ON THE MECHANISM OF MORPHOLOGICAL DIFFERENTIATION IN THE NERVOUS SYSTEM

I. THE TRANSFORMATION OF A NEURAL PLATE INTO A NEURAL TUBE

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THREE FIGURES

I. INTRODUCTION

The early development of the vertebrate nervous system is among the commonplaces of descriptive embryology. Every elementary text-book tells us that the first clearly recognizable rudiment is a flat patch of cells ectodermal in nature; how in due course of time after the appearance of a longitudinal furrow, the edges of this plate rise, meet in the mid-dorsal line, and fuse to form a tube, enclosing the neurocoel. What however are the forces at work when the primitive plate changes into a tube?

This question was in the mind of His[1] when he wrote his classic letters, "Unsere Körperform." In the fourth of these lucid epistles, His shows that the nervous system, during the period of folding, grows faster than the surrounding tissues with which it is continuous. On the assumption that these resist any increase in the width of the neural plate, he shows that the latter, must fold under the mechanical necessities of the case. Models, in which the entire process could be simulated at will, helped to emphasize the argument.

The well-known experiments of Roux,[2] however, proved that this view of the origin of the neural groove is mechanically impossible, despite the inequalities of growth emphasized by His.

[1] Unsere Körperform, und das Physiologische Problem Ihrer Enstehung. F. C. W. Vogel, Leipzig, 1874.

[2] Die Entwicklungsmechanik. W. Engelmann, Heft 1, Leipzig, 1905.

Roux indeed was able to produce a fold in the neural plate of the chick by pressure from the sides, but when this pressure was released, the plate instantly returned to its original flat condition. Pressing upon it through the lateral extra-neural membranes, these instead of transmitting the pressure, collapsed, a result which might have been foreseen when their thickness is compared with that of the plate (loc. cit., p. 45).

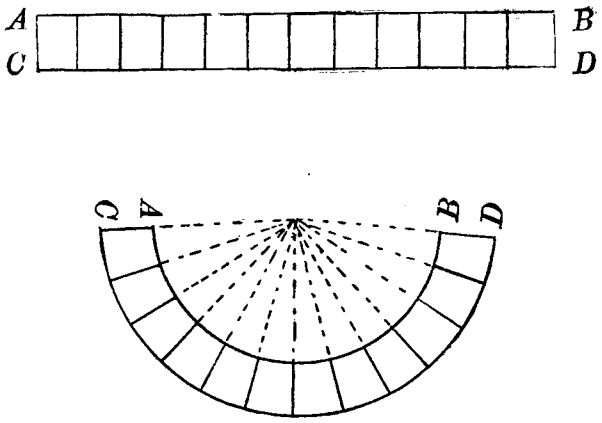

Figure 1

Most conclusive have been Roux's isolation experiments. By cutting the neural plate from its surroundings he found that it still folded in the normal manner, "und zwar geschah dies noch rasher als es normalerweise der Fall ist" (loc. cit., p. 451). Folding occurred even when in addition to lateral isolation, the neural plate was cut transversely into a number of segments. From these experiments the conclusion was drawn that the nervous system is self-differentiating.

Not only do the lateral membranes, as implied in the remark quoted, contribute nothing toward converting the plate into a tube, but they are actually an hindrance, for they exert a pull away from the mid-dorsal line where fusion of the neural folds finally takes place. I have convinced myself by a few simple experiments on the embryos of Amblystoma punctatum, that such pull away from the median axis actually exists. In these,

if a longitudinal cut be made in the neural plate, the wound gapes widely for twelve hours or more if the extra-neural ectoderm has been uninjured, but if this is also cut on each side of the nervous system parallel with the incision in the plate, the wound in the latter after twelve hours, is very much smaller than in the first experiment, or has practically disappeared.

If we accept, as it seems to me we must, Roux's conclusion that the nervous system is self-differentiating, we must ask what this statement means. Evidently for the stages of development under consideration here, self-differentiation is identical with self-folding. The question to be answered therefore is how the neural plate can autonomously fold itself.

II. THE CHANGE IN THE SHAPE OF THE CELLS DURING FOLDING

Rhumbler's[3] very complete analysis of the geometrical relations in invaginate gastrulation finds its application to the case in hand. For the sake of simplicity let us imagine it possible to cut through a neural plate a rectangular cross-section, $ABCD$, in which each component cell is itself a rectangle. If this section is folded symmetrically about an axis, AC and BD, and all cell-boundaries parallel with them, will radiate toward a center, and AB and CD will become respectively the circumferences of two spheres, one with the radius, R, the other with the radius, $R + AC$. It follows from the geometrical necessities of the case, not only that circumference CD is greater than AB, but that each cell, from being a rectangle, has become a trapezium whose lower boundary is greater than its upper.

Although actual conditions in the nervous system are more complicated, nevertheless, sections through the two stages under consideration approximate the ideal case very closely. An examination of any section, or any one of the thousands of published figures, will disclose many cells in which the change in shape here emphasized is strikingly shown. Indeed, being a geometrical necessity, the case cannot be otherwise, but whether the change in shape is the result of folding, or folding the result

[3] Zur Mechanic des Gastrulationsvorganges; Arch. f. Entwicklungsmech., Bd 14.

of the change in shape, remains unanswered. One thing however appears certain; we must seek the answer in the nervous system itself, for the neural plate is self-folding.

