertrophy of various regions of the brain; various degrees of anterior approximation and fusion of the eyes; ventral fusion of the eyes; absence of one eye, the other remaining normal in size and position; eyes of unequal size; optic cups not closed; various degrees of albinism never quite complete; absence of certain types of pigment cells; atypical concentrations of pigment cells; greatly dilated pericardial cavity; heart abnormally placed, posterior or lateral to its normal location; two hearts dissimilar in size and form, asymmetrically located and with different rates of contraction; absence of heart; heart short and 'telescoped,' heart elongated and thread-like; heart long and dilated; heart flat and plate-



like; heart undifferentiated into chambers; only the sinus end of heart contractile; well-marked rhythms in rate of contraction of heart, sometimes occasionally stopping; no circulation, either on account of absence, or proximal or distal closure of the heart; few vessels over the yolk; yolk-vessels abnormally arranged; heart thread-like but with a good and complete circulation; very few erythrocytes but abundant plasma circulating freely; erythrocytes in masses on postero-dorsal surface of yolk; large masses of erythrocytes antero-ventral to heart or along the anterior margin of pericardial cavity (after hatching this latter mass was in a median ventral position); dense mass of erythrocytes collected in tail, *i.e.*, caudal aorta and vein anastomosing at base of tail.

Many more abnormalities might be mentioned and of course innumerable minor details of abnormal character might be cited from the material. What variety of conditions might be revealed by the thorough study of sections of these embryos can only be imagined.

One further point might be mentioned. Stockard observes ('15, p. 26) that embryos developing without a circulation are not able actually to hatch. As a rule I had a similar experience but one exception was so remarkable that it seems worth noting. After eleven days at 10–11°C., one lot of eggs which had developed in the laboratory for twenty-three hours before cooling, showed many normal embryos, for the most part at a stage when the optic vesicles were just forming. Others were grossly abnormal or entirely dead. Among those appearing normal upon removal from the cold, many abnormalities appeared later and one of these, which was found hatched twelve days after removal, or twenty-four after fertilization, had no circulation whatever, although the heart was present and pulsating weakly. This, however, was not its only defect. It was below average size, had but a single median anterior eye and correspondingly the nose and upper jaw region were, as usual in cases of cyclopia, narrow and elongated, the tail was short and ended in an undifferentiated mass, pigment cells were largely massed on the ventral surface of the yolk. It seemed unable to direct its swim-

ming movements (it was probably blind) and both in swimming and in resting it turned on either side instead of maintaining a normal position. This fish hatched sometime during the twenty-eight hours preceding the notice of its having hatched, and after living in this condition for thirty hours longer, its heart seemed somewhat more feeble, its fin motions became slower and it was killed.

That fish lacking so physiologically important a process as the circulation may develop to the point of hatching is alone a most remarkable fact. But that such an organism can actually hatch from its egg-membrane and, in spite of the other abnormalities noted, remain alive and active for between thirty and fifty-eight hours, can not fail to arouse many questions regarding the general problem of embryonic adaptations. If a fish can live for weeks, and then hatch and remain active for hours longer, entirely lacking a whole physiological system commonly regarded as so essential, one may certainly be permitted to doubt whether slight details in the arrangement of this or of other parts, may have the functional importance often assumed.

In connection with the observation of the irregular, only partially cellular protoplasmic masses mentioned above, it is interesting to notice that we have here an illustration of the fact that the protoplasmic substance of a highly specialized form may still be capable of existence as protoplasm, though not showing any of the normal morphogenetic processes characteristic of the living substance at that age; and may remain able for days and weeks to carry on some of its vital processes. Yet in spite of the fact that these protoplasmic masses were so widely aberrant in form and appearance, exhibiting none of the usual morphological characteristics of the protoplasmic portions of fish-eggs, nor of any other kind of vertebrate egg, save the primary differentiation into nuclear and cytoplasmic materials, they were still undoubtedly living. They remained free from bacterial infection, form and appearance slowly changed, coagulation did not occur, in many instances for weeks. Moreover it is not true that in all such cases death was the inevitable result, for when this condition was not too long maintained, such irregular,

largely though not wholly non-cellular masses occasionally gave rise to true embryos exhibiting the usual developmental processes. Such embryos usually showed various degrees and kinds of abnormality, but some few ultimately produced normally hatching larvæ. These masses were therefore more than merely living; their later history shows that even in that state there must have remained present, in many instances, some kind or remnant of an underlying construction or organization that determined either directly or through some regulatory process, development of parts, at least, of Teleost embryos, and in a few instances of essentially normal *Fundulus* embryos.

My chief object in describing, at this time, the effects of low temperature upon the development of *Fundulus* is not to give a morphological or histological description of the malformations and abnormalities produced, but to suggest the bearings of these results upon certain current hypotheses as to the real causes of such defects and the way in which the unusual physical conditions may have affected the morphogenetic properties of the ovum. I shall not attempt to review extensively the various suggestions as to the causes of monstrous development, but some of the more recent hypotheses only may be examined from the point of view of the results described here. For this purpose I shall refer chiefly to the suggestions made by three recent workers in this field.

