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EXPERIMENTAL EMBRYOLOGY

BY

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OXFORD
AT THE CLARENDON PRESS
1909

HENRY FROWDE, M.A.
PUBLISHER TO THE UNIVERSITY OF OXFORD
LONDON, EDINBURGH, NEW YORK
TORONTO AND MELBOURNE

PREFACE

For the biologist there are, I conceive, in the main two problems. One is to give an account of those activities or functions by means of which an organism maintains its specific form in an environment. The other is to find the causes which determine the production of that form, whether in the race or in the individual. The solution of the first of these problems is the business of physiology, in the usual sense of the term. The second falls to morphology.

It is with the origin of form that we are here concerned, and in particular with its origin in the individual. The endeavour to discover by experiment the causes of this process—as distinct from the mere description of the process itself—is a comparatively new branch of biological science, for Experimental Embryology, or, as some prefer to call it, the Mechanics of Development (*Entwicklungsmechanik*), or the Physiology of Development, really dates from Roux's production of a half-embryo from a half-blastomere, and the consequent formulation of the 'Mosaik-Theorie' of self-differentiation. That hypothesis has been the focus of much fruitful criticism and controversy, the experiment has been followed by many others of the same kind, and the present volume is an attempt to sketch the progress of these researches and speculations on the nature and essence of differentiation, as well as of those which deal with growth, cell-division, and the external conditions of development.

In writing this review I have had the very great advantage of an excellent model in the textbook of Korschelt and Heider (*Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere*, Allgemeiner Theil, Jena, 1902). I have

indeed followed the general arrangement adopted by these authors fairly closely except in one respect. I believe so strongly that the processes of growth and cell-division, though they always (in the Metazoa) accompany, are yet distinct from, differentiation, that I have felt justified in treating them in a chapter apart from the other internal factors of development. The external factors—whether of growth, cell-division, or differentiation—are discussed in Chapter III, and the ground is thus cleared for a consideration of the real problem —the differentiation of specific form.

The last chapter is devoted to the theories, scientific and philosophical, of Hans Driesch. I sincerely hope that Herr Driesch will allow my great admiration for the former to atone in some measure for my inability to accept the tenets of neo-vitalism.

It is a very great pleasure to me to acknowledge my indebtedness to the Delegates and Secretaries of the Clarendon Press, and in particular to Professor Osler, for undertaking the publication of this book, as well as for the pains which have been expended in its preparation. Dr. Osler also took the trouble to read through the whole of the manuscript, and Mr. G. W. Smith and Dr. Haldane have been kind enough to look through certain chapters.

To Dr. Ramsden I am under great obligations for his assistance in that part of Chapter II, Section 1, in which surface-tensions are discussed; to Dr. Vernon for calling my attention to Roberts's work on Anthropometry, and to Mr. Grosvenor for the information embodied in the foot-note on p. 89. Mr. A. D. Lindsay has given me invaluable assistance in those sections of Chapter V which deal with the philosophy of Kant, while, for Aristotle, I was fortunately able to attend Professor Bywater's lectures on the *De Anima*.

I can hardly express the debt I owe to Mr. J. A. Smith for much friendly counsel and criticism, although he is, of course,

in no way responsible for the philosophical speculations in which I have ventured to indulge.

The illustrations are largely borrowed from Korschelt and Heider's work, and I must thank Herr Gustav Fischer, of Jena, for his readiness in supplying the blocks. Others are from the original publications[1], and I am obliged to the proprietors for permission to make use of them. A few are my own.

In the appendices will be found an account of some recent work on the relation between the symmetry of the egg and that of the embryo in the Frog, and on the part played by the nucleus in differentiation.

[1] *Proceedings of the Boston Society of Natural History*, the *Journal of Experimental Zoology* (Williams & Wilkins, Baltimore), the *American Journal of Physiology* (Ginn & Co., Boston), *Zellen-Studien* (Fischer, Jena), *Verhandlungen der Anatomischen Gesellschaft* (Fischer, Jena), *Ergebnisse über die Konstitution der chromatischen Kernsubstanz* (Fischer, Jena), *Archiv für mikroskopische Anatomie* (Cohen, Bonn), *Archiv für Entwicklungsmechanik* (Engelmann, Leipzig), and the *Popular Science Monthly* (Appleton & Co., New York).

Recte ponitur: *Vere scire esse per causas scire.*

BACON.

Πᾶν γὰρ τὸ γινόμενον ἔκ τινος καὶ εἴς τι ποιεῖται τὴν γένεσιν, καὶ ἀπ' ἀρχῆς ἐπ' ἀρχήν, ἀπὸ τῆς πρώτης κινούσης καὶ ἐχούσης ἤδη τινὰ φύσιν ἐπί τινα μορφὴν ἢ τοιοῦτον ἄλλο τέλος· ἄνθρωπος γὰρ ἄνθρωπον καὶ φυτὸν γεννᾷ φυτὸν ἐκ τῆς περὶ ἕκαστον ὑποκειμένης ὕλης. Τῷ μὲν οὖν χρόνῳ προτέραν τὴν ὕλην ἀναγκαῖον εἶναι καὶ τὴν γένεσιν, τῷ λόγῳ δὲ τὴν οὐσίαν καὶ τὴν ἑκάστου μορφήν.

ARISTOTLE.

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CHAPTER I

INTRODUCTORY

THAT living creatures reproduce their kind is a fact which is familiar to us all, but it is the peculiar privilege and province of the embryologist to observe and to reflect upon that marvellous series of changes whereby, out of a germ which is comparatively structureless and unformed, a new organism is developed which is, within the limits of variation, like the parents that gave it birth.

Development is the production of specific form. From a particular kind of germ only a particular kind of individual will normally arise, though unusual conditions may lead to the formation of an abnormality or monstrosity. Thus, while the germ is the material basis, development is the mechanism of inheritance. The student of heredity seeks to express in terms which shall be as exact as possible, ultimately mathematically exact, the degree of similarity between the offspring on the one hand, and parents and more remote ancestors on the other. The embryologist has under his very eyes the process by which that similarity is brought about, and even when the resemblance shall have been stated with all possible precision, it will still remain for him to give an explanation of those changes whereby the inheritable peculiarities of the species are handed on from one generation to the next.

Used in the widest sense of the word, development includes not merely the formation of a new individual from a single cell, whether fertilized or not, but also the phenomena of budding and regeneration. In a narrower sense, however, the term is restricted to the first of these processes, and a corresponding distinction is made, however artificially, between Experimental Embryology and Experimental Morphology, when the subject is treated from a physiological point of view.

In development two factors are obviously involved. One is growth, or increase of volume, more correctly increase of mass; the other is differentiation, or increase of structure; and, in multicellular organisms, both these factors are accompanied by division of the nucleus and the cell.

Segmentation is the first sign, or almost the first sign, the developing ovum gives of its activity; and this cutting up of the egg-cell into parts, which marks the beginning, is also continued during the later stages of ontogeny, and goes on as long as the life of the organism endures.

Growth is especially characteristic of the embryonic and of the adolescent organism. It occurs at different rates in the different cells, and indeed the growth of a group of cells is in itself often an act of differentiation. Growth may depend upon the absorption of water or the assimilation of other substances, and this may lead simply to an increase in the size of internal cavities, as in the blastula of Echinoderms or the Mammalian blastocyst; to an increase in the volume of the living protoplasm; or to the secretion of intracellular or intercellular substances, either organic (for example, the notochordal vacuoles, the matrix of cartilage and bone) or inorganic (the skeletal spicules of Echinoderm larvae, Sponges, and Coelenterata). This increase of mass is not only conditioned by the presence of food in the form of substances found in the environment, but depends on such external circumstances as temperature, atmospheric and osmotic pressure, and so forth.

But while the embryo is dividing up its material—a material which is already to a certain extent heterogeneous, composed, for example, of protoplasm and deutoplasm or yolk—while it is increasing its mass, it is also undergoing a process of differentiation; and, as even a superficial acquaintance with embryology will inform us, one of the most characteristic features of differentiation is that it occurs in a series of stages which follow upon one another in regular order and with increasing complexity. When segmentation has been accomplished—sometimes, indeed, during segmentation—certain sets of cells, the germ-layers, become separated from one another. Each germ-layer contains the material for the formation of a definite set of

organs—the endoderm of a Vertebrate, for instance, contains the material for the alimentary tract and its derivatives—gill-slits, lungs, liver, bladder, and the like; the germ-layers are therefore not ultimate but elementary organs, and elementary organs of the first order. In the next stage these primary organs become subdivided into secondary organs—as the archenteron of an Echinoderm becomes portioned into gut and coelom-sac, or the ectoderm of an Earthworm into epidermis, nervous system, and nephridia—and in subsequent stages these again become successively broken up into organs of the third and fourth orders and so on, until finally the ultimate organs or tissues are formed, each with special histological characters of its own. This end is, however, not necessarily reached by all the tissues at the same time. Indeed, it is no uncommon thing for certain of them to attain their final structure while the others are yet in a rudimentary condition; thus, in some Sponges the scleroblasts begin to secrete spicules in the larval period, nematocysts may be formed in the Planula of the Coelenterates, notochordal tissue is differentiated in the newly hatched tadpole of the Frog; and, speaking generally, larval characters are developed at a very early stage.

To this regular sequence of ontogenetic events Driesch has applied the term 'rhythm', the rhythm of development. The organs of the body are, however, by no means all formed of single tissues—bone, epithelium, blood, and the rest—but are compounded, frequently of very many tissues, and this 'composition', to quote a term of Driesch's again, is another of the obvious features of organogeny.

While, therefore, in the last resort all differentiation is histological, that final result, the assumption by the cells of their definitive form, is only achieved after many changes have taken place in the position of the parts relatively to one another while the organs are being compounded, and so its specific shape conferred upon the whole body.

It is possible to find a few general expressions for the manifold changes that take place in the relative positions of the parts. Several years ago, in 1874, His compared the various layers of the chick embryo to elastic plates and tubes; out of these he

suggested that some of the principal organs might be moulded by mere local inequalities of growth—the ventricles of the brain, for instance, the alimentary canal, the heart—and he further succeeded in imitating the formation of these organs by folding, pinching, and cutting india-rubber tubes and plates in various ways. This analysis, however, deals only with the foldings of flat layers, and must be supplemented by a more exhaustive catalogue of the processes concerned in ontogeny, such as that more recently suggested by Davenport. Davenport resolves the changes in question into the movements of cells or cell aggregates, the latter being linear, superficial, or massive, and within the limits of these categories the phenomena are susceptible of further classification. The catalogue proceeds as follows :—

I. The Movements of Single Cells.

1. Migration of nodal thickenings in a network of protoplasm: e. g. the migration of the 'cells' to the surface of the Arthropod ovum to form a blastoderm, the movements of vitellophags, and yolk-nuclei (Fig. 1).

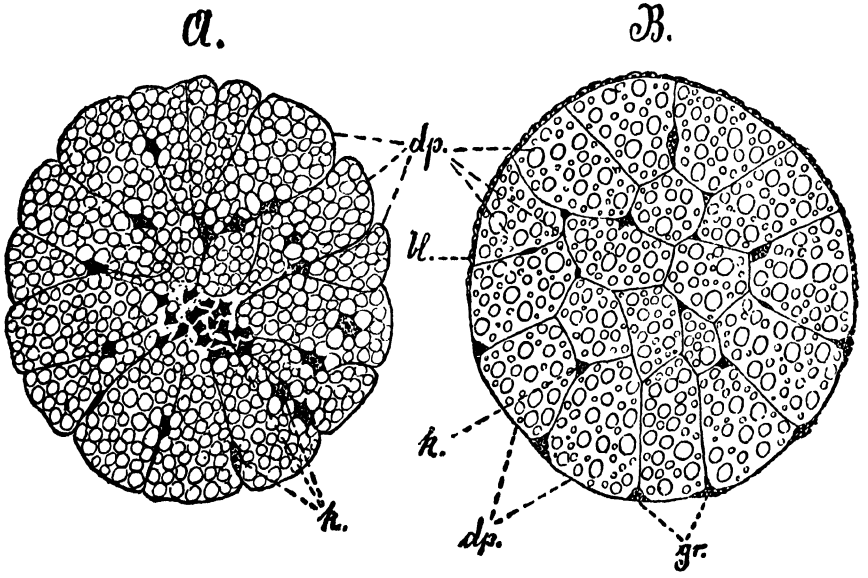


Fig. 1.—Sections of the egg of *Geophilus ferrugineus* showing two stages in the formation of the blastoderm: *bl*, blastoderm; *dp*, yolk pyramids; *gr*, groups of blastoderm cells on what will be the dorsal side; *k*, nuclei surrounded by masses of protoplasm. (After Sograff, from Korschelt and Heider.)

2. Migration of free amoeboid bodies: e. g. the mesenchyme cells in the Echinoderm gastrula, the lower layer cells of Elasmobranchs, the blastomeres amongst the yolk-cells in Triclads and Salps.

3. Aggregation of isolated cells.

a. Linear aggregates: e. g. the kidney of Lamellibranchs, the yolk-gland of Turbellaria, capillary blood-vessels.

b. Superficial aggregates: e. g. the blastoderm of Arthropods, the formation of the imaginal gut-epithelium in some Insects.

c. Massive aggregates: e. g. the gemmule of Sponges, the spleen of Vertebrates.

4. Attachment of isolated cells to another body: e. g. the union of muscles to the shell in Mollusca and Arthropoda, of tendon to bone in Vertebrates, the application of skeletal cells to the notochord.

5. Investment and penetration by isolated cells: e. g. the follicle cells between the blastomeres in Tunicata, the muscles of the gut in various animals, the septa of the corpus luteum, the formative cells of the vitreous body of the Vertebrate eye, the immigration of the nephric cells in the Earthworm.

6. Transportation of bodies by wandering cells: e. g. of the buds in Doliolidae.

7. Absorption by wandering cells: e. g. phagocytosis in Insect pupae and in the tadpole's tail.

8. We may place here the frequent alterations in the shapes of cells, which do not apparently involve growth: e. g. when flat cells become columnar.

II. The Movements of Cell Aggregates.

A. Linear Aggregates.

1. Growth in length: e. g. the growth of the roots and stems of plants, of the stolons and hydranths of Hydroids, the outgrowth of nerves, of the necks of unicellular glands, the growth of the blood-vessels from the area vasculosa into the body of the Chick embryo, of blood-vessels towards a parasite, the growth of mesoblastic and other germ-bands in Annelids, the back-growth of the Vertebrate segmental duct, and the like.

2. Splitting.

α. At the end, that is, branching: e. g. of nerves, blood-vessels, kidney tubules, glands, tentacles.

β. Throughout the length: e. g. the segmental duct of Elasmobranchs, the truncus arteriosus of Mammalia.

3. Anastomoses: e. g. of the dorsal and ventral roots of the spinal nerves, of nerve plexuses, of capillaries, of bile capillaries, of the excretory tubules of Platyhelmia.

4. Fusion with other organs: e. g. of a nerve with its end-organ, of the vasa efferentia with mesonephric tubules, of nephridia with the coelom in Annelida.

B. Superficial aggregates.

i. Increase of area.

a. Growth of a sphere.

1. Equal in all directions: e. g. the blastula of Echinoderms.

2. Unequal.

α. Unequal in different axes: e. g. the conversion of a spherical blastula into an ellipsoid Planula in Coelenterata, or into an ellipsoid Sponge larva, or of the spherical into the ellipsoid blastocyst in Mammalia.

β. Unequal at different poles: e. g. the formation of ovoid forms, such as Planulae, the club-shaped gland of *Amphioxus*, the auditory vesicle of Vertebrata.

b. Growth of a plane surface.

1. Equal in all directions: e. g. the growth of the blastoderm over the yolk in Sauropsida, or Cephalopoda.

2. Unequal.

α. When parts lying in one plane move out of that plane: e. g. invaginations and evaginations of all descriptions. (Fig. 2).

β. When parts—e. g. a row of cells—lying in one plane are moved in that plane: e. g. the germ-bands of *Clepsine*, by the growth of the epiblast (Fig. 3).

ii. Alterations of thickness.

a. Increase: thickenings: e. g. the formation of the central nervous system in Teleostei, the formation of gonads from the

coelomic epithelium, the development of hair follicles, the trophoblast in the Mammalian placenta (Fig. 4).

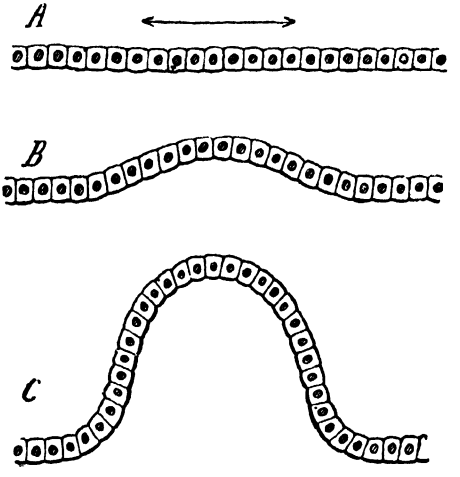


FIG. 2.—Three stages of an invagination or evagination. (After Korschelt and Heider.)

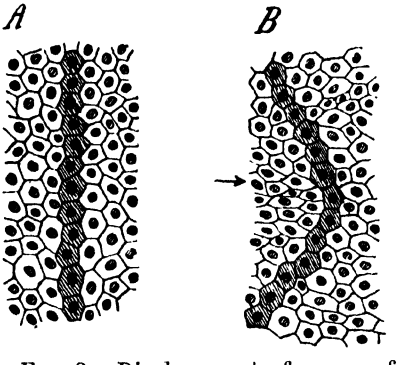


FIG. 3.—Displacement of a row of cells in an epithelium. (After Korschelt and Heider.)

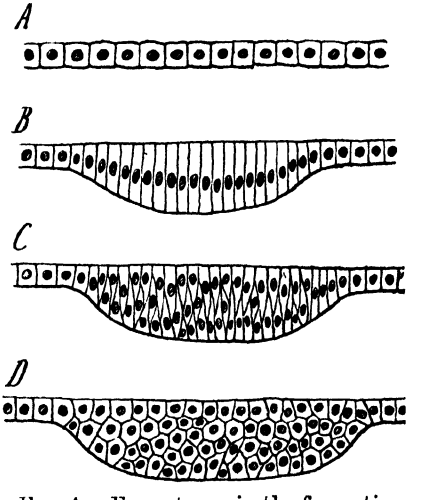


FIG. 4.—Four stages in the formation of an epithelial thickening of many layers. (After Korschelt and Heider.)

b. Decrease: thinnings: e.g. in the roof of the thalamencephalon and medulla, the outer layer of the lens, the trophoblast of the Mammalian blastocyst.

iii. Interruptions of continuity.

a. By the atrophy of part of a layer: e. g. when the floor of the archenteron together with the underlying paraderm disappears in Amniota (Fig. 5).

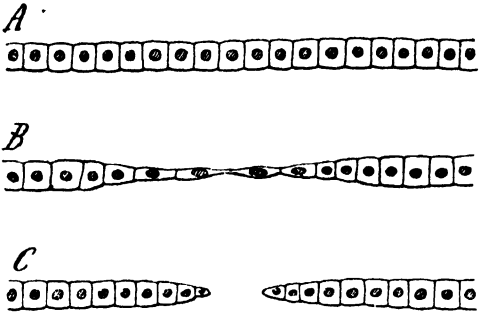


FIG. 5.—Three stages in the development of an interruption of continuity perpendicular to the surface of an epithelium. (Perforation.) (After Korschelt and Heider.)

b. By the detachment of a part: e. g. of the medullary plate from the ectoderm in *Amphioxus* (Fig. 6), of the notochord from the roof of the archenteron in Urodela and *Petromyzon*.

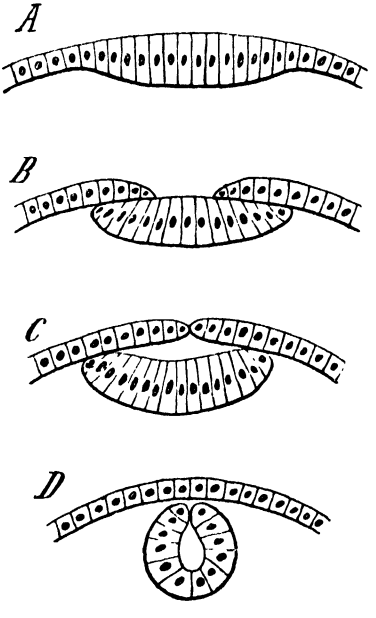


FIG 6.—Scheme of the formation of the medullary canal in *Amphioxus*. (After Korschelt and Heider.)

iv. Concrescence of layers.

a. By their margins: e. g. the edges of the ectoderm over the medullary plate, the edges of the embryonic ectoderm inside

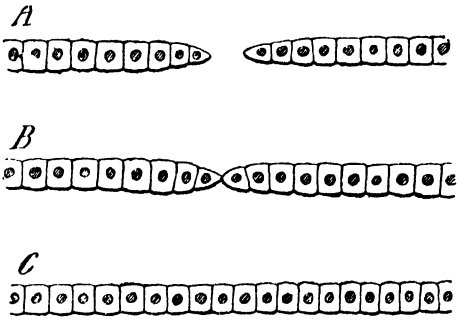


FIG. 7.—Fusion of two cell plates by their margins.
(After Korschelt and Heider.)

the serosa of *Sipunculus*, the embryonic plate with the trophoblast in some Mammals (Figs. 7, 9).

b. By their surfaces (Figs. 8, 9, 10): e. g. when the stomodaeum or proctodaeum open into the gut, when the medullary

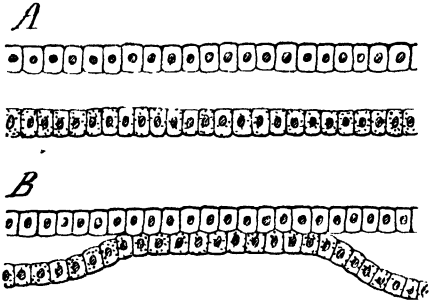


FIG. 8.—Fusion of two cell plates by their surfaces.
(After Korschelt and Heider.)

folds meet, when the edges of the peritoneal groove close to form the canal of the oviduct in Amphibia and Amniota.

This concrescence is commonly followed by a communication of the cavities on opposite sides of the adherent layers, as when the stomodaeum opens into the gut, or the amnion-folds unite; but not necessarily, as when the somatopleure fuses with the trophoblast, or the allantois with the somatopleure in Mammalia.

v. Splitting of a layer into two: e. g. the inner wall of the pineal vesicle in Lacertilia (Fig. 11).

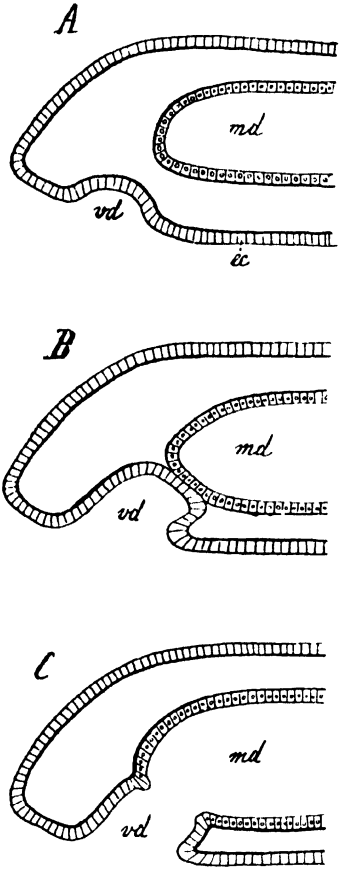


FIG. 9.—Diagram to illustrate the formation of the fore-gut (stomodaeum) and its opening into the mid-gut: *ec*, ectoderm; *md*, mid-gut; *vd*, stomodaeum. (After Korschelt and Heider.)

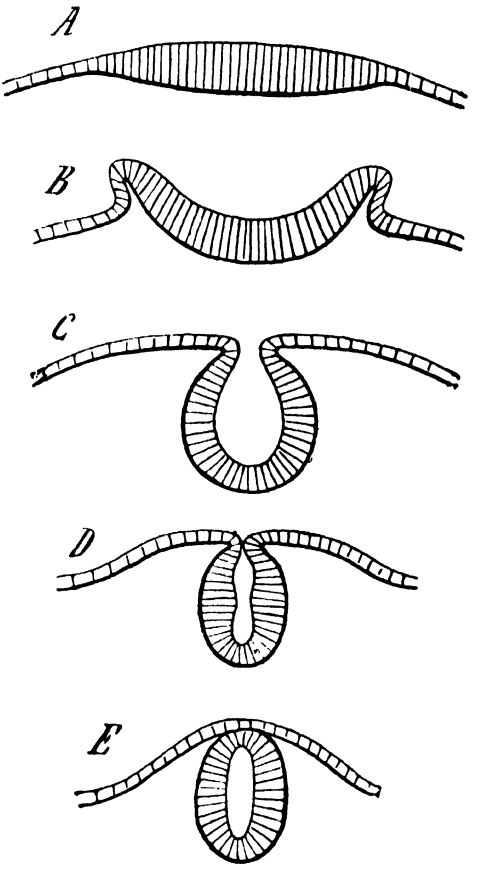


FIG. 10 – Scheme of the formation of the medullary canal in a Vertebrate. (After Korschelt and Heider.)

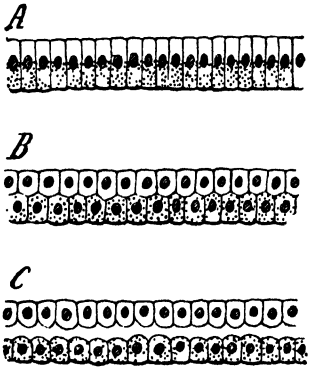


FIG 11.—Three stages in the development of an interruption of continuity parallel to the surface of an epithelium. (Delamination) (After Korschelt and Heider.)

C. Massive aggregates.

i. Changes in volume.

α. Unequal in different axes: e. g. when the spherical larva becomes cylindrical in Dicyemidae.

β. Unequal at different points: e. g. the outgrowth of limb-buds of Vertebrates and other forms, of the buds of plants.

ii. Rearrangement of material.

a. Simple rearrangement of cells: e.g. in the formation of the concentric corpuscles of the thymus, in the development of kidney tubules in the metanephric blastema of Amniota, in the grouping of the cells to form ectoderm, gut and atrium in the Salps.

b. Development of an internal cavity: e. g. segmentation cavities, lumina of ducts and blood-vessels, of the coelom and many generative organs.

c. Dispersion of the elements of an aggregate: e. g. in gemmule formation in certain Sponges, in unipolar immigration in some Sponges and some Coelenterates, in the liberation of the germ-cells.

iii. Division of masses.

a. By constriction: e. g. the segmentation of the mesoderm and neural crest.

b. By splitting: e. g. the nervous system from the ectoderm in Teleostei and many Invertebrates, the notochord from the roof of the archenteron.

iv. Fusion of masses: e. g. of originally separate nerve ganglia (Vertebrates, Arthropods, Annelids), of myotomes, of somites in Arthropods.

v. Attachment of one mass to another: e. g. of sclerotome to notochord.

It will be seen that this *résumé* of the principal kinds of movement executed by the developing parts extends His's principle of the local inequality of growth from flat layers to linear and massive aggregates and at the same time includes the movements of isolated cells. Davenport, however, is not content merely to give a simple classification of the phenomena; he goes further, and endeavours to express them in terms of responses to stimuli, an idea due in the first instance to Herbst.

Thus he suggests that the migrations of vitellophags and mesenchyme cells, the thickenings, thinnings, and perforations of flat layers, the rearrangements of cells in a massive aggregate, their dispersion, the constriction, and splitting and fusion may be regarded as tactic responses, the growth in various ways of linear aggregates, the concrescence of layers and masses as so many tropic responses to stimuli which may be positive or negative and exerted by other organs or by agents in the world outside.

Now it is clear that the analyses both of His and Davenport aim at something more than a mere description of ontogenetic events, for a serious attempt is here made to give a causal, if you will a mechanical, explanation of those events, and the subject thereby raised at once from the level of mere morphology or morphography to a loftier, aetiological point of view.

There are, indeed, two methods by which embryology, like any other branch of zoology, may be investigated. One is purely descriptive, anatomical, morphological. By this method, truly, great results have been achieved. The life-histories of members of all the most important groups of the animal kingdom have been worked out, and the science of Comparative Embryology has been built up. Nor has an explanation of the process been lacking. For ontogeny is, the fundamental Biogenetic Law assures us, a recapitulation of and therefore explicable in terms of phylogeny; and since on this principle the individual repeats in its development the ancestry of its race, embryology affords a means of tracing out the relationships of the organism and establishing the homologies of its parts.