III. THE NUMBER OF NUCLEI IN COMPARABLE SECTIONS OF THE NERVOUS SYSTEM AT THE BEGINNING AND AT THE END OF THE FOLDING PROCESS

In order to discover how the neural plate folds itself, we must first find what rôle, if any, cell-division plays in the process. Determinations capable of answering this question could be made if we could count the number of cells in comparable sections at different stages in the development of the tube. However, the nervous system is so largely syncitial in nature, and cell-boundaries, even where present are often so ill defined, that this direct method would surely lead to error. Another road is open however, for we may count accurately the number of nuclei. For this purpose the most satisfactory material I have found are some embryos of Cryptobranchus allegheniensis, for the possession of which I am indebted to Prof. Bertram G. Smith of the Michigan State Normal College.[4]

The period of folding was arbitrarily divided into three, beginning with the flat neural plate, and ending with the neural tube just prior to fusion. Between these extremes is the half-folded plate. For the stages in question I selected ten comparable but unconsecutive sections from the middle point of each series. The sections were all 10 micra in thickness, and the number of nuclei in each was carefully counted. The results are given in table 1, in which each column is headed by a diagram representing the stage of development referred to. Examination of this table indicates that during folding the number of nuclei, and hence of cells per section, does not increase. At the beginning of the period there are, on the average, 62 cells per section, in the middle, 61, and at the end, 59.

[4] For details on the development of Cryptobranchus, not dealt with in the present paper, see the excellent communications of B. G. Smith: Biol. Bull., vols. 11 and 26, and Journ. Morph., vol. 23.

Naturally these values are not absolute. Lack of uniformity in the distribution of nuclei in the syncitial system is responsible for considerable variations in individual sections, and may have affected the averages. Some errors no doubt have crept in on account of faulty enumeration, and also because the sections are necessarily from different individuals. The seriousness of the first source of uncertainty seems to me largely offset by the remarkable constancy of the averages; against the second source

TABLE 1

Number of nuclei in comparable sections

STAGE I	STAGE II	STAGE III
		
63	56	55
53	64	60
58	50	73
69	56	47
72	50	69
58	82	59
59	70	64
58	74	51
58	58	52
68	51	55
Ave. 62	61	59

of error I guarded by reflecting the nuclei on paper, dotting each one as counted, and then recounting the dots as a check; the third difficulty was met as completely as possible by choosing embryos from eggs of uniform size laid by a single female.

The conclusion that cell division or better, multiplication, does not occur during the process of folding, although possibly correct, cannot be drawn without a certain reservation, for the nervous system increases in volume during this period (see Section V). With this fact in view one cannot consider the constancy in the number of nuclei per section sufficiently conclusive to warrant the statement that cell multiplication does not occur

during the period of folding, and hence can play no part in transforming the neural plate into a tube. However if it does occur, the number of nuclei produced in this way is too small to overcome the 'nuclear dilution' brought about by the volumetric increase of the system as a whole. Relatively, therefore, even if not actually, the number of cells per section remains constant, and we can make no great mistake by assuming that the rôle of cell-multiplication is practically negligible.

IV. THE DISTRIBUTION OF NUCLEI IN COMPARABLE SECTIONS OF THE NERVOUS SYSTEM AT THE BEGINNING AND AT THE END OF THE FOLDING PROCESS

If we mark off a series of points midway between the upper and lower surfaces of the neural plate, a line connecting them will divide the nervous system into an upper and a lower zone. A corresponding line drawn in the half, or fully, folded stages, gives us inner and outer zones. Since morphologically upper and lower are identical with inner and outer respectively, it will be advantageous to use the latter terms also for the unfolded neural plate.

The nuclei of the sections which served as the basis for table 1, are distributed in the inner and outer zones in the proportions given in table 2. Comparing the three stages, we find that the distribution of nuclei in the inner and outer zones is quite different at the beginning and at the end of the folding, and that Stage II occupies an intermediate position. Roughly, the inner zone looses one-half its nuclei and the 'nuclear concentration' in the outer zone increases by this amount. Absolute correspondence between the nuclear loss of one zone, and the gain in the other, cannot be expected. Not only must we recall the sources of error mentioned in section III, but we must also remember that the division into inner and outer zones is a somewhat arbitrary expedient, and furthermore that the neural plate, although spoken of as flat in Stage I, is in reality quite irregular in detail, and moreover exhibits, more or less, the general curvature of the sphere of which it is a part. Without attempting any special

refinements which seem to me quite unnecessary, it is obvious that during folding there is a marked outward migration of nuclei (see text-figure 2). With this outward migration, there is associated, from the geometrical necessities of the case, a distinct increase in the volume of the outer zone. Both these changes in the folding nervous system would occur if this tissue were suitably pressed upon from without, but since the neural plate folds itself, the forces that result in the translocation of materials and the change in the shape of the cells must be sought within the autonomous system.

V. THE INCREASE IN THE VOLUME OF THE NERVOUS SYSTEM DURING FOLDING

Change of shape in the cells of a folding tissue may occur with constant volume. In the neural plate, for instance, the inner zones of the cells might decrease by an amount exactly equalled by the increase in the outer zones. This, however, is not true.

TABLE 2

Distribution of nuclei in inner and outer zones

STAGE I		STAGE II		STAGE III	
					
Inner	Outer	Inner	Outer	Inner	Outer
32	31	31	25	15	40
32	21	22	42	15	45
34	24	16	34	21	52
55	14	27	29	13	34
39	33	18	32	22	47
38	20	27	55	16	43
31	28	33	37	26	38
33	25	29	45	13	38
37	21	21	37	20	32
44	24	19	32	20	35
Ave. 38	24	24	37	18	39

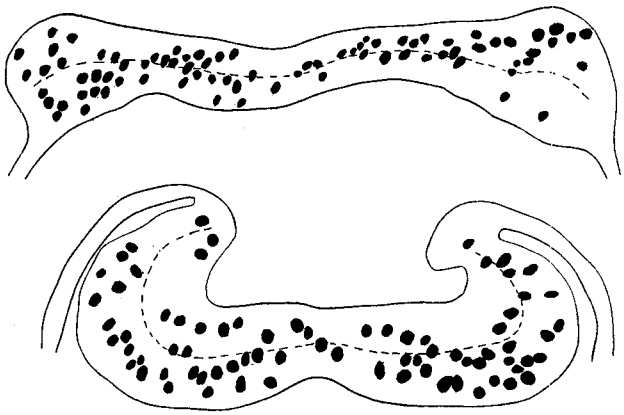

Fig. 2 Two sections through the embryonic nervous system of Cryptobranchus allegheniensis, showing the nuclear distribution in Stages I and II. The sections are from the same series and regions as those dealt with in the tables but contain for Stage I, six, and for Stage II, one nucleus more than the maximal number recorded in table 1. In the unfolded plate there are in the present case, 78 nuclei, of which 47 are in the inner zone, and 31 in the outer; in the half-folded plate, there are 75 nuclei, 21 in the inner zone, and 54 in the outer. Nuclei which happen to fall on the line separating the two zones are ascribed to the one into which the greater portion of their mass projects.