As a preliminary word I should note that such observations as these show that "the idea that the low temperature only retards the chemical reactions underlying development" (Loeb '15, p. 59) is, for the *Fundulus* egg, true only to a certain point. When temperatures are lowered below say 12–14° C. the orderly developmental processes are not only slowed but may be actually modified so that some of the consequent processes are rendered abnormal. This may, of course, be due primarily to the fact that certain processes are slowed more than others, but the result is a disturbance of normal development which is different from a mere slowing down of the entire mechanism, the component processes remaining normally related to one another. Apparently the precise temperature when this effect is produced

varies with same internal or fluctuating condition of the egg and also with its stage of development.

We should also point out here that Lillie and Knowlton ('97) found that eggs of the frog (*Rana virescens*) at 2-3° C. always developed abnormally, if at all, the abnormalities usually appearing in the region of the blastopore.

In his classic "Study of the Causes Underlying the Origin of Human Monsters," Mall ('08) included an extensive survey of the more important results in the whole field of experimental teratology, particularly among the vertebrates. He concludes that even in such dissimilar instances of teratogeny as those in the toad resulting from fertilization with X-rayed spermatozoa (Bardeen, '07), those in the Teleost following chemical treatment during cleavage (Loeb, '93, Stockard, '06, '07) and the human instances described by himself, "although the methods employed are very different, the principle involved and the results obtained are much the same" (p. 24). And, "In general the methods employed by experimental teratologists is to subject the eggs to various insults which affect the nutrition and impair the growth of the embryo" (p. 52). "A monster is due to the influence of external substances which retard the growth of the embryo, usually one portion more than the other" (p. 36). In brief Mall's conclusion regarding the causes of human monsters is that "faulty implantation of the ovum, which naturally affects the growth of the embryo" (p. 25), by interfering with the normal nutritional relations leads to monstrous development; "that certain parts of the embryo are more susceptible to insults than others" (p. 32), and consequently it is these that are affected first (p. 16), though subsequently the faulty implantation must be remedied so that the embryo may continue to grow (p. 25). "But in order to produce a finished monster the nutrition must not be impaired too much" (p. 31). Many other quotations from the same work might be added to show that the words nutrition and growth are used with their customary significance, implying only the supply of materials and energy necessary for the usual processes of extension of at least partially differentiated structures or anlagen. Disturbances of

the general chemical reactions underlying development but not directly related to the income of the embryo, such as nuclear differentiation, mitosis, distribution of nuclear and cytoplasmic substances, specification of protoplasmic areas, et cetera, are evidently not included under the terms nutrition and growth as used here.

Essentially the same idea is contained in Stockard's hypothesis of the causes of the defects observed in *Fundulus* after chemical treatment, namely, that such treatment tends to lower the developmental vigor of the embryo, this lack of vigor being subsequently shown by the failure of certain structures to develop normally. This hypothesis is formulated chiefly in connection with the explanation of optic defects, particularly cyclopia. "A certain amount of energy is necessary for differentiation of the eye to take place . . . but when the required energy for any reason is not available the eyes are incapable of any differentiation" ('13 b, p. 271). In the absence of any other evidence for such a deficiency in available energy on the part of the embryo, such an explanation comes to little more than a statement that the eyes do not develop. I can not be certain whether or not it is intended that this explanation should be applied to the other abnormalities observed by him, regarding which, however, no other suggestion is made. But as I shall show there are some difficulties in making a general application of this idea in explaining all of the defects noted in his experiments with various chemical substances.

Using a butyric acid or acetone treatment, Werber ('15 a, '16) secured not only abnormalities similar to those recorded by Stockard, but also a wide range of the same general types, and in many instances the same specific types, as those which I have found to follow the action of a low temperature. Werber believes that there are some factors common to the morphogenesis of all the diverse abnormalities and monstrosities observed in a given experiment. He applies the term 'blastolysis' collectively to the factors involved and believes that there occurs, as a consequence, a destruction or dispersal of parts or all of the germ's substance.

My own findings reported here certainly tend strongly to confirm the idea that the causes underlying abnormal development are to be sought in some derangement of the fundamental developmental mechanism or relations of the diverse components of the very early stages of the organism. In the first place it should be noted that by using a low temperature as the abnormal stimulus I have eliminated the necessity of supposing that there are any specific chemical alterations, such as precipitating or solvent effects, due to the use of chemical stimuli, which is so important an element of Werber's hypothesis. There may be some osmotic changes in the cooled eggs and there certainly is an increase in oxygen tension, but McClendon ('12) was unable to find that such conditions affected the frequency of cyclopia in *Fundulus*. On the other hand some of the effects of cold may be directly observed in the eggs during treatment, so that the character of the disturbance is not wholly left to be inferred from the study of later development.

