

CHAPTER XII

INTERPRETATIONS OF THE EXPERIMENTS; AND CONCLUSIONS

THE results of the experiments of Pflüger, of Roux, and of others have given rise to much discussion in respect to the relation existing between the unsegmented egg and the embryo. The old questions of evolution and epigenesis have been once more brought into the foreground, but divested of their historic meaning.

The results of the experiments on the frog's egg are, however, in the first place, too insufficient in themselves, and in the second place are as yet too uncertain on many points, to warrant general conclusions based on these results alone. The experiments can only be understood if considered in connection with similar experiments on other groups of animals.

ROUX'S MOSAIC THEORY OF DEVELOPMENT

Roux's discussion of the problems of development is deserving of most careful examination, for even in his earliest papers we see foreshadowed many of the possible interpretations that have later been accepted in one or another form. Roux pointed out that the known facts of development showed that a certain *formal self-differentiation* of many parts of the egg takes place during development. This self-differentiation may result from an unequal growth of different substances in the egg which come into activity at different times; and if so, it should be our aim to discover the stimuli that bring these different substances into action, and thus cause the consecutive series of events. The stimuli must come either from without at each stage of development, or the egg may contain within itself the power of progressive development as soon as it is once set into activity. That the egg needs during its development certain things from

its environment is self-evident; a certain amount of warmth and of oxygen, etc., must be present. These, while necessary for the development of the egg, do not necessarily determine the sequence of events; for under the same external conditions, eggs of different animals develop very differently. The results obtained by placing the frog's egg under different conditions also show that the power of progressive development must lie within the egg itself. Roux compared the egg, in this respect, to a complicated piece of machinery which, when once set in motion, would go through a long series of changes depending on its internal structure.

If so much be granted, the next question to be answered is this: do all the parts of the dividing egg work together, *i.e.* interact to form the result, or have the parts of the egg separated from one another by the cleavage the power to develop independently? The first alternative Roux called *the differentiating interaction of the parts*, and the latter alternative, *the self-differentiation of the parts*. With reference to the results of the experiment in which one of the first two blastomeres of the frog's egg was killed or injured, Roux concluded that each of the first two blastomeres shows in this experiment the power of self-development: *i.e.* each half is independent of the other and we may legitimately infer that when both blastomeres are alive, as in the normal development, the same self-differentiation of each blastomere takes place. This independent development goes on till the organs of the body begin to form. Whether the limit of independent development is then reached we do not know, for it is possible that in the complicated series of movements that take place in the formation of some of the organs, the power of independent development may be supplemented or replaced by the action resulting from the *correlation of the parts* to one another, *i.e.* by a mechanical interaction of different parts. Each of the first two blastomeres contains not only the building-material for the corresponding parts of the embryo, but also *the differentiating and formative forces* for those parts. The cleavage in the direct, or normal development of the individual, divides *qualitatively* the "germ-plasm," and, in particular, the nuclear material. The development of the frog's gastrula and of the embryo

immediately resulting from the gastrula is, from the second furrow on, a mosaic work of at least four vertical, independent pieces. How far this mosaic work of four pieces is altered by later changes in the position of the material, and by differentiating correlation, is not known.

Roux also stated clearly the relation that exists between the method of self-differentiation, and the method of interaction of the parts on one another, and the bearing of these questions on the older problems of evolution and epigenesis. If many portions of the egg are differentiated owing to their inherent power, and produce in this way the manifold differentiations seen in the embryo, then the egg must have been composed in the beginning of many parts bound up together, and the development is a metamorphosis or an unfolding of its peculiarities; *i.e.* the development is an evolution. Further, the cleavage not only divides the egg into smaller parts, but at the same time *localizes* the differentiated material, so that this material is arranged definitely in relation to later development. This result appears possible only through a qualitative separation of the material during the course of the cleavage. If this is true, we see that the development depends on the molecular structure of the egg, and therefore further analysis is beyond our reach. The *segmented* egg would be then only the *sum of its independent parts*, and during the period of the self-differentiation of these parts, there has been no united action to form a whole. Therefore the whole can have no regulating or formative influence on the parts.