Unfortunately a more intimate acquaintance with the facts has made it abundantly clear that development is no mere repetition of the ancestral [illegible] that the organism has manifold ways of attaining its [illegible] that those resemblances in early stages which were held to [illegible] the most triumphant vindication of the Biogenetic Law bear no constant relation to the similarities of adult organization, that the attempt to find in development an absolute criterion of homology is vain.

The facts thus remain unexplained, as in truth it was only to be supposed that they would. A method, however comparative, which relies on mere observation, and is content to wait for

Nature's own experiments, cannot hope to arrive at sound inductions, or to establish general laws of causation.

There is, however, another way. Development, the production of form, may be regarded as one of the activities, one of the functions of the organism, to be investigated like any other function by the ordinary physiological method of experiment; and the ideal of the experimental or physiological embryologist is to give a complete causal account, whether the causes are external or internal, of each stage, and so of the whole series of ontogenetic changes, his weapon, to borrow Roux's splendid phrase, 'die Geistesanatomie, das analytische causale Denken.'[1]

This effort is, of course, no modern one. Speculation into the nature and essence of development begins, indeed, with the Greeks, and theories of fertilization and development are to be found in the writings of Aristotle.[2] In fertilization the male element, which, according to Aristotle, provides the formal and efficient causes in providing the necessary perceptive soul, acts upon the mere matter, endowed only with a nutritive soul, which is given by the female, in the same sort of way, to use his own illustration, as rennet coagulates milk. In the germ thus formed the parts of the embryo, which can only be said to pre-exist potentially, arise not simultaneously but in gradual succession, first the heart, then the blood, the veins from the heart and the various organs about the veins by a process of condensation and coagulation, the anterior parts of the body being built up first.

This Aristotelian doctrine appears to have persisted through the Middle Ages; it reappears in the seventeenth century in the pages of Hieronymus Fabricius ab Aquapendente and his pupil William Harvey in essentially the same form, although both authors differ from Aristotle in certain matters of observational detail. Thus Fabricius[3] states that 'ope generatricis facultatis pulli partes, quae prius non erant, produci atque ita ovum in pulli corpus migrare', while Harvey[4] gives to development as thus conceived of the name of 'Epigenesin sive partium superadditionem', though he believes that in some cases (Insects) the

[1] Roux, 1885.

[2] Aristotle, *De Gen.*, i. 20. 18; ii. 4. 43; 5. 2, 3, 10; 6. *De An.*, ii. 4. 2, 15; 5; 6.

[3] Fabricius, l. c., p. 22.

[4] Harvey, l. c., Ex. 44.

process is one of 'metamorphosis' or the simultaneous origin of all parts. In generation properly so called, however—in the development of the Chick, for example—the process 'a parte aliqua, tanquam ab origine, incipit; eiusque ope reliqua membra adsciscuntur: atque haec per epigenesin fieri dicimus: sensim nempe, partem post partem', and this 'pars prima genitalis' Harvey held, in opposition to Aristotle, to be the blood.[1]

But in spite of the exact observation and brilliant exposition of his followers, the teaching of Aristotle was destined to be overshadowed and eclipsed, temporarily at least, by a new hypothesis which, appearing first towards the end of the seventeenth century, swept the schools and universities, and dominated biological speculation for a hundred years.

This was the theory of Evolution or Preformation. According to it the future animal or plant is already present in miniature in the germ with all its parts complete, invisible or hardly visible it may be, but still there, and not merely 'potentiâ'; and in development there is no such thing as 'generation', but only growth, whereby that which was before impalpable and invisible becomes tangible and manifest to our eyes. A further and logical outcome of the hypothesis was the doctrine of 'emboîtement', enthusiastically described by Bonnet as 'une des plus belles victoires que l'entendement pur ait remporté sur les sens'.[2] The organism present already in the germ, with all its parts complete, possesses of necessity the germs of the next generation, and so on in indefinite though not in infinite regress, for as Bonnet is careful to tell us, 'Il ne faut pas supposer un emboîtement à l'infini, ce qui seroit absurde. La divisibilité de la matière à l'infini par laquelle on prétendroit soutenir cet emboîtement est une vérité géométrique et une erreur physique.'[3] Swammerdam solved the difficulty in another way. All the germs of the human race must have been present in the bodies of our first parents, and 'exhaustis his ovis humani generis finem adesse'.[4]

The theory became widely held. First put forward by Marcello

[1] Harvey, l. c., Ex. 50.
[2] Bonnet, *Cont. de la nat.*, 7me partie, c. ix; *Œuvres*, vol. iv, p. 270.
[3] Bonnet, *Consid. sur les corps org.*, c. viii; *Œuvres*, vol. iii, p. 74.
[4] Swammerdam, 1679, pp. 21, 22.

Malpighi in the memoir, 'De formatione pulli in ovo,' which he presented to the Royal Society of London in 1673, it was not only adopted by biologists of prestige, by Swammerdam, Haller, who in his early days had been an advocate of Epigenesis, de Buffon, and Bonnet, but secured the adherence of philosophers of such eminence as Malebranche and Leibniz.

In some cases it was accepted as a result of observation. Thus Malpighi,[1] in the treatise referred to, asserted that he had himself observed the chick in the unincubated egg, 'inclusum foetum animadvertebam, cuius caput cum appensae carinae staminibus patenter emergebat,' while de Buffon[2] expresses himself even more categorically. 'J'ai ouvert,' he says, 'une grande quantité d'œufs à différens temps, avant et après l'incubation, et je me suis convaincu par mes yeux que le poulet existe en entier dans le milieu de la cicatricule au moment qu'il sorte du corps de la poule.' To others, however, it was rather a matter of theoretical necessity. Haller explains his conversion from the contrary opinion by asking the very pertinent question, 'Cur vis ea essentialis quae sit unica tam diversas in animali partes semper eodem loco, semper ad eundem archetypum struit; si materies inorganica mutabilis et ad omnem figuram recipiendam apta est? Cur absque ullo errore ex gallinae mista materie ea vis semper pullum, ex pavone pavonem fabricatur?'

'Nil nisi vis dilatans et progrediens recipitur. Ab ea nihil sperarem nisi vasorum rete tamdiu continuo amplius futurum quamdiu vis expandens resistentiae superandae par est. Cur loco eius retis cor, caput, cerebrum, ren struuntur? Cur in singulo animali suus ordo partium? Ad eas quaestiones nulla datur responsio,' a charge which is, of course, perfectly just.[3]

Bonnet's argument is different. The heart of the chick, he points out, is already present in the egg; and since anatomy teaches that all the parts of an animal 'doivent avoir toujours coexisté ensemble', preformation follows as a matter of course.[4]

The belief in preformation continued paramount till towards the end of the eighteenth century, nor was it till the publication

[1] Malpighi, l. c., p. 4. [2] de Buffon, l. c., p. 351.
[3] Haller, 1778, VIII. i. 29, p. 121.
[4] Bonnet, *Cont. de la nat.*, 7me partie, c. ix; *Œuvres*, vol. iv, p. 261.

in 1774 of Caspar Friedrich Wolff's *Theoria Generationis* that the evolutionists were aroused from their dogmatic slumbers. Putting speculation on one side, Wolff returned to the method of Harvey, Fabricius, and may we not say also of Aristotle, the method of exact observation. He demonstrated the presence in the unincubated egg not of a complete organism, but of 'globules'; 'partes enim constitutivae, ex quibus omnes corporis animalis partes in primis initiis componuntur, sunt globuli,'[1] and described the epigenetic formation of the heart and blood-vessels, the central nervous system, the limbs and the 'Wolffian' bodies from these primary elements.

Development thus consists of the gradual production and organization of parts; 'embryonis partes sensim produci, mea observata suadent,'[2] and again, 'suppeditari prius partem, deinde eam organisari intelligitur'.[3]

The ground was thus taken from beneath the feet of the preformationists, and Epigenesis restored to its former place of honour as the fundamental expression of developmental fact.

Tacitly accepted by all the great embryologists of the nineteenth century—Pander, von Baer, Reichert, Bischoff, Remak, Kölliker, Kowalewsky, Haeckel—the epigenetic idea continued to control the progress of research. These were men who set themselves to describe the sequence of changes that the embryo passes through with all possible accuracy, and over as wide a range as might be of animal form. They made Comparative Embryology. On the facts that they discovered new light was shed by the doctrine of descent with modification, or evolution in the wider sense of the word. Von Baer had pointed out that in any group of animals the embryos were more like one another than were the adult organisms, and this now became easily translated by Haeckel into the idea that the form which is in every group—ultimately in all groups—the common starting-point of individual development is representative of the common ancestor of the race. Ontogeny was thus not merely expressed but explained in phylogenetic terms.

Now that, as we have already seen, the proposed explanation

[1] Wolff, l. c., *Praemonenda*, lxviii. [2] Id. ib. lxxiii.
[3] Id. ib., *De Gen. An.*, § 240.

has very largely broken down, Epigenesis, taken by itself, remains, not a theory in terms of cause and effect, but a mere description of what occurs, and it is the crying need for such a theory that has given birth to modern experimental embryology.

The new era opens with the publication by Wilhelm His in 1874, just a hundred years after the appearance of the *Theoria Generationis*, of a remarkable series of essays entitled *Unsere Körperform und das physiologische Problem ihrer Entstehung*. In these essays His, who was already in revolt against the 'Biogenetic Law', not only sought to give a mechanical explanation of differentiation, but also laid down his famous 'Prinzip der organbildenden Keimbezirke.' According to this principle of 'germinal localization', every spot in the blastoderm corresponds to some future organ: 'das Material zur Anlage ist schon in der ebenen Keimscheibe vorhanden, aber morphologisch nicht abgegliedert, und somit als solches nicht ohne Weiteres erkennbar.'[1] Conversely, every organ is represented by some region in the blastoderm, and 'wenn wir consequent sein wollen' in the fertilized, or even unfertilized, egg. In other words, although the parts of the embryo cannot be said to be preformed in the germ, the materials for those parts are already there, prelocalized, arranged roughly, at least, as the parts themselves will be later on. In this material unequal growth produces the form of the parts, and so of the whole body. Whether there is a strict causal connexion between each material rudiment and the organ which arises from it, whether these rudiments could be interchanged without prejudice to the normality of subsequent development, is a question which is not touched upon by His. It was reserved for another anatomist, Wilhelm Roux, to raise what in His's hands had been merely a principle to the rank of a theory, the 'Mosaik-theorie', or theory of self-differentiation.

For Roux, no doubt, the 'Mosaik-theorie' was in part the outcome of the theoretical necessity of explaining the specific nature of development; but it rests also upon a basis of observation and experiment. The coincidence in a majority of Frogs' eggs of the first furrow with the sagittal plane, the production of local defects in the embryo by local injuries to the egg, the

[1] His, l. c., § ii.

occurrence of certain natural monsters (*Hemitheria anteriora*, for example) in which one half of the body is normally developed, the other entirely suppressed, and the experimental demonstration of the formation of a half-embryo from one of the first two blastomeres of the Frog's egg when its fellow had been killed, all led Roux to regard the development of the whole and of each part as essentially a process of self-differentiation, a process, that is to say, of which the causes reside wholly within the fertilized ovum and within each part as it is formed, though allowance was made for the possible formative influence of the parts on one another in later stages. External conditions, though they may be necessary in the same sense as they are generally necessary to the maintenance of life, are yet of no importance for differentiation regarded as a specific activity of the organism.

In the meantime, an experiment of Pflüger's had apparently shown that, however obviously each part of the egg-cell might be related to the production of a particular organ, the relation was no necessary one, but that, on the contrary, the parts were all equivalent and the ovum 'isotropic'. Pflüger demonstrated that in a Frog's egg which had been prevented from assuming its normal position with the axis vertical, the planes of the segmentation furrows bore no constant relation to the original egg-axis, that is to say to the structure of the egg, though they exhibited the same relation to the vertical as when developing in the normal position. Further, in such forcibly upturned eggs the plane which included the original egg-axis and the present vertical axis became the median plane of the embryo, whose axes were disposed with regard to the vertical as in normal cases. Any part of the egg, therefore, might give rise to any part of the embryo, according to the extent to which and the direction in which the egg-axis had been diverted out of its original vertical position, and hence the egg-substance was 'isotropic'; the planes of segmentation and the embryonic axes being determined by gravity. In fact, Pflüger went so far as to say that an egg only becomes what it does become because it is always placed under the same external conditions.

Nor was this conception of the isotropy of the ovum invalidated by Born's proof that in these eggs there is a redistribution

of yolk and protoplasm owing to the sinking of the former to the lower, the rising of the latter to the upper side of the egg. For though the egg thus comes to acquire a secondary structure about an axis which is vertical, still the arrangement of the parts of the supposed rudiments of the organs must have been disturbed. Yet from such ova normal tadpoles are developed.

It became necessary, therefore, to locate the self-differentiating substance, the 'idioplasma', mainly, at any rate, in the nucleus; and this idioplasma was imagined as composed of dissimilar determinant units, each representative of some part or character of the organism and arranged according to a plan or architecture which corresponds in some way with the architecture in the embryo of the organs represented. All that is necessary, then, and all that happens, at least in the early stages of development, is the gradual sundering of these units from one another by successive qualitative divisions of the nucleus and their distribution to the cytoplasm, where each determines the assumption by the cell to which it is allocated of that character which it represents.

Roux's 'Mosaik-theorie' and Weismann's very similar but more elaborate hypothesis of the constitution and behaviour of the germ-plasm both frankly involve the belief that every separately inheritable quality of the body has its own representative in the germ, with the difference, however, that this preformation, extended by Weismann to the adult characters, is limited by Roux to those of the embryo. The renewed inquiry into the nature and essence of development has thus simply resulted in the resuscitation of the eighteenth-century doctrine of evolution, though in a far more subtle form. Once again we find ourselves face to face with the old alternative, Preformation or Epigenesis; and it is to the desire of solving this problem that a very considerable proportion of modern experimental research is attributable. Though much of this has been directed against and been destructive of the 'Mosaik-theorie', which as far as the nucleus is concerned has now been abandoned by Roux himself,[1] renewed investigation has proved the existence in many cases of definite and necessary organ-forming substances in the cytoplasm, while the necessity for finding a causal explanation

[1] Roux, 1903.

of what is obviously in some sense a predetermined process, without presupposing the preformation in the germ of morphological units representing every possible inheritable character, has issued in Herbst's and Driesch's conception of the events of ontogeny as so many responses to stimuli exerted by the developing parts on one another. At the same time the need for inquiry into the external conditions and the part they may play in growth and differentiation has not been forgotten.

Thus though it would be vain to pretend that the ideal of a complete causal explanation has yet been realized, still some material has been gathered for an answer to each of the two main questions: what are the internal, what the external conditions that determine the course of development? These questions we shall discuss in the following pages. It will then only remain to inquire whether a causal explanation—in the accepted sense of the phrase—a mechanics of ontogeny which resolves the single occurrences first into general physiological laws, and these in the last resort into the generalizations of physics and chemistry, can ever afford a theory which may be said to be complete either from a scientific or from a philosophical point of view. Should the mechanical explanation prove to be scientifically insufficient, it may be necessary, with Driesch and the neo-vitalists, to invoke a consciousness of the end to be realized to guide and govern the merely material elements; but even were this not so it would still be incumbent upon us to consider whether the end itself—not the consciousness of it—is not the final, and yet none the less the principal determining cause of the whole process.

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CHAPTER II

CELL-DIVISION AND GROWTH

1. CELL-DIVISION

In a future chapter we shall see that there is no necessary connexion between segmentation and differentiation. Nevertheless, since cell-division is the first sign, or almost the first sign, that a developing organism gives of its activity; since, moreover, cell-division accompanies the later processes of growth and differentiation, we may briefly discuss what is known of those factors which determine the direction of division in general, and in particular the pattern of segmentation.

We shall first presume that segmenting ova may be grouped under several distinct types, as follows :—

1. *The radial type.* Here the first division is meridional, the second meridional and at right angles to the first, the third equatorial—or more often latitudinal—and at right angles to both the preceding, the fourth meridional and at forty-five degrees to the first two, the fifth latitudinal. What is characteristic above all of this type is, first, that four surfaces of contact between cells meet in one line; for example, the four surfaces between the first four blastomeres meet in the egg-axis, while each pair of animal cells lies exactly over each pair of vegetative cells after the third division; and secondly, the blastomeres are radially arranged about the axis. This type has been observed in Sponges (*Sycon*[1] (Schulze)), in Coelenterates, in Crinoids, Holothurians, and Echinoids (Fig. 12) amongst the Echinoderms, in Ectoproctous Polyzoa, in *Amphioxus* and the Vertebrates, and in some Crustacea—*Cetochilus* (Grobben), *Lucifer* (Brooks), *Cyclops* (Häcker), *Branchipus* (Brauer) and some Cirrhipedes. Certain of these cases present special peculiarities.

In Echinoids micromeres are formed at the vegetative pole by the division of the fourth phase (Fig. 12, *f*).

[1] In *Sycon* the third cleavage is meridional, the fourth latitudinal.

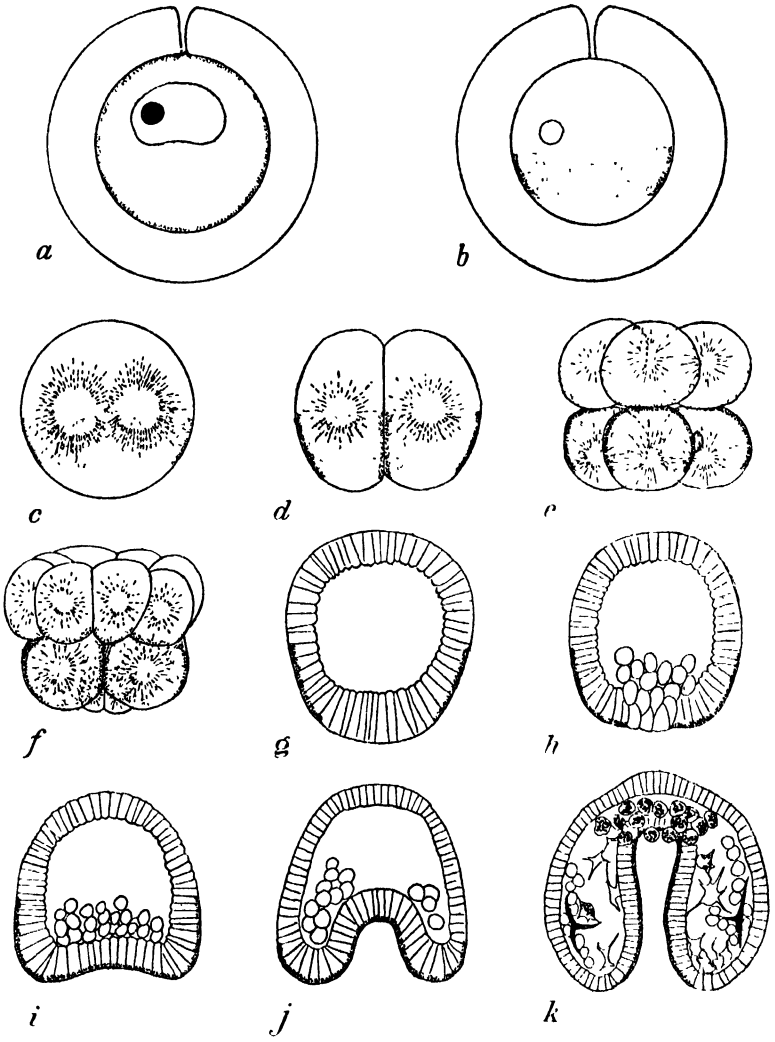


FIG. 12.—Normal development of the sea-urchin *Strongylocentrotus lividus*. (After Boveri, 1901.)

The animal pole is uppermost in all cases, and in the first two figures the jelly with the canal (micropyle) is shown.

a, primary oocyte, the pigment is uniformly peripheral.

b, ovum after extrusion of polar bodies. The pigment now forms a subequatorial band. The nucleus is ex-axial.

c, *d*, first division (meridional).

e, 8 cells, the pigment almost wholly in the vegetative blastomeres.

f, formation of mesomeres (animal cells) by meridional division: the vegetative cells have divided into macromeres and micromeres.

g, blastula. *h*, mesenchyme blastula.

i, *j*, *k*, invagination of the pigmented cells to form the archenteron of the gastrula. In *j* the primary mesenchyme is separated into two groups, in each of which, in *k*, a spicule has been secreted. In *k* the secondary, pigmented mesenchyme is being budded off from the inner end of the archenteron.

In Asteroids and Ophiuroids the division is at first tetrahedral, and to be classed, therefore, with those of the following type; after the second furrow, however, the blastomeres are rearranged, and division thenceforward is radial.

In Vertebrata segmentation is altered in megalecithal eggs by the amount of yolk present. It becomes meroblastic; still the radial type is preserved, though the sequence of the furrows is often altered, the third, for instance, being frequently meridional, and the fourth latitudinal. Amongst the Ascidians *Pyrosoma* has a large-yolked, telolecithal, and radially segmenting egg. In the Placental Mammals the first two divisions may conform to this type; but segmentation soon becomes irregular. The accumulation of yolk in the Arthropod egg has resulted in a totally different type of meroblastic segmentation. The yolk is here uniformly distributed about the central protoplasm. In the latter is placed the segmentation nucleus, and this central mass divides into a number of cells, which subsequently migrate to the surface and form a blastoderm; the egg is then centrolecithal (Fig. 1). The stages of the development of this modification may be seen in the Crustacea. In certain forms—those alluded to above (with the exception of the Cirrhipedes)—division is holoblastic and radial. In *Gammarus*, *Branchipus* (Brauer), *Peltogaster* (Smith) segmentation is at first total, but the inner yolk-containing ends of the cells subsequently fuse. In *Crangon*, *Moina*, *Daphnella*, *Daphnia*, *Orchestia* segmentation is superficial. In Isopods and in Decapods segmentation is internal. In all cases the result in the end is the same, a peripheral blastoderm, a central yolk. But the blastoderm is not always, though it is often, formed simultaneously over the whole surface. There are cases in which it appears first on the ventral side, and by what may be described as a still more precocious formation of the blastoderm, segmentation may begin at this, the future ventral, point, as in *Mysis* and *Oniscus*. In these cases the egg is telolecithal.

In the Insects, Arachnids, Myriapods, and *Peripatus novae-zealandiae*, the segmentation is meroblastic and the egg comes to be centrolecithal. In *Peripatus capensis* and in some other species it would appear that the yolk has been secondarily lost.

2. The second type is the so-called spiral form of cleavage (Fig. 13). This is especially characteristic of the eggs of Polyclads, Nemertines, Molluscs (except Cephalopods), Annelids, and Sipunculoids (*Phascolosoma*). The peculiarity of this mode of division is that after the four-celled stage the blastomeres usually known as the macromeres—give off 'quartettes' of micromeres towards the animal pole, the first quartette being given off dexiotropically (except in cases of reversed cleavage),

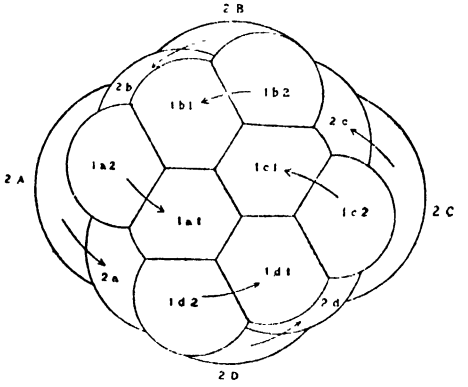


FIG. 13.—Diagram of a 'spirally' segmenting egg in the 16-cell stage. 2 A–2 D macromeres; 2 *a*–2 *d* micromeres of second quartette; 1 *a* 1, 1 *a* 2–1 *d* 1, 1 *d* 2 micromeres of first quartette.

the second laeotropically, and so on in regular alternation, until four quartettes have been produced. The cells of each quartette divide meanwhile in conformity with the same law of alternation of direction of cleavage. The direction of division is thus always oblique to the egg-axis, and this obliquity can be observed in the division of the first two blastomeres, the result of which is that of the two sister cells A and B A is nearer to the animal pole than B, while in the other pair C is nearer that pole than D; A being to the left of B and C to the left of D (to an observer standing in the axis with his head to the animal pole), the division is laeotropic. The arrangement of cells approaches the tetrahedral, especially when, as occurs very frequently, A and C are united by a cross, or polar, furrow above, B and D by a polar furrow at the vegetative pole, as in *Nereis*, *Ichnochiton*, *Limax*, *Planorbis*, *Lepidonotus*, *Discocelis*, and others. In *Unio*, however, it is B and D that are in contact at the animal, A and C at the vegetative, pole. In other cases

(*Ilyanassa*, *Capitella*, *Umbrella*, *Crepidula*, *Amphitrite*, *Arenicola*, for instance) the furrows are parallel, the same two blastomeres, B and D, being in contact at both poles. In *Trochus* both the 'parallel furrows' and the 'crossed furrows' conditions are found. A similar disposition is to be observed amongst the micromeres of the first quartette. These micromeres, also, alternate with the macromeres. Not more than three contact surfaces between blastomeres, therefore, meet in one line.

The eggs of certain Lamellibranchs—*Teredo*, *Cyclas*—in which the 'spiral' arrangement is obscured by the large size of the D macromere, and possibly the ova of the Rotifera, are to be referred to this type.

The tetrahedral arrangement of the first four cells is conspicuous in Asteroids and Ophiuroids, where the planes of division of the first two cells are at right angles to one another. Before the next division, however, the cells shift their positions and come to lie in one plane, in which, however, the sister cells are not adjacent, but opposite, to one another.

The eggs of *Amphioxus* sometimes segment spirally (Wilson).

After the completion of the spiral period of division, segmentation becomes radial and then bilateral.

3. The third type of cleavage is the bilateral. The first two divisions intersect in the axis; the next may be equatorial, as in Ascidians. In this case the bilaterality becomes evident in the succeeding phase, in which the divisions in two adjacent cells of the animal hemisphere meet the first furrow, while in the other two they meet the second. The bilaterality is marked in the reverse way in the vegetative half of the egg. The egg is thus divided into what will be anterior and posterior, dorsal and ventral, and right and left halves. In future divisions the bilateral symmetry is retained.

The egg of *Amphioxus* may divide in this fashion (Wilson), and this is the normal method, according to Roux, in *Rana esculenta*.

In the Teleostei and some Ganoids (*Lepidosteus*) the bilaterality becomes evident in the third division, which is parallel to the first, the fourth being parallel to the second division. The egg is in fact iso-bilateral.

The Ctenophore egg also possesses two planes of symmetry,

for the third division is meridional and unequal in such a manner that the next stage—eight cells—is composed of two opposite pairs of small and two opposite pairs of large cells.

The mesomeres in the sixteen-celled stage of Echinoids are bilaterally arranged.

In the Cephalopoda the egg is large-yolked, and segmentation consequently meroblastic. After the first two meridional divisions

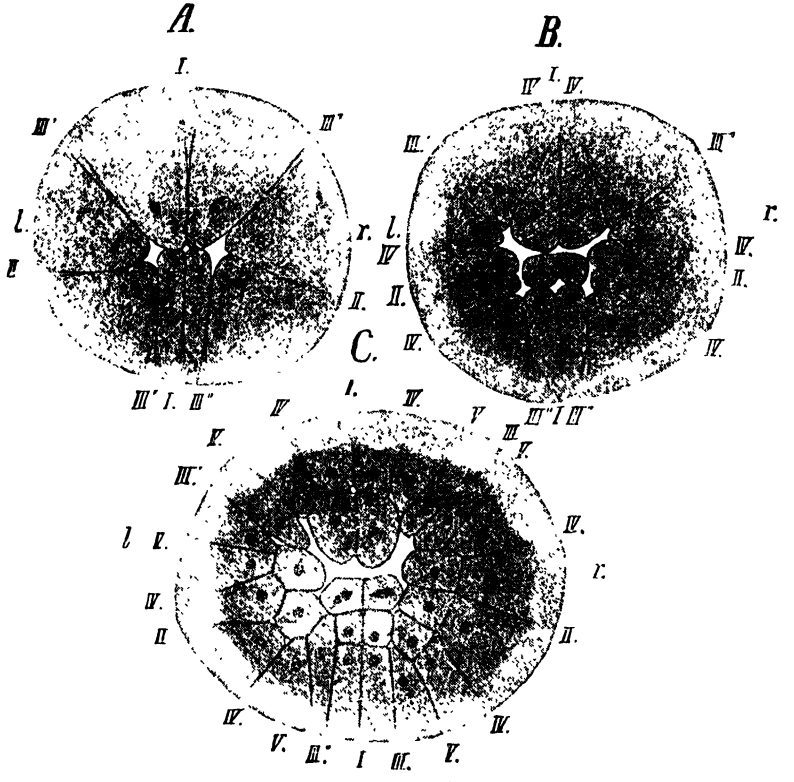


FIG. 14.—Three segmentation stages in the blastoderm of *Sepia officinalis*; the segmentation is of the bilateral type. *l*, left; *r*, right; *I–V*, first to fifth cleavages. The top sides of the figures are anterior. (After Vialleton, from Korschelt and Heider.)

the bilateral disposition sets in, for the furrows of the third phase are unequally inclined to the first furrow in two halves—the future anterior and posterior halves—of the egg (Fig. 14).