Adequate measurements cannot be carried out directly. Instead, we may compare the areas of the two regions, in section, for area is definable as volume in one plane. On this basis, determinations capable of giving some insight into the volumetric relations during involution are easily made; all that is needed is to draw the sections at constant magnification with the aid of a camera lucida, and then by means of a planimeter, trace the relative areas of the inner and outer zones. The results of such measurements, carried out on the same sections used in making the nuclear counts, are presented in table 3. From this table it is apparent that the areas of the two zones are related during the process of folding by the following ratios:

	STAGE I	STAGE II	STAGE III
Inner zone	4.8	4.3	7.4
Outer zone	4.4	6.8	12.3

In other words, while inner and outer zones are approximately equal in Stage I, in Stage II, the outer zone exceeds its original size by half, and the inner remains practically unchanged. In Stage III on the other hand, both zones show an increase, but the inner, roughly, has doubled, the outer, trebled, its volume. Although it is impossible under these circumstances to determine how much of the increase in the volume of one zone is due to the migration of nuclei and cytoplasmic materials from the other, it by no means follows that the outer zone does not gain at the expense of the inner. Indeed such translocation is definitely proved in the case of the nuclei, and appears inevitable for the other cell contents.

VI. ON THE CAUSE OF THE INCREASE IN VOLUME

Since cell-multiplication during the process of folding appears negligible, it cannot be concerned practically with the increase in volume which takes place at this time. It follows that the cells of the nervous system must individually increase in volume,

TABLE 3

Relative areas of inner and outer zones

STAGE I		STAGE II		STAGE III	
					
Inner	Outer	Inner	Outer	Inner	Outer
4.6	4.8	5.3	8.4	6.6	10.9
4.4	3.7	4.1	7.2	6.9	12.6
6.7	3.9	4.6	6.8	6.9	12.6
5.2	4.7	4.3	5.8	7.8	12.8
5.0	4.1	4.1	6.0	7.3	13.2
4.8	4.8	4.0	6.9	6.8	11.3
4.6	4.5	5.0	6.5	7.7	11.3
3.3	4.9	4.2	7.8	7.7	10.8
4.4	3.9	3.7	6.5	7.3	11.6
4.9	5.0	3.8	6.3	8.5	10.3
Ave. 4.8	4.4	4.3	6.8	7.4	12.3

and this might be due to the absorption of water. Such absorption would be capable of direct demonstration if it were possible to compare the water content of the neural plate with that of the neural tube, but very serious obstacles of a technical nature stand in the way of the required determinations. It would be exceedingly difficult to isolate neural plates in sufficient numbers, or with sufficient accuracy, to make the necessary weighings reliably. Another method of procedure is open.

The rate of growth in these early stages of the nervous system is known to differ from that of the surrounding tissues. Since growth in general is measurable in terms of water absorption it follows that the differential growth of the neural plate and tube must be the reflection of a differential absorption of water on the part of their component cells. If this is correct then at the stage of complete involution, the water content of the neural tube should differ from the water content either of the larva, taken as a whole, or of certain portions, for unless this were true, the embryo, instead of having grown in complexity, would simply have grown in size.

The most satisfactory material available for the study of this question proved to be the eggs of the frog, Rana pipiens, and of the salamander, Amblystoma punctatum. Development in both cases was allowed to proceed normally until the bodies of the embryos could be conveniently cut from the yolk-sac, an operation easily carried out with a minimal loss of tissue. Unfortunately the separated portions cannot be further divided, and even if the isolation of their constituent layers were possible the inclusion of yolk within the cells of the nervous system would leave an uneliminated and unavoidable error. However it is safe to assume that our operation results in the separation of two tissue masses, one predominantly yolk, the other predominantly nervous.

The fresh weight of the entire larvae as well as that of the separated tissue masses was in each case ascertained after carefully removing the superficial water. Following this, dry-substance determinations were made in the usual manner by complete dessication at 60°C. *in vacuo*, over P_2O_5. For the sake of easy

comparison, all the results are assembled in table 4. From this table it is at once apparent that the water content of the frog and salamander embryos at this stage of development belong to the same order of magnitude, and further that the water content of the yolk-sac of the frog larvae is not very different. Corresponding determinations for the yolk-sac of Amblystoma proved impracticable as the consistency of the yolk in these embryos is such that considerable losses occur when the sac is removed. However, the values for the entire larvae are identical in the two cases.

Comparing the embryonic nervous systems of these two forms with the entire larvae or the yolk-sacs, it is seen at once that the water content of the first belongs to a totally different order of magnitude, for the nervous system is a tissue which, within the limits of error may be said to contain 80 per cent of water and 20 per cent of dry substance.