If this view be true, His's principle of germinal localization in the egg has not only a descriptive worth, but also expresses a causal relation, so that organs can be referred to parts of the fertilized egg, and even to the unfertilized egg.¹

If, on the other hand, development takes place as a result of the interaction of all or many parts on one another, then the fertilized egg may be composed of a very few differentiated parts, which by their interaction produce a greater and greater

¹ We could explain those exceptional cases in which two embryos arise from one egg, if we supposed that after the first cleavage there was a sort of doubling, in each blastomere, of the primary constituents of the body (Roux).

complexity. The development would then be due to the production of many parts out of a few primary ones, *i.e.* the development is a process of epigenesis. There would thus result an ever-changing interaction of the parts to form the whole, by which means there would be also brought into play a regulating influence of the whole back again on the parts, *i.e.* correlation of the parts under the influence of the whole. His principle of germinal localization would, therefore, have a causal meaning only in so far as it points out the place in the egg where the resulting formation of many-sided changes takes place; and it would be of only secondary value to be able to refer the place of action of these changes to the undifferentiated plasm or to the unfertilized egg.¹

In conclusion, it should be noted, Roux said, that self-differentiation of the parts and dependent differentiation of the parts, *i.e.* evolution and epigenesis, may be combined in a many-sided activity or *union*, and it would then be our duty, in order to interpret these problems, to use a double foresight and a double care, to make out the part played by each of these factors in the development.

THEORY OF DRIESCH AND OF HERTWIG OF THE EQUIVALENCY OF THE EARLY BLASTOMERES

Studies on other forms show that great care must be taken in interpreting the results of the experiments on the frog's egg. In 1891 Driesch made a series of most important experiments on the eggs of the sea-urchin.² The blastomeres were isolated by shaking them apart, and it was found that although each blastomere segmented as a part, *i.e.* as if still in contact with the missing half, yet the open side of the blastula closed over very soon, and a gastrula and embryo having the normal form were produced. Driesch concluded from this and similar experiments that all the blastomeres are equivalent, and that the position of each blastomere in the segmenting egg determines in

¹ The formation of two embryos from one egg would take place, on the theory of interaction of the parts, at the time when the median axis of the body is formed. Two such axes would be laid down instead of one.

² Fiedler had made, in 1891, a somewhat similar experiment, but it was not carried sufficiently far to be of great value.

general the fate of the blastomere. If the blastomeres could be interchanged as can the individual marbles of a heap, then the fate of each would be determined by its new position in the whole. This conclusion is directly opposed to Roux's theory of a qualitative division of the blastomeres during the early cleavage.

Hertwig had also stated shortly before Driesch that in his opinion the egg divides quantitatively, and that Roux's experiments did not touch the cardinal point of this problem, because the other injured half of the egg remained in contact with the developing half. Hertwig expressed his belief that if the first two blastomeres of the frog's egg could be separated from each other, each would develop into a whole embryo. Further, he thought that the development of an organism is not a mosaic work, but that the parts develop in relation to one another, *i.e.* the development of a part is dependent on the development of the whole. Wilson ('93) also, from the results of a most careful and important series of experiments on the egg of *Amphioxus*, concluded that the division of the egg is not qualitative. He found that isolated blastomeres give rise to larvæ smaller in size than the normal, but having the normal form. The differentiation of the blastomeres, Wilson thought, takes place in the later periods of cleavage.

ROUX'S SUBSIDIARY HYPOTHESIS

Roux replied to the criticisms that Driesch, Hertwig, Wilson, and others have made of his theory, and attempted to show that his view is fully compatible with the results that Driesch and others obtained.