As mentioned above, eggs which were placed in the refrigerator within a few minutes after insemination were found, some days or weeks later, whether a few cleavages had appeared meanwhile or not, to contain at least three classes of materials. 1) Not only nuclei of the usual appearance but also irregular, large and small masses and fragments of nuclear material scattered indiscriminately through the cytoplasmic parts of the egg. There were ordinarily no cellular outlines corresponding with these masses of nuclear substance. 2) Masses of granular cytoplasmic substance somewhat resembling the material of the greater part of the normal uncleaved germ-disc. 3) Masses of clearer, possibly protoplasmic substance usually in the form of vacuoles, slightly resembling in physical appearance that mass of clear cytoplasm that in normal development forms, for a brief period preceding cleavage, a lens-shaped disc on the lower side of the central part of the germ-disc. I am not yet in position to identify these forms of cytoplasmic substance with the two chief forms observable in normal development; but the similarity is suggestive. Eggs in this state are still capable of some sort of developmental process when returned to ordinary temperatures,

but abnormalities are very frequent and show the remarkable range of characters described above.

It seems, then, that the causes of the observed abnormalities in development may be referred to these conditions: (a) abnormalities in nuclear composition and in the distribution of differentiated nuclear substances; (b) abnormal distributions and associations of two more evidently differentiated cytoplasmic substances; (c) abnormal associations between nuclear and cytoplasmic substances, either or both of which may be abnormal in its own composition as compared with the corresponding materials in the regularly developing egg.

In a word this means that the organization of any part of the early organism may be disturbed, and that such disturbances are the causes of abnormal and monstrous development. That development can occur at all under such circumstances indicates remarkable regulatory properties of the egg substance. The complete lack of specificity in the effects of the cold indicates that the disturbance is profound and that it affects the fundamental organization of the ovum rather than any especially differentiated representative substances or anlagen, which, moreover, have been shown by other evidence not to exist in the Teleost ovum (Morgan, '93, Sumner, '04).

Going a step back of these observations to the query as to just how the low temperature can produce such disturbances, we can at this time make suggestions only in very general terms. It is evident that not all of the processes of development or parts of the developmental mechanism are affected similarly, for that would lead to development normal in all save its rate. Some physical or chemical processes or structures must be more extensively interrupted or altered than others, as a result of the lowered temperature. They are thrown out of their normal relations to the other processes or parts of the mechanism and sooner or later a whole train of consequences may become evident. It is very important to note, however, that the substances or processes thus affected are not to be thought of as specific tissue—or 'organ-forming substances' nor as differentiated, cellular or formed rudiments or anlagen, but as elements or factors

in that whole complex mechanism which as an entire system epigenetically gives rise to such substances or rudiments. It is quite likely that, in part at least, these altered reactions are such as may lead to disturbances of the mitotic processes of maturation and fertilization, such as were described in such great variety in *Ascaris* by Sala ('95) or of nuclear division during cleavage, as described by Conklin ('12) in *Crepidula*, whereby abnormal nuclear structures may be formed. It is also not unlikely that the observed failure to form cell-walls in many parts of the cytoplasm, or the abnormal location of cell-walls when formed, may open the way to the possibility of abnormal nuclear and cytoplasmic associations. Another possibility lies in the physical slowing of the translocatory movements of the gradually differentiating cytoplasmic materials. In eggs subjected to cold immediately after fertilization the normal flowing together of the cytoplasm to form the germ-disc and the subsequent rearrangement and redistribution of different cytoplasmic substances, often appear superficially to be seriously interrupted. But all such conditions are themselves to be regarded as consequences of antecedent modifications of some more elementary chemical or physical organizational processes as yet beyond analysis and description.

The great variety in the results following treatment is just what would be expected on the basis of such a disorganizational effect. Both nuclear and cytoplasmic materials are themselves so complex, and the complete system of relations, both material and energetic, between them and among the various component parts of each, which as a whole we term the 'organization' of the ovum, is so extremely complicated, that it affords almost unlimited possibilities for modification and disturbance. Without knowing very much more than we do about the physics and chemistry of this organization and about its regulatory capacity, it would seem largely or wholly a matter of chance, what would be the precise later results of any single modification or simple group of disturbances in this system. The results of such disturbance are entirely unpredictable in individual instances at present.



We should recognize, as preliminary to much of the discussion that follows, that either the morphological or the chemical extent of the initial disturbances may not be the only condition correlated with the extent of the later derangements. A comparatively slight modification in an essential or highly important organizational factor would have a more marked result in later development, than would a more extensive alteration in factors of lesser importance. The quality of the disturbance rather than its extent is primarily involved. It is further likely that no relation could be determined between the extent of the initial disturbance and the final effect on account of the possibility of regulatory action<sup>1</sup> and because comparatively slight initial alterations might give a wholly abnormal trend to a long series of consequent processes finally resulting in very pronounced abnormalities.

With this general statement of the essential nature of the disorganization hypothesis we may turn to an examination of the widely current nutrition hypothesis, based largely upon the results following chemical treatment, in order to draw attention to certain difficulties in its application to some of the observed facts and to inquire whether the suggestions made here avoid any of these difficulties without creating others.