TABLE 4

Showing water content four to five days after fertilization

MATERIAL	FRESH WEIGHT	DRY WEIGHT	DRY SUBSTANCE	WATER
R. Pipiens	*grams*	*grams*	*per cent*	*per cent*
38 Larvae	0.1278	0.0557	43.8	56.2
50 Larvae	0.1718	0.0722	42.0	58.0
39 Larvae	0.1815	0.0630	40.2	59.8
Average			**42.0**	**58.0**
24 Yolk-sacs	0.0440	0.0204	46.4	53.6
31 Yolk-sacs	0.0585	0.0264	45.2	54.8
Average			**45.8**	**55.2**
24 Nervous systems	0.0464	0.0098	19.1	80.9
31 Nervous systems	0.0714	0.0149	20.8	79.2
Average			**19.9**	**80.1**
A. Punctatum				
16 Larvae	0.0955	0.0399	41.8	58.2
15 Larvae	0.0992	0.0406	40.9	59.1
Average			**41.4**	**58.6**
125 Nervous systems	0.3914	0.0785	19.9	80.1
52 Nervous systems	0.1756	0.0400	22.8	77.2
15 Nervous systems	0.0524	0.0106	20.2	79.8
69 Nervous systems	0.2039	0.0363	17.8	82.2
Average			**20.2**	**79.8**

The essential correctness of these values for the nervous system is guaranteed by the identity of the averages, the relative constancy of the individual observations, and finally by comparison with the adult condition. This comparison[5] can be made in the case of Rana pipiens, because Donaldson[6] has given us certain standard values for this form. In making this comparison it is more correct to use the water content of the adult cord, for this is less differentiated than the brain and hence more nearly in the larval condition. According to Donaldson's figures (loc. cit.) the average water content of 12 cords of Rana pipiens is 80.5 per cent, a value identical with mine of 80.1 per cent for the larval system.

Since the rate of cell-multiplication during folding is either zero or practically negligible; since this period, moreover, coincides with a great increase in volume, and finally, since the water content of the neural tube differs so radically from that of either the yolk-sac or the larva taken as a whole, it seems scarcely doubtful that the differential absorption by the nervous system occurred during the process of involution. This, however, does not prove that the differential absorption is responsible for the folding.

VII. ON THE THEORY OF FOLDING

Since every metazoan body arises from germ-layers having a common origin in the egg, and connected with one another without interruption, it follows that even a complicated organism, theoretically at least, could be unravelled and spread out in the form of a continuous membrane. It has been evident for many years that in embryogenesis, the commonest occurrence leading to increased complexity of form, is the folding process, but its causes have not been adequately analysed, nor have attempts at analysis received the recognition from anatomists and embryologists, which they deserve.

[5] Otto Glaser, The water content of the embryonic nervous system. Science, vol. 39.

[6] Donaldson, Henry H., Further observations on the nervous system of the American leopard frog, Rana pipiens, etc. Jour. Comp. Neur., vol. 20; see also numerous earlier papers.

From the standpoint of static morphology, the analysis of an organism into a system of folds may appear satisfactory enough, and constitutes a step forward. From the same standpoint, however, every fold is like every other, a complication, the contemplation of which can give us no idea as to how it was produced. From the dynamic point of view, the mode of production is the important thing, and a moment's reflection is enough to show that significance of one fold may be quite different from that of another.

Experimental analysis of the early stages of the nervous system has gone far enough to show that here the folding process is autonomous. The same effect could be achieved by coercion, and would be morphologically indistinguishable. But coercion is a very different process from the one under consideration, and certain foldings of the heart and digestive system in the embryo, have only a superficial resemblance to the autonomous folding of the nervous system. Nevertheless, we are not without analogies, for it follows from Rhumbler's work (loc. cit.) that invaginate gastrulation belongs to the same category.

In the first place, Rhumbler draws attention to the significance of the change in shape undergone by the entoderm cells during infolding. Before as well as after gastrulation the cells are wedge-shaped, but in the blastula, the narrow ends of the wedges point inwards, as they do in all the other cells of the spherical larva, whereas during and after invagination the wedge-shape of the entoderm cells is completely reversed and their narrow ends now point outward. This change in shape, which has also been emphasized by Conklin,[7] occurs, as we have seen in the nervous system. According to Rhumbler, a translocation of materials within each of the involved cells is a mechanical necessity. "Die Umgestaltung der Zellen der Entodermplatte, d. h. die Verbreiterung der Entodermzellenkeile auf der Blastocölseite, *erfordert unbedingt Zellensubstanz-verlagerung innerhalb jeder einzelnen Entodermzelle nach der Blastocölseite hin, um verbreitern zu können was vorher zugespizt war*" (loc. cit. p. 432).

[7] Mosaic development in Ascidian eggs. Jour. Exp. Zoöl., vol. 2, p. 163.

The migration of the nuclei which I have described is in strict harmony with this idea, and if the nuclei migrate it is probable that other portions of the cell contents also undergo a change in location.[8] The occurrence of the same changes in shape on the part of the infolding cells in the two cases, however, is in itself not enough to show that invaginate gastrulation and the formation of a neural tube are fundamentally identical, for gastrulation by coercion would force upon the entoderm cells the shape which they would assume autonomously, if invagination were an autonomous process. There are cogent reasons for believing that this is true.

According to Rhumbler (loc. cit., p. 410) there are conceivable three ways in which the entoderm cells might possibly be coerced from without. In the first place, differential growth on the part of the ectodermal and entodermal elements of the blastula might result in invagination; in the second place, the blastula, growing inside the egg-membrane, might have invagination forced upon it if the entodermal plate were less resistant to mechanical pressure than the ectoderm; and finally, the decrease in the volume of the blastocoelic fluid during invagination might result in a suction which would be followed by a caving-in of the weakest region in the wall.

The second and third possibilities are readily disposed of. It is only necessary to recall that invaginate gastrulation takes place perfectly well in the absence of an egg-membrane (Rhumbler). Furthermore, the entodermal plate cannot be the weakest region in the wall of the blastula since in general its component cells are the largest which the larva possesses. Mechanically the relation between the entoderm and the ectoderm of the blastula, must be the same as that between the neural plate and

[8] In this connection, figure 2 in a recent paper by J. F. Gudernatsch in The Anatomical Record (vol. 7, p. 416) is interesting. In this picture the nuclei are located in the inner ends of the gastral plate cells *before* invagination. The author imagines that this localization renders the inner ends of the cells less compressible than the outer, and that invagination is caused by pressure exerted by the ectoderm on the more compressible outer ends of the gastral cells. It seems curious that the work of Rhumbler and Roux, mentioned in the very extensive bibliography attached to this paper, should have made so little impression.

the extra-neural membranes. Suction due to a decrease of the blastocoelic fluid would bring about the invagination not of the entoderm, but of the ectoderm.