The egg of *Ascaris megalocephala* also exhibits a bilateral cleavage, but not on the plan just described. The first division is equatorial. Then the animal cell divides meridionally, and, as it will prove, transversely, the vegetative cell latitudinally.

Before the next division the most vegetative cell (P_2) slips round to what will be the posterior side. All four cells are bilaterally arranged about the plane in which they all lie, and this will become the sagittal plane of the embryo. The anterior and posterior ends, and therewith the right and left sides, are likewise now determined. The bilateral symmetry is preserved in future divisions, at least in the vegetative hemisphere; in the animal part of the egg the blastomeres of the left side become tilted forwards, those of the right side backwards (Fig. 155, p. 255).

4. In the Triclad Turbellarians, in Trematoda and Cestoda, segmentation is irregular, the blastomeres separate from one another and lie amongst the yolk-cells. The same phenomenon may be witnessed in the Salps, and the separation and subsequent reunion of the blastomeres has also been described in Coelenterates and in Asteroids.

Although these types of segmentation are distinct enough from one another, intermediate conditions are readily found. The radial easily passes into the spiral type for example, for in many eggs of the former kind the 'cross furrows' have been observed at either one or both poles, while the animal blastomeres may be rotated slightly on the vegetative, and so lie not over, but in between, them. The radial symmetry again may become bilateral, as when the meridional furrows of the fourth phase, instead of passing through the animal pole, meet the first or second furrow, symmetrically on either side of one of these divisions; this occurs as a variation in *Rana fusca* and (normally (Roux)) in *Rana esculenta*.

In Ophiuroids and Asteroids the tetrahedral arrangement is lost, and the egg segments radially. In *Amphioxus* all three types occur.

All three forms may therefore have been derived from one, though what that one was we do not know. In any case, however, one feature is common to them all; in all cases successive divisions are at right angles to one another. This is the law formulated by Sachs long since for the divisions of the cells of plants. It holds good for the segmenting animal ovum, though exceptions may, of course, be found. The alternation of dexiotropic and laeotropic divisions, for instance, in spirally segmenting ova continues for a long period with striking regularity,

and it is comparatively rare for a cell to disobey the rule. The rule is, however, no universal law of cell-division. Every embryologist will recollect the continued division of a teloblast in the same direction to form a germ-band, which is such a conspicuous fact in the development of Molluscs, Annelids, and Arthropods. The four polar nuclei of Insect eggs, lying in one straight line, may also be cited.

The direction of division and the size of the blastomeres are not, however, the only factors which determine the actual pattern of segmentation. The cells can, and do, shift their positions on one another. This is of common occurrence, and a few examples will suffice. The rearrangement of the tetrahedrally disposed cells in Asteroids and Ophiuroids has been noticed already. In many 'spiral' ova the micromeres have been observed to rotate on the macromeres, or one quartette to be pushed out of position by the cells of another. In *Ascaris* the cell P_2 slips to one side. Further, cells change their shape.

Two factors are therefore involved in the production of the pattern of cleavage, the direction of division, and the movements of the cells, and these factors in their turn demand explanation. To these must be added the shape, the size, and the rate of division of the cells.

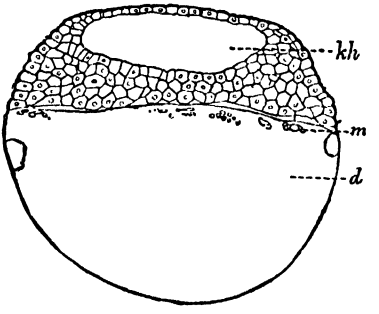


FIG. 15.—Segmentation of the Frog's egg under the influence of a centrifugal force (from Korschelt and Heider, after O. Hertwig). The egg consists of a blastoderm and an undivided yolk (yolk-syncytium): *kh*, blastocoel; *m*, yolk-nuclei; *d*, yolk.

The two latter depend very largely upon the amount of yolk present in the egg; yolk-cells are large, the yolk divides slowly, or not at all. This was expressed long since by Balfour in the formula, 'The velocity of segmentation in any part of the ovum is, roughly speaking, proportional to the concentration of the protoplasm there; and the size of the segments is inversely proportional to the concentration of the protoplasm.'[1] The rule has been vindicated by O. Hertwig experimentally. If the egg of the Frog be centri-

[1] *Comp. Emb.* i. c. 3.

fugalized with sufficient force the yolk is driven still more towards the vegetative pole, while the protoplasm is accumulated in the animal half of the egg. Such eggs segment meroblastically, a cap of cells or blastoderm being formed lying on the surface of a nucleated but undivided yolk. The yolk-nuclei, moreover, are enlarged, as in megalecithal fish eggs (Fig. 15).

The rule is, of course, only applicable to telolecithal eggs, and for many of these it holds good, notably for Vertebrates. In other classes there are, however, exceptions, which are best known in those whose segmentation has been most carefully studied, the 'spiral' eggs of Turbellarians, Annelids, and Molluscs. Large cells, in these ova, often divide more quickly than small ones; the second quartette of micromeres, for instance, is formed before the first quartette divides in *Crepidula*, *Unio*, *Limax*, *Trochus*, *Aplysia depilans*, *Discocelis*, and the cells of the third quartette before the first products of division have had time to divide again in *Limax*, *Umbrella*, and *Aplysia limacina*. 4*d* is often formed before the corresponding cells in the other quadrants (in *Unio*, for example), but in *Crepidula* this is in accordance with the rule, since 4*a*, 4*b*, and 4*c* contain more yolk than 4*d*. In *Arenicola*, though the yolk is uniformly distributed, the cells are still unequal. Other exceptions are to be found in the continued unequal division of teloblasts, in the formation of the micromeres in Echinoids, and in the unequal division of the blastomeres in the third and fourth phases in Ctenophors. According to Ziegler the formation of the micromeres in Ctenophors cannot be due to the presence of yolk, since they are still formed when part of the vegetative hemisphere is removed, as Driesch and Morgan have also found.

Ziegler indeed puts forward another hypothesis to account for unequal division; he supposes that the centrosomes are heterodynamic. So far there appears to be little evidence in support of this view. It is quite true that in many cases of unequal division the asters—not the centrosomes—vary in size with the size of the cells. This occurs, for instance, in the division of the first micromeres and of the first somatoblast in *Nereis*, in the formation of the first and second quartettes, and in the division of the first somatoblast in *Unio*, in the division of the cell CD in *Asplanchna*,

and in the division of the pole cells of Annelids (Wilson and Vejdovský). It is doubtful, however, whether it is not the inequality in the cells that is responsible for the inequality of the asters, there being more room in a large cell for the outgrowth of the astral rays. At any rate, there are many cases of unequal cleavage—in polar body formation—where the asters are of the same size. Until evidence is brought forward of a difference in the size of the centrosomes the hypothesis is no more than a conjecture.

Before quitting this subject we should refer to a rule which Zur Strassen has found to hold good for the rate of segmentation of *Ascaris megalocephala*. The cells do not all divide at the same rate, but in certain groups of cells division is found to occur simultaneously. These cells are related, descended from some one cell, and the more nearly related the cells are, the more nearly together do they divide. Coincidence in time of division depends therefore on the degree of cell-relationship.

The direction of division of the cell depends upon that of the nucleus, since, speaking generally, the division occurs in the equatorial plane of the spindle, or, in other words, the plane of division is at right angles to the direction of elongation of the spindle or separation of the centrosomes. The latter again depends on the relation between the nuclear spindle and centrosomes on the one hand, and on the other the cytoplasm and its contents, more particularly the yolk. The relation between the (resting) nucleus and the cytoplasm has been expressed by O. Hertwig in the following empirical rule: 'The nucleus always seeks to place itself in the centre of its sphere of activity.' The sphere of its activity being not the inert yolk but the cytoplasm, we find, in accordance with this rule, that the nucleus places itself in the centre of the egg where the yolk is uniformly distributed (isolecithal), nearer the animal pole but still in the axis where the yolk is on one side (telolecithal). Examples of the former condition are to be found in Echinoids (the fertilization nucleus is nearly, but not quite, central) and large-yolked Arthropod ova, of the latter in the eggs of Vertebrates, Molluscs, and many others.

The nucleus, however, may wander from this position, as occurs,

for instance, in the egg of Echinoids after the expulsion of the polar bodies and before fertilization. Apart from such exceptions, due very likely to some temporary alteration in the relations of yolk and cytoplasm, the rule is a reliable one.

The relation between the dividing nucleus, the spindle and centrosomes and the cytoplasm has been stated by O. Hertwig in his second empirical rule 'that the two poles of the division figure come to lie in the direction of the greatest protoplasmic mass', by Pflüger in the formula, 'the dividing nucleus elongates in the direction of least resistance.'

The objection that has been urged to this latter expression, that in a fluid the pressure is equal in all directions, may be set aside. For though the cytoplasm is fluid it is an extremely viscid fluid, and the presence of the suspended yolk granules

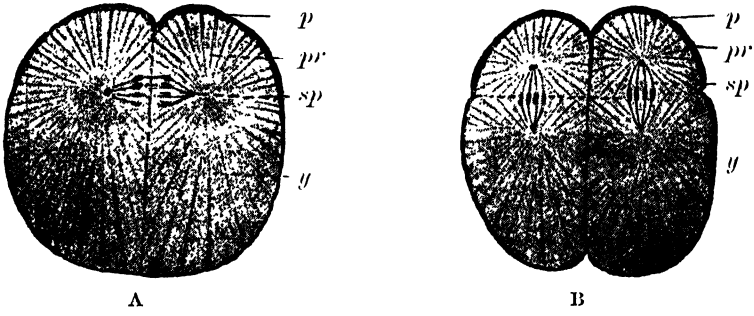


FIG. 16.—Diagram of the segmentation of the Frog's egg (after O. Hertwig, from Korschelt and Heider). A, first (meridional); B, third (latitudinal) phase of segmentation; *p*, superficial pigment of animal hemisphere; *pr*, protoplasm; *y*, yolk; *sp*, spindle.

must certainly offer a greater resistance than the fluid itself, and greater in proportion to their number and size. Pflüger's formula, therefore, if not merely a truism, resolves itself into a restatement of Hertwig's rule. This rule certainly holds good for a large number of cases, for it explains, for instance, the two first meridional divisions of all spherical telolecithal and radially segmenting eggs, the third, latitudinal (in small-yolked eggs[1]), and possibly also subsequent meridional and latitudinal divisions (Fig. 16). It will not, however, in the present state of our knowledge, explain the obliquity of the spindles to the egg-axis in spirally dividing ova, nor cases of bilateral division;

[1] In *Sycon* the third is meridional, the fourth latitudinal.

here, it is evident, other factors must come into play, in the second case probably a bilateral symmetry in the constitution of the cytoplasm. These exceptions may, however, ultimately prove to be special cases of Hertwig's rule.

A very striking confirmation of the rule is to be found in the division of the egg of *Ascaris nigrovenosa* (Figs. 17, 18). The

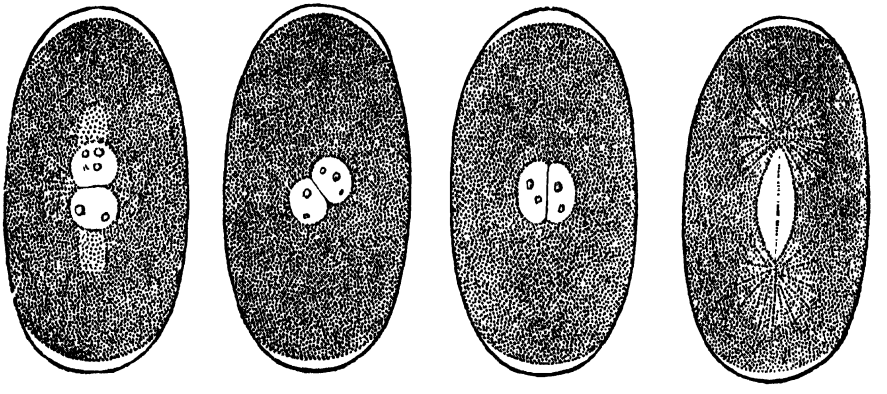


FIG. 17.—Four stages in the fertilization of the egg of *Ascaris nigrovenosa*. (After Auerbach, from Korschelt and Heider.)

egg of this worm is ellipsoid. At one end (that turned towards the upper end of the ovary) the polar bodies are extruded, and here the female pronucleus is placed. The spermatozoon enters at the

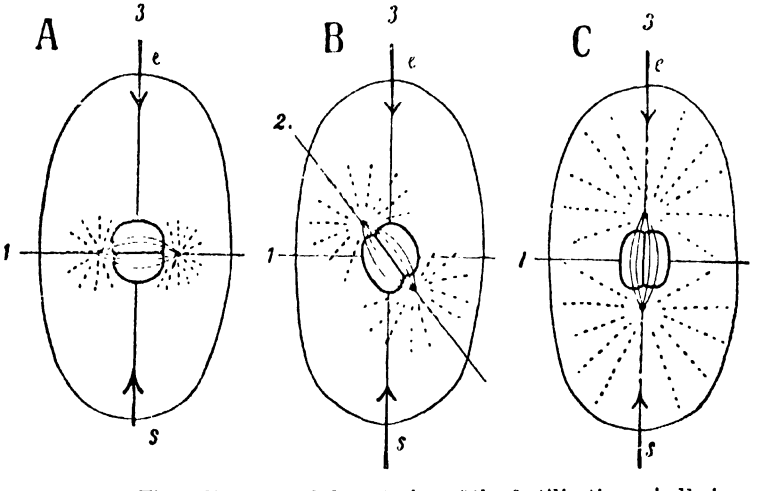


FIG. 18.—Three diagrams of the rotation of the fertilization spindle in the egg of *Ascaris nigrovenosa*. *e*, *s*, the directions in which the female and male pronuclei approached one another in A; 1, 2, 3, successive positions of the spindle. (From Korschelt and Heider, after O. Hertwig.)

opposite end. The line of union of the two pronuclei therefore lies in the long axis of the egg. Nevertheless the fertilization spindle is not formed in the minor axis of the ellipsoid as one might expect. The two pronuclei rotate together through 90°, the spindle is developed, as usual, in a direction at right angles to their line of union, that is to say the axis of the spindle lies in the major axis of the egg, and the rule is confirmed. There is a similar rotation of the fertilization spindle in the egg of another Nematode, *Diplogaster* (Ziegler), and in the Rotifer *Asplanchna* the spindle, at first oblique, becomes later coincident with the long axis of the ovum (Jennings).

Curiously enough, this rotation of the pronuclei does not occur in another ellipsoid egg, that of the Rotifer *Callidina*. According to Zelinka, the polar body is formed at one end of the long axis, but the fertilization spindle lies in the minor axis, the first division includes the major axis, and the law is disobeyed. After the division, however, the cells rotate, and the plane of contact is then, as in *Ascaris nigrovenosa*, transverse.

Again, all polar divisions violate the rule, as also does the first division of the fertilized egg of *Ascaris megalocephala*, and the division of the cells of the germ-bands of Crustacea parallel to their length (Bergh).

On the other hand, Hertwig has brought forward experimental evidence in support of his generalization. In the eggs of the Frog the directions of some of the divisions were altered by compression between glass plates. The eggs were just allowed to assume their normal position with the axis vertical. They were then placed between glass plates and compressed.

In the first series of experiments the plates were horizontal. In such eggs the first furrow was meridional and vertical, the second meridional and vertical and at right angles to the first. So far, therefore, division was as in the normal egg. In the third division the furrows were, however, not latitudinal and horizontal, but nearly vertical, being parallel to the first furrow above, to the second furrow below. The surface of contact, therefore, formed by the furrows of this phase must pass through a meridional position in the interior of the egg. The fourth furrows are latitudinal. Born has repeated the experiment and confirmed this result (Fig. 19). He adds, however, that the furrows of the third division pass

towards the vegetative pole below, or may even remain parallel to the first furrow throughout. The fourth furrows, Born says, are parallel to the second. I have myself observed that this division may be either parallel to the second, or latitudinal, even in different

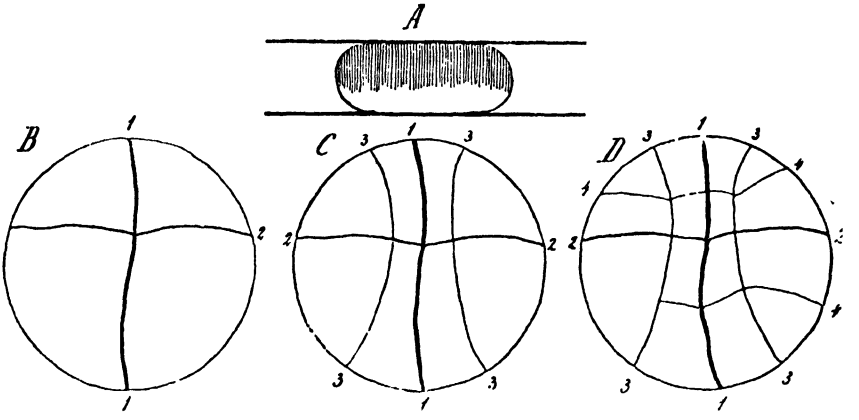


FIG. 19.— Segmentation of the Frog's egg under pressure. The compression is in the direction of the axis.

A, view of the egg between horizontal plates; the animal part is shaded. *B*, *C*, *D*, first (1), second (2), third (3), and fourth (4) divisions as seen from the animal pole. (After Born, from Korschelt and Heider)

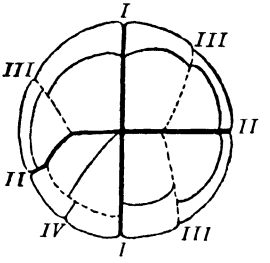


FIG. 20.—The first four divisions (*I*, *II*, *III*, *IV*) in a Frog's egg compressed between horizontal plates in the direction of the axis. The third furrow is more or less meridional and vertical in three quadrants, horizontal in the fourth, and this a smaller quadrant. The fourth furrow is meridional in this quadrant, horizontal in the remaining three.

quadrants of the same egg (Fig. 20). It will be observed that the quadrant in which the third furrow is latitudinal is smaller than the others. It is of great interest to observe the striking similarity between the direction of the third and fourth furrows in these eggs and the corresponding divisions in the Teleostean egg where the blastodisc is compressed by the chorion.

In the second series of experiments made by Hertwig the glass

plates were vertical, the eggs, therefore, compressed not, as before, in, but at right angles to, the axis.

The first furrow was meridional, and therefore vertical, and at right angles to the plates. The second was latitudinal and horizontal, and also at right angles to the plates. The furrows of the third phase were parallel to the first, those of the fourth, in the four upper animal cells, parallel to the plates. Born again

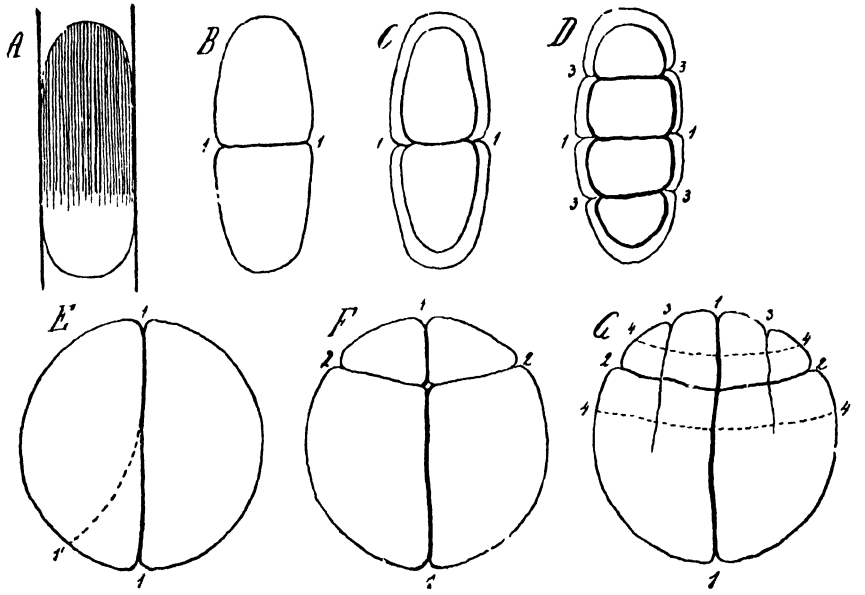


FIG. 21.—Segmentation of the Frog's egg under pressure. The pressure is at right angles to the axis.

A, view of the compressed egg. The pigmented animal portion is shaded.

B, *C*, *D*, views of the egg from the animal pole after the first (1), the second (2), and the third (3) divisions.

E, *F*, *G*, views of the egg from the compressed side after the first (1), the second (2), the third (3), and the fourth (4) divisions. The first furrow may pass as (1′) in *E*.

(From Korschelt and Heider, after Born.)

confirms this account (Fig. 21). The direction of the furrows of the third phase is, however, variable; it may be not parallel to the first, but perpendicular to it. In this case it may be parallel to the second, or so oblique to it as to become nearly parallel to the glass plates. The direction of the fourth division depends on that of the third, to which it is at right angles. It may, therefore, be either oblique and nearly parallel to the plates, as described by Hertwig, or parallel to the second furrow and perpendicular to the plates.

In a third series of experiments Hertwig placed the plates

obliquely, at 45°. In these eggs the yolk sinks slightly from the upper to the lower side, while the cytoplasm rises in the opposite direction; in other words, a bilateral symmetry is conferred upon the egg by the combined action of pressure and gravity. The plane of this symmetry is midway between and parallel to the plates. The first furrow is at right angles to the plates and to the plane of symmetry.

We are indebted to Driesch for a similar series of experiments on Echinoderm eggs. Driesch compressed the eggs of *Echinus* under a cover-glass supported by a bristle. The direction of the egg-axis with regard to the pressure was not known, but the

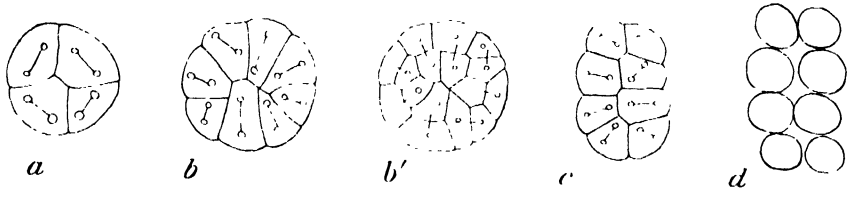


FIG. 22.—*Echinus*: segmentation under pressure.
a, preparation for third division (radial); *b*, preparation for fourth division (tangential); *b'*, after fourth division; *c*, another form of the 8-cell stage (third division parallel to first); *d*, the same after removal of the pressure. (After Driesch, 1893.)

Echinoid egg is nearly isolecithal. When the egg membrane remained intact the first two furrows were vertical, that is, in the direction of the pressure, since the slide and cover-glass were horizontal, and at right angles to one another.

The spindles for the next division are again horizontal, and usually tangential, sometimes, however, radial. The eight-celled stage consists, therefore, of a flat plate of cells. At the next division the formation of micromeres—which would ordinarily occur at this moment—is suppressed; the spindles are horizontal and radial, the furrows, therefore, vertical and tangential (Fig. 22 *a*, *b*, *b'*).

In certain cases cell-formation is wholly or partially suppressed. When the pressure is less (in those eggs which lie nearer the bristle) the micromeres may be, but generally are not, formed. The spindles are no longer horizontal. Similar results are obtained when the eggs are released from strong compression.

In another experiment the eggs were first deprived of their membranes. The first and second furrows are vertical and generally at right angles to one another. Sometimes, however,

the second is parallel to the first, or one blastomere may lie apart from the other three. Should the eggs be now released from the pressure, each blastomere becomes rounded off, and—after two more cleavages—the sixteen-celled stage consists of two plates of eight cells lying over one another. But if the pressure is maintained, the spindles are horizontal and the blastomeres lie all, or nearly all, in one plane (Fig. 22 *c*, *d*).

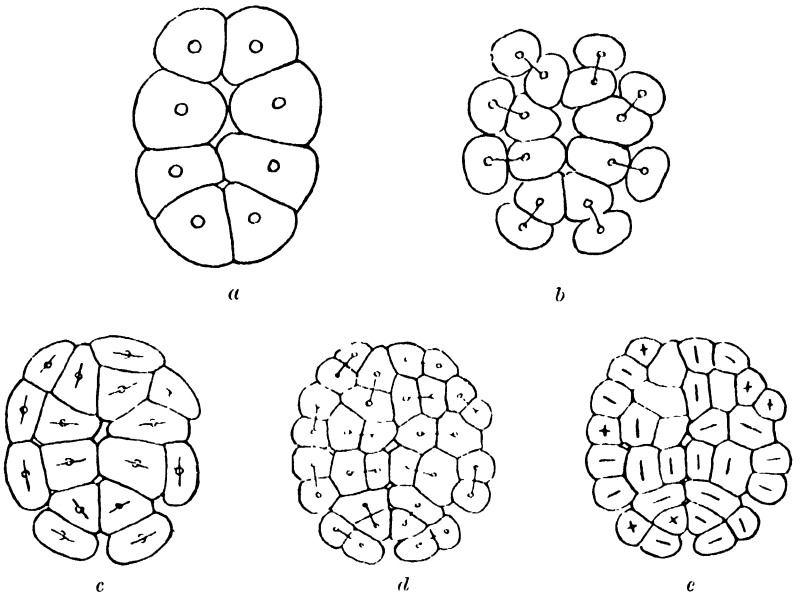


FIG. 23.—Segmentation of the egg of *Echinus microtuberculatus* under pressure. (After Ziegler, 1894.)
a, 8 cells in one plane; *b*, 16 cells, the last division having been tangential; *c*, *d*, 16-32 cells: the direction of the spindles in *c* is shown by the line: it is in the greatest length of each cell; *e*, 64 cells: a cross signifies a vertical or oblique division, a line a horizontal division.

Ziegler has followed the segmentation of the compressed eggs a step further (Fig. 23). As the figures show, the first two divisions are at right angles to one another, while the furrows of the next two phases are, roughly, parallel to the first and second. In the next division—sixteen to thirty-two cells—the outer cells divide radially, the inner more or less tangentially, these divisions being, like the previous ones, at right angles to the compressing plates. In the following phase, some cells (those marked with a line) still divide in the same direction as before; but in others (distinguished by a cross) the spindle is perpendicular to the

plates and the division horizontal. Ziegler points out that, in the former cases, the cells have greater dimensions in the horizontal plane than in the latter. This, however, may be the effect, not the cause, of the direction of the spindle-axis.

Two other pressure experiments may be mentioned here. In *Nereis* Wilson produced a flat plate of eight equal cells by applying pressure in the direction of the axis. The formation of the first quartette of micromeres was thus suppressed. On relieving the pressure eight micromeres were formed. For the Ctenophora (*Beröe*) Ziegler has shown that the normal inequality of the third and fourth divisions is not altered by pressure.[1]

The foregoing experiments all agree in demonstrating the perfectly definite effect produced by pressure upon the segmenting egg. The nuclear spindles place themselves at right angles to the direction of pressure, the divisions fall at right angles to the compressing plates. This holds good for the first three or four divisions, at least, and sometimes for later phases still. In all these cases, therefore, the nuclear spindle elongates in a direction of least resistance, and, in the normal uncompressed egg, we may argue, with Hertwig, the least resistance is offered by the greatest protoplasmic mass.

Even in the compressed eggs, however, the greatest extension of the protoplasm, or the least extent of the yolk, is a factor which must in some cases come into play. When the egg of the Frog is compressed between vertical plates, the nuclear spindle does not elongate in any direction at right angles to the pressure, but in one only, a horizontal; and this is the direction of the greatest protoplasmic mass, since the egg-axis is vertical.

Speaking generally, therefore, experiment has upheld Hertwig's contention that the direction of nuclear division, and therefore of cell-division, is determined by the relation between the nucleus with its centrosomes and the cytoplasm with its yolk.

There are one or two experiments which do not support Hertwig's view. Boveri stretched the eggs of the sea-urchin *Strongylocentrotus* in the direction of the axis. The fertilization spindle lay in the usual equatorial position, occupied, that is, the minor axis of the ellipsoid.

[1] I have recently had occasion to notice that when the egg of *Antedon* is compressed in the direction of the axis the third division is meridional instead of latitudinal.