If any justification seems necessary for the attempt to criticize, from the viewpoint of the results of the action of low temperatures, the hypothesis based upon chemically produced effects, it is to be found in the essential identity of the consequences of these different modes of treatment. Indeed I should go further and bring under this same point of view the abnormal types of development following certain other experimental conditions, such as heterogeneous hybridization, and the subsection of gametes or zygote to radium radiations, which will be discussed later.

The nutrition hypothesis as stated by Mall, and to some extent adopted by Stockard, has for some time now largely been held to account for such illustrations of teratogenesis as those described here, but it seems open to certain serious objections. In the first place it might be pointed out that the work of Pack-

ard ('14) on the effects of radium radiation upon the eggs and sperm of *Nereis*, has largely rendered untenable the suggestion of Mall that the effects of such treatment are nutritional in character. Packard concludes upon very clear evidence that there are strong reasons for believing that the radium radiations act indirectly upon both the chromatin and cytoplasm of either or both germ cells or the zygote, by bringing about in these, destructive chemical processes. Development is thus rendered abnormal both by the destruction of the normal chemical and physical mechanism of early development, and also possibly by the toxic presence of the abnormal substances thus formed and present in the cleaving egg. That is, the abnormalities found in later development may be referred to abnormal nuclear and cytoplasmic behavior during the cleavage processes, before the specific germ-layer or tissue differentiations are inaugurated. This clearly removes the results from the category of nutritional effects and affords an explanation of the same general character as that which I have suggested above, with this difference, however, that I am not inclined to stress the possibility that the effects are due to the presence or absence of specific chemical substances, but rather hold them to be due to unusual combinations of differentiated materials both nuclear and cytoplasmic, in a word to a disturbance of the 'organization' of the ovum or of certain parts of it; and this difference seems to me of quite an essential character.

How such results as those of Lewis ('09) and Spemann ('03, '04) and some of Stockard's ('13 b) who caused cyclopia in *Fundulus* and *Amblystoma* by the actual physical destruction or even removal of the differentiated anterior end of the central nervous system, can be interpreted as due to a nutritional effect is even less clear than the possibility of such an interpretation of the effects of radium upon the spermatozoa previous to fertilization. To compare and identify the cyclopia in *Fundulus* resulting from such removals of already differentiating structures, with the cyclopia resulting from chemical treatment of four to eight cell stages, involves the assumption that during early cleavage in *Fundulus* there is already present some repre-

sentative, definitive, organized rudiment of the anterior tip of the central nervous system, and further that it possesses a sensitivity to general nutritional disturbances which is also specific, *i.e.*, not shared equally by other rudiments which must also be assumed to be present at this time. But it is well known that the Teleost ovum is of the indeterminate type, and injury or removal of whole blastomeres of the normal cleavage group causes no later defects or abnormalities in the developing embryo. The assumption of specificity in the action of nutritional effects in connection with cyclopia, is further negated by the great variety of the results following chemical treatment. I have already pointed out the great variety of abnormalities and monstrosities observed by Stockard, Werber, and others, appearing under the same experimental conditions. The only suggestion of actual specificity is that of Stockard ('10, p. 369) that treatment with certain percentages of alcohol gives, among the surviving embryos 90–98 per cent with abnormal eyes, generally cyclopean. But he does not say that other defects may not also be found in these or other embryos similarly treated, indeed on the contrary he mentions a great variety of ear, brain and other defects which may either accompany eye-defects or appear independently of them, under similar treatment. Moreover, the expression 'eye defects' covers a number of different conditions, not all of which can be referred to the cause which he assumes for cyclopia, namely, the inhibition of the development of the median anterior tip of the central nervous system. To find a great variety of abnormalities among embryos subjected to the same treatment leads definitely to giving up the idea of specific reactions of the rudiments of such structures to the unusual conditions. To relate the appearance of such a variety of abnormalities during later development of the embryo, to the effects of the treatment upon some specific parts of the materials of the cleavage group is, therefore, to assume the existence of differentiations during cleavage which have been shown not to exist, and then to require further that each or any or all of these may be specifically affected, which is to say that there is no specificity at all in the action or in the reaction.

Stockard ('13 b) explains "the fact that a number of eggs when subjected to the same solution do not all respond in a like manner" merely by regarding this as "a typical case of differences in individual resistance and vigor which is observed among any one hundred individuals of any living species" (p. 282). But the kinds of differences found here are not at all such that they may be regarded as illustrations of that normal fluctuation in all characteristics which represents the reactions of organisms to the incidence of environing conditions. It is probably true that eggs and embryos do differ in those complex conditions which we summarize in the words 'resistance and vigor.' But these qualities do not determine whether an embryo shall or shall not have eyes, hearts, pigment cells, and so forth. We are dealing with a wholly and fundamentally different phenomenon.