Differential growth as a factor in invagination is not so easily disposed of. Rhumbler's analysis deals with the following possibilities:

Case I. Cell multiplication in the ectoderm proceeds at a higher rate than in the entoderm. The result is pressure upon the entodermal plate from the periphery, and this pressure may be sufficient to bend the plate. However, not only is this region of the blastula the least likely of all, to give way, but as long as the broad ends of the entodermal wedges point outward (Rhumbler) any folding produced by the means imagined would necessarily result in evagination, not invagination.

Case II. Cell multiplication in the entoderm proceeds at a higher rate than in the ectoderm. The effect would be the same as in Case I. A pressure on the entodermal plate would result, but no matter how produced, the plate could not fold inwards.

Consideration of these two possibilities of differential growth is worth while as an aid to clearing the ground, although Case II was unnecessary even at the time that Rhumbler wrote, for Morgan[9] had found that during gastrulation

> Karyokinetic division is no more frequent in the cells involved than elsewhere. At the end of the period, if the posterior hemisphere be examined, it will be found that the plate of cells has disappeared from the surface, and that the surface nuclei are little more frequent than the surface nuclei of the anterior hemisphere. Karyokinetic division therefore plays no part in the development of the archenteron of the gastrula of Sphaerechinus (loc. cit., p. 85).

Although my conclusion with regard to the rôle of cell-multiplication during the folding of the neural plate is stated more conservatively, Morgan's result with respect to the same factor in the invagination of the gastral plate, is practically identical.

With respect to the change in shape, the consequent translocation of cell-contents, the influence of external pressure, and

[9] Studies of the 'partial' larvae of Sphaerechinus. Arch. f. Entwicklungsmech., Bd. 2.

finally, the rôle of cell-multiplication, invaginate gastrulation is identical with the folding process undergone by the nervous system. We may, therefore, safely attribute to the gastral plate an equal degree of autonomy.

But the elimination of cell division does not necessarily eliminate differential growth. We have already seen that an increase in the volume of the nervous system takes place at the time of folding, and that this growth, occurring with a negligible rate of cell-multiplication, is the result of water absorption. If such absorption were demonstrable for the gastral plate we should have one more point of identity between the two processes under comparison.

Unfortunately I have no trustworthy direct measurements. Nevertheless, there are considerations which seem to bear closely on the point at issue. Thus Morgan (loc. cit., p. 85) says:

> The nuclei in the walls of the archenteron enlarge during the invagination period and lie further apart than they did in the plate. This might give us some clue as to the mechanical principles involved in the process if we could estimate the volumes of the cells (protoplasm) before and after the process, but this it is impossible to do.

I have little doubt that the methods which I have employed for detecting the change in volume during the folding of the neural plate will be applicable to the process of invaginate gastrulation. What is of more immediate concern, however, is the increase in the volumes of the nuclei, and the fact that after invagination they "lie further apart than they did in the plate."

Morgan proved that there is no increase in the number of entoderm cells during invagination. The only factor which can be concerned in separating the nuclei is an increase in the volume of the extra-nuclear material. If such increase were the result of water absorption one would expect the increase in the size of the nuclei which was observed.

While this reasoning may be valid, nevertheless it is desirable to know whether nuclear size is a reliable index of the relative amount of water which the cell contains. That this actually is the case is indicated by certain measurements which I have

made on the unfertilized ova of Asterias forbesii.[10] These eggs constitute a very favorable material for the solution of this problem, not only on account of their size and shape, but also because their nuclei (germinal vesicles) are easy to see in the living cells and like these are spheres. In table 5 are given the diameters of eggs and nuclei which were first measured in normal sea-water, and afterwards in hypotonic.

From the figures in table 5 it is at once apparent that the cells absorb water in the hypotonic solution, and moreover that when they increase in volume, their nuclei do so likewise. It follows

TABLE 5

Showing relative diameters of Asterias ova and their nuclei. The hypotonic sea-water was made by diluting six volumes of the normal sea-water, with four volumes of distilled water. The units of measurement are the same in all cases, but the exact absolute value cannot be given at this time. With greater as well as with smaller dilutions measurable changes in the same sense can be detected

		NORMAL SEA-WATER	HYPOTONIC SEA-WATER
		units	*units*
Lot I	47 eggs	152.0	188.0
	47 nuclei	0.7	0.8
Lot II	49 eggs	154.0	170.0
	49 nuclei	0.7	0.8

from this that the volume of the nucleus may be taken as an index of the relative amount of water held by the cell, and that the increase in the size of the nuclei noted by Morgan during gastrulation, indicates an increase in the water content of the gastral plate. In this tissue, therefore, we have differential growth just as in the nervous system, and instead of being the outcome of the synthetic processes involved in growth by cell-multiplication, the increase in bulk is here also the result of water absorption.

The question as to the rôle of this absorption in the process of folding remains to be answered. As far as invaginate gastrulation is concerned, so long as the entoderm cells continue to have

[10] For an account of the methods employed see, Glaser, The change in volume of Arbacia and Asterias eggs at fertilization. Biol. Bull., vol. 26, p. 84.

the shape they possess in the blastula, an increase in volume could result only in stretching the ectoderm, and bending the gastral plate outward. If the increase in volume were to continue, the blastula would flatten, and finally the ectoderm, unable to stretch further would tear away from the entoderm. In the nervous system the situation would be exactly the same. The differential growth of neural and gastral plates alone therefore could never convert these structures into tubes, a conclusion reinforced by Roux's isolation experiments, for when a neural plate, completely isolated from other tissues folds itself, differential growth is certainly excluded. What significance then are we to attach to the water absorption, and how are we to explain the folding?