Again, Roux observed that Frogs' eggs sucked up into a tube with a narrow bore became elongated either parallel or transverse to the length of the tube, the axis of the egg lying in each case lengthways. In the first case the division was at right angles to, in the second usually parallel to, the tube in accordance with the rule; but exceptionally, in the transversely stretched eggs, the division was not perpendicular to, but coincided with, the extent of the greatest protoplasmic mass.

However important a factor the disposition of the yolk may thus be in deciding the direction of cell-division, it is certainly not the only factor. In the eggs pressed between horizontal plates there are many—an infinite number—of directions of least resistance. In one of these the segmentation spindle elongates, and at right angles to this the first furrow falls. This is probably determined—as it is determined in the normal Frog and Sea-urchin egg—by the point of entrance of the spermatozoon, or at least by the direction of the sperm-path in the egg. The second division is at right angles to the first, and here the direction may very possibly be decided on Hertwig's principle. But why, in the next phase, should the furrows be at right angles to the second rather than to the first, for the extent of the protoplasmic mass is as great in each of the four cells, in a direction parallel to the first as to the second furrow? Here, it is clear, some other reason must be found for this succession of divisions at right angles to one another. The cause is probably to be sought for in the direction of division of the centrosomes; for these divide—frequently soon after the telophase—at right angles to the axis of the previous figure. We thus gain a new expression for Sachs' Law.

The original direction of divergence of the centrosomes is, however, by no means always the ultimate one, for the growing spindle may be twisted out of its original position. Conklin has made a careful study of this phenomenon in *Crepidula*, in which egg he finds that vortical movements are set up in the cytoplasm by the escape of nuclear sap at the beginning of mitosis. The movements are in opposite directions in sister cells, centre in the spindle poles, and often carry both nucleus and spindle into a fresh place. These currents, which had been noticed previously by other observers (by Mark in *Limax* and by Iijima in *Nephelis*), may

thus play an important part in the production of the cell pattern. We shall see elsewhere that they, and other protoplasmic movements, are also of the very greatest significance in differentiation.

There remains now to be noticed another principle, which is especially applicable to plant-cells with fixed walls, though it may possibly be used for the phenomena of animal segmentation as well. Berthold has pointed out that when a newly formed cell-wall places itself perpendicular to the previously existing walls it is—at least in a good many instances—simply obeying the laws of capillarity, it merely conforms to the principle of least surfaces formulated by Plateau. This principle is as follows: 'Homogeneous systems of fluid lamellae so arrange themselves, the individual lamellae adopt a curvature such that the sum of the (external) surfaces of all is under the given conditions a minimum.'

A fluid lamella, of soap solution, for example, placed across the interior of a hollow, rigid cylinder, or parallelepiped, or cube, is, with the film coating the internal surface of the vessel in which it lies, a special case of such a system of lamellae, and, in obedience to the principle, the lamella places itself at right angles to the walls of the cavity and transverse to the long axis.

In the case of the plant-cell, the cell-plate, formed by solidification of the spindle fibres in the equator of the mitotic figure, represents the soap-lamella, and like the latter in its parallelepiped, the cell-plate, or new cell-wall, places itself perpendicular to the old one, and transverse to its length.

There are very numerous cases in which the law is obeyed, but it is not so in all. Under certain conditions the lamella should be not at right angles, but oblique to the wall of the chamber across which it is stretched. If, to take a concrete case, the lamella be made to move (by abstracting air) towards one end of its receptacle (a cube or parallelepiped), it will reach a critical position in which the principle of least surface can only be satisfied by its occupying an oblique position. The point at which this occurs is when the lamella is distant $\frac{a}{\pi}$ from the end, where a is the length of the side of the cube (short side of the parallelepiped). The lamella now forms the fifth side to a wedge-shaped space (quadrant of a cylinder, whose radius is $r = \frac{4}{9}a$), but as more air is abstracted, and it moves still

further toward the end, it comes to another critical position when it must lie across one corner, forming so the base of a pyramid, or octant of a sphere. This position is defined by the equation $r_1 = \frac{2}{3}a$, where r_1 is the radius of this sphere. It is impossible, therefore, for a very flat cell, or short cylinder, to be divided in conformity with the principle parallel to its longest side, and yet this occurs, as, for instance, in cambium cells.

It will also be noticed that this principle does not explain why one particular direction is selected when many are apparently equally possible.

We turn now to a consideration of the remaining factor which assists in determining the shape of the cells and so the geometrical pattern of segmentation; this is the movement of the cells upon one another.

That such movement does occur we have already seen; the question which immediately suggests itself is whether in taking up their new positions the cells obey the laws of capillarity as enunciated for systems of fluid lamellae such as soap-bubbles by Plateau in his principle of least surfaces.

This principle, as we have seen, demands that the sum of the external surfaces should be, under the conditions, a minimum, or, expressed in physical rather than in geometrical language, that the total surface energy should be minimal. In accordance with this doctrine of minimal surface energy a drop of fluid floating in a fluid medium assumes, as need hardly be said, the form of a sphere. In a system of drops contact surfaces will be formed between the drops, provided that each possesses a coating film which has a positive energy with the media it separates; a film, that is, of such a nature that the total surface energy would be diminished by apposition, without, however, involving the disappearance of the separating film and fusion of the drops. In other words, the film must be insoluble in both the external and the internal media. A simple example of this is afforded by the behaviour of the spheres of jelly covering the eggs of the Frog, when taken from water and floated between chloroform and benzole. Two or more such drops of jelly cohere by their coating films, and form systems of lamellae—the films, that is, at the external surfaces and between the opposed surfaces of the

drops—in which the principle of least surfaces is obeyed. Soap-bubbles form similar systems. But where this condition is not fulfilled, as in oil-drops floated, for instance, between alcohol and water, the drops either unite or separate, each retaining its spherical form.

The geometrical analysis of such systems given by Plateau is as follows. In a system of two bubbles the curvature of the surface of contact is given by the equation $r = \frac{\rho\,\rho'}{\rho - \rho'}$, where r is the radius of that surface, ρ, ρ' the radii of the larger and smaller bubbles. Since the pressure varies inversely with the radius, the surface of contact is convex towards the larger bubble. When $\rho = \rho'$, $r = \alpha$, and this surface is plane. Since there is equilibrium the external surfaces of the bubbles and their common surface meet at angles of 120°.

In a system of three bubbles there are three contact surfaces; these meet in one line and make angles of 120° with one another. When there are four bubbles, however, the four contact surfaces cannot meet in one line except for an inappreciable instant; they immediately shift their positions in such a way that two opposite bubbles meet and separate the other two from one another. There are thus five surfaces of contact, and these make angles of 120° with one another as before. This is the arrangement when four bubbles—whether equal or unequal is no matter—are placed side by side in the same plane. When, however, one bubble is placed in a different plane to the remaining three, four surfaces are formed and disposed in such a manner that the four lines, each formed by the intersection of three of these surfaces, meet in one point, making with one another angles of 109° 28′ 16″, the angles at the centre of a tetrahedron. In short, the four are now tetrahedrally arranged. The systems of drops of jelly alluded to above arrange themselves as do soap-bubbles under similar circumstances. What holds good of four holds good of an assemblage of any number of bubbles. The size of the bubbles is a matter of indifference, except to the curvature of the surfaces of contact, and, to a certain extent, to the arrangement. Thus, if four equal bubbles be placed in a plane, they will form together five surfaces of contact, one of which will be between two opposite

bubbles. If these two be now diminished, or the opposite two enlarged, the surface of contact will be between the opposite pair of larger bubbles. On the other hand, it is possible to bring smaller opposite bubbles into contact, while the larger ones remain apart. Again, on four bubbles lying in one plane, four small ones may be superimposed in such a fashion that while two lie at either end of the surface of contact, the other two lie over between the two opposite large bubbles below. If now the two latter small bubbles be enlarged, they will displace the other two until all four come to lie not over but between the

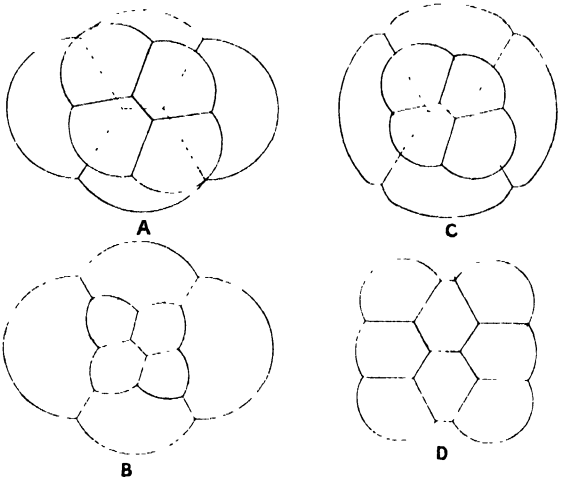


FIG. 24.—Diagrams of systems of soap-bubbles.

A–C, four small bubbles superimposed on four large ones. In A and B the bubbles are not compressed; in C the lower bubbles have been circumscribed by a cylindrical vessel. In B the upper bubbles are small enough to show the surfaces of contact between each and the two adjacent large bubbles below. These surfaces are invisible in A and C.

D is a system of eight bubbles in one plane, four forming a cross in the centre.

In all figures notice the fifth contact surface or 'polar furrow'.

bubbles below, the usual arrangement when four are superimposed on four (Fig. 24 A–C).

The final disposition must depend, therefore, not merely on the principles of least surfaces, but also, provided that the conditions of that principle are fulfilled, on the sizes and initial arrangement of the bubbles.

It will hardly need pointing out that very many ova adopt the form which presents the least external surface, that of a

sphere, when placed in a fluid medium, and it is also a familiar fact that after the first (and subsequent) divisions the blastomeres are flattened against one another (Cytarme, to use Roux's term), and that whether they are compressed by an egg membrane or not (examples of the second alternative are to be found in *Unio*, *Dreissensia*, *Umbrella*, *Crepidula*, *Aplysia limacina*, *Asterias*), though the surface of contact is not always curved when the cells are unequal. The two cells, however, often become rounded off and partially separated from one another prior to the next division. Such a separation (Cytochorismus) has also been observed by Roux in the case of cells of the Frog's egg, which, having been isolated in albumen or salt solution, have subsequently reunited.

That the cells flatten against instead of repelling one another, as free oil-drops would do, suggests that they, like soap-bubbles, are provided with an insoluble coating-film, while their subsequent separation may be provisionally explained by supposing that this coating-film becomes temporarily dissolved under the action of some substance formed in the cell. This idea is borne out by a striking experiment of Herbst's, who found that in sea-water deprived of its calcium the blastomeres of the sea-urchin egg came apart and resumed their spherical shape. At the same time the surface membrane underwent a visible alteration, becoming radially striated. It seems reasonable to conclude that there is a membrane by which contact is normally effected, and that this is soluble in sea-water devoid of calcium. On the addition of calcium the cells cohere again.

It may be mentioned that when systems of drops of jelly, floating in a medium of oil and united by their coating-films of water, are removed to alcohol, in which both oil and water are soluble, the films disappear and the drops separate.

In the next stage (four cells) the type of segmentation in which the laws of capillarity are most strictly obeyed is obviously that which we have distinguished above as the spiral or tetrahedral type, and Robert has been able to show that successful imitations of the four-, eight-, twelve-, and sixteen-celled stages of the egg of *Trochus* may be made with soap-bubbles.

Four equal bubbles were placed in a porcelain cup, which held them together in the same way that the actual cells are held

together by the vitelline membrane. Five surfaces of contact were formed, that between two opposite bubbles representing the cross furrow or polar furrow in the egg. In the *Trochus* egg, however, the polar furrows need not be parallel at the animal and vegetative pole; they may be at right angles to one another, and this tetrahedral arrangement of crossed polar furrows may be imitated by lifting up one of the bubbles and bringing it into contact with its opposite, one pair of bubbles being now in contact below, the other pair above. This arrangement is, however, unstable while the four bubbles remain in one plane, the two bubbles soon coming into contact both above and below. When the bubbles are not confined within a cup the instability of the 'crossed-furrow' condition is extreme.

By reducing the volume of the bubbles that are in contact the other two may be brought together; as the polar furrow changes positions there is at least a temporary condition when they are crossed.

As we have already pointed out, both conditions—the 'parallel furrows' and the 'crossed furrows'—are met with in the eggs at the four-celled stage of Molluscs, Annelids, and marine Turbellarians. Whether both opposite pairs or only one opposite pair of blastomeres are in contact does not, however, appear to depend upon whether the vitelline membrane is close to and compresses the egg or not. In most cases of crossed furrows the membrane fits, it is true, quite closely (*Nereis*, *Ichnochiton*, *Podarke*, *Lepidonotus*, *Discocelis*, *Physa*, and possibly *Limax* and *Planorbis*, if there is in these two, as in *Physa*, a very fine membrane between the albumen and the ovum); so also, speaking generally, where the furrows are parallel the membrane is absent (*Umbrella*, *Aplysia*, *Dreissensia*, *Crepidula*), but in *Amphitrite* and *Clymenella* it is lightly applied to the egg.

It is remarkable that when the furrows are crossed, it is the A and C cells which meet at the animal pole, the B and D cells at the vegetative (except only in *Unio*), and this must depend on other properties of the cells than their surface tensions. But it may be very plausibly suggested that the explanation of the fact that it is the cells B and D which meet to make the 'parallel' furrows is to be looked for simply in the large size of D.

Robert has indeed shown that by simply altering the sizes of

the bubbles the conditions observed in the four-celled stage of other types—*Nereis*, *Arenicola*, *Unio*, *Aplysia*, *Discocelis*—may be faithfully copied.

It only remains to be added that the contact surfaces of the cells, like those of the bubbles, make angles of 120° with one another.

Robert has also imitated the eight-celled stage (the four micromeres alternating with the four macromeres), the stage of twelve cells (division of the micromeres), and that of sixteen cells (second quartette formed). The bubbles of the second quartette may be made to slide in between the macromeres and so rotate the whole first quartette, as happens in the egg. The division of the micromeres in the egg results in the arrangement of four cells crosswise in the centre, four others occupying the spaces between the arms of the cross. The bubbles behave in the same manner.

In the eight-celled stage the micromeres alternate with the macromeres. In the case of the bubbles this is not necessarily so; the two sets of bubbles may be superposed if the 'polar furrow' in one tier is at right angles to that in the other, or if, as pointed out above, the upper bubbles are small. Otherwise superposition is a very unstable condition.

It would appear then that many of the patterns exhibited by eggs with a spiral cleavage are explicable by reference to the laws of surface tension. The principle of least surfaces may be extended to other cases. The first four blastomeres of Ophiuroids and Asteroids form a perfect tetrahedron, though this arrangement is subsequently discarded for one which could not be imitated with soap-bubbles (we may notice in passing that in the first case the egg is tightly invested by its membrane, in the second it is perfectly free). In *Ascaris megalocephala* the four cells come to lie, as do four bubbles, in one plane, and polar furrows have been seen in many eggs which belong to another type of segmentation (in Coelenterates (*Hydractinia*), Sponges (*Spongilla*), Crustacea (*Branchipus*, *Lucifer*, *Orchestia*), Vertebrates (*Petromyzon*, *Rana*), Ascidians, and *Amphioxus*).

The principle of least surfaces—not more than three surfaces meeting in a line, not more than four lines meeting in a point—is, however, not of itself sufficient to explain the whole of the phenomena even in this most favourable tetrahedral type;

other factors must intervene, just as other factors intervene in a mass of soap-bubbles—their size and initial arrangement—in the determination of the actual pattern. These other factors are the direction of cell-, that is of nuclear, division, and the magnitude of the cells; and these, as we have seen, in turn depend upon the relation between the nucleus and the cytoplasm with its included yolk. Thus it is the direction of the spindles which determines whether the micromeres of the first quartette shall be given off laeotropically or dexiotropically; the direction of division, oblique to the egg-axis, again determines that the micromeres shall alternate with the macromeres and not be superimposed upon them; the size of the cells and the direction

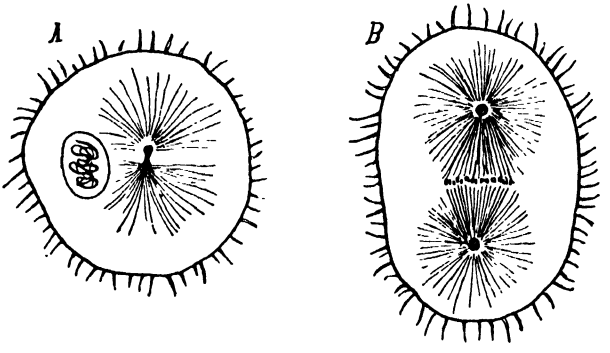


FIG. 25.—Mitotic division with elongation of the cell-body in a protozoon, *Acanthocystis aculeata*. (After Schaudinn, from Korschelt and Heider.)

of division may determine the position of the polar furrow, while the rate of division will also not be without effect, since the whole arrangement at any stage depends in part on the disposition at the stage before.

There is one other point that is worthy of notice. The mitotic spindle possesses considerable rigidity, and is able as it elongates to materially alter the shape of the cell. This may be seen in many cases in Annelid, Molluscan and other eggs—the division of the first micromeres in *Nereis* is an instance—and in the Protozoa (Fig. 25). Another interesting case is the Rotifer *Asplanchna*, where, preparatory to the fourth division, the shortest axis of the cells—in which the spindles are placed—becomes by the elongation of the spindles the longest. This alteration of shape is itself an important factor in deciding the positions to be taken up by the daughter cells.

In the other types—radial and bilateral—the principle of least surfaces is obviously disobeyed, for here four or more surfaces meet in one line and at angles other than 120°.

Roux (1897) has, however, shown that if a certain condition be imposed on the system of lamellae, figures may be produced which very closely resemble the patterns presented by radially and bilaterally segmenting ova. This indispensable condition is that the system shall be surrounded by a rigid boundary, as the eggs themselves are by a membrane. Roux's system was made by dividing into two, four, and eight a drop of paraffin oil suspended in a closely fitting cylindrical vessel between alcohol and water. To this medium was added calcium acetate to prevent the drops reuniting. The drop was divided with a glass rod.

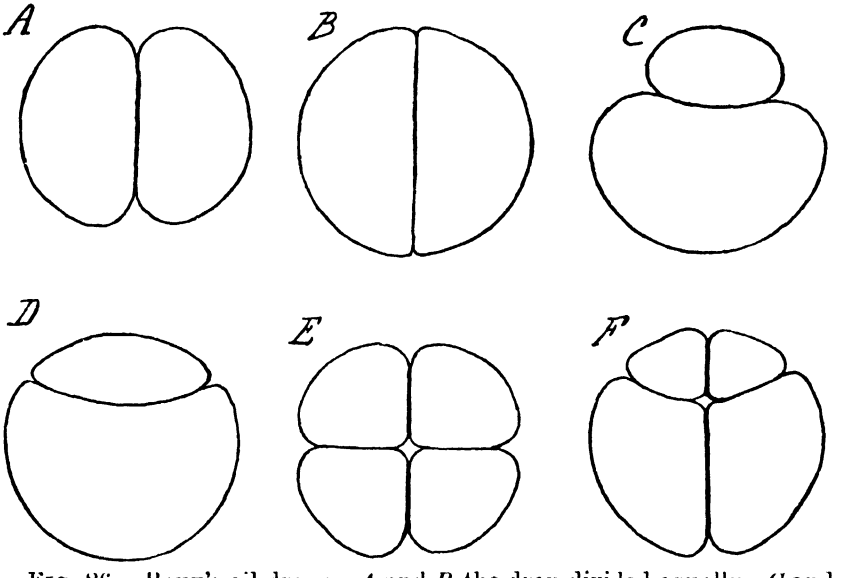


FIG. 26.—Roux's oil-drops. *A* and *B*, the drop divided equally; *C* and *D*, unequally. Each of the two equal drops divided equally in *E*, unequally in *F*. (From Korschelt and Heider, after Roux.)

When the two drops formed by the first division were equal the surface of contact was flat, when unequal convex towards the larger one, in accordance with the rule (Fig. 26 A–D).

When the second was also equal, four drops were formed with four surfaces of contact meeting in one line, or enclosing between them a small 'segmentation' cavity. If the division of the two equal drops was unequal, and the smaller cells adjacent, they pushed into the larger ones; the result, in fact, was the same

as would have been produced by an equal following on an unequal division, the four surfaces meeting in one line as before (Fig. 26 E, F). The appearance presented is like a side view of a radially

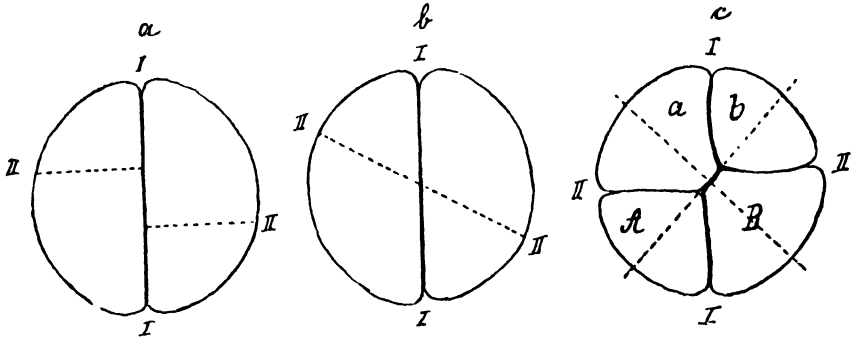


FIG. 27.—Arrangement of four oil-drops produced by unequal division of two equal drops, the small and large drops alternating. The first division is shown by *I*: the second (*II*) may pass as in *a* or in *b*, but the result is always as in *c*, the two large drops meeting in a polar furrow and excluding the small drops from the centre; the system is symmetrical (iso-bilateral) about the dotted lines in *c*. (After Roux, from Korschelt and Heider.)

segmenting egg after the third division. When, however, the smaller drops were not adjacent, but opposite, five surfaces of contact were formed, a polar furrow appearing between the two larger and joining the centres of mass of the two smaller drops, whether these are unequal or not. The direction in which the division of the drops is performed is irrelevant; the final result is always the same. Should two adjacent drops be equal, the polar furrow is still formed by the union of those two which have together the larger mass (Fig. 27).

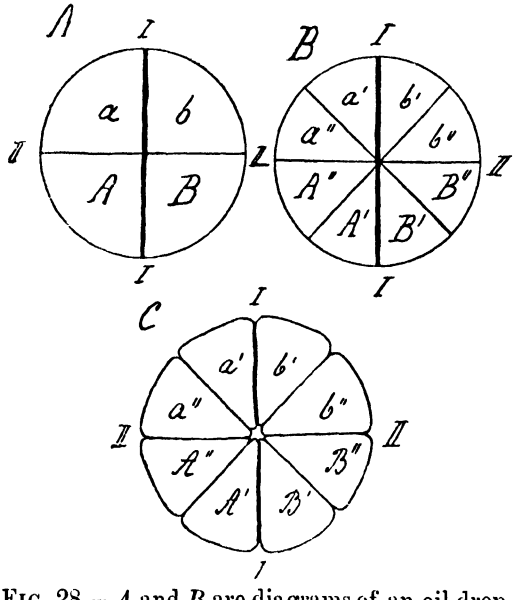


FIG. 28.— *A* and *B* are diagrams of an oil-drop divided into four and eight to explain Roux's notation. *C* is a figure of the oil-drop divided into eight equal parts. (From Korschelt and Heider.)

The length of the polar furrow varies directly with the size of the drops which unite to form it; its direction makes an angle with the plane separating the first two, which varies

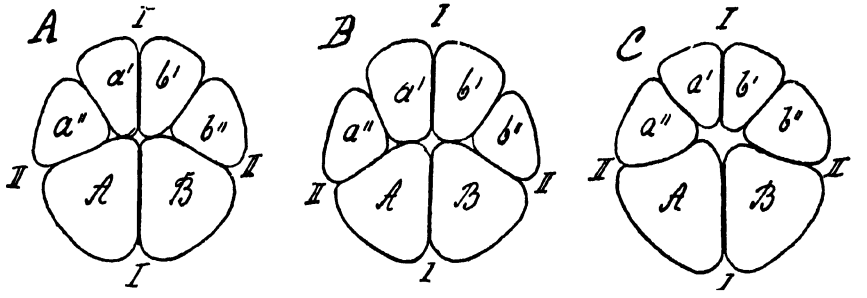


FIG. 29.—Arrangements of six oil-drops. In all cases $A = B = a = b$. In A, $a' = a''$, $b' = b''$. In B, $a' > a''$, $b' > b''$. In C, $a' < a''$, $b' < b''$. *I*, first furrow; *II*, second furrow. (From Korschelt and Heider, after Roux.)

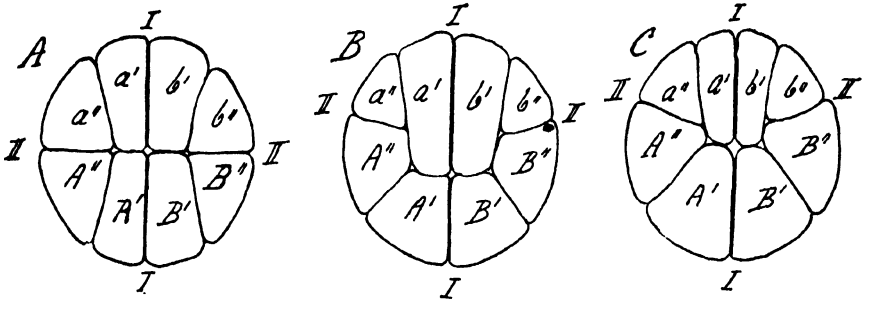


FIG. 30.—Various arrangements of eight oil-drops, all bilaterally symmetrical about the first furrow (*I*). In all cases the first division has been equal. In *A* and *B* the second division (*II*) has also been equal, but in *C* a, b are smaller than A, B. In *A*, $a'', b'', A'', B'' < a', b', A', B'$. In *B* and *C*, $a'', b'' < a', b'$, but $A'', B'' = A', B'$; hence $a'', b'' < A'', B''$. (From Korschelt and Heider, after Roux.)

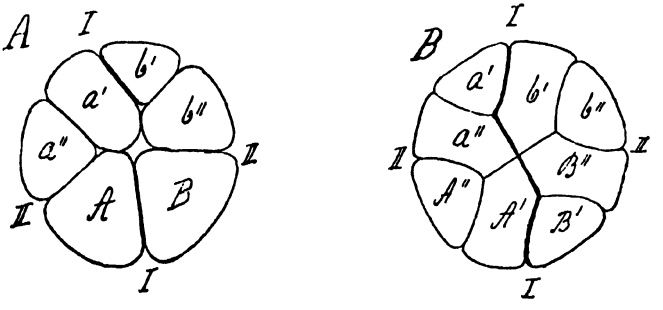


FIG. 31.—Arrangement of six (*A*) and eight (*B*) oil-drops, after unequal division of four equal drops ($A = B = a = b$), the smaller and larger drops regularly alternating. (From Korschelt and Heider, after Roux.)

inversely with its length, so that when all the drops are equal the cross furrow lies in the same plane with the first division, and so disappears.

By another division it is possible to make a ring of eight drops whose surfaces of contact all meet in one line, or in a 'segmentation' cavity (Fig. 28). To realize this condition, however, it is necessary that the division should be equal, and its direction accurately radial. If unequal, the larger drop invariably passes towards or wholly into the inside. If oblique or tangential the inner drop passes into the segmentation cavity (Fig. 32).

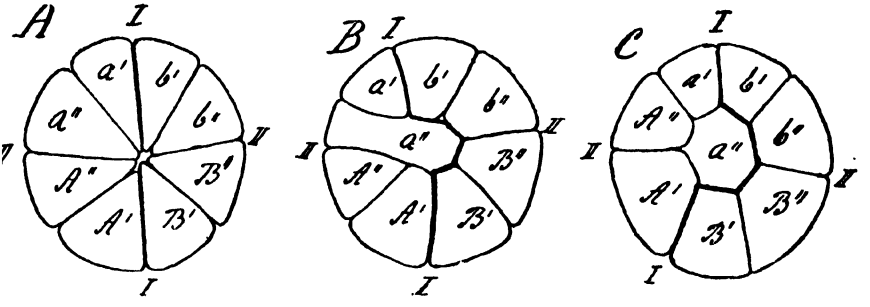


FIG. 32.—Three stages in the passage of a large drop (a'') into the centre of the system. The first stage extremely unstable. (From Korschelt and Heider, after Roux.)

Unequal division of all four equal drops produces very interesting patterns, some of which recall the appearance of bilaterally segmenting ova, when the divisions are correspondingly unequal on each side of the first or second division (Figs. 29, 30), while others resemble certain phases of 'spiral' division when small and large cells regularly alternate (Fig. 31). It is a rule for the smaller of the two drops to go to the periphery, while the larger assumes an oblong or wedge shape, passing towards the centre if it does not slip entirely inside. The latter occurs with clean oil, when the large drop is flanked by small ones on both sides.