An important objection to this nutrition hypothesis, it seems to me, is that there is little or no actual evidence given that the nutritional conditions of the egg or embryo are directly affected. The evidence offered is that defective and monstrous embryos result from various methods of chemical treatment; but it is these defective and monstrous embryos that are to be explained. In the few instances where there is evidence of abnormal relations between embryonic and vitelline portions of the egg, it is more reasonable, in view of the great variety of other conditions found, to regard this too as a result of a primary disturbance, rather than as the cause of a variety of conditions which may also occur in its absence.

The suggestion as to the responsibility of abnormal nutritional relations as the cause of abnormal and monstrous development was made primarily in connection with the human embryo and then extended to other forms. It is quite possible that in the human and other placental mammalian embryos such effects may in some cases be found to be specific and to be related to nutritional abnormalities due to disturbances in the parental organism. However, the mammalian embryo develops for some hours or days before implantation occurs and becomes effective as a nutritional factor so that it is only subsequently to that time that the effects of faulty nutrition might be exercised.

Embryonic differentiations having been begun before that time, the way might be opened to a specificity of action upon some part requiring a large supply of energy and material. The later the abnormal conditions act, the more likely are they to be specific in their action. But in the absence of definite proof of such a cause, it is on the whole much easier to interpret monstrosities, even among the Mammals, as resulting from organizational disturbances produced by the presence or absence of some definite chemical environment, whether that be nutritional or not. And such a chemical stimulus might be operative not only after, but also before implantation, when the extent of the resulting abnormality might be very great, so great as to result in the formation of a 'complete monster.' I should not, however, oppose the idea that mammalian monsters may be due, in some cases to nutritional defects: in this connection I should merely suggest that the observed facts do not exclude the possibility of a general organizational disturbance as the cause of monstrous development in this group, and that even in the placental mammalian embryo, although with less likelihood than in oviparous forms, the nutritional disturbance, when it is known to exist, may itself be a result rather than a cause of the deranged organization.

But it seems, in the light of subsequent observations, that it was a mistake to extend this interpretation of the causes of abnormal development so generally to other classes of vertebrates and to say that such abnormalities result from conditions "which affect the nutrition and impair the growth of the embryo" (Mall '08, p. 52) or which "tend to lower the developmental vigor of the embryo" (Stockard, '13 a, p. 83), or that "a certain amount of energy is necessary for differentiation of the eye to take place . . . but when the required energy for any reason is not available the eyes are incapable of any differentiation" (Stockard, '13 b, p. 271). Such a statement seems to leave unexplained such cases as have been observed both by Werber and myself, where portions of eyes, fragments of optic cups, lenses, or even fairly complete eyes, may be found either without other true tissues or organs being differentiated, or with scattered parts of other organs and bits of tissue.

The difficulty of explaining such an abnormality as monophthalmia asymmetrica, or other asymmetrical abnormalities, on this nutritional basis is also apparent. Stockard recognizes this and merely suggests that "It might be that at some critical point in development one of the future eye centers is affected after the growth centers had begun to localize in more or less lateral positions" ('13 b, p. 278). Such asymmetrical abnormalities offer no special difficulties of interpretation on the disorganizational hypothesis.

Stockard ('13 b, p. 281) has also noted that the earlier the treatment is administered during the development of Amblystoma the more extensive are the resulting abnormalities: "the developmental period of administration is of as high importance in determining the result as is the nature of the stimulus used." This is readily understood upon the hypothesis that the unusual stimuli act by disturbing the normal organizational relations of nucleus and cytoplasm, since a simple or localized disturbance at an early stage would be followed by much more widespread effects than would an equal disturbance at a later stage, when many of the differentiations might be already determined and the effects consequently more localized. After the essential differentiations of the organism have been made, the effects of external stimuli would be likely to have relatively slight morphogenetic results. (Compare the morphogenetic results of stimulation in embryo and in adult organisms.)

Stockard believes that "all of the eye conditions [in *Fundulus*] may be interpreted as arising through developmental arrests ('13 a, p. 83); and throughout his papers the abnormalities observed are continually referred to as 'defects,' an interpretation that is cited in support of the nutrition hypothesis. While most of the abnormalities observed have the nature of defects, if by defect we mean only failure to differentiate, yet not all of the abnormalities noted in *Fundulus*, to mention but this case, are of this nature. In my own observations I might mention for example, the development of two complete and separate embryos on a single yolk; the development of two separate hearts, not paired but in different regions of the embryo; the

proliferation of ectoderm cells, the formation of large masses of erythrocytes, et cetera. Apparently the only way to interpret such conditions as due to some defect is to have recourse to the interpretative method employed by some geneticists (with the difference, however, that there it may be clearly justified) and say that when abnormal development occurs it is due to a defect in that which would have kept it normal. But that would merely be to say that abnormal development occurs. And it should be recalled just here that Loeb ('15) noticed that eyeless embryos developing from a cross between *Fundulus* and *Menidia* reached that condition after passing through earlier stages in which the eyes appeared to be normal. In such instances anophthalmia is certainly not due to any original destruction of 'ophthalmoblastic anlagen.'