Rhumbler (loc. cit.) has pointed the way to an answer. The cells of the gastral and neural plates change in shape during invagination and folding. Since both processes are autonomous, an explanation would be found if we could show how the cells of themselves could change in form so that no other arrangement except that of the folded or invaginated state is possible. The factor possessing the necessary requirements, is, according to Rhumbler, differential surface tension.

Three cases are considered, as follows:

Case A. In the spherical blastula, all the cells are wedges whose smaller ends point toward the center. The outer poles of the entoderm cells are bathed by an external medium of relatively constant composition; the inner poles on the other hand project into the blastocoelic liquid whose composition is not only different, but no doubt changes during the course of development. For instance, the concentration of CO_2 alone must be greater in this liquid than in the external medium which not only has a far greater volume, but into which ciliary convection currents are constantly driving the CO_2 from the surface of the larva. Now the surface tension of a cell depends upon the nature of the surface and this is determined equally by the characters of two media which it separates. Rhumbler imagines that in the gastrula we have conditions under which the tension of the inner surfaces of the entoderm cells may well be different from that of the outer, and if it were less, then the outer surfaces

with their higher tension would, like elastic bags, squeeze the cell-contents inward. The inner surface under the circumstances would give way until an equilibrium resulted in which the entodermal wedges would be reversed. Theoretically such reversal is all that is needed to insure invagination or folding.

But why does this not apply equally well to the other cells of the blastula? Why do the entoderm cells alone invaginate? For the sake of simplicity, Rhumbler (loc. cit., p. 448) considers first the mechanical conditions which may be supposed to obtain if the surfaces of the ento- and ectoderm cells are identical in physico-chemical composition. From the greater size of the entodermal elements it follows that their surface tension is less than that of the smaller ectoderm cells.[11]

If now the blastocoelic fluid lowers surface tension, it would bring about, per unit area, an equal lowering in all the cells, but in the entoderm, because of its absolutely greater surface, the absolute lowering would exceed that in the ectoderm.[12]

[11] The phenomenon of exo-gastrulation in lithium larvae (Herbst, Arch. f. Enwicklungsmech., Bd. 2, p. 455, referred to by Gudernatsch, loc. cit., p. 417) harmonize with the opinions of Rhumbler as expressed here, for in these embryos all the blastomeres are swollen and vacuolated, but just before exo-gastrulation the entoderm cells decrease in volume and become actually smaller than the ectoderm. This experimental fact harmonizes equally well with the somewhat freer interpretation which I shall present later on. In this connection it is interesting to recall the 'exo-neurula' of the frog, also produced by the use of lithium (Hertwig, Morgan). Gudernatsch (p. 423) also refers to Driesch's observation that gastrulation occurs in Sphaerechinus blastulae (Mitteil. Zool. Stat. Neapel, Bd. 11, p. 221), no matter whether they are derived from the micomeres or macromeres of the early cleavages. This fact does not preclude minute differences in the size of the cells in these 'partial' larvae, nor necessarily even differences in their chemical composition.

[12] The passage in Rhumbler (p. 449) in which this matter is discussed, reads as follows: "Die Entodermzellen sind aber grösser und haben desshalb auch eine 'absolute' grössere Oberfläche, so dass bei ihnen die Spannungserniedrigung wohlgemerkt 'pro-Einzelzelle' viel erheblicher ausfallen muss als bei den einzelnen Ectodermzellen, zumal die kleineren Ectodermzellen (weil sie von Haus aus eine grössere Oberflächenspannung pro Flächeneinheit besitzen), an sich zur Erziehlung des gleichen Effektes nicht die gleiche, sondern eine entsprechend beträchtlichere Herabminderung der Oberflächenspannung pro Flächeneinheit verlangen würden, während ihnen doch nur eine gleiche geliefert wird." Gudernatsch (loc. cit., p. 419) writes: "Rhumbler however believes that chemotaxis alone is the inducing factor of gastrulation."

Under these circumstances the entodermal cells would move into the blastocoel before the ectoderm, and the rise in internal pressure owing to the incompressibility of the blastocoelic fluid would render impossible any immigration or invagination on the part of any other cells.

Case B. The conditions of Case A are needlessly difficult, and were assumed simply to show that invagination is conceivable from this standpoint even under the most adverse circumstances. But the physico-chemical constitution of the surfaces of neither the gastral nor neural plate cells can be identical with that of the neighboring ectoderm for they do not enclose identical chemical systems. This is indicated not only by the localization of yolk and 'organ-forming' substances in the entoderm, and by the well-known cytological specificity of the early nerve cells, but especially by the specific water-holding capacity of the embryonic nervous system,[13] a specificity, identical with that of the adult system, and conceivable only as the outcome of a specific physical-chemical organization.

Rhumbler, for similar reasons, considers as a second possibility, "dass die *Zelloberflächen der sich einstülpenden Zellen so stark anders beschaffen wären* als diejenigen der sich nicht einstülpenden Ectodermzellen" (loc. cit., p. 450), that only the former react toward the blastocoelic fluid in the manner necessary to insure invagination.

Case C. Finally Rhumbler suggests that the greater size of the entoderm cells and their greater readiness to react appropriately to the influence of the blastocoelic fluid, might coöperate, and together bring about the decrease in surface tension required for the change in shape.

Although Rhumbler seems to me to place the emphasis where it belongs, certain qualifications appear to be either necessary or pertinent. In the first place, while the incompressibility of the blastocoelic liquid follows necessarily from the physical properties of fluids in general, it cannot be employed to explain

[13] See Glaser, The water-content of the embryonic nervous system. Science, vol. 39, p. 730.

in Case A and subsequent cases, why the ectoderm cells fail to invaginate, for if the fluid remained within the blastocoel, its incompressibility would of course render the invagination of the entoderm cells equally impossible.