It is also possible to divide four equal drops horizontally into two tiers. The upper drops, however—unless absolutely undisturbed—quickly come to alternate with the lower.

In these systems of drops the final arrangement is due to, first, the principle of least surfaces; secondly, the circumscribing boundary; thirdly, the size of the drops; and fourthly, in some cases, the direction in which they are divided.

It only remains for us to consider, with Roux, to what extent

the cells of a radially segmenting egg, such as that of *Rana fusca*, are governed by the same influences as determine the pattern of the drops.

The resemblances, it will be conceded, are often very close. There are also important differences. The polar furrow, which is often present in the Frog's egg, is not necessarily between the cells with the greatest mass. Again if, in the four-celled stage, with no polar furrow, one of the cells be diminished by puncture, a polar furrow does not always appear, as it would with oil-drops, nor, if it does, is it always formed by the union of the larger cells. Or, if when a polar furrow is present between the larger cells, one of these is diminished by puncture until it, together with its opposite, is less than the other two, the polar furrow nevertheless retains its position.

In the sixteen-celled stage the animal cells together form a ring of eight around the axis. The cells are not necessarily equal, and a small cell may be compressed by, instead of compressing, adjacent large ones, while they, not it, move away to the periphery.

Other differences are that large cells bulge into small, that cells are elongated tangentially instead of radially, that there are amoeboid processes at the inner ends of the cells, and intercellular spaces between them.

Further, Roux has examined the behaviour of the isolated cells of the Frog's egg in the morula stage. The cells were separated in a medium of albumen, or salt-solution, or a mixture of the two. They first approach and then flatten against one another (Cytarme), as do the blastomeres in the egg, completely or incompletely. The contact surface is generally symmetrical to the line joining the centres of mass of the two cells; it may be concave towards either the small or the large cell. More than two cells may unite to form rows or heaps. The angles made by the surfaces of contact may be 120°, or have other values. Four surfaces may meet in one line; at other times the arrangement is tetrahedral. In a 1·25 % solution of salt the cells are elongated, and united end to end in long branching strings. The pigment, diffused through the cell, later returns to its original position at the surface, or usually to the middle of the free surface of each.

The cells may also move over one another (Cytolisthesis) by

sliding or rotation, or both. Even two cells will glide on one another, as two soap-bubbles will not. In complexes two three-surface lines may unite to form one four-surface line, a behaviour the very opposite of that exhibited by soap-bubbles.

It appears, then, that in the living egg of the Frog (and other radial and bilateral types) there are factors which overcompensate, to use Roux's expression, the purely physical factors by which the behaviour of the oil-drops is governed. These organic factors are that division is slow, and begins on the outside; that the direction of division—determined by the yolk—is persistent; that the cell contents are neither perfectly fluid nor perfectly structureless; that the cells being different, their surface tensions may be of different magnitudes, and the whole system, therefore, not homogeneous; and that the cells possess a more or less solid rind or membrane, the rind which becomes wrinkled transversely to the furrow when the cell divides.

It would seem that this rind is an important factor, for if Roux's experiments be repeated with drops of albumen suspended between xylol and oil of cloves, to which a little alcohol has been added, it will be seen that each drop gets a superficial membrane, and that by these membranes adjacent drops adhere. In fact, such drops behave more like the cells of the egg than do the oil-drops. Thus, a small cell goes towards the inside, or the outside, according to the way in which the division is made, and, after a horizontal division of four equal cells, the upper remain superimposed upon the lower.

At the same time, it is apparently because the cells have this surface film, which the oil-drops have not, that they are able to flatten against one another as soap-bubbles do; while, on the other hand, it is because the film is solid that the cells are unable to move upon one another and adopt the geometrical arrangement seen in systems of soap-bubbles.

There is still another kind of cell-movement to which brief reference must here be made, since it is found in one type of segmentation at least. In the segmenting eggs of some Platyhelmia (Triclads), Ascidians (Salps), Echinoderms (Asteroids), and Coelenterates (*Oceania*), the blastomeres have been seen to completely separate from one another, afterwards reuniting. Roux

has observed a similar reunion of the artificially isolated cells of the Frog's egg. This Cytotropism, as Roux calls it (Cytotaxis would be a preferable term), is noticed when the slide is kept perfectly horizontal and streaming movements of the medium (albumen) are rigidly excluded. The cells become rounded, and then approach one another in, more or less, a straight line, oscillating slightly backwards and forwards. The cells must not be too far apart, not further than a radius of small, or less of large cells. Groups of two or more cells behave in the same way.

The movement may be simply a surface-tension phenomenon, or, as Roux suggests, more complex, of the nature of a response to a mutual chemotactic stimulus.

These various kinds of cell-motion are also an important feature in such processes of differentiation as the union of cells to form muscles, tendon, epithelia, and so forth.

A review of all the facts thus leads us to conclude that while some of the phenomena of segmentation—the flattening of cells against one another, the pattern made by the cells in cleavage, especially of the spiral type—are largely referable to the action of the purely physical laws of surface tension, there are many cases, the radial and bilateral types, and the radial and bilateral periods of spirally segmenting eggs, in which the operation of these laws is restricted and confined by other causes. But in any case those laws can only co-operate with other factors, which are to be looked for in the rate and direction of division, and in the magnitude of the cells, factors which themselves are dependent on the relation between the cell and its nucleus.

Before concluding this section we have to call attention to some experiments which may possibly throw some light on an event of fairly frequent occurrence in ontogeny—the division of the nucleus without the division of the cell,[1] as in the formation of coenocytia such as striated muscle fibres and the trophoblast of the placenta; or the fusion of distinct cells into a syncytium, as in the trophoblast again; or the secondary union of yolk-cells.

[1] In the Alcyonaria the nucleus may divide three, four, or five times before the egg simultaneously breaks up into eight, sixteen, or thirty-two cells. See especially E. B. Wilson, 'On the development of Renilla,' *Phil. Trans. Roy. Soc.*, clxxiv, 1883.

Driesch has observed that in the egg of *Echinus* cell-division may be wholly or partially suppressed by pressure, and also by diluting the sea-water. Nuclear division continues (Fig. 33).

Morgan has found that the egg of another sea-urchin (*Arbacia*) will not segment in a 2% solution of salt in sea-water; on replacing the eggs in sea-water, however, the nucleus divides with great rapidity several times, and this is followed by cell-division. So Loeb notices that the eggs when treated in this way, and brought back to their normal medium, divide simultaneously into four. The egg of the fish *Ctenolabrus* (according to the same author) behaves in a similar fashion when first deprived of, and then restored to oxygen.

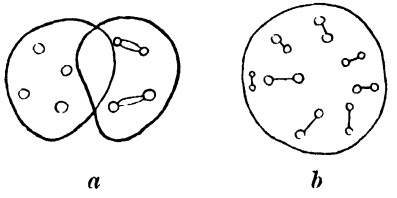


FIG. 33.—Echinus: suppression of cell-division by pressure, *b*, and by heat, *a*. Nuclear division continues. (After Driesch, 1893.)

Graf, again, has seen the reunion of sister cells and nuclei in the eggs of *Arbacia* released from pressure.

Three distinct agencies—mechanical pressure, increase of osmotic pressure, and decrease of osmotic pressure—are all capable of effecting this interesting change in the usual relations of cell and nucleus. We can only guess at the real cause, and surmise that it will be found in an alteration of internal and external surface tensions.

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2. GROWTH

Following Davenport we define growth as increase in size or volume. Since, therefore, growth is increase in all three dimensions of space, it is most accurately measured not by increase in some one dimension—such as stature—but by increase of mass or weight.

Growth depends upon the intake of food and the absorption of water and exhibits itself in the form of increase in the amount of living matter or of secretions of watery or other substances, organic or inorganic, intra-cellular or extra-cellular, such as chondrin, fat, mucin, cellulose, calcium phosphate, and the like.

That growth depends—in later stages at least—upon the intake of food is obvious. That it is due to the absorption of water has been demonstrated effectively by Davenport for the tadpoles of Amphibia (*Amblystoma*, *Rana*, *Bufo*). The method employed was to weigh known numbers of the tadpoles at different ages, desiccate and weigh again. The results of the investigation are shown in the accompanying figure (Fig. 34), from which it will be seen that the percentage of water rises with remarkable rapidity—from 56% to 96% during the first fortnight after hatching. After that point the amount of water present slightly but steadily declines.

The same result is brought out by an analysis of the terminal buds and successive internodes of plants. It is found in *Heterocentron* (Kraus) that the percentage of water rises rapidly from the terminal bud to the first internode, more slowly from the first to the second internode, and then remains constant.

It would thus appear that during the period of most rapid growth, growth is effected by imbibition of water rather than by assimilation, since the weight of dry substance in the tadpole during this period does not increase at all.

In later development the proportion of water slowly falls. This may be seen not only in Davenport's table of the growth of Frogs but in the data furnished by Potts for the Chick and by

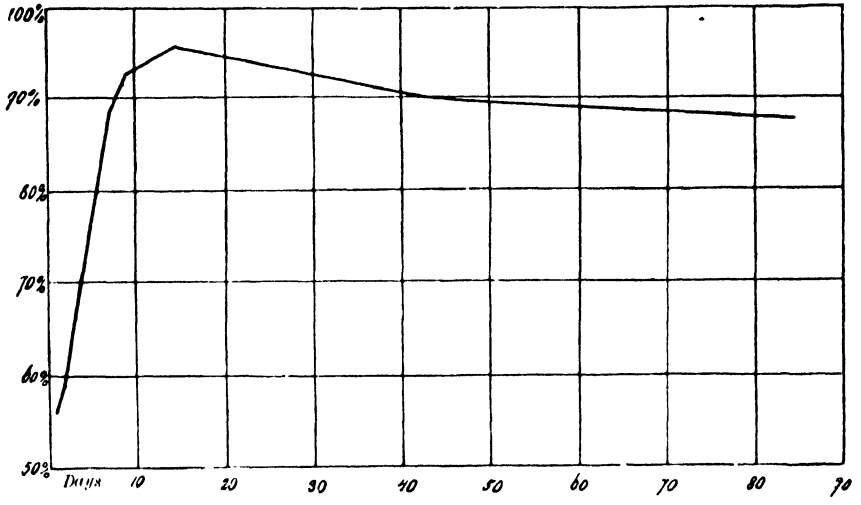


FIG. 34.—Curve showing change in percentage of water in Frog tadpoles from the first to the eighty-fourth day after hatching. Abscissae, days; ordinates, percentages. (After Davenport, from Korschelt and Heider.)

Fehling for the human embryo. These data are given in the accompanying tables (Tables I, II). The percentage of water, at first high, slowly falls in both cases; conversely, the percentage of other substances increases.

'These results indicate that during later development growth is largely effected by excessive assimilation or by storing up formed substance' (Davenport).

There are other external agencies by which growth may be affected in various ways—such as heat, light, and atmospheric pressure. These will be discussed in another chapter. For the present let us confine our attention to certain features which are characteristic of growth in general, of the growth of the animal organism under normal conditions. These are the changes that

take place during growth in the rate of growth itself, in the variability of the organism and in the magnitude of the correlations between its various parts.

TABLE I

Showing the percentage of water in Chick embryos at various stages up to hatching. (From Davenport, 1899 (2), after Potts.) The table also shows the hourly and daily percentage increments of weight.

Hours of brooding.	Absolute weight in grammes.	Increase.	Hourly percentage increment.	Daily percentage increment.	Percentage of water.
48	0·06				83
54	0·20	0·14	38·3	919·2	90
58	0·33	0·13	16·0	384·0	88
91	1·20	0·87	7·9	189·6	83
96	1·30	0·10	1·7	40·8	68
124	2·03	0·73	2 0	48·0	69
264	6·72	4·69	1 6	38·4	59

TABLE II

Showing the percentage of water in the Human embryo at various stages up to birth. (From Davenport, 1899 (2), after Fehling.) The table also shows the weekly percentage increments of weight.

Age in weeks.	Absolute weight in grammes.	Increase.	Weekly percentage increment.	Percentage of water.
6	0·975			97·5
17	36·5	35·525	331·2	91·8
22	100·0	63 5	34·8	92·0
24	242·0	142·0	71·0	89·9
26	569·0	327·0	67 6	86·4
30	924·0	355·0	15·6	83·7
35	928·0	4 0	0·1	82 9
39	1640·0	712·0	19·2	74·2

We follow Minot and Preyer in measuring the rate of growth by the percentage increments of weight (or of other measurements where weight is not available) during a given interval of time; that is to say, by expressing the increase in weight during a given period as a percentage of the weight at the beginning (or end) of that period. The change of rate, if any, is found by taking such percentage increments for successive equal increments of time.

As a first example let us consider the data furnished by Minot

himself for the rate of growth, after birth, of guinea-pigs (Table III, Fig. 35).

TABLE III

Showing the change of rate of growth in male and female Guinea-pigs, as measured by daily percentage increments of weight. (From Minot, 1891.)

Age in days.	Average daily per cent. increments.		Age in months.	Average daily per cent. increments.	
	Males.	Females.		Males.	Females.
1–3	0·0	2·1	8	0·05	0·2
4–6	5·6	5·5	9	0·3	0·2
7–9	5·5	5·4	10	0·1	0·1
10–12	4·7	4·7	11	0·04	0·1
13–15	5·0	5·0	12	0·1	0·05
16–18	4·1	4·3	13	−0·2	0·3
19–21	3·9	3·5	14	0·5	−0·03
22–24	3·1	1·7	15	0·2	0·00
25–27	2·8	1·9	16	0·07	0·2
28–30	2·8	2·6	17	−0·1	−0·02
31–33	1·9	1·8	18	−0·05	−0·2
34–36	1·7	1·6	19–21	0·006	−0·1
37–39	1·9	1·8	22–24	0·02	−0·05
40–50	1·2	1·1			
55–65	1·3	1·3			
70–80	1·2	0·8			
85–95	0·9	0·9			
100–110	0·7	0·8			
115–125	0·6	0·5			
130–140	0·1	0·2			
145–155	0 4	−0·03			
160–170	0·3	0·5			
175–185	0·2	0·2			
190–200	0·2	0·2			
205–215	0·4	0·3			

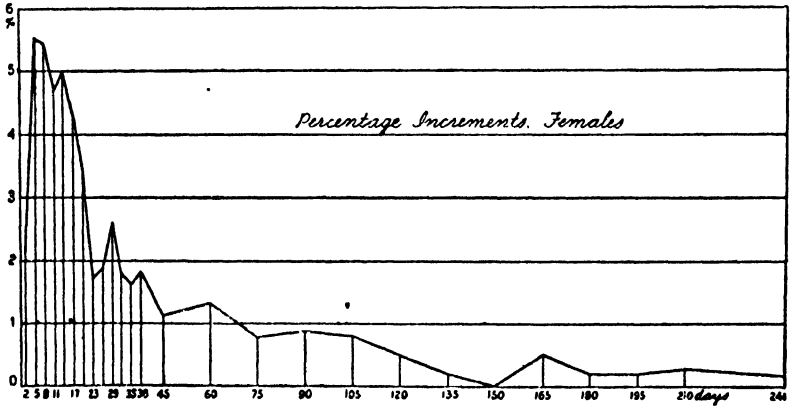


FIG. 35.—Curve showing the daily percentage increments in weight of female Guinea-pigs. (From Minot, 1907)

An inspection of the accompanying table and figure in which Minot's results are reproduced will show at once that there is in both sexes, almost from the moment of birth, a decline in the growth-rate. The decline is not, however, uniform. The rate falls rapidly between about the fifth day (when it is from 5% to 6%) and the fiftieth, from the fiftieth day onwards more slowly, becoming eventually very small, zero or even negative. The younger the animal, therefore, the faster it grows; the more developed it is the more slowly it grows. The rate of growth in fact varies inversely with the degree of differentiation. A mammal, therefore, which is born in a less developed condition than is the guinea-pig ought to grow at first more rapidly still. The rabbit is such an animal, and Minot has been able to show that on the fourth day after birth the young rabbit adds 17% to its weight. The curve also shows the same rapid decline in the growth-rate as was observed in the guinea-pig, followed by a period of gentle decrease.

Accurate observations on the prenatal rate of growth of these two mammals are lacking, but Hensen's few observations (quoted by Preyer) on the weight of guinea-pig embryos show that the daily percentage increase descends from 220% on the twenty-first day to 116% on the twenty-ninth day, to 33% on the forty-third day, and again to 6% on the sixty-fourth day, that is just after birth, the moment at which Minot's observations begin. Again, Minot has found, as a result of the investigation of the weight

TABLE IV

Showing the decrease in the rate of growth of the Human embryo before birth. Percentage increments calculated from the figures given by Hecker, Toldt, and Hennig. (From Preyer.)

Month.	Average monthly percentage increments of Weight. (Hecker.)	Length. (Toldt.)	Length. (Hennig.)
1	—	—	—
2	—	133·3	433 0
3	—	100·0	110·0
4	418·2	71·4	92·8
5	398·2	66·7	69·8
6	123·2	50·0	28·2
7	92·2	16·7	14·2
8	28 8	14·3	11·9
9	25·6	12·5	6·3
10	—	11·1	4 3

of spirit specimens of rabbit embryos that the mean daily percentage increment is 704 between the ninth and fifteenth days, but between the fifteenth and twentieth days only 212.

The postnatal decline in the growth-rate is therefore only a continuation of a process which has been going on for some time, perhaps from the first moment at which growth began.

The human being forms no exception to this rule. Data of the growth of the human embryo before birth are somewhat meagre, but an inspection of the tables will show that whatever the discrepancies may be between the results obtained by Fehling (Table II) and Hecker (Table IV), they agree in this, that the growth-rate falls with great rapidity between the fourth and the sixth months, thereafter more slowly till the end of pregnancy. This is graphically represented in the curve (Fig. 36). It will be observed from the table (Table IV) that the rate of increase of stature also declines, but less abruptly. This is a point to which we shall return. For the study of the postnatal growth of man very numerous data have been collected by various observers. Measurements of the body weight have been made on Belgians by Quetelet, on Boston school children by Bowditch, on the school children of Worcester, Mass., and Oakland, Mass., by Boas, and on English of the artisan and the well-to-do classes by Roberts. It is unnecessary to reproduce all these data here, for they all show the same decline in the growth-

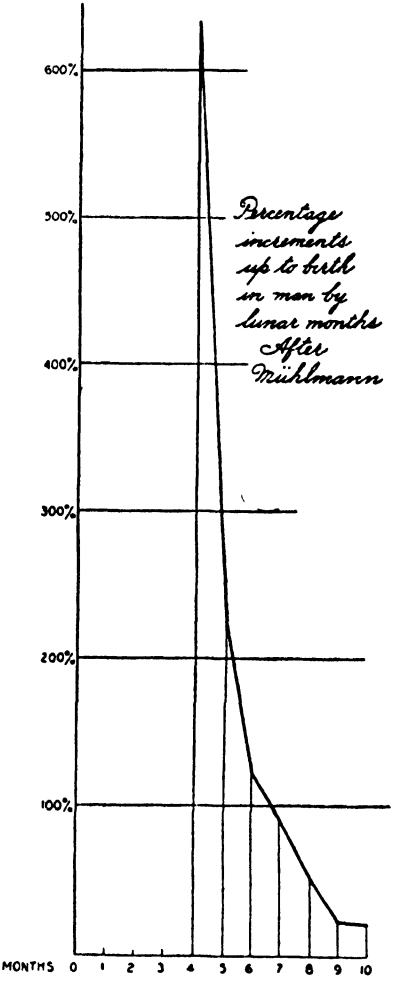


FIG. 36. — Curve showing monthly prenatal percentage increments in Man. (From Minot, 1907.)

rate, but Quetelet's measurements for males, being the completest series, are given in the accompanying figure (Fig. 37). The figure shows that at the end of the first year after birth the per-

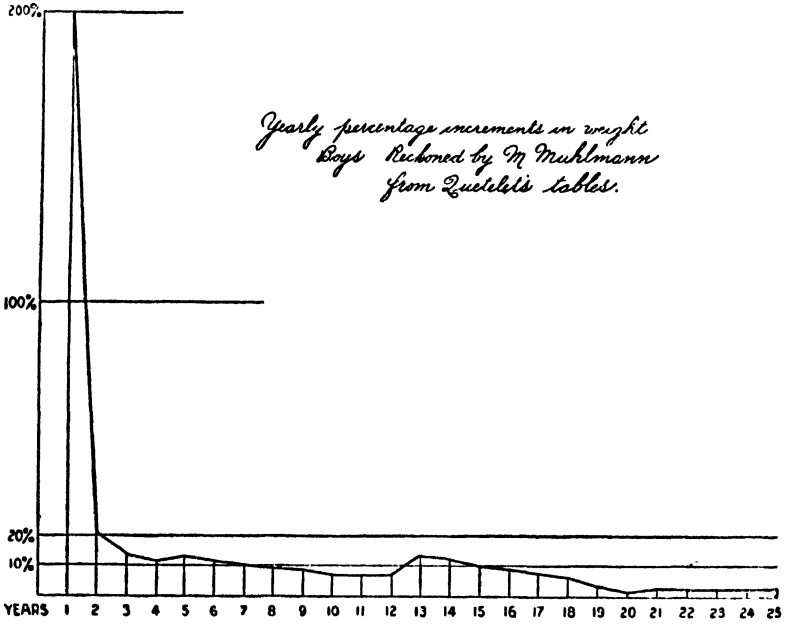


FIG. 37.—Curve showing the yearly percentage increments in weight of Boys. (From Minot, 1907.)

centage increment is as high as 200% (or nearly), but that then this increment drops to just over 20% at the end of the second year. From this point the decline is slow but sure, until at the thirtieth year the annual percentage increase is only 0·1%. The change of rate of growth in females is practically the same as in males.

The monthly percentage increment immediately before birth is about 20% according to Mühlmann's curve (Fig. 36); this represents an annual percentage increment of, say, 250%, and the annual increase at the end of the first year is about 200%. The postnatal decrease of growth is, therefore, as in other mammals, a continuation of the prenatal change. Further, there are two points at which the rate diminishes with great rapidity—between the fourth and sixth months of pregnancy and between the first and second years after birth. It would be of the greatest interest to discover the causes of these sudden decreases. Elsewhere the

diminution is gradual. A point of importance is that in both years there is a slight temporary rise in the growth-rate about the time of puberty (see the curve, Fig. 37). This has been noticed by all observers, but the actual time of its occurrence differs in different cases; the rise is invariably earlier in females than in males.

A comparison of the growth of the three mammals considered is interesting.

A Guinea-pig	reaches	775	grammes in	432	days.
A Rabbit	,,	2,500	,,	395	,,
A Man	,,	63,000	,,	9,428	,,

or the average daily increment is for a

Guinea-pig	1·82	grammes.
Rabbit	6·30	,,
Man	6·69	,,

Hence 'men are larger than rabbits because they grow longer, but rabbits are larger than guinea-pigs because they grow faster'. Minot, however, distinguishes between the 'rapidity' of growth, the average actual increment, and the 'rate' of growth, the percentage increment. The average percentage increments for these mammals are

Guinea-pig	0·47
Rabbit	0·50
Man	0·02

The rate is, therefore, much slower in man than in the other two. These percentages Minot calls the coefficients of growth. Together with the duration of growth they determine the ultimate size of the organisms.

The progressive loss of growth-power Minot speaks of as 'senescence', and compares to the loss of the power of cell-division in the 'senile decay' of Protozoa. The same author has also brought forward evidence to show that during differentiation there is an increase in the amount of cytoplasm in the cell, a decrease in the size of the nucleus, and a decrease in the 'mitotic index', that is in the proportion, in any tissue, of dividing cells. During segmentation, of course, the reverse of this is taking place, since cell-division is rapid and the protoplasm per cell is being constantly diminished until a fixed ratio between

nucleus and cytoplasm is reached (Boveri) (see below, p. 268). Minot suggests that 'senescence' and differentiation alike depend on an increase in the protoplasm.

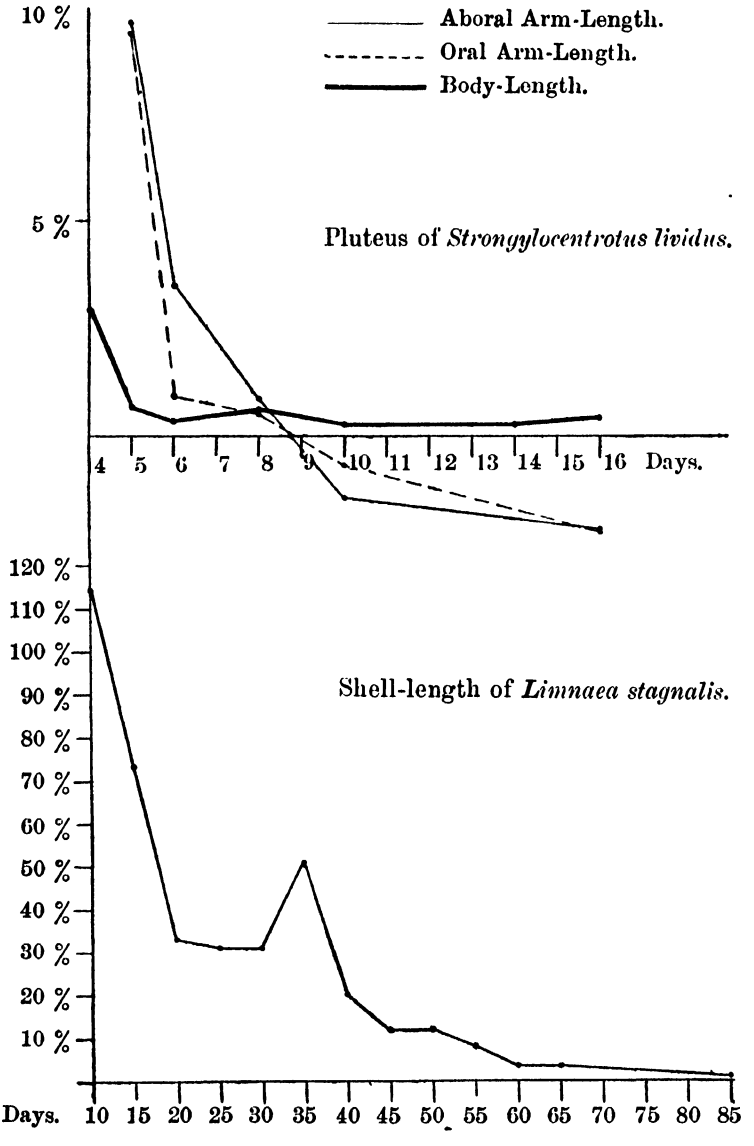


FIG. 38.— Curves showing the change with age of the rate of growth in the larva of the sea-urchin *Strongylocentrotus* (from Vernon's data), and the pond-snail *Limnaea* (from Semper's figures). The abscissae are days, the ordinates percentage increments.

The decline of the growth-rate may also be seen in Pott's weighings of the Chick embryo before hatching (Table I) and Minot's weights of young chickens. It appears from these that the daily percentage increment is 919% at the beginning of the third day of incubation, 189% at the end of the fourth day; at this point there is a sudden drop to 40%, which is still the rate of growth after eleven days of incubation; eight days after hatching the rate is 9% in the male, not quite 9% in the female, and then comes a period of more or less gradual decline, until when the chicken is 342 days old it is able to add less than 0·5% to its weight per diem.

Semper's observations on the pond-snail, *Limnaea*, and Vernon's on the sea-urchin, *Strongylocentrotus*, are other examples which

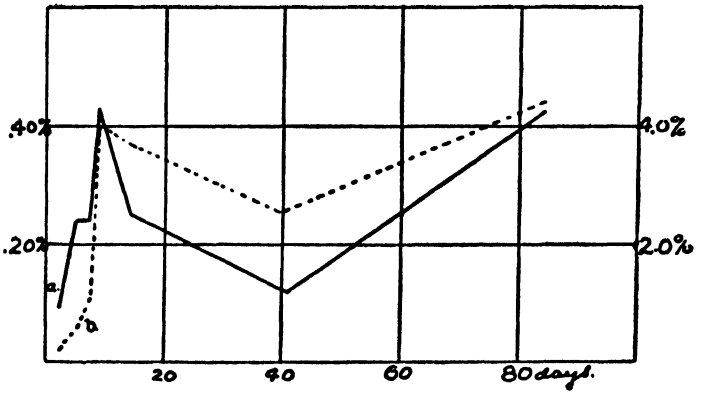


FIG. 39.—Daily percentage increments of weight in tadpoles: the continuous line (*a*) gives the whole weight, the broken line (*b*) the dry weight. (After Davenport, 1899.)

may be mentioned. The results of these authors are shown in the accompanying charts (Fig. 38). Their measurements are of lengths, not of weights.