Loeb occasionally inclines toward this nutrition hypothesis in explanation of some abnormalities and points out that, since in most of his observed instances of anophthalmia circulation also is lacking, "the inference is possible that the anomalous condition of the eye may be due to lack of circulation" ('15, p. 67). But the large number of instances in which the eyes may develop normally in the complete absence of circulation renders such an inference untenable.

A rather significant test of the nutrition hypothesis, or at least of certain phases of it, can be found in those cases where the embryo develops without a heart or without a circulation. Here is certainly a profound disturbance of the nutritive relations of the entire embryo. 'Whatever the primary cause of such a lack, the conditions should, on this hypothesis, be accompanied or followed by marked and varied abnormalities. If nutritional disturbances so easily affect development as the general hypothesis requires, such embryos should certainly produce 'complete monsters' capable of but a brief existence. It is often the case that embryos lacking these organs are also defective in various other ways, but it not infrequently happens that such embryos may develop with a high degree of normality in all other respects and when removed from the egg membranes may continue to live and react almost normally for some time.

The main objections to the nutrition hypothesis of the causes of embryonic abnormalities and monsters as it has been stated, are, then, the following: it does not afford an interpretation of the results, such as those of Bardeen and Packard, following treatment of the sperm with radium radiations; it does not afford any suggestions as to the nature of the underlying disturbances through which the abnormalities are produced by the unusual conditions used or assumed to be present; it does not explain why the action of the experimental conditions during cleavage should not produce visible results until much later; it does not explain why monsters of the same parentage are diverse (see Mall '08, p. 12); it does not explain why the effects of treatment are greater when applied during the earlier stages of development; it does not explain the production of abnormalities which are not defects or developmental arrests; it does not avoid the necessity of assuming a degree of differentiation during cleavage and a specificity of the action of the external conditions, both of which have been shown not to exist.

On the other hand, the hypothesis suggested earlier in this paper, that the causes of abnormal and monstrous development are to be found in the disturbance of the normal organization of the fertilized ovum or cleavage group, as evidenced by the abnormal characters and distribution of the nuclear and cytoplasmic substances, avoids these objections and affords an easy interpretation of the observations mentioned.

It seems to have been premature to have assumed (Mall '08) that all the classes of embryonic abnormalities and monstrosities described by Mall, Bardeen, Spemann, Lewis and Stockard, are really of the same essential nature and due to similar causes. I see no reason for not admitting that such embryos may result from different causes in different cases, whether they be (1) the abnormal characters of the gametes before fertilization, or disturbances of the early cleavage processes as these concern both nuclear and cytoplasmic constituents, (2) the mechanical removal of differentiated rudiments, or (3) the lack of energy and materials ordinarily supplied through nutritional pathways, including the pathways within and among the parts



of the egg and embryo themselves; and I believe that these are not merely different phases of the action of a single principle, certainly not all due to a lack of 'developmental vigor' although that is a pretty general phrase and one that might cover a multitude of varied conditions, individually unlike and due to varied primary causes.

Turning now to a much briefer consideration of Werber's hypothesis, as stated above, I shall refer only to his latest papers ('15, '16). While admitting that the nutrition hypothesis may account for the production of certain types of human monsters, Werber rejects its general validity in favor of the view that modifications in the physical or chemical environment, for example in the blood of the parent organism, affect directly either the germ cells before fertilization or the fertilized ovum or later developmental stages ('15 a, p. 530). Thus he agrees with Spemann in opposing the hypothesis of Stockard mentioned above, regarding the cause of cyclopia, and believes that the eye-defects, which form the main subject of disagreement here, are really due to morphological defects of some kind, and not to an inhibition resulting from lack of developmental vigor or energy. He goes farther than this, however, and suggests that the substances used in his experiments, namely, butyric acid and acetone, caused "an elimination of materials of the blastomeres or of the germ-disc and probably also of the yolk-sac." "Blastolysis either destroys part or all of the germ's substance, or it may split off and disperse parts of the latter" ('16). And further "This elimination of material may be due either to the precipitating or solvent effect respectively of the chemicals which were used" ('15 a, p. 559). From the examination of very extensive material he concludes that "either the blastomeres or the germ-disc had been blastolytically fragmented owing probably to both physical and chemical factors" (p. 559), and that whatever scattered parts survive this fragmenting and blastolytic process "may go on developing into a whole defective [*sic*] or a meroplastic, embryo, or even into an isolated organ" (p. 559). And in another place ('15 b) he adds that an increased imbibition of water following an increase in the per-

meability of the egg membrane is another factor in producing blastolysis. Thus through the operation of such factors together, some parts of the germinal substance may "be entirely destroyed owing to the increase in osmotic pressure, while the remainder may go on developing and eventually give rise to various monstrosities" (p. 240).