Roux ('92, a, '93, b) pointed out that the results of Chabry,¹ Fiedler, and Chun show that in ascidians, sea-urchins, and ctenophors a half-development takes place when one of the first two blastomeres has been removed, and that the experiments of Driesch also showed that an isolated blastomere of the egg of *Echinus* cleaved as a half, and not as a whole, and that a half-blastula also developed. These results indi-

¹ Later experiments have shown that this statement is not true for ascidians, as Chabry's work might seem, in part, to show.

cate that a certain formal self-differentiation of many parts of the segmenting egg has taken place. On the other hand, the fact of postgeneration shows that in each of the first blastomeres a power sufficient to complete the whole must also be potentially present. In order to awaken this potential power of a blastomere, a disturbance in the development must occur. This latent activity may be only slowly awakened in the development, sometimes sooner, sometimes later.

We have, therefore, to distinguish two sorts of development, —the normal “direct” development, and an “indirect” post-generative (or regenerative) development. The first or direct is the result of the self-differentiation of the early blastomeres, and of the complexity of their derivatives. The second or indirect is the result of a profound correlation which adds to an imperfect whole the lacking parts. Should the postgeneration set in immediately after the isolation of the blastomere and so convert the blastomere at once into an actual whole, then we should not have found out that each blastomere is really a self-differentiating cell, but we should have erroneously concluded that the first (four) cleavage-cells are qualitatively equivalent. Into this error Roux believed Driesch and Hertwig to have fallen. In the frog, ascidian, and ctenophor each of the first blastomeres is specifically different from the others, but in respect to postgeneration we find that each blastomere has the same potentiality, and each is in reality totipotent. The “idioplasm” in direct (*i.e.* normal) development, called into activity by the process of fertilization, is divided qualitatively and *unequally* during the cleavage, while the material which may later serve for postgeneration and regeneration (which is not active during the normal development) is always equally or quantitatively divided.

According to Roux, the nucleus represents the controlling power of the cell, but the protoplasm acts as a stimulus to the nucleus and hence may indirectly regulate the process of cleavage. “In the telolecithal frog’s egg the position of the food-substances and formative substances stands in strict causal relation to the position of the main axes of the embryo.” The nuclei of eggs in which the normal arrangement of the contents has been disturbed will be influenced during the first cleavage-

period, so that a qualitative division of the nucleus may result different from the corresponding normal qualitative division. The second cleavage, for instance, may come first (qualitatively) as a result of the position of the nucleus in the protoplasm.

Roux further suggested that the consecutive series of nuclear divisions must be different *in kind* in the normal and in the compressed eggs, and that an "anachronism" has taken place in the latter case. By this "anachronism" Roux has tried to save his theory of qualitative division of the nucleus during the cleavage-period.

To sum up Roux's later position, we may say that in order to vindicate his earlier theory of a *qualitative* division of the nucleus and a resulting self-differentiation of the first-formed blastomeres, he has been obliged in the first place to bring forward his theory of postgeneration, assuming that along with the qualitative division of the nucleus a parallel quantitative division of the germ-material also occurs. Further, Roux assumes that the kind of qualitative *division* of the nucleus is directly influenced by the arrangement of the protoplasm, and, as we have seen above, he is unable to explain satisfactorily the results of the experiment of the compressed egg, except as an "anachronism." These complications into which Roux has been forced are largely the outcome of the primary assumption of a qualitative division of the nucleus. This Roux-Weismann hypothesis of qualitative nuclear division has, however, no known histological facts in its favor. On the contrary, all we know of nuclear divisions speaks clearly in favor of an exact division of the chromatin-material, and a most elaborate mechanism is present to bring about this result.