In the second place, the explanatory value of 'surface tension' does not seem to me acceptable without certain reservations. That it may be the important factor, cannot be denied, neither can its importance be considered as demonstrated. Surface tension, together with the Gibbs-Thompson principle, gives an adequate physical explanation for the concentration of certain substances in the surface of the cell, but inasmuch as the substances so concentrated undergo changes in aggregation resulting in the formation of solid films, the application of the laws of surface tension meets with some difficulties. As long as we have no clear conception of the order of magnitude of the 'surface tension' of cells, and moreover lack really adequate methods of measurement, it seems rather questionable to lay too much emphasis on this particular factor. As Loeb[14] has pointed out, even in the relatively simple cases of amoeboid movement, mere changes in surface tension hardly seem adequate, for

> If it is true that the Amoeba is covered with a solid film, one condition for the formation of a pseudopodium must be a local liquefaction of protoplasm. In consequence of such liquefaction new protoplasm must flow out, which subsequently will form a new solid film at its surface. This may again be liquefied, and a new streaming may occur, etc. Such liquefactions can be caused by lack of oxygen . . . .; but they may also be caused by other chemical changes. I am inclined to believe that phenomena of liquefaction play at least some rôle in the processes of protoplasmic motion.

If such liquefactions occur in the neural and gastral plates, they, rather than alterations in surface tension, might be the important factors.

Until the possibilities have been experimentally limited or defined, it seems unwise to specify too carefully which particular surface effect, or combination of surface effects, is responsible for the change in shape undergone by the cells in the folding plates.

[14] Jacques Loeb, The dynamics of living matter, p. 57.

Nevertheless, even without this specificity, desirable as it would be, it is possible to apply Rhumbler's general thesis to the folding of the neural plate.

Let $ABCD$ (fig. 3) represent a cell in the neural plate. Along the two sides AD and BC the cell is bounded by others like itself. The side AB limits the system toward the external world, the side DC toward the internal, but nevertheless extra-neural, environment. The shape and position of the cell is the expression of a state of equilibrium which depends not only on the nature of the physical-chemical system, $ABCD$, but equally upon conditions outside.

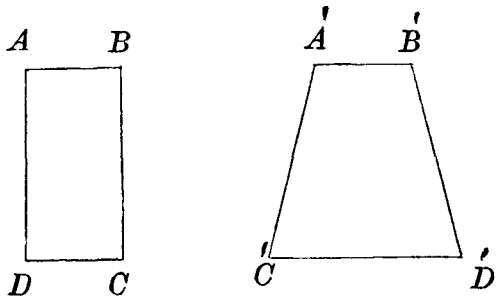

Figure 3

In the neural tube the shape of this cell is represented by $A'B'C'D'$, and this also is the expression of an equilibrium dependent on internal and external conditions. Obviously, however, since the shape and perhaps also the position of the cell differ from those it originally had, the new equilibrium is not indentical with the old. Now the chances of a disturbance of equilibrium along the lines DA and CB, are not very great for the cell is bounded on these sides by chemical systems like itself. Along the line AB also the chances of disturbance are small, because here the cell abuts upon an external environment whose constancy is relatively high. Along the line DC, however, the cell is subjected to influences due to what is going on in the rest of the embryo, and as important changes are constantly occurring within every developing organism, it is certainly not

unreasonable to imagine that the internal environment, compared with the external, is relatively unstable. From the morphological standpoint, this is only another way of saying that development is taking place, from the physiological it is almost self-evident, and with reference to one chemical factor, at least, I know it to be true, for whereas *the frog's egg is neutral in its reaction to litmus, the contents of the young larvae, not yet hatched, are distinctly acid.*

Whether the transition from neutrality to acidity, or some other chemical change, is important, certainly the relative inconstancy of the internal extra-neural environment is no assumption. Rhumbler attributes a similar instability to the blastocoelic fluid, and imagines that when certain substances have reached a certain concentration, a lowering of the surface tension in the entodermal plate will result. Correspondingly, if such a lowering should occur in the neural plate, the side AB would lengthen, the cell would assume the shape $A'B'C'D'$, and the observed translocation of intra-cellular contents, and necessarily also the observed folding, would be accounted for.

For reasons already given, I prefer to assume, instead of a lowering of the surface tension, simply 'a surface effect.' This does not exclude the factor emphasized by Rhumbler, but leaves room for such other possibilities as liquefaction, 'etching' and changes in permeability. Any of these, singly or in combination might result in a weakening of the internal surface, and though the effect of such weakening would be identical with that brought about by a lowering of the surface tension the actual mechanism might nevertheless be quite different. But what evidence have we for this 'surface effect'?

A surface effect necessarily involves a change in permeability. and a change in permeability may be followed either by a gain or loss of water.[15]

Since now the nervous system demonstrably, and the entoderm, probably, increase in volume during folding, and since

[15] Glaser, On inducing development in the sea-urchin (Arbacia punctulata) together with considerations on the initiatory effect of fertilization. Science. vol. 38, p. 446.

this increase is the outcome, not of cell-multiplication, but of water absorption, a surface effect, involving a change in permeability is practically certain. Since the sense of this change, for water at least is positive, it seems likely that the affected surface would be mechanically weakened by it.[16] With the initiatory changes in folding accounted for on this basis, the possibility that the absorption of water has after all a 'formative' influence, once more arises, for even if an increase in volume cannot of itself induce folding, it might accelerate or retard the process when initiated by other forces.