The first part of Werber's hypothesis, namely that the effects are due to a physical and chemical modification of the germ cells, cleavage group or germ-disc, is clearly in accordance with the more general disorganization hypothesis. But in the absence of direct evidence, which it would be extremely difficult to secure, of the destruction of certain specific materials and not of germinal substance in general, it seems more nearly in accord with the general conception of development to believe that what is effected is a disturbance or disarrangement of the constituents of nuclei or cytoplasm, or both, or of the normal relations between and among these materials. Otherwise, and in accordance with the latter part of Werber's hypothesis, it is necessary to assume in the germ cell or cleavage group, a whole series of unlike substances whose differentiations are already specific and definitely necessary, not as organizational factors, but as the rudiments or anlagen of the later differentiating tissues and organs of the embryo. It is here that I should take exception to the hypothesis, for there is as yet no direct evidence for the existence, in such early stages, of such differentiated rudiments. It seems much more likely that it is just the mechanism of differentiation that is disturbed by these abnormal environments, and not even the earliest formed results of the operation of such a mechanism, although it is quite possible that there may also be an added actual destruction of specific materials that as such are necessary to normal development. This difference between Werber's view and that stated here is not a minor one; it is the difference between the predeterminational and the epigenetic views of development.

The fact is an important one, that results exactly parallel to those of Werber (and Stockard) follow upon the mere lowering of temperature, a condition which eliminates the possibility of a

direct chemical action, such as precipitation or decomposition, and which can also produce disturbances in the arrangement of nuclear and cytoplasmic substances that are actually visible, but which may only be assumed to have destroyed any chemically and developmentally differentiated and specifically necessary materials. In this respect my experiments afford, I think, a valuable check on the results following chemical treatment and indicate the more general validity of the disorganization hypothesis.

And further, since I have observed several instances where eggs exhibiting rather extensive fragmentation and dispersal of protoplasmic parts, were still able to form normal embryos, I am led to believe that such conditions in themselves need not lead to abnormal development, but that some other condition must be primarily responsible. If certain essential organizational conditions may remain present or susceptible of restoration, development may proceed normally even though some considerable parts of the egg protoplasm may actually have been destroyed; a conclusion which is also indicated by the results of removal and pricking experiments on single blastomeres of the Teleost ovum (Morgan '93).

Most of Werber's discussion of the action of his mode of treatment centers about the actual mode of the production of eye-defects and of the respective merits of the 'fusion' and the 'inhibition' hypotheses. But such a discussion seems concerned rather with the after effects than with the real causes of the abnormality. That is, while it is very important to know the proximate causes of such a condition as cyclopia, neither of the suggested causes is really fundamental, and either fusion or inhibition may result from a primary organizational disturbance, in the same way that varieties of nutritional abnormality may also result from a similar underlying cause.

In one respect Werber seems to fall into the error made by Stockard in assuming that such conditions as cyclopia result from a specific effect upon a differentiated rudiment of the anterior end of the central nervous system, already differentiated in the early cleavage group. This leads him ('15, pp. 557-8) to

support Child's 'axial gradient' theory in explanation of the frequency with which there occur defects of the tissues and organs developing in the anterior end of the embryo. "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" (p. 557). "The size of the injured area at the anterior end is probably subject to considerable variation," and thus the effects may be limited to the future interocular area, or they may include parts, varying in extent, of the potential optic anlagen, or one optic anlage only, and so on. In addition to these assumptions, it is further necessary to assume (p. 558) not only the existence of differentiated ophthalmoblastic anlagen in the very early cleavage group, but definite and symmetrically placed double ophthalmoblastic anlagen with different degrees of susceptibility to the chemical substances in solution. By means of these and other assumptions (p. 558) Werber is able to reconcile the two hypotheses of the causes of cyclopia mentioned above, but in accomplishing this he runs contrary to the demonstrated lack of specification or determination in the cleavage group of the Teleost. Eggs were treated in the one- to sixteen-cell stages, and as Werber himself remarks (pp. 531-2) it is probable "that it is mainly the initial effect of the toxic solution on the ovum that causes it to develop in an atypical manner." There is no anterior end of an embryo represented by any differentiated material in the sixteen-cell stage of the Teleost, no ophthalmoblastic anlage; but there is an organization or developmental mechanism capable of producing these parts, much later, a mechanism that is interfered with and upset, and there is no specificity in the result of the derangement. This lack of specificity is directly opposed to the application of the axial gradient hypothesis, for as a matter of fact, any part, posterior as well as anterior, may become abnormal following this or other modes of treatment.

The explanations for the observation that all of the organs of the anterior end of the Teleost embryo—eyes, brain, heart, etc.,

are the more likely to exhibit abnormality seem much less rec-ondite. In the first place, from the mode of its formation by confluence (concrecence) the head end of the Teleost embryo is the first part to be formed and differentiated, and any disturbances of the normal processes of tissue and organ differentiation are much more likely to be exhibited first in the region earliest differentiating. And in the second place, the organs of the head region, especially the sense organs, brain and heart are in general more highly and therefore more extensively differentiated, their normal development involves the interaction of a larger number of factors, than in most other parts of the embryo where muscles and the connective tissues form the chief constituents. Hence a slight initial disturbance would produce a more frequent as well as more marked result in the anterior part of the embryo, where a more precise arrangement of the underlying conditions of differentiation is necessary, than in the more posterior parts where the tissues and organs are simpler and possess greater regulatory properties.