EXPERIMENTS ON OTHER FORMS

The results obtained from a study of the development of fragments of the unsegmented egg and of isolated blastomeres of ctenophors¹ have a direct bearing on our interpretation of the experiments on the frog's egg. When the first two blastomeres are separated from each other by a sharp needle or cut apart by a pair of small seissors, each continues to cleave as a half, *i.e.*

¹ Chun ('92). Driesch and Morgan ('95).

as though it were still in contact with its fellow-blastomere. When the organs appear in the larva, only half the full number of rows of swimming-paddles appear. Each row, however, has its full complement of paddles. The invagination of ectoderm to form the "stomach" is very excentric in the half-larva, but forms a *closed* tube running from the mouth-opening to the excentric sense-plate. In several respects, therefore, the larvæ were distinctly half-larvæ. But in another respect they were more than half-larvæ. The endodermal cells of the normal larva arrange themselves into four hollow pouches, and the "stomach" invagination passes in the central line of the four pouches. In the half-larva, on the contrary, the endodermal mass forms more than two pouches (*i.e.* more than half the normal number in the whole larva). Two distinct pouches are present and in addition, generally, a third smaller pouch is formed. The latter lies excentrically. In the meeting-point of the three pouches is the excentric "stomach" invagination.

The isolated one-fourth blastomere segments also as a part of a whole, and develops in some cases into a one-fourth larva, having only *two rows* of paddles (*i.e.* one-fourth the normal number), but with *two* endodermal pouches (*i.e.* with one more than one-fourth the normal number). The three-fourth embryos develop six rows of paddles (*i.e.* three-fourths of the normal number) and *four* endodermal pouches. The problem is here a complicated one, for while in one set of organs we find a half-development, in other organs we find more than a half, but yet not the whole development.

The results show, however, beyond question, that, even when isolated from its fellow, the one-half blastomere may give rise to a larva that is in many respects only one-half of the normal larva.

There is yet to be described another series of experiments that have a direct bearing on the interpretation of the preceding results. Roux showed that if a part of the protoplasm be removed from the *unsegmented frog's egg*, the egg may continue in many cases to develop into a normal embryo. The eggs of the sea-urchin lend themselves much more readily to this experiment. They may be broken up into fragments of all sizes

if shaken in a small tube. Those fragments which contain the egg-nucleus may be fertilized and will develop. If the pieces are large enough a gastrula is formed, and still larger pieces develop into normally formed larvæ.

When the *unsegmented egg of the ctenophor* is cut into pieces, there may result either a whole larva or a larva lacking certain parts, and, further, the study of the cleavage of these egg-fragments shows that if the fragment cleaves like the whole egg (but with smaller blastomeres) then a whole larva results, while if the cleavage is irregular the larva is also imperfect. Presumably, in the first case the egg has been cut symmetrically, but in the second case unsymmetrically. Or we might assume that in the one case the egg-fragment rearranged its protoplasm into a new whole, while in the second case it was unable to do so. On either alternative we must conclude that a defect in the protoplasm often brings about a modified cleavage and also a defective embryo, and this takes place even *although the whole of the nuclear material of the unsegmented egg remains present*. There seems, therefore, no escape from the conclusion that *in the protoplasm and not in the nucleus lies the differentiating power of the early stages of development*.

GENERAL CONCLUSIONS

We have seen that one of the first two blastomeres of the frog's egg may develop into a half-embryo, or into a whole embryo of half-size, according to the conditions of the experiment. So long as the first two blastomeres remain in contact without any disturbance of the cell-contents, each blastomere develops its half of the body. On the other hand, if the protoplasm is disturbed by reversing the position of the egg after the first cleavage, there generally results a whole embryo from each blastomere. Unfortunately, it has not been found possible to separate completely from each other the first two blastomeres of the frog's egg, so that we do not know whether a whole embryo of half-size or a half-embryo would result. In other animals (Echinodermata, Hydromedusæ, Teleostei, Amphioxus, Ascidia, and Salamandra) each of the first two blastomeres, if separated from its fellow, develops into a whole embryo, regardless of the means employed to separate the

blastomeres.¹ On the other hand, the *isolated blastomere* of the ctenophor-egg develops into a half-embryo. These experiments show that the half-development of the frog's egg need not be the result of the presence of the other blastomere, as has been suggested. This is also shown by Schultze's experiment, in which, although both halves are present and in contact, each blastomere develops into a whole embryo.