A colloidal rod or tube which bends in boiling water can easily be shown not to have a uniform dry substance, and a board may warp through differential water absorption. Since the water must enter the neural plate via the affected surface, a differential localization would greatly facilitate folding.[17]

I have tried many experiments for the purpose of detecting such differences in the intra-cellular concentration of water, but have not succeeded in finding any evidence of differential distribution. Indeed on physical-chemical grounds, such an arrangement of the water does not appear very likely. It is of course still less likely to be distributed so as to oppose the folding. Most probably the distribution is what one might expect, an arrangement, which indeed affects the size of the cells, but has no other formative, or morphogenetic significance at this particular stage of development.

[16] See Höber, Physikalische Chemie der Zelle und der Gewebe, 3rd ed.

[17] In the case of the gastral plate, the increase in size must be due to the absorption of water from the blastocoel. As a matter of fact the blastocoelic fluid decreases in amount, and this decrease is absolutely essential for invagination, not because the absorbed water is differentially distributed in the entoderm cells, or because its removal from the cavity produces a suction, but because in this way an insurmountable obstacle, an incompressible liquid, is removed. The following experiment is not without interest in this connection. Normal free-swimming Arbacia blastulae placed in sea water diluted with an equal quantity of distilled, absorb a great amount of water of which part enters the blastocoel and part remains in the cells themselves. The development of such inflated blastulae is arrested, but if returned at the end of 24 hours to normal sea water, they instantly regain their former size, and development once more proceeds normally.

VIII. SUMMARY

We may summarize the foregoing considerations as follows:

1. The folding process by which the neural plate becomes converted into a neural tube, does not depend on coercive pressure from without, and in this sense is autonomous (Roux).

2. The cells of the neural plate, actively engaged in folding undergo the change in shape emphasized by Rhumbler in the invaginating gastral plate.

3. During the period of involution in Cryptobranchus cell-multiplication appears to proceed at a negligible rate, a conclusion practically identical with the inference drawn by Morgan from a study of gastrulation in Sphaerechinus.

4. If the folding nervous system be divided into an inner and outer zone, an outward migration of nuclei during involution is demonstrable. Translocation of intra-cellular contents also occurs in invaginate gastrulation (Rhumbler).

5. During involution no doubt the outer zones increase at the expense of the inner, but the exact extent of this is difficult to determine since the inner zones also increase.

6. This 'growth' of the nervous system is not the result of the synthetic processes ordinarily associated with cell-multiplication, but is the outcome of water absorption.

7. The folded nervous system contains 80 per cent of water and 20 per cent of dry substance; the entire embryo, on the other hand, has only 58 per cent of water and 42 per cent of dry substance. For the isolated yolk-sac of the frog's embryo the corresponding figures are 55 per cent and 45 per cent respectively. During involution, therefore, differential water absorption takes place in the nervous system.

8. Such differential absorption can also be inferred for the entoderm from Morgan's observations on the gastral nuclei of Sphaerechinus, for these increase in volume and lie further apart after gastrulation than before. These changes would be expected if the gastral plate absorbs water at this time.

9. As shown by the behavior of Asterias ova in normal and hypotonic sea water, nuclear size may be used as an index of the relative water content of the cell.

10. The 'morphogenetic' or 'formative' effect of the water absorption is in all probability zero. The size of the cells is of course increased, but such increase can only affect the process of involution if the absorbed water is differentially distributed in the cell. For this there is no evidence, and little probability. The real significance of the water absorption seems to lie in the fact that it is a symptom of a surface effect which involves apparently a change in the permeability of the neural plate cells.

11. The surface affected is more likely to be the one bounded by the extra-neural, intra-embryonic environment than any other.

12. The contents of the frog's egg are neutral to litmus; those of the larva not yet hatched, acid.

13. On the basis of these facts and certain other considerations, it is proposed to modify Rhumbler's theory of autonomous folding by substituting 'surface' effect for 'surface tension.' This does not exclude surface tension from the list of possible factors, but leaves room for others such as liquefaction of the surface, 'etching,' and changes in permeability, all of which are possible in solid films.

14. The surface effect indicated by the absorption of water during folding, may very possibly result in a mechanical weakening from which the involution of the neural, and the invagination of the gastral plates follow, not only automatically, but with the demonstrated autonomy.

POSTSCRIPT

After this manuscript had been completed, I discovered the very recent and important contribution of Gurwitsch, "Der Vererbungsmechanismus der Form" (Arch. f. Entwicklungsmech., Bd. 39, p. 516).

This work falls naturally into two divisions, one theoretical, the other dealing with concrete observations. Inasmuch as the theoretical discussion involves the conceptions of 'Partialzweck' and the 'dynamisch präformirte Form' I must postpone, perhaps indefinitely, the attempt to enter these difficult regions.

With respect to the concrete results of Gurwitsch, it is to be noted that the distribution of the nuclei in the folded regions studied by him, is identical with the distribution I have found. However, there are also significant differences, particularly in connection with the rôle assigned to cell-multiplication and cell-migration, but these 'discrepancies' are not necessarily indicative of errors, instead they may be only the inevitable results of dealing with two quite different periods of development as well as with different materials. The neural plate of Cryptobranchus shows that folding can take place without cell-multiplication, and the conclusion that it does so, is in no wise affected by the frequent occurrence of mitoses in the corresponding stages of other forms. These constitute a less favorable material inasmuch as they do not present the simplest case. However, even these cases may prove to be instructive for the localization of the mitoses in the mammalian neural plate is such that cell-multiplication, if effective at all, would not facilitate, but oppose the process of involution.

I am inclined to hazard the guess even now, that the cell-migrations in the folds studied by Gurwitsch are effects rather than causes, but as I shall approach these stages from another angle, in a forthcoming paper, I shall reserve until that time a full discussion of the bearing of Gurwitsch's basic observations. In the meantime, in order to avoid misunderstanding and anticipate its attendant needless difficulties, I should like to impress on the reader as strongly as possible, that the mechanism of the process so carefully analysed by Gurwitsch, involves factors which are not concerned in the autonomous folding of the neural plate. In the present instance, the assumption that the mechanics of every folding process are identical with those of every other, would certainly lead to erroneous conclusions.