So far we have found no exception to the law of the decline in the rate of growth as development proceeds. Davenport's measurements of tadpoles will not, however, conform to the generalization. As the figure shows (Fig. 39), the daily percentage increments, whether of the whole weight or of the weight of dry substance only, first rise abruptly, then descend and then rise again. An explanation of this anomaly may possibly be found in the fact that Davenport's measurements are

taken during that early period when growth is due in the main to absorption of water, the other measurements (as may be seen from Tables I, II) during the later period when the percentage of water has already begun to decline and growth is effected by other means.

It is, of course, a commonplace of embryology that the growth of all the organs of the body does not occur at the same rate.

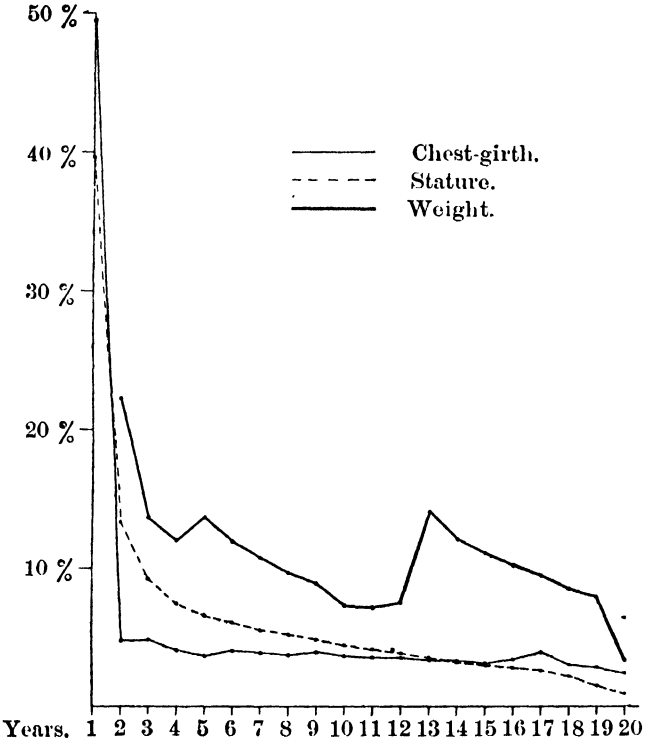


FIG. 40.—Curves showing the alteration during the first twenty years of life of the rate of growth as measured by weight, stature, and chest-girth in the human being (males). (Constructed from Quetelet's data.) The abscissae are years, the ordinates percentage increments. (The percentage increment of weight for the first year could not be included in the figure. It is given in Fig. 37.)

There are nevertheless few cases in which the exact difference in rate has been ascertained. From those few cases, however, it appears that the individual parts, though they do not grow with equal rapidity, still obey the same law as the whole.

Thus human stature exhibits the same loss of growth-power as is shown by the weight of the whole body, with this

difference, however, that the rate is not so high in early stages, the descent in later stages less abrupt. This will be seen in Table IV, in which such figures as are obtainable for the prenatal growth-rate are given, and in Fig. 40, in which the curve of change of growth-rate in human stature has been constructed from Quetelet's data (male Belgians). The percentage

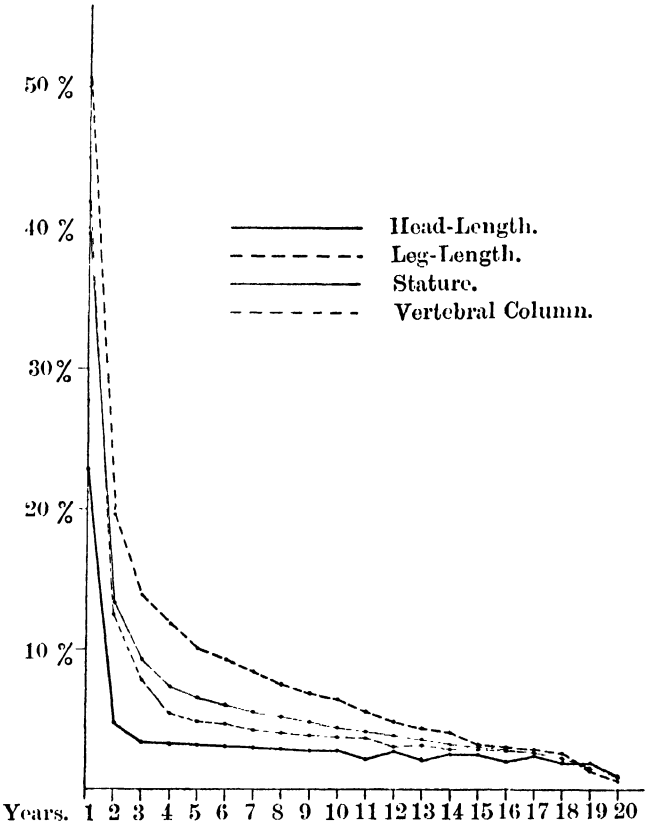


FIG. 41.— Curves showing the alteration during the first twenty years of life of the rate of growth of stature, length of head, length of vertebral column, and length of leg in the human being (males). (Constructed from Quetelet's data.) Ordinates, percentage increments; abscissae, years.

increment in the first year is only 39·6 as against 190·3 for weight, in the second year 13·3 as against 22·2 for weight. Thenceforward the rate slowly declines, until at the fortieth year it is zero, and after the fiftieth year increasingly negative. The rate of increase of stature is always slightly less than that of

weight. Quetelet's figures do not show the rise in rate about the time of puberty, but this phenomenon is apparent in the data furnished by Bowditch, Boas, and Roberts (see Fig. 42). The change in the growth-rate is practically the same in women as in men. As with weight, the rise of rate at the time of puberty is earlier.

The decline in the growth-rate of chest-girth is shown in the same figure (Fig. 40). It will be noticed at once that in this case the drop in the first year is very great indeed, from nearly 50 % to nearly 5 %, and that the rate is only diminished by another 2·4 % in the next nineteen years. The weight will depend upon the volume and the volume on both stature and girth; in fact a rough weight-curve might be constructed from the measurements of stature and girth. It is evident that the sudden loss in the rate of total growth after the first year is due to the very rapid decrease in the percentage increment of girth.

It may be mentioned that other measurements of girth—girth by the sternum, the waist, the hips, the neck, the biceps, the thigh—show the same exceedingly abrupt decrease, almost to the minimum rate, between the first and second years.

In other cases—distance between the eyes, width of mouth, length of hand, length of foot, arm-length, leg-length, length and breadth of head, distance from the crown of the head to the first vertebra, length of the vertebral column—the change is more gradual; the rate of change, however, differs in different cases. As an instance of this let us consider the measurements—from the crown to the first vertebra, the length of the vertebral column, and the leg-length—which together make up the total stature. The growth-curves of these three and of the whole stature are presented in the figure (Fig. 41), from which it will be seen that the growth of the leg is faster than that of the vertebral column (until the eighteenth year), and this than that of the head. Increase in stature takes place at nearly the same rate as that of the vertebral column, but is on the whole a little faster.

There are few cases—besides man—in which we possess information as to the growth of the parts. In the sea-urchin, *Strongylocentrotus*, Vernon has shown that the growth-rate of the oral and aboral arms of the Pluteus diminishes rapidly from

the third to the fifth days, more slowly from the fifth to the eighth days. After this the rate becomes negative, as the skeleton of the Pluteus is used up by the developing urchin. The curves of change of rate of growth—as constructed from Vernon's figures—are shown in the chart (Fig. 38).

In *Carcinus moenas* a gradual decrease in the growth-rate of the frontal breadth can be ascertained from Weldon's data.

We have next to consider another feature of growth, the alteration of variability. The facts at our command are derived from a study of Echinoid larvae (Vernon), Duck embryos (Fischel), Guinea-pigs (Minot), the Periwinkle (Bumpus), the Crab, *Carcinus* (Weldon), and the human being (Bowditch, Pearson, Roberts, and Boas). Vernon has shown that in the Pluteus of *Strongylocentrotus* the variability of the body-length increases regularly up to the fifth day, and then decreases regularly again to the sixteenth day. So Fischel's measurements of Duck embryos seem to establish a greater variability in younger than in older stages. This is true of the whole length, the head (as far back as the first somite), the hand and trunk together, and the total length exclusive of the primitive streak. The data are, however, too few to be treated statistically; the variability can only be roughly estimated from the extent of the limits within which the part varies at each stage.

Minot, who expresses the variability of guinea-pigs by the difference between the mean weight and the mean weight of the individuals above, and of those below, the mean, likewise finds that the range in variation diminishes with age, and further that, in the case of the males, there is a period—from about the fourth to the ninth months—when the variability is very much less than at any other time. No such sudden fall is observed in the female, only a steady diminution.

A more satisfactory calculation of the alteration of variability may be made from the measurements taken by Bumpus of the 'ventricosity' (ratio of breadth to length) of the shell of the Periwinkle, *Littorina littorea*. The series of observations is very large, and includes both British and American forms. In the accompanying table (Table V) the coefficients of variability (the standard deviation expressed as a percentage of the mean) are

given for each age, as determined by length of shell, for the English and American periwinkles separately and also for the complete series. It will be seen that the variability increases slightly and then diminishes again. This is the case also in the American examples, where the fall at the end of growth is greater still, but in the British specimens there is only a slight fall, at 20–21 mm., followed by a considerable rise. The possible significance of this difference in the behaviour of the same species on the two sides of the Atlantic we shall discuss in a moment.

TABLE V

Showing the alteration in the variability of the ventricosity of the shell of the Periwinkle (*Littorina littorea*) during growth.

Coefficient of variability $\left(\frac{\sigma}{M} \times 100\right)$.

Length in mm.	British.	American.	All.
–15	2·77	3·27	3·25
16–17	2·94	3·41	3·34
18–19	3·02	3·39	3·35
20–21	2·93	3·03	3·13
22	3·25	2·84	3·02

In the meantime let us consider another case, the Crab, *Carcinus moenas*, the variability of the frontal breadth of which was examined by the late Professor Weldon. Weldon found that the variability, as measured by the quartile error (Q), first increased and then suddenly diminished with age (as determined by carapace length). If the variability is measured by the coefficient of variability (easily calculated from Weldon's data) the result is the same. This will appear from the table (Table VI).

TABLE VI

Showing the change in the variability of the frontal breadth with age in *Carcinus moenas*. (After Weldon.)

Carapace Length in mm.	Q.	$\frac{\sigma}{M} \times 100$.
7·5	9·42	1·64
8·5	9·83	1·76
9·5	9·51	1·73
10·5	9·58	1·78
11·5	10·25	1·93
12·5	10·79	2·06
13·5	10·09	1·95

For the calculation of the variability at different ages in man data have been provided by Roberts, Bowditch, and Boas. Some

of these results are collected in the following table (Table VII), from which it may be gathered that the variability diminishes at first, then rises until it attains a maximum at about the time of puberty, and then diminishes again, reaching finally a value which is lower than the original. The values for the coefficient obtained by the different investigators are fairly similar, and agree very well with those first given by Pearson (for male new-born infants, weight 15·66, stature 6·50; for male adults, weight 10·83, stature 3·66). It may be seen from the values for the new-born that the variability has already undergone a diminution before the age at which the other observations begin.

TABLE VII

Showing the change in the value of the coefficient of variability in the male Human being during growth.

Coefficient of variability $\left(\frac{\sigma}{M} \times 100\right)$.

Years.	Weight.			Stature.		
	Boston (Bowditch).	Worcester, Mass. (Boas).	English Artisans (Roberts).	Boston (Bowditch).	Toronto (Boas).	Worcester, Mass. (Boas.)
4			14·00			
5	11·56		11·48	4·76	4·82	
6	10·28	12·04	10·08	4·60	4·34	5·40
7	11·08	11·87	10·29	4·42	4·35	4·24
8	9·92	11·83	10·78	4·49	4·58	4·32
9	11·04	12·29	10·85	4·40	4·41	4·30
10	11·60	12·92	11·06	4·55	4·68	4·44
11	11·76	14·45	11·90	4·70	4·53	4·51
12	13·72	15·56	11·48	4·90	4·85	4·49
13	13·60	18·07	11·76	5·47	5·36	5·21
14	16·80	16·80	12·74	5·79	5·64	5·43
15	15·32	18·28	14·00	5·57	5·71	5·19
16	13·28	13·95	12·95	4·50		3·92
17	12·96	11·23	11·55	4·55		3·32
18	10·40		12·18	3 69		
19			10·29			
20			9·03			
			10·50			
			10·92			
			12·04			

Further, the variability does not merely diminish as the animal grows older. Its diminution accompanies the diminution in the rate of growth, and when—as at the time of puberty in man—that rate increases, the variability increases too.

The variability of such parts as have been examined for the

purpose alters in the same way as that of the whole body. Besides weight and stature Boas has recorded measurements of height sitting, head-length, head-width, length of fore-arm, and hand-width.

Though the evidence, it must be admitted, is scanty, it is none the less a remarkable fact that in all the cases we have examined the variability, whether of the whole organism or of its parts, decreases with the decrease in the rate of growth. We seem to be in the presence of a phenomenon of general occurrence, though what the significance of the phenomenon is is not at present clear.

As is well known, Weldon has argued that the decline in the variability of the older crabs is due to a selective death-rate, an argument which is supported by the same author's observations on the snail *Clausilia*, since in this form the variability of the adult was found to be the same as the variability of the same individuals when young, but less than the variability of the general population of young. It is possible that the marked decrease in the variability of the American as compared with the British periwinkles may also be attributable to the same cause, since this animal has only recently been introduced into America, and may, therefore, be subjected to a more severe struggle for existence in its new environment.

It is doubtful, however, whether this explanation will fit all cases.

Vernon has suggested that at periods of rapid growth the effect produced upon the organism by a change in its environment must be much greater than at other times, and, since he has further shown that one of the effects of an adverse change of circumstances is an increased variability, he argues that an increase in variability would naturally accompany a high growth-rate.

Lastly, Boas points out that the rate of growth is itself a variable magnitude, and this 'variation in period' may, with other causes, be a factor in producing the actual variation at each stage. Should that be so, the variability would necessarily increase and diminish with an increasing and diminishing growth-rate, since those that are above the mean would tend to remove

themselves further from the mean than those that are below could approach it, and the more so the faster they were growing, and conversely.

We have finally to consider very briefly what little is known of the alteration with growth in the value of the correlation between various organs. Such data as we have indicate that, like variability, this value rises and falls with the growth-rate.

Boas has ascertained the correlation coefficient (ρ) in man between weight, stature, height sitting, length and width of head at different ages. Some of these results are tabulated below (Table VIII); from this table it will be evident that the value of ρ decreases, increases, and decreases again. The values are for girls, and the period of increase is earlier than that found for boys. In the chart (Fig. 42) are given the successive values of growth-rate (stature), variability (height), and correlation coefficient (height sitting and head-length) for boys; the three magnitudes rise at about the time of puberty, and subsequently decline together. Boas urges that if the actual variability is in part the effect of variation in period, this effect will be greater during periods of rapid development. It follows from this that if the various organs of the body are equally affected by a change in the growth-rate, correlations would be closer during periods of rapid growth than at other periods.

TABLE VIII

Values of the correlation coefficient, ρ, during growth for four different correlations. Girls, Worcester, Mass. (Boas).

Years.	Stature and Weight.	Stature and Height sitting.	Stature and Length of Head.	Stature and Width of Head.
7	·73	·74	·30	·21
8	·76	·79	·36	·15
9	·80	·82	·35	·16
10	·83	·83	·37	·16
11	·81	·84	·37	·25
12	·77	·82	·38	·27
13	·73	·83	·38	·37
14	·67	·82	·30	·25
15	·65	·79	·26	·22
16	·60	·74	·25	·10

It will be noticed that the value of ρ for the different organs is different, being greater between axial organs—stature and height sitting, stature and length of head—than between longi-

tudinal and transverse parts, such as stature and width of head. The correlation between stature and weight is high.

To whatever cause it may be due this diminution of correlation with age is of the greatest interest, since it points to an increasing power of self-differentiation in the parts of the body. From other sources also there is evidence of a progressive loss

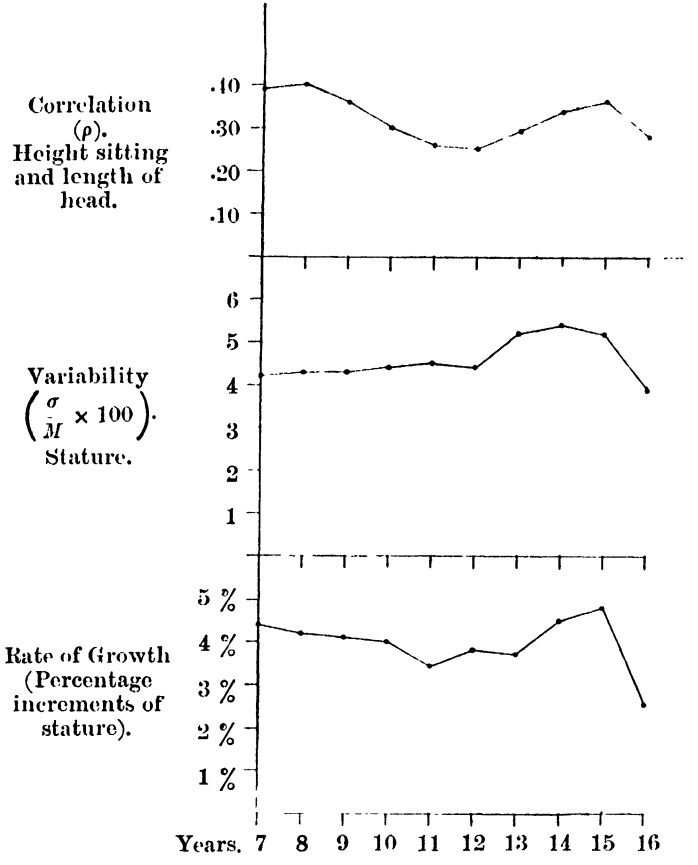


FIG. 42.—Figure to show how the rate of growth (percentage increments of stature), the variability (of stature) and the correlation coefficient (between height sitting and length of head) rise together at the time of puberty in man and then fall together. (Constructed from the tables of Boas.)

of totipotentiality of the parts, of an increasing independence of the parts, of a tendency to be increasingly governed in their development by factors that reside wholly within themselves. But this evidence must be discussed elsewhere.

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CHAPTER III

EXTERNAL FACTORS

1. GRAVITATION

In the large majority of cases there is no definite relation between the vertical and either the axis of the egg, the planes of its segmentation furrows, or the position of the development of the embryo in it. Thus the eggs of insects are laid with the axis making any angle with the vertical, and the same may be said of Crustacean ova. In such eggs as develop freely in the sea (some Mollusca, for example, Polychaeta, Coelenterata, Ctenophora) the axis and the planes of segmentation undergo a perpetual change of position, and Oscar Hertwig has shown that in the eggs of Echinoderms there is no necessary fixed relation between the direction of the planes of segmentation and the vertical. In these cases it is clear that the features of development referred to cannot depend upon the force of gravitation.

There are, however, instances in which it seems possible that the directions of the planes of segmentation—bearing as they do a constant relation to the axis of the egg—may depend upon gravity, since the axis is normally vertical. It was Emil Pflüger who in 1883 first brought forward experimental evidence to show that this was indeed the case.

It is well known that the yolk of the hen's egg always turns over so that the germinal disc is uppermost, and the egg of the Frog, free to rotate inside its jelly membrane, invariably takes up a position with the black pole uppermost, the white pole below.

This property of the Frog's ovum is exhibited alike by the ovarian, the coelomic, the uterine, and the freshly laid egg, by the living egg and the dead egg, by the whole egg, and by portions that contain both kinds of egg-substance, the yolk and the cytoplasm, as Roux showed by floating eggs or fragments in a medium of the same specific gravity. It is simply due to the fact that in the spherical telolecithal egg the heavier yolk

is placed mainly on one side, while on the other the lighter protoplasm is more abundant, the yolk granules far smaller and more sparse. The distribution of these two substances determines indeed the axis about which the egg has a 'rotation structure' or is radially symmetrical. The symmetry is further marked by the disposition of the pigment and the position of the nucleus. The pigment is placed in a thick superficial layer in the protoplasmic portion, it extends over rather more, sometimes considerably more, than a hemisphere, for there is much variation in this respect, and its boundary is a circle whose plane is at right angles to the egg-axis—the line which passes through the centre of the egg, the centre of the pigmented portion or animal pole and the centre of the unpigmented portion or vegetative pole. There is also an axial, less-deeply pigmented plug in the animal hemisphere. The nucleus is placed axially, but excentrically, very much nearer the animal than the vegetative pole, in a pigment-free spot or 'fovea germinativa'.

The egg is invested with a layer of jelly (mucin), inside which it becomes eventually free to rotate. This, however, is not possible when the egg is first laid, for the jelly is at that time closely adherent to it. In water, however, the jelly swells up, and a narrow cavity is formed in about three hours between it and the egg, and the egg then turns over until its axis is vertical. The formation of the cavity is much more rapid if fertilization (insemination) has taken place; in this case the egg turns over in half an hour. The rapid formation of the perivitelline fluid is the first effect of insemination, and is due to some substance secreted by or accompanying the sperm, since the spermatozoon does not reach the egg for another quarter of an hour (O. Hertwig). A second effect is an alteration in the viscidity or cohesion of the egg-contents; for while in the ovarian or uterine egg no alteration occurs apparently in the disposition of yolk, cytoplasm, and pigment, although the egg-axis may make any angle with the vertical, such an alteration is undoubtedly produced by gravitation (see below) after fertilization has occurred. Another effect noted by Roux is that fertilized (not merely inseminated) eggs turn over more rapidly in a medium of like specific gravity than do unfertilized.

The most important first result of fertilization is, however, the replacement of the radial by a bilateral symmetry. About two or three hours after insemination a certain portion of the border of the pigmented area, crescentic in shape and extending over about half its periphery, becomes grey by retreat of the pigment into the interior (Roux). The egg can now be divided into similar halves by only one plane, the plane of bilateral symmetry, which includes the axis and the middle of this grey crescent (Fig. 43).

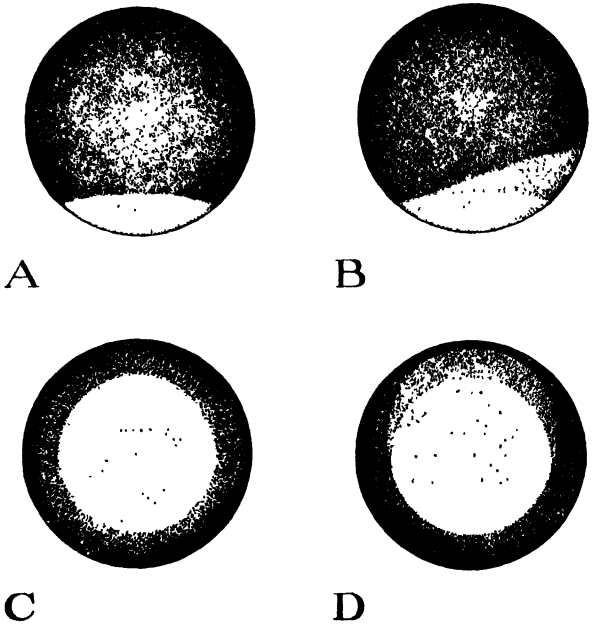


FIG. 43.—Formation of the grey crescent in the Frog's egg (*R. temporaria*). A, B from the side; C, D from the vegetative pole. In A, C there is no crescent, in B, D a part of the border of the pigmented area has become grey.

The middle of the grey crescent is always diametrically opposite to the point of entry of the sperm (Roux and Schulze); the crescent has hence been held by Roux to be directly caused by that entrance.

The plane of symmetry, as we shall see in another connexion, becomes in most cases the sagittal plane of the embryo, since the dorsal lip of the blastopore arises in the region of the grey

crescent. This side becomes the dorsal side of the embryo, while the animal pole marks, approximately, the anterior end.

By complete disappearance of the pigment the grey crescent becomes added to the white vegetative area of the egg.

The foregoing account applies in particular to *Rana temporaria* and *R. fusca*; *R. palustris* appears to be similar, but in *R. esculenta* it is stated that the egg-axis is eventually not vertical but oblique (Fig. 44). It seems, however, doubtful whether this obliquity is not rather apparent than real. The grey crescent has apparently not been recognized as such—the pigment is not so deep as in the other species—but included, nevertheless, in the white area, with the result that the centre of this, the definitive

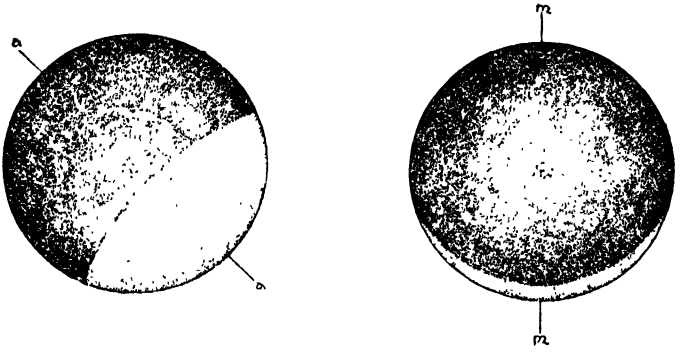


FIG. 44.—Egg of *Rana esculenta* after fertilization, in its normal position with the axis oblique (?). *A*, from the side; *B*, from above; *a a'*, egg-axis; *m m*, plane of first furrow. (After Korschelt and Heider.)

white area, has been confounded with the centre of the original unpigmented area or vegetative pole of the vertical egg-axis. (Compare Fig. 44 A with Fig. 43 B.)

As is well known, the planes of division during the first few regular phases of segmentation bear a perfectly definite relation to the axis. The first two, at right angles to one another, are meridional and therefore also vertical, the third furrows are parallel to the equator, therefore also horizontal; the furrows of the fourth phase are again meridional, and hence vertical, those of the fifth once more latitudinal and horizontal. It is this obvious relation of the planes of cleavage to the direction of gravity which has raised the question whether there is not a causal connexion between the two, the question which Pflüger attempted

to answer by experiments, performed, however, not on the eggs of the Frog but on those of a Toad, *Bombinator igneus*.

The close adherence of the unlaid egg to the glutinous jelly, which in its turn could be easily fixed to some object, provided a simple method of keeping the egg in any desired position. The eggs were removed from the uterus, attached with the axis at various angles to the vertical to watch glasses and fertilized with just enough sperm-water to allow of development, but not enough to permit of the formation of the perivitelline space and rotation of the egg into the normal position with the axis vertical. In these forcibly inverted eggs it was found that the furrows of segmentation bore the same relation to the vertical as in the normal egg; that is to say, the first was vertical, the second vertical and at right angles to the first, the third horizontal and nearer the upper pole, whatever the inclination of the egg-axis to the vertical, except in the extreme case where the white pole was exactly uppermost (180° of inversion), when segmentation did not occur at all. There was, however, no definite relation between the plane of the first (and therefore of subsequent) furrows and the original axis of the egg; the angle between this axis and the plane of the first furrow, as also that between the first furrow and the plane including the original and the actual vertical axes of the egg, might, it was found, have any value.

Except in a few cases, and where the white area was nearly exactly uppermost, these eggs gave rise to normal embryos. The upper smaller cells divided more rapidly than the lower ones, whether pigmented or unpigmented, and the blastula stage was reached; the dorsal lip of the blastopore appeared on one side a little below the (actual) equator, and the lower surface was covered over by the blastoporic fold in the ordinary way. Only in the failure of the whole egg to rotate after the closure of the blastopore (owing to the close adherence of the jelly) and in the irregular pigmentation (according to the original degree of inclination) did these embryos differ from the normal. One other point is worthy of notice. In the majority of cases the dorsal lip of the blastopore, marking the sagittal plane, appeared on the unpigmented side and lay in the plane including the

original, now inclined, and the actual axis, or vertical line of intersection of the first two furrows. While, therefore, the cleavage planes are definitely related to the vertical but not to the original axis of the egg, the median plane of the embryo appears to be jointly determined by both.

From these experiments Pflüger drew remarkable and far-reaching conclusions. He conceived of the egg as being meridionally polarized, composed of a large number of rows of molecules placed meridionally with regard to the original egg-axis. Each row is equivalent developmentally to every other row, but within the limits of each the molecules are of different value, since one end, for example, is anterior, the other posterior. Which of these equipotential rows shall lie in the sagittal plane of the embryo is decided by gravity, and by gravity alone. Similarly the vertical direction of the first two furrows, the horizontal direction of the third, is due to the operation of some general, though at present unknown, law, in accordance with which 'die Schwerkraft die Organisation beherrscht'.[1]

The original structure of the egg, on the other hand, has no definite relation either to segmentation or to the symmetry of the embryo, except, of course, in so far as the original axis together with the actual vertical axis determines its sagittal plane, the white side its dorsal side.