The results show that in general the first blastomeres are totipotent, *i.e.* each has the power to produce a whole embryo if separated from its fellow, even although it may under certain conditions produce only a half-embryo, as in the frog. Nevertheless, in most forms each isolated blastomere continues to *segment* as though still in contact with the other half. This latter phenomenon shows that the egg-protoplasm has a definite arrangement according to which the cleavage peculiar to each kind of egg is brought about, and there is sufficient evidence to show, I think, that this is a cytoplasmic phenomenon, and is not the result of nuclear interference. We have also seen that some of the isolated blastomeres that cleave as a part may later develop into whole larvæ (echinoderms), while other blastomeres that cleave as a part may give rise to half-larvæ (ctenophors). That these phenomena too are dependent directly on the cytoplasm is shown by the experiment of cutting a piece from the *unsegmented* egg. Under these circumstances, the nucleated fragment of the echinoderm-egg gives rise to a whole embryo, although it segments as a part, while in the ctenophor an imperfect embryo is *generally* formed. The results in these two cases are nearly the same as when the blastomeres of the respective eggs are isolated, although in the latter experiment the entire segmentation-nucleus is present. In the ctenophor the process of self-regulation seems to be

¹ Roux ('95) has stated that the development of a half or a whole embryo may depend upon the method employed to separate the blastomeres. If shaken apart, whole embryos result; if cut apart, half-embryos. Zoja's results ('95) refute such an interpretation. He cut apart echinoderm and hydroid eggs and yet got whole embryos. On the other hand, when the blastomeres of the ctenophor are cut apart, half-embryos result. It must, however, be admitted that disturbance of the contents of an isolated blastomere might be favorable to whole development, as in the frog.

largely absent, either because the blastomeres cannot be brought into a new whole, or because the protoplasm is so fixed, so stiff, that it cannot readily rearrange itself. If from either of these conditions, or from some other, the blastomeres are not capable of rearrangement or reconstruction, an imperfect embryo results.

How far does the totipotence of the blastomeres reach? Does it end with the two-cell, four-cell, or later stages of cleavage? Probably this varies in different eggs. The one-fourth blastomere of *Echinus* can form a perfect embryo, and even the one-eighth blastomere may develop into a gastrula. The same is true for the egg of *Amphioxus*. For the frog it is not yet possible to say where the limit lies. In this connection the following facts are of importance. The isolated blastomere of the sea-urchin's egg runs through the same number of divisions that it would have done had it remained in contact with its fellows.¹ Hence the half-embryo has only one-half the number of cells of the normal embryo, and the one-fourth embryo has only about one-fourth the full number. This seems to give, in part, an explanation of the statement made above, viz., that the one-half embryo develops further than the one-fourth, and the latter further than the one-eighth, since the smaller the isolated blastomere the fewer are the cells it produces from which the embryo is formed. The lack of power of development of the small isolated blastomere is not, therefore, dependent on its differentiation. This is also shown by the following experiment. In the blastula-stage of the sea-urchin's egg, pieces may be cut or shaken from the blastula-wall, and, if large enough, they develop into small larvæ. Here also we find that the large pieces can go further in the ontogeny than the smaller pieces, probably owing to the presence of a sufficient number of cells or of sufficient material to form the necessary organs of the embryo.²

If the early blastomeres are totipotent, what brings about the later differentiation of these cells? There are sufficient reasons,

¹ Morgan ('95).

² The same experiment cannot be made on the frog's blastula, because, if cut, the pieces immediately disintegrate.