And it should not be forgotten that as a matter of fact every part of the embryo is subject to abnormality following treatment with chemicals or low temperature; no part has been found to be wholly free from abnormality in every case. This is an observation, by the way, which has a decided bearing upon the use of experimentally treated material as an aid to the solution of problems in the normal development of *Fundulus*. If any part, organ or tissue, whatever, may be affected abnormally by such treatment, an extreme degree of caution should be exercised in applying to the interpretation of the events of normal development, the evidence drawn from the histogenesis of embryos developing from treated eggs.

In conclusion I should like to refer briefly to the bearing of the hypothesis stated here, upon a group of observations of a wholly different kind. I refer to certain results of Teleost hybridization described by Moenkhaus ('10), Loeb ('12), and Newman ('14). Moenkhaus found that in such hybrids "development in its early stages proceeds normally, *i.e.*, when superficially viewed the deleterious effects of the two strange

sex products upon each other showing only at later cleavage and subsequently;" that gastrulation, *i.e.*, the formation of the germ layers—the most marked of the earlier differentiations of the embryo, was a period of high mortality; that numerous abnormalities appeared in the hybrid embryos surviving this period. He interpreted this to signify that the sex cells exercised a poisonous action upon each other, preventing normal development, and suggested as an analogy, merely, the toxic effects of transfused bloods. Loeb also noted that various abnormalities were not infrequent among heterogeneous hybrids, especially in respect to the circulatory system and eyes, and suggested that the small size of the embryos thus produced might be due to their failure to digest the yolk as rapidly as the pure bred embryos.

Newman similarly finds the period of gastrulation one of high mortality, but he shows that the effect of the 'foreign' sperm may frequently be detected even during the earlier cleavage period. He also refers to the variety of abnormalities that may appear during the later development of those embryos surviving the gastrulation period, and partially relies upon the nutrition hypothesis to explain them. However, since the problem he was investigating was an entirely different one, he does not attempt a careful analysis of the probable causes of abnormal development, and it would be unfair seriously to criticise his suggestion that death or abnormality during gastrulation is due to failure to establish nutritive relations with the yolk, which is to be regarded rather as a passing suggestion than as a definite opinion. I do not understand that he, or anyone, has demonstrated that this period is especially characterized by the establishment of such relations with the yolk.

It is of considerable importance from my point of view to note that Newman finds that one of the common effects of hybridization is a disturbance in the time relations of various processes of development, both acceleration and retardation being quite common consequences. This means that not only is there a disturbance of the normal morphological sequences in such hybrid organisms, but that the whole organizational, in-

tegrational mechanism is or may be affected. Several obscure relations noted by Newman might be profitably discussed from the viewpoint of the general hypothesis which I am suggesting, for example, his statement (p. 469) that "It is difficult to imagine what factors underlie this wide range of success of individual hybrids of the same parentage," an explanation of which I think these suggestions afford.

However, it is not my intention, at this time, to attempt a general application of this disorganizational hypothesis to such phenomena, and the work of Moenkhaus and Newman is mentioned in this connection chiefly to show that such observations are not counter to this general explanation of the causes of abnormal development. For it is, I take it, a strong point in favor of any suggested cause of a restricted group of phenomena, that it is not opposed by the facts concerning nearly related phenomena. Such seems to be the case with these suggestions as to the causes underlying the formation of abnormal and monstrous embryos in *Fundulus*; for it not only accounts for the results following treatment with low temperatures and chemical substances, but it is not opposed, to say the least, by observations on the causes of similar developmental phenomena following treatment of the germ cells by radium radiations and following hybridization, and further, it accords with what is known of the early development (cleavage) of the Teleost and with the current general conceptions of the developmental process.

#### SUMMARY

1. By subjecting the eggs of *Fundulus*, immediately after fertilization, to the temperatures of the ordinary refrigerator, many of them are caused to develop abnormally when returned to the laboratory temperature.

2. The abnormalities observed after such treatment cover a very wide range and no characteristic, externally observable, is found not to be affected to some degree in some embryo.

3. Similar treatment after the embryo has become well-formed also leads to similar results, though with lesser frequency.

4. The effects of the low temperature which may be actually observed in the treated individuals, take the form of irregularities in the distribution and combinations of both nuclear and cytoplasmic substances, and in the formation of cell-walls.

5. The results obtained by this method are essentially similar to those already known to follow chemical treatment and heterogeneous hybridization.

6. It is suggested that the cause of abnormal and monstrous development here and in other similar instances, is to be found in a disturbance of the normal organization of the ovum, as expressed by the unusual characters and distributions of the differentiated materials of the egg protoplasm.

7. This 'disorganization' hypothesis seems to afford a better explanation of many instances of abnormal and monstrous development among the vertebrates, than does the current 'nutrition' hypothesis, which is in many particulars opposed by the results reported here.



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