'. . . das befruchtete Ei gar keine wesentliche Beziehung zu der späteren Organisation des Thieres besitzt, sowenig als die Schneeflocke in einer wesentlichen Beziehung zu der Grösse und Gestalt der Lawine steht, die unter Umständen aus ihr sich entwickelt. Dass aus dem Keime immer dasselbe entsteht, kommt daher, dass er immer unter dieselben äusseren Bedingungen gebracht ist.'[2]

It will certainly be agreed that so sweeping and revolutionary a dogma as this is in need of very substantial support; and though the facts as stated by Pflüger are incontrovertible, as the repetition of his experiments has shown, it is unfortunate that he did not also take into consideration the internal changes occurring in his forcibly inverted eggs. The deficiency has been made good by Born. Like Pflüger, Born found that in the

[1] l. c. infra, xxxii. p. 24.

[2] l. c. infra, xxxii. p. 64.

forcibly inverted eggs the cleavage planes had the normal relation to the vertical, but not to the egg-axis; he observed, however, that the first furrow was usually in or at right angles to the streaming meridian, the plane, that is, including the original and the secondary vertical axes. The recent examination of 215 cases by the writer has shown that the first furrow tends to lie either in, or at right angles to, or at an angle of 45° to this plane. Subsequent development was normal, and the sagittal plane coincided with the streaming meridian. The dorsal lip appeared on the white side, which is thus anterior (anterodorsal). The examination of sections, however, showed that in these

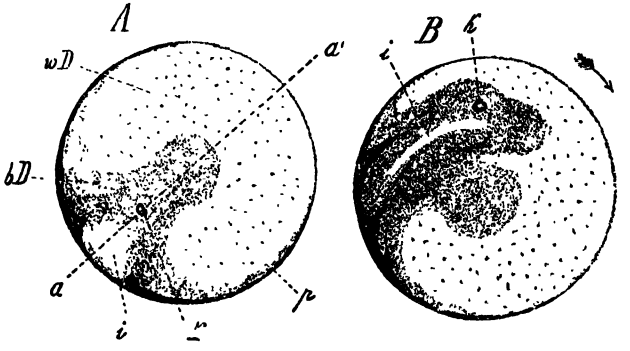


FIG. 45.—Sections through forcibly inverted Frog's eggs. In *A* the egg has just been inverted, in *B* the streaming of protoplasm upwards and yolk downwards has begun. Both sections are in the streaming meridian or gravitation symmetry plane, including both the axis ($a\,a'$) and the vertical. *bD*, pigmented animal protoplasm; *wD*, unpigmented vegetative yolk. *a*, animal pole; *a'*, vegetative pole; *i*, pigment-free clear area; *k*, nucleus; *p*, superficial pigment. (After Born, from Korschelt and Heider.)

inverted eggs there had been a redistribution of the contents, the heavy yolk sinking to the lower side, the lighter protoplasm, with the pigment and the nucleus, and the spermatozoon rising to the upper side (Fig. 45). The movement is rotatory, the cytoplasm and yolk ascending and descending in opposite directions; and it also takes place naturally parallel to, and in a similar manner on each side of, the plane in which the primary and secondary axes lie, hence known as the streaming meridian. That end of this plane towards which the protoplasm moves in its ascent, the end, that is, marked by the primary vegetative pole,

is anterior (more correctly, anterodorsal), for it is here that the dorsal lip of the blastopore appears; the opposite end is posterior (more correctly, postero-ventral).

The pigment moves with the cytoplasm; it is, however, unable to completely displace that yolk which remains at the upper surface in consequence of the greater viscosity of the superficial rind, and here a 'white plate' or 'grey patch' is formed. Similarly, at the lower surface the pigment is not necessarily wholly displaced by the descending yolk.

There is one special case that may be noticed. When the inversion is complete (180°) the yolk flows radially and peripherally away from the upper pole while the cytoplasm ascends in the axis.

Born's observations make it perfectly clear that gravity rearranges the contents of these inverted eggs, and so confers upon them a secondary structure like that of the normal, and symmetrical about a secondary axis which is likewise vertical. To this secondary axis the direction of elongation of the karyokinetic spindles, and consequently the cleavage planes, bears the same relation as in the normal egg; and there is certainly no more need to explain these directions by reference to gravity, to suppose, in fact, a causal connexion between the two, in the one case than in the other. The planes, indeed, may fall where they do simply because the mitotic figures elongate in the direction of least resistance (Pflüger) or (O. Hertwig) in that of the greatest protoplasmic mass, or may be related, in some similar way, to the structure of the egg alone.

The point can only be determined by an experiment in which the directive influence of gravity is eliminated. This experiment has been made by Roux. The eggs were fastened in small vessels, at distances of from one to eight centimetres from the centre, to a wheel rotating continually about a horizontal axis, but so slowly (one revolution in from one to two minutes) that the centrifugal force developed was insufficient to make the eggs turn with the white pole outwards, and therefore negligible. The direction of the force exerted by gravity upon them from moment to moment was thus not constant. Of the eggs some were free to rotate inside their jelly, others were fixed. To anticipate the objection that the plane of rotation,

the plane of the wheel, is constant, a third set were packed loosely in test-tubes, and so able to roll over one another in all directions as they fell from one end of the tube to the other with each revolution. The first furrow appeared in all these eggs at the normal time and it was meridional, as in the normal egg; similarly the second was meridional, the third latitudinal; but the egg-axes exhibited no definite relation either to the vertical or to the plane of the wheel. The eggs were allowed to continue their development on the apparatus, and gave rise to normal tadpoles.

From this experiment Roux drew the conclusion that it is not gravity which determines the direction of the planes of cleavage, and that gravity is not an indispensable necessity for the normal development of the egg of the Frog.

Incontrovertible though this conclusion appears to be on the evidence, it has nevertheless been disputed by certain embryologists, Schulze and Moszcowski, the controversy between whom and Roux upon the subject has now extended over many years.

Schulze has urged (1) that eggs placed on such a machine do not develop normally, and (2) that the rotation of the eggs in their jelly exactly compensates for the rotation of the wheel. With regard to the latter point Roux has replied that on this supposition the egg-axes ought to be, at any moment, vertical, which is not the case. To the first objection it is a sufficient answer that not only Roux, but subsequent investigators (Morgan, Kathariner and the present writer) have been able to produce normal tadpoles from such rotated eggs.

It may be noticed here that Kathariner has repeated Roux's experiment with a slight variation. The eggs were kept constantly rotating, not in one but in an indefinite number of planes by a stream of air-bubbles passing through a glass vessel filled with water. Development was normal. This result does not differ materially from that obtained by Roux with the test-tube eggs referred to above, which has indeed been also independently corroborated by Morgan.

The criticisms of Moszcowski take a different form. This author urges that gravity always exercises an influence upon the egg in determining the bilateral symmetry of both egg and

embryo. The grey crescent which appears soon after fertilization and is regarded by Roux as a direct effect of this process, is supposed by Moszcowski to be produced by the action of gravity upon the egg-contents during the short interval before the perivitelline space is formed and the egg able to turn over, to be comparable, in fact, to the grey area or white plate described by Born in his forcibly inverted eggs. Every normal egg, therefore, has a 'gravitation' plane of symmetry which later on becomes, as in inverted eggs, the median plane of the embryo; nor are the eggs on the rotatory apparatus exempt, for it is held that the work of gravity can be accomplished on them even in the few moments before they are placed on the machine.

With regard to the latter point both Kathariner and Morgan have demonstrated that eggs kept in a state of perpetual rotation in all directions, from the very moment of insemination develop into perfectly normal, bilaterally symmetrical embryos, while Roux has replied to the first part of the criticism by pointing out that the grey area observed by Moszcowski was not the normal grey crescent produced by the entering spermatozoon, but the 'white plate' of Born due to the incomplete rearrangement of yolk and cytoplasm in an egg which had been quite unintentionally prevented from assuming its normal position. The grey crescent, indeed, Roux argues, could not possibly be due to gravity in a normal egg, for it does not appear until some time after the axis has become vertical.

There seems, therefore, to be little room for doubt that Roux's original contention, that gravity does not determine the symmetry of the egg and embryo in the Frog, is correct, although it remains a result of considerable importance that this external factor may be made artificially to induce a bilaterality in the egg which is sufficiently strong to persist as the symmetry of the embryo.

There is one other matter of interest in this connexion. It is obvious, and has been experimentally shown by O. Hertwig, that a centrifugal force can replace gravity. On a wheel rotated with sufficient velocity the eggs turn with their axes radial, their white poles outermost. If the velocity is great enough (145 revolutions a minute, radius from 24 to 32 cm.) the yolk is driven

inside the egg towards the vegetative pole, and the distinction between it and the protoplasm accentuated. The segmentation of such eggs is meroblastic; a cap of small cells is formed, a blastoderm, resting upon an undivided, though nucleated, yolk, and these yolk-nuclei are large and irregular, resembling the giant nuclei of the large-yolked eggs of Elasmobranchs and other forms (Fig. 46). An experimental confirmation is thus afforded of Balfour's hypothesis, put forward on comparative grounds, that it is on the varying quantity of yolk that differences in the segmentation of eggs primarily depend.

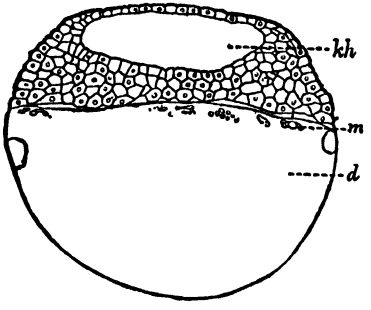


FIG. 46.—Segmentation of the Frog's egg under the influence of a centrifugal force (from Korschelt and Heider, after O. Hertwig). The egg consists of a blastoderm and an undivided yolk (yolk-syncytium): *kh*, blastocoel; *m*, yolk-nuclei; *d*, yolk.

If removed from the centrifuge in time, such eggs may continue to develop, though they frequently give rise to monstrosities (*Spina bifida*).

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2. MECHANICAL AGITATION

The necessity of perpetual and violent agitation for the very numerous pelagic ova which are ordinarily exposed to the stress of wind and weather is well known to every zoologist who has attempted to rear such forms in an aquarium, and need not be further insisted on.[1]

There are also other eggs which require a small amount of movement. The Hen turns her eggs every day, and the operation has to be artificially performed in an incubator. Its omission leads to serious consequences, for, as Dareste has shown, the allantois sticks to and ruptures the yolk-sac in unturned eggs, the ruptured yolk-sac cannot be withdrawn into the abdomen, and the Chick cannot hatch out. Death may ensue at an early stage.

A violent agitation of the Hen's egg, on the other hand, is equally fatal.

Dareste subjected the unincubated eggs to violent shocks at the rate of 27 a second for varying periods (from $\frac{1}{4}$ hour to 1 hour). The percentage of monstrosities observed after three or four days of incubation was very high indeed, except when

[1] It seems probable that the principal value of the mechanical agitation to the larvae is to prevent the Diatoms and Algae, of which their food consists, from sinking to the bottom.

the eggs were placed vertically with the blunt pole uppermost, the blastoderm therefore resting against the shell membrane.

Marcacci has exposed the eggs, inside the incubator, to continual rotation for 48 hours. The eggs were fastened to horizontal and vertical wheels rotating 40, 80, and 60 times a minute. At the last mentioned rate of revolution, the direction of rotation was reversed half-way through the experiment.

Many of the eggs actually hatched out, but the chickens were feeble and liable to disease, and exhibited malformations of the muscles or skeleton. Others, however, died before hatching, in some cases at an early stage, and death seems to have been due to rupture of the vitelline membrane; this was always fatal. The vertical motion was, on the whole, more harmful than the horizontal, owing to the perpetual see-saw.

It may be noted here that Féré has succeeded in producing retardation and abnormality of development in the Chick by means of short exposures to sound-vibrations.

Mathews has shown that mechanical agitation — violent shaking in a test-tube—is sufficient to provoke development (artificial parthenogenesis) of the unfertilized eggs in *Asterias*, but not in *Arbacia* (see, however, below, p. 124).

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3. ELECTRICITY AND MAGNETISM

An external agent, to which all eggs are inevitably exposed, is the natural magnetism of the earth. No evidence has, however, as yet been brought forward that this agent exercises any directive influence upon them, although their development may be distorted by excessive exposure to it.

Thus Windle placed a number of Hens' eggs between the poles of a large horse-shoe magnet. Over 50 % of these, when incubated, gave rise to abnormalities, the area vasculosa being affected in most cases. No relation could be detected between the position of the egg in the magnetic field and the kind of monstrosity produced.

In the case of Trout ova similarly treated a very high death-rate was observed, but this was attributed by the experimenter to the action of the electric currents set up by the running water between the poles of the magnet. Weak electric currents had less effect.

Silkworms' eggs, however, suffered no harm.

The effects of the electric current upon the eggs of Amphibia and Birds were tested by some of the older observers. Rusconi, Lombardini, and Fasola all found that the development of the Frog's egg could be accelerated by weak currents. Lombardini produced monstrosities in the case of the Chick by this method. More modern experiments are due to Windle, Dareste, Rossi, and Roux.

Windle observed a fairly high death-rate amongst Trout eggs exposed to the action of the current. Dareste has found a large percentage of monsters among embryos developed from Hens' eggs subjected for from one to three minutes to the electric spark (12 cm. long from Bonnetty's machine, 3·35 cm. long from a Rhumkorff coil). Development was, however, normal in the case of eggs placed for an hour in a Tesla's solenoid traversed by a discharge of 500,000 periods a second. Rossi employed a continuous current passing through the eggs (of *Salamandrina perspicillata*) in the direction of the axis. Both yolk and pigment became aggregated at the animal pole, leading to the formation there of a grey raised area surrounded more or less

completely by a furrow. When segmentation occurred the first two blastomeres were unequal and detached; the vegetative hemisphere was hardly segmented at all in later stages, the previous divisions having disappeared. The nuclei were affected in various ways, and the directions of the cleavage spindles altered. The capacity for resistance to these evil effects was noticed to increase as development advanced.

The polar area produced in these experiments recalls the polar areas observed by Roux in Frogs' eggs exposed to a horizontal current, at right angles, therefore, to the axis. Alternating currents of 50 and 100 volts were employed. The eggs were fertilized two or three hours before the commencement of the experiment. In from fifteen to thirty seconds after exposure two polar areas appeared in each egg. The polar areas were turned towards the electrodes. They were marked, dotted in various ways, and flecked with white extruded drops of yolk, and separated by furrows from a middle or 'equatorial' zone, the width of which varied directly with the distance of the egg from the electrode, inversely with the strength of the current and the duration of exposure.

Unfertilized ova were found to react in the same way. So also eggs in which segmentation had begun, and in those cases where the furrow cut the equatorial zone obliquely, the two halves of the latter turned away from one another.

The polar areas appear too in eggs which are exposed in the 'morula' stage, each cell having in addition a polar area of its own. The latter, however, do not appear in enfeebled eggs, but only the former.

In the gastrula and later stages the reaction occurs, but less markedly.

None of the eggs which have been exposed to the current develops any further. They stick to the jelly, and consequently lose their power of rotation.

Similar results were obtained by the use of the continuous current (43 volts), but the anodic and the kathodic areas usually differed from one another in certain details.

It is important to notice that neither in these experiments, nor in another in which the eggs were placed inside a glass

tube surrounded by a coil, could any definite relation be satisfactorily made out between the direction of the first furrow and that of the current. Indeed, though intrinsically interesting, the experiments throw no particular light upon the problem of development. Rather should they be classed with the investigations of Verworn and others upon the behaviour of Protozoa in the electric current, investigations which promise to contribute to the understanding of the structure and movements of living substance. It may be noted here that Roux has himself produced these polar areas on such structures as the heart and gall-bladder of the Frog and other vertebrates.

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4. LIGHT

As Roux pointed out long ago in the case of the Frog, light exercises no directive influence upon the development of the ovum. Blanc, indeed, has attempted to prove that the direction of the embryonic axis in the egg of the Hen may be made to depend upon the direction of the incident light-rays, but the experiments are hardly conclusive. The method employed was to blacken the shell of the horizontally placed egg with the exception of one spot to right or left of the blastoderm. On this spot a beam of light was kept directed during incubation. In some cases, but not in all, the embryonic axis was found to deviate from its normal position at right angles to the long axis of the shell. Further, the head of the embryo might be turned towards or away from the source of light. There was

no relation between the amplitude of the deviation and the length of the exposure.

Nor are the processes of growth and differentiation necessarily affected in any way at all by the presence or absence of light, or by the kind of light to which the eggs are subjected.

Thus Driesch, who has experimented with the eggs of *Echinus*, *Planorbis*, and *Rana*, maintains that neither red, yellow, green, blue, nor violet light has the slightest effect upon the eggs during the early stages of segmentation and gastrulation, in what he calls the organ-forming period of development; and Loeb has asserted that the development of the embryos of the fish *Fundulus* is as rapid in darkness as in the light, except that on the yolk-sac (not in the embryo) far fewer pigment-forming cells are produced.

Yung, on the other hand, has brought forward evidence to show that in later stages, at any rate, the embryos of the Frog react differently to lights of various wave-lengths, some of which are harmful, others, apparently, beneficial.

Yung obtained his colours from solutions of fuchsin (red), potassium bichromate (yellow), nickel nitrate (green), bleu de Lyon (blue), and viole de Parme (violet). The colours, it may be noticed, are not absolutely monochromatic.

Freshly laid eggs of *Rana temporaria* were placed under the influence of these lights. After one month, samples of the tadpoles were measured, with the following result in millimetres:—

TABLE IX

	Red.	Yellow.	Green.	Blue.	Violet.	White.
Length	21·58	25·91	18·83	26·83	29·66	25·75
Breadth	4·83	5·58	4·16	5·75	6·83	5·25

The mortality in the green light was great.

After two months the dimensions were as follows:—

TABLE X

	Red.	Yellow.	Green.	Blue.	Violet.	White.
Length	26·25	31·83	All	33·50	41·30	31·00
Breadth	6·00	7·50	dead.	8·00	10·16	7·33

All the tadpoles in the red light eventually died.

White and yellow light gave the greatest number of perfect frogs, but, as will be seen, those in the violet were larger. They

were, however, less differentiated, for they did not acquire their hind legs so soon as did those in the white light. It may be mentioned, however, that when the tadpoles reared under these conditions are replaced in ordinary light and starved, those from the violet exhibit a greater power of resistance.

Experiments with *Rana esculenta* gave the same result. In this case the effect of darkness was also tried and found to be distinctly unfavourable. Thus after one month the lengths in darkness and white light were respectively 19·66 mm. and 23·10 mm., the breadths 4·66 mm. and 5·50 mm.; after two months the difference was intensified, the lengths being 21·50 mm. and 32·16 mm., the breadths 7·16 mm. and 7·66 mm. The death-rate in the dark was exceedingly high.

The eggs and embryos of the Trout were likewise found by Yung to be highly sensitive to green and red light, while the larvae reared in violet hatched out rather more quickly than those from yellow, blue, or white light.

In an experiment on the eggs of *Limnaea stagnalis*, due to the same investigator, the effect is measured by the time required for the young to hatch out, as the following table shows :—

TABLE XI

Light.	Time to hatching in days.
Red	36
Yellow	25
Green	The heart is formed, then death occurs.
Blue	19
Violet	17
White	27
Dark	33

Green light is evidently fatal; development is retarded in red light, less so in darkness; yellow has about the same effect as white light, while there is a considerable acceleration in blue and violet.

The relative effect produced by the various lights is as in the preceding experiments.

The results obtained by Vernon for Echinoid larvae are, however, not quite consonant with this, as may be seen in the table (Table XII), where the colours are arranged in the order of the effect they produce. It will be observed that yellow is

more harmful than red, while green exerts about the same effect as blue (copper sulphate). The author states, however, that in two other experiments the larvae were entirely killed off by the green light though developing perfectly in the white. He also adds that in violet light no development was possible owing to the swarms of bacteria.

TABLE XII

	Percentage change of size.
Semi-darkness	+2·5
Absolute darkness	−1·3*
Blue (copper sulphate)	−4·5
Green	−4·8
Red	−6·9
Blue (bleu de Lyon)	−7·4
Yellow	−8·9

* Almost within the limits of experimental error.

In the Pluteus Vernon found that both the oral and the aboral arm-length decreased in darkness, green and blue (bleu de Lyon) lights, while blue (copper sulphate), yellow, and red light exerted little influence on this magnitude.

It only remains to be added here that Blanc and Féré have brought forward some not very satisfactory evidence to show that white light is favourable to the development of the Chick. Féré has also stated that red and orange lights are more harmful than white, while violet has about the same effect. The experiments are, however, vitiated by the fact that the eggs were not turned over.

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5. HEAT

As is very well known, those activities by which every organism maintains its specific form can only be carried on within certain definite limits of temperature. So also a certain degree of heat is necessary for the due performance of the functions of growth and differentiation; above or below certain limits—more or less definite for each organism, but varying in different organisms—development is unduly accelerated or retarded, or brought to a standstill, while its form is frequently distorted as well.

To Oscar Hertwig we are indebted for a careful inquiry into the conditions of temperature under which the development of the Frog's egg takes place.

In the case of *Rana fusca* Hertwig has found the cardinal temperature-points to be as follows:—The normal is about 15°–16° C.; above this up to 20°–22° C. development is accelerated without being otherwise altered; this temperature is therefore the optimum (Fig. 51). Above this point the form of development is altered, and at such a high temperature as 30° C. death follows very quickly. At low temperatures (6°–1° C.) there is considerable retardation, and at the zero-point a complete cessation of segmentation; the eggs are often permanently injured.

At the high temperatures referred to—from 23° C. upwards—it is the yolk-cells which are primarily affected. At from 29·6° to 27·5° the yolk is unable to divide, though it is nucleated, and segmentation is confined to the animal hemisphere, and soon ceases even there (Fig. 47). At 26·5° the first furrow indeed passes through the yolk, but subsequent segmentation is meroblastic, with the resulting formation of a cap of cells or blastoderm lying upon and separated by a segmentation cavity from the

nucleated yolk. The eggs then die. At lower temperatures—25°–23°—the yolk is also affected, and many eggs die in the 'morula' stage; such as do survive give rise to distortions or monstrosities (Figs. 48, 49). The injury to the yolk interferes

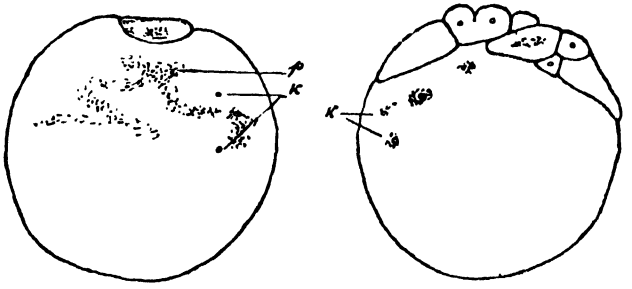


FIG. 47.—Meridional sections of eggs of *Rana fusca* developed (A) at 29·5° C., (B) 26·5° C. Five hours ten minutes after fertilization. *k*, nuclei; *p*, pigment.

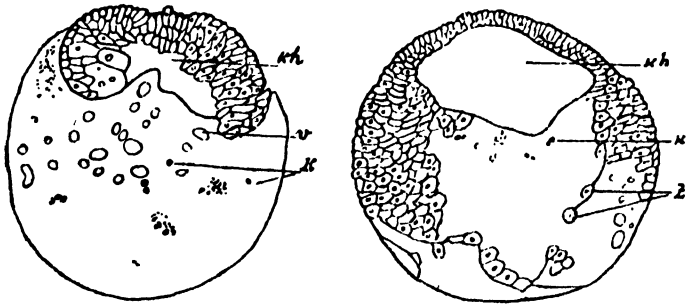


FIG. 48.—Meridional sections of eggs of *Rana fusca* developed at 26·5° C. One day after fertilization. *k*, nuclei; *kh*, blastocoel; *z*, cells imbedded in unsegmented yolk.

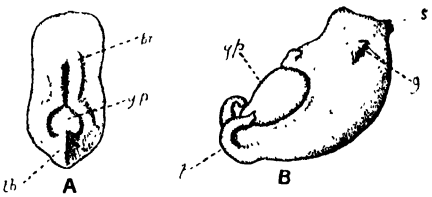


FIG. 49.—Abnormal embryos of *Rana fusca*, produced by heat. A, embryo two days old developed at a temperature of 24° C.; B, embryo three days old, reared at a temperature of about 25° C. *br*, brain; *y.p*, yolk-plug; *t.b*, tail-bud; *t*, tail; *s*, sucker; *g*, gill.

with the proper closure of the blastopore; there is consequently a large, persistent yolk-plug surrounded by a thickened blastoporic rim into which the separated halves of the medullary plate and notochord are differentiated (spina bifida) (Fig. 50). The

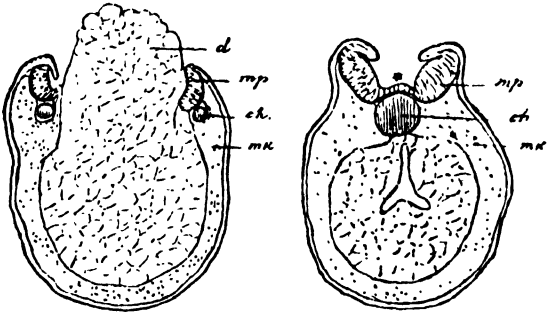


FIG. 50.—Two transverse sections through the embyro shown in Fig. 49 A. A, passes through the blastopore and yolk-plug; B, through the anterior end. *d*, yolk-plug; *m.p*, medullary plate; *ch*, notochord; *mk*, mesoblast.

front end of the archenteron is, however, normally developed if the temperature is not too high, and in this case the anterior portion of the nervous system and notochord are undivided; posteriorly, however, their right and left halves diverge round the blastopore, and are continued into the halves of the double tail when the latter is formed. Gill slits, protovertebrae, striated muscle-fibres, the pronephros and its duct, and the tail fin may all be differentiated.

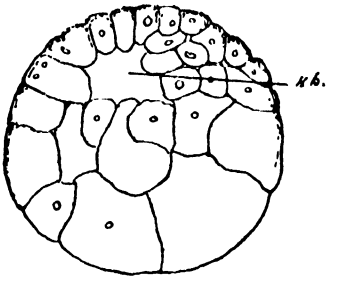


FIG. 51.—Meridional section of an egg of *Rana fusca*, developed at a temperature of 22° C. Six hours fifty minutes after fertilization. *kh*, blastocoel.

The development of the organs of the two sides of the body is frequently unequal.

At low temperatures segmentation and the closure of the blastopore take place very slowly, and at 0° cease altogether. The eggs are not, however, dead, but will resume their development when replaced under ordinary temperature conditions. They show abnormalities, however, due to injury of the yolk;

Morgan has similarly found that the fertilized (not, however, the unfertilized) eggs of an American species (*R. palustris*) which have been subjected to a temperature of 1° C. and then allowed to develop under normal circumstances exhibit spina bifida and persistent yolk-plug.

Schulze has also observed these abnormalities as the result of excessive cold. On one point, however, this author is not quite in agreement with O. Hertwig, for he states that eggs and embryos exposed to 0° in various stages do continue to develop, though of course very slowly. Thus, in the case of eggs exposed shortly after fertilization, the blastula stage was only reached in ten days, while a month elapsed before the blastopore was formed. Lillie and Knowlton, however, state that in *R. virescens* and *Amblystoma tigrinum* segmentation is totally inhibited at 0°. In another species of Frog (*R. esculenta*) which spawns much later in the year—in May and June—the cardinal points were found by Hertwig to be much higher, and the eggs endured a temperature of 33° C. without injury. They are, in fact, acclimatized to a higher temperature, and it is very interesting to notice that Davenport and Castle have succeeded in artificially acclimatizing the eggs of another Amphibian (*Bufo lentiginosus*) to a considerable degree of heat. Eggs were reared at 15° C. and 24°–25° C. After four weeks the heat rigor temperature was 40° C. for the former, 43·2° for the latter; and in another experiment the temperature was raised to 43·5° by allowing the eggs to develop at 33°–34° for seventeen days.

A similar lowering of the minimum seems to have been observed by Lillie and Knowlton in the case of *Amblystoma tigrinum*. In this form, which spawns much earlier than *Rana virescens*, there is considerably less retardation of development at 4°.