I think, to conclude that the power of differentiation lies within the egg itself, and does not depend directly on external stimuli. We have seen that Roux and Weismann (particularly the latter) explain this differentiation of the cells as a result of the qualitative division of the nucleus from the very beginning of the cleavage. The nucleus, unravelling its qualities at each division, sends into each cell the proper constituents, and the nuclei, then acting on the cell-protoplasm, cause it to differentiate. On the other hand, Hertwig contends that the early blastomeres are equivalent, and that *differentiation is brought about* by the *interaction of the blastomeres*. In other words, any blastomere that has come to occupy a given position has its fate sealed, because in this position it bears a certain relation to the other blastomeres of the whole; the whole being simply the sum-total of the blastomeres present. But it is impossible to imagine that the interaction of strictly equivalent blastomeres could bring about a self-differentiation. If it is assumed that the gross-contents (such substances as yolk, etc.) determine the differentiation of each part, still the hypothesis is obviously insufficient for all cases, because, as we have seen, fragments of *any part* of the egg of echinoderms develop into whole embryos, and fragments even of the blastula form new blastulæ, gastrulæ, and embryos. Some of these small blastulæ represent only the "animal" half of the original blastula, and the cells will not, therefore, contain any of the protoplasm or yolk that the cells usually contain that are invaginated, for all this portion of the blastula has been cut off. And since these "animal" pieces gastrulate, we must infer that the gross-contents of the blastomere, or collection of blastomeres, do not *necessarily* cause the differentiation. If, then, neither qualitative division of the nucleus, nor cellular interaction, nor the gross-contents of the blastomeres can be the cause of differentiation of the embryo, what does bring about the differentiation? There are certain facts of inheritance that also have a bearing on this question. The characters of the male are known to be transmitted by means of the spermatozoön. The latter carries into the egg mainly the male nucleus. Therefore, many embryologists have turned to the nucleus as the originator of the differentiation of the cell. Various suggestions have been offered as to the way

in which such an influence could be transmitted from the nucleus to the cytoplasm. Strasburger supposes the nucleus exerts a dynamic influence on the cell-plasm. De Vries and others imagine that organized particles, "pangens," pass out of the nucleus to transform the cytoplasm. Driesch suggests that the nucleus secretes ferments which change the cell-plasm. These hypotheses are purely imaginary, for at present we know almost nothing of the function of the nucleus; and even if we suppose the differentiation comes in some unknown way from the nucleus, still we do not know what could start the process in isolated nuclei that are after the cleavage-period assumed to be equivalent. There is, however, one series of experiments which seems to throw some light on the present problem, although the interpretation is extremely difficult and hazardous. I refer to the experiment on the ctenophor-egg, in which a part of the cytoplasm was cut from the unsegmented egg, and the latter gave rise in most cases to an imperfect embryo. Here, although the entire segmentation-nucleus is present, yet by loss of cytoplasm defects are produced in the embryo. The form, therefore, of the early embryo would seem to result from the structure of the protoplasm, or from the arrangement of the blastomeres after cleavage. In either case the phenomenon is in the first instance cytoplasmic. How can this conclusion be brought into harmony with the facts, stated above, of inheritance of characters through the male pronucleus? Let us assume an imaginary case to show how this union of the two conceptions is possible. If we had used the spermatozoön of one species (or variety) of ctenophor and the egg of another species, and then after fertilization had removed a part of the egg-cytoplasm, we should expect to find the embryo defective, but the organs that were formed we should expect to show a combination of male and female characters. In other words, the imperfect embryo would have resulted from the arrangement of the protoplasm into an imperfect form, but the *kind of organ* would have depended on the structure of the nucleus in each cell. After cleavage, the cytoplasm of each part differentiates into this or that organ, but the kind of differentiation of each part is determined by the nucleus of that part.

If the argument given above should prove true, then the

origin of the differentiation is to be found in the ultimate structure of the cytoplasm of the egg or embryo, although even then we do not know how this mechanism could be started. Whitman ('95) has stated his conviction that it is erroneous to think of the embryo as only the *sum-total* of cells interacting upon one another, but that the embryo itself is to be thought of as a whole, which regulates its parts regardless of cell-boundaries. According to this view, each portion of the embryo has its fate sealed, not because the given portion forms a member of the community of cells, but because the whole directs the fate of each special part. Driesch has pointed out that the egg seems to act like an intelligent being. If so, are the causes of differentiation and of regeneration the same in kind as physico-chemical causes, or do they belong to the category of intelligent acts, and can these latter be accounted for by the *known* principles of chemistry and physics? The plain answer is, we do not know.