That temperature markedly influences the rate of development, or, as Hertwig puts it, that the quantity of developmental work performed per unit of time is a function of the temperature, is abundantly clear, and is well shown in the annexed diagrams (from Hertwig), in which the curves show the times taken to reach various stages at various temperatures (Figs. 52, 53).

It will be seen that as the temperature sinks the rate of development, or rather of differentiation, decreases, but at an

increasing rate. Lillie and Knowlton have made the same observation for the species investigated by them.

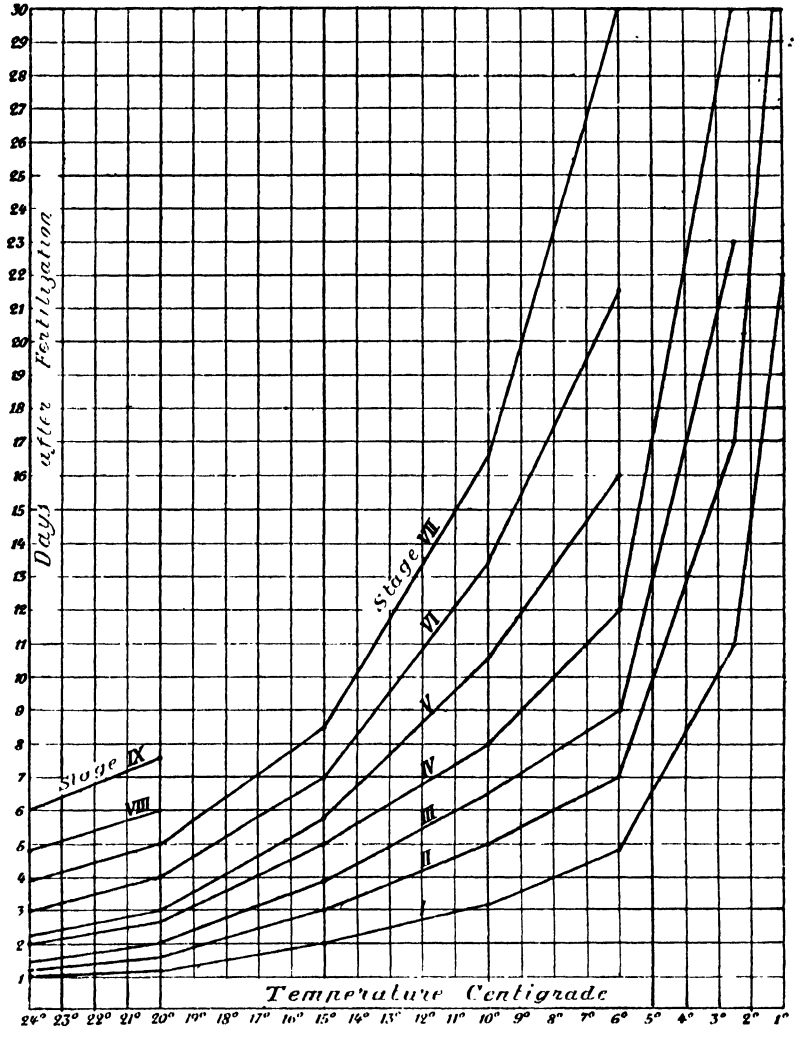


FIG. 52.—Curves showing the effect of temperature upon the rate of development of the Frog (*Rana fusca*). The abscissae give the temperature in degrees Centigrade, the ordinates the days required to reach each of the stages I to IX. I, gastrula; II, medullary plate; III, medullary folds closed, suckers; IV, tail-bud; V, tail and gills; VI, tail fin; VII, operculum beginning; VIII, operculum closing; IX, rudiments of hind legs. (After O. Hertwig, 1898.)

The rate of growth, however, may increase at an increasing (or decrease at a decreasing) rate, as Lillie and Knowlton found

for the tadpoles of *Rana virescens* and *Bufo lentiginosus*. The same authors state that at low temperatures (below 3° in the case of the Frog, below 6° in the case of the Toad) growth was altogether inhibited, while at 2° there was an actual shortening in length in the case of the Frog tadpole, due, it is suggested, to a diminution in the turgor of the cells.

The cardinal points have also been determined for the Hen's egg. According to Kaestner normal development occurs only

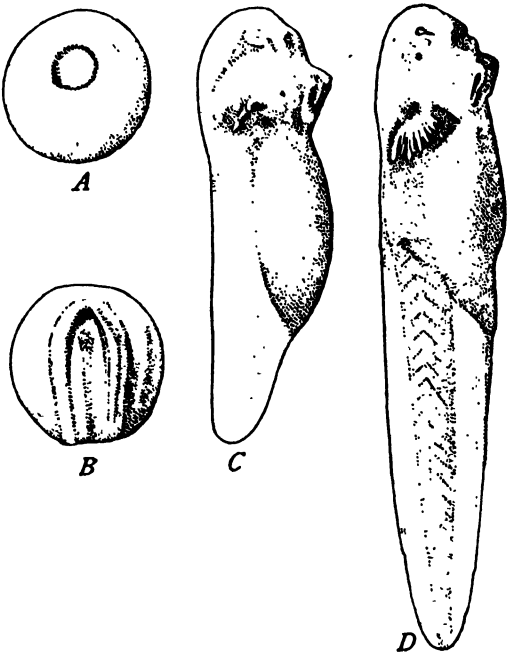


FIG. 53.—Effect of temperature upon the growth of the tadpole of the frog (*Rana fusca*). *A*, *B*, developed at a temperature of 14·5°–15° C.; *A*, two days old, circular blastopore (Stage I in Fig. 52); *B*, three days old (Stage II in Fig. 52); *C*, *D*, developed at a temperature of 20° C.; *C*, three days old (Stage V in Fig. 52); *D*, four days old (Stage VI in Fig. 52). (From Minot, 1907, after O. Hertwig, 1898.)

between 95° and 102° F. (35° and 39° C.). The maximum, the temperature above which the embryo dies, is 43° C.; the minimum, at which development stands still, 28° C. Edwards, however, fixes the minimum or physiological zero at 20°–21° C., for, as the annexed diagram shows (Fig. 54), development may continue between 20° and 29°, though it is, of course, very much retarded.

Edwards has further made the highly interesting observation

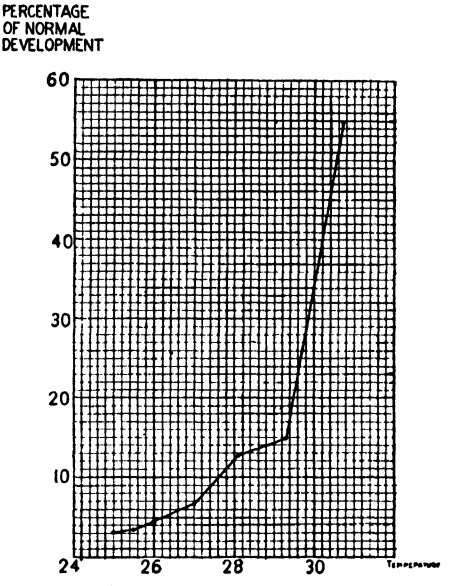
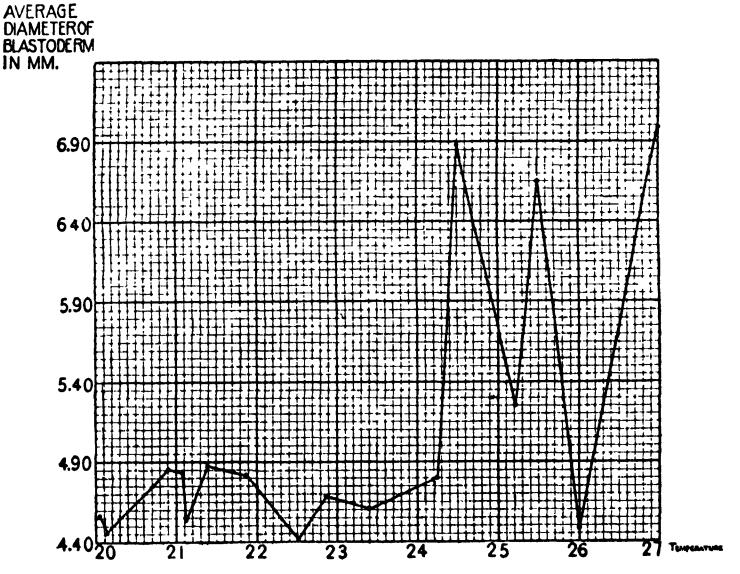


FIG. 54.—The index of development (percentage of normal development) for the egg of the Hen at temperatures varying from 20° C. to 30·75° C. (After Edwards, 1902.)



RELATION OF TEMPERATURE TO GROWTH OF BLASTODERM

FIG. 55.—Growth of the blastoderm of the Hen's egg independently of the appearance of the primitive streak, at low temperatures. (After Edwards, 1902.)

that at the low temperatures in question growth may occur without differentiation (Fig. 55). Thus in one series of experiments at 24·5° for six days the blastoderm increased in diameter from 4·4 mm. (the average diameter of the blastoderm in unincubated eggs) to 6·9 mm. The primitive streak was, however, not formed.

A temporary exposure to low temperatures often inflicts a permanent injury on the egg and leads to malformations. Kaestner, by subjecting the eggs at many different stages to temperatures of 15°–25°, 10° and 5°, has discovered that the capacity of resistance decreases as development proceeds (though not with absolute regularity). Thus the maximum exposure to 21° consonant with subsequent normal development was 192 hours for embryos of six hours, 96 hours for embryos of one day, 72 hours for embryos of two to six days, 49 hours for embryos of eight days, and 24 hours for embryos of 20 days.

At these low temperatures development is stated to be completely arrested, though the heart never ceases beating, irregularly and convulsively. The cooling process may be repeated over and over again without altering the capacity for future growth and differentiation, or reducing or increasing the maximum capacity of endurance of cold.

Malformations, as stated above, are of frequent occurrence, but only in those cases in which the embryo has been exposed in an early stage, during the first two or three days of incubation, and only after long exposures.

The medullary folds may remain unformed anteriorly, the two halves of the heart may remain widely separate, the head amnion fold may be absent and abnormal gill slits be formed; the heart and blood-vessels are often enormously distended, and haemorrhages are frequent. Kaestner attributes these monstrosities not directly to the cold but to the pressure of the blastoderm against the shell, for in the cooled eggs, owing to some change in the specific gravity of the albumen or yolk, the latter rises up; if the egg is placed with the blunt end uppermost, so that the embryo is pressed against the shell-membrane, no monsters are produced.

Mitrophanow is another observer who has utilized low temperatures to cause malformations. High temperatures also give

rise to abnormalities accompanied by acceleration (Féré, Mitrophanow).

The effects of extremes of heat and cold upon the ova and embryos of certain Invertebrates have been studied by Driesch, the brothers Hertwig, Vernon, Sala, and Greeley.

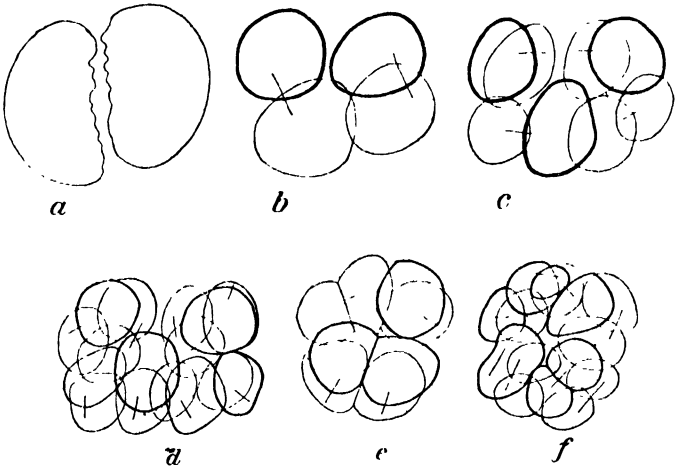


FIG. 56.—The effect of heat upon the segmentation of the Echinoid egg. *a*, *b*, *c*, *d*, four successive stages in the segmentation of the same egg of *Echinus*; *e*, *f*, two successive stages in the division of the same egg of *Sphaerechinus*. (After Driesch, 1893.)

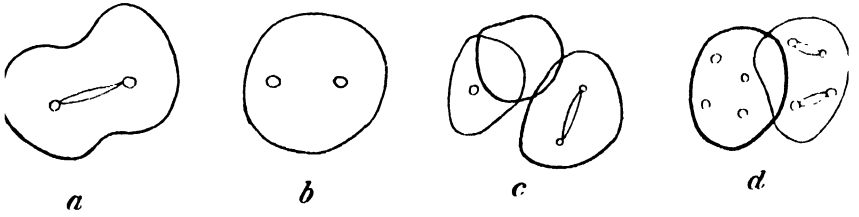


FIG. 57.—Suppression of cell-, but not of nuclear, division by heat (*Echinus*). (After Driesch, 1893.)

The first-named observed that by subjecting the fertilized ova of *Sphaerechinus* to a temperature of 30°–31° (the normal is 19°) development was accelerated and segmentation abnormal (Fig. 56).

After the first furrow—though not after subsequent divisions—the blastomeres separated and sometimes remained apart, a fact which provided a means of watching the independent development of the first two blastomeres. After the four-celled stage the direction of division became irregular, one spindle being

perpendicular, instead of parallel, to the other three, or two perpendicular to two, or all irregular; in the next phase the formation of micromeres was partially or wholly suppressed. Nevertheless these abnormally segmented eggs produced perfectly normal Plutei.

It is also possible for nuclear division to continue while cell-division is suppressed, as a result of exposure to high temperatures (Driesch) (Fig. 57).

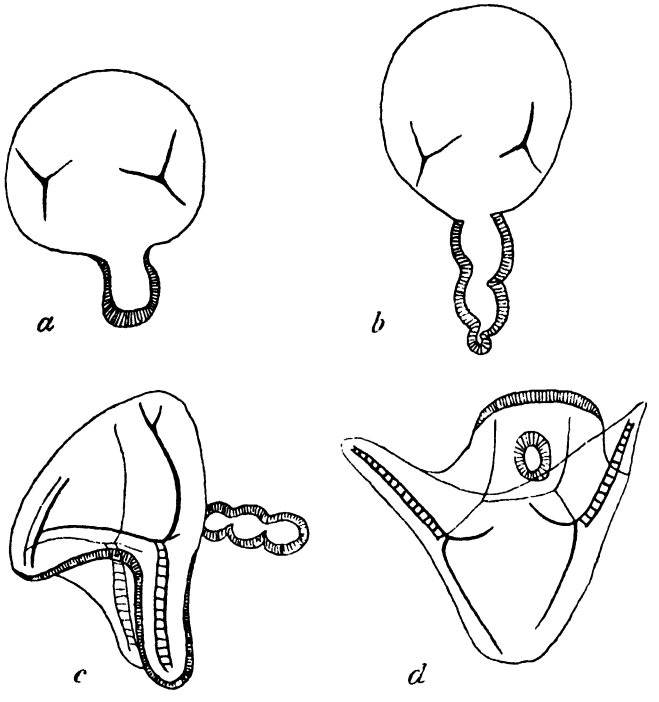


FIG. 58.—The effect of heat upon the development of *Sphaerechinus granularis*. *a*, exogastrula; *b*, exogastrula, with tripartite gut; *c*, Pluteus, with tripartite gut; (*d*) Anenterion, with stomodaeum, but no gut. (After Driesch, 1895.)

By exposing the blastulae to the same high temperature Driesch brought about a very interesting malformation, an Anenterion (Fig. 58). The archenteron was formed and constricted into the normal three portions, but it was evaginated instead of invaginated. Later on it shrank up and disappeared; the rest of the embryo, however, became a Pluteus, with a stomodaeum.

Vernon finds the optimum temperature for Echinoid larvae

to be from 17·5° to 21·5° C. Exposure to high or low temperatures after fertilization, either for longer or shorter intervals or continuously, produced a decrease in body-length of the Plutei. The arm-length, however, increased with increasing temperature.

Vernon has also made the most interesting observation that the variability alters with the temperature. Eight-day larvae were measured, and the mean variability (Galton's Q) of the body-length was found to be at 16° to 18°, 22·2, at 18° to 20°, 26·3, at 20° to 22°, 24·8, and at 22° to 24°, 24·0. Thus the variability is greatest at the temperature most favourable for development, and conversely.

It is also possible for the cell processes that occur during fertilization itself to be seriously affected by heat and cold, as the researches of O. and R. Hertwig have shown.

Moderate exposure (twenty minutes) of the eggs of *Strongylocentrotus* to a temperature of 31° C. so weakens the cytoplasm that many spermatozoa are enabled to enter. Each sperm forms its own aster, and these combine with one another to form various irregular mitotic figures (triasters, tetrasters, and so on). The segmentation of such eggs is very irregular. With longer exposures the cones of entrance become feebly developed and the asters are not formed, while the numerous sperm-nuclei remain unaltered. Greater heat—over 40° C.—prevents the entrance of the spermatozoa altogether.

This pathological polyspermy may also be produced by cold; in this case also excessive exposure prevents the formation of the vitelline membrane, the cones of entrance, and the sperm-asters, while the spermatozoa remain in the peripheral layer of the egg. The effect of a low temperature on eggs which have already been normally fertilized is seen in the reduction of the astral rays and spindle fibres, though not of the spheres, and in the thickening and irregular aggregation of the chromosomes. At a normal temperature the achromatic figure reappears.

Very similar phenomena have been described by Sala in *Ascaris*. This author kept the eggs (the females, that is, containing eggs in all stages of development) at low temperatures —from 3° to 8° C.—for from half an hour to five hours and longer. The effect of a short exposure to a very low tem-

perature is not so harmful as a longer exposure to a less degree of cold. The processes of maturation and fertilization were both abnormal. Granules of chromatin took the place of the tetrads and were unequally distributed to the spindle-poles; or, if the chromosomes (tetrads) had been normally formed before the commencement of the experiment, their division was irregular, in extreme cases all passing to one pole and into the first polar body. Again, the formation of the polar bodies might be suppressed altogether, or abnormal, the second only being formed, or both as one, or the first polar body might be as large as the egg itself. The achromatic figure was also deformed, the spindle being split at one or both poles (pseudo-triaster, pseudo-tetraster), and centrosomes appeared instead of the usual centrosomal granules. The cytoplasm became granular, the vitelline membrane was not formed, two or more eggs frequently fused together. Polyspermy, with consequent multiplication of asters and centrosomes, was very noticeable, and, in fertilization stages, a separate pronucleus may be formed from each female chromosome, or fragment of a chromosome.

Closely connected with the cytoplasmic effects brought about by these temperature changes is the phenomenon of artificial parthenogenesis, produced by Morgan and Greeley in *Arbacia* and *Asterias* by lowering the temperature of the sea-water to the freezing-point. Greeley has also shown that a lowering of the temperature, like the raising of the osmotic pressure, results in a withdrawal of water, the cause to which, as is well known, Loeb attributed the development of unfertilized ova in his experiments.

Greeley has shown that by the combination of a low temperature with a chemical reagent a higher percentage of swimming blastulae can be obtained.

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6. ATMOSPHERIC PRESSURE. THE RESPIRATION OF THE EMBRYO

The respiratory exchange, which is so characteristic a function of adult organisms, is a necessity for the embryo also, and in some cases can be detected in very early stages indeed.

In the case of the Chick this need of oxygen is shown by the arrest or distortion of development, or the death of the embryo when the egg is placed in too confined a space, or when the shell is varnished, wholly or above only (Mitrophanow, Féré), though a coat of varnish on the lower side has no effect according to the latter author; or again, when the egg is placed in an atmosphere of hydrogen, or when the pressure of the ordinary atmosphere is reduced (Giacomini).

Giacomini found that at a pressure of about 600 mm. the

embryos were small and abnormal in respect of the medullary tube and amnion; the optic vesicles and cranial flexure were absent, and there were serious disturbances in the area vasculosa, where, though the blood islands were present, the capillaries were either not formed or failed to reach the embryo. No haemoglobin was produced. Embryos exposed at a later stage (four days) nearly all died in two days of asphyxia, the blood being dark red and haemorrhages numerous. That these effects were due not to the reduced pressure but to the want of oxygen was shown by the complete normality of embryos reared in an atmosphere of pure oxygen at the same pressure (except in certain characters always exhibited by such embryos; see below).

Similar methods may be employed to demonstrate the necessity of oxygen for the Frog's egg, a necessity which is indeed patent to any one who has observed the inferior development, accompanied by spina bifida and open blastopore (Morgan) of the eggs in the middle of a mass of spawn.

Thus, according to Rauber, development is retarded at a pressure of $\frac{3}{4}$ atmosphere of ordinary air, and the mortality high, while at pressures of $\frac{1}{2}$ or $\frac{1}{4}$ atmosphere death very rapidly ensues. As a result of four days' exposure to pure hydrogen or nitrogen (ordinary air from which the oxygen had been removed) Samassa observed retarded segmentation, and subsequently irregularities in development of the type already referred to. Carbon dioxide produced irregular segmentation and death in twenty hours.

Godlewski's experiments are perhaps more thorough. The eggs subjected to ordinary air at a greatly reduced pressure (2 mm.), as well as those kept in thoroughly boiled water, segmented but little, and cell-division was confined to the animal hemisphere. In an atmosphere of pure oxygen at the same low pressure, however, development was, in many cases at least, neither retarded nor abnormal. Further experiments with pure oxygen, pure hydrogen, and an atmosphere composed of oxygen and carbon dioxide in equal parts, gave the same result, as the subjoined table shows (Table XIII). It is also clear that the absence of oxygen makes itself felt almost from the beginning, while pure oxygen accelerates development.

TABLE XIII

Hours	Oxygen.	Hydrogen.	Oxygen and Carbon Dioxide.	Controls.
3	First furrow in some	No furrow	No segmentation.	No furrow
$3\frac{1}{2}$	All but one with first furrow	One-half with first furrow		Most with first furrow
4	All but one 4 cells	The same, 2 cells		All with 2 cells
5	All with 4 cells	Most with 4 cells		Most with 2 cells; a few with 4
$17\frac{3}{4}$	Blastomeres smaller than in controls	Blastomeres smaller than in controls		Normal
$22\frac{1}{4}$	Blastomeres very small	Segmentation ceases		Normal
47	Blastopore closed			White hemisphere visible
73	Medullary folds			Blastopore closed

This method has given similar results for the eggs of the fishes *Ctenolabrus* and *Fundulus* (Loeb). One or two points are, however, worthy of especial notice.

The former develops at the surface of the sea, and is more sensitive to a lack of oxygen than the latter, the segmentation of whose egg will indeed continue for twenty-four hours in pure hydrogen, though an embryo is never formed. In *Ctenolabrus*, on the other hand, segmentation never advances further than the eight-celled stage, and the cell-boundaries already formed subsequently disappear, though they can be restored on removal to pure oxygen. In *Fundulus* the capacity for enduring a lack of oxygen decreases (or the need of oxygen increases) with the progress of development; the fatal exposure for a newly fertilized egg is four days, for a newly formed embryo thirty-two hours, for an embryo with the circulation established twenty-four hours, and for the newly hatched larva shorter still. Carbon dioxide is quickly fatal to both species.

The lack of oxygen has also a noteworthy effect on the pigment cells which are found, especially round about the blood-vessels, on the yolk-sac of *Fundulus*. These pigment cells are of two kinds, black and red, and when the embryo is deprived of oxygen the former disappear, the latter diminish only a little.

It has been noticed elsewhere that this pigment is less abundantly formed in darkness than in light, and Loeb has suggested that light may promote oxidation.

The ova of Echinoids also require oxygen from the beginning of their development (Loeb). Without this element segmentation is impossible, or, if segmentation has already begun before they are deprived of it, the blastomeres swell up and fuse. According to Lyon the eggs of *Arbacia* are only sensitive to a want of oxygen for from fifteen to twenty minutes after fertilization. Vernon has shown that water saturated with carbon dioxide and mixed in the proportion of 20 % or more with sea-water is fatal to the development of these forms.

Exact quantitative determinations of the oxygen absorbed and the carbon dioxide excreted have been made by Godlewski for the Frog and by Pott and Preyer for the Chick. The results are shown in the tables annexed (Tables XIV, XV).

TABLE XIV

Showing the result of one experiment on the respiration of the Frog's egg (Godlewski).

Days after fertilization.	Amount in grammes per 24 hrs. and per 100 eggs of	
	O absorbed.	CO_2 excreted.
1	0·03908	—
2	0·4502	0·0995
3	0·7033	0·2131
4	1·0539	0·4193

It thus appears to be the very general rule that the egg begins to respire at an early age. There is a case, however, *Ascaris*, in which not only can the egg endure an atmosphere of nitrogen or carbon dioxide or nitric oxide for prolonged periods and still develop, but is actually killed by pure oxygen (at $2\frac{1}{4}$ atmospheres) (Samassa). The adult worm, of course, is an endoparasite, and Bunge has shown that it can manage to produce carbon dioxide though denied access to free oxygen.

The effect of pure oxygen has also been tried on various embryos. In such an atmosphere (at ordinary pressure) the development of the chick is normal, except that skin, allantois, limbs and amniotic fluid are all very red with oxyhaemoglobin; an excessive amount of carbon dioxide is produced. The amount of this gas

excreted by the undeveloped though incubated egg in pure oxygen is, however, less than in air.

TABLE XV

Showing the oxygen absorbed and carbon dioxide excreted by the Hen's egg during incubation. (After Pott and Preyer, from Preyer, *Spez. Phys.*)

Days of incubation.	Amount in grammes per 24 hrs. of O absorbed.		Amount in grammes per 24 hrs. of CO_2 excreted.	
	Developed.	Undeveloped but incubated.	Developed.	Undeveloped but incubated.
1				
2				
3				
4				
5		·08		
6		·10		·08
7	·09	·10	·09	·08
8	·10	·11		·15
9		·11		
10	·11	·11	·11	·17
11		·11		·17
12				
13	·24	·14	·24	·33
14		·15		·35
15	·40	·15	·40	·36
16	·42	·15	·42	·36
* 17	·59	·15	·53	·39
18	·65	·15	·52	·39
19	·67		·54	
20	·68	·16	·55	·41
21	·86	·16	·68	·43

* Pulmonary respiration begins.

According to Samassa and Rauber the development of the Frog's egg in pure oxygen is normal, but Godlewski states, as

we have seen, that it is somewhat accelerated. At a pressure of $2\frac{1}{2}$ atmospheres, however, segmentation is arrested and death ensues (Samassa).

When the tadpoles, newly hatched, are exposed to its influence, the hyoid becomes immensely thickened and the branchial chamber completely closed; the internal gills are weak (Rauber), and the same author states that 'gastrulae' subjected to three atmospheres of ordinary air had their development temporarily arrested, while later embryos, in the stage of the medullary folds, became small and immobile in air at twice the atmospheric pressure. This result seems to be due to pressure, not to the oxygen.

In the foregoing the general necessity of respiration for the life of the developing organism has alone been taken into consideration, but it should not be forgotten that oxygen may exert a stimulus on some part, the response to which results in a process of differentiation. Thus His has suggested that the growth of the blastoderm over the yolk is oxygenotropic, and Herbst that the migration of the blastoderm-forming cells to the surface in Arthropod ova, and the migration of spicule-forming cells in Echinoid larvae are cases of definite reaction to oxygenotactic stimuli. Loeb, we may note, has found that the regeneration of the head of *Tubularia* will only take place when the stem is supplied with fresh water, and the same author has suggested that the accumulation of the pigment cells round the blood-vessels on the yolk-sac of *Fundulus* is also an oxygenotaxis.

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7. OSMOTIC PRESSURE. THE RÔLE OF WATER IN GROWTH

That growth seems to depend in many cases on the absorption of water or a watery fluid—in the swelling of the Echinoderm blastula, for example, or the enlargement of the Mammalian blastocyst—has been noticed by several observers; in a few instances experimental proof has been given of the relation between the two.

Although, as is very well known, the Hen's egg loses weight daily throughout incubation by loss of water, this loss is due almost entirely to the slow evaporation of the albumen, and a humid atmosphere is necessary for development, as Pott and Preyer have found. Féré's experiments with eggs incubated in desiccators demonstrated, during later stages, a slight retardation accompanied by abnormalities and a high death-rate; in earlier stages, up to about the fourth day, there was on the contrary an acceleration of development.

Davenport has shown for tadpoles of various Amphibia (*Amblystoma*, Toads, Frogs) that increase in weight is very largely due to increase in weight of water. Known numbers of tadpoles, from which superficial water had first been carefully removed, were placed over sulphuric acid in a desiccator. Re-

peated weighings were made until a constant minimum was reached. The results are set forth in the accompanying table and figure (Table XVI, Fig. 59). It will be seen that the percentage of water rises very rapidly in the first fortnight, from 56 % to 96 %, then decreases slightly, afterwards becoming nearly constant.

TABLE XVI

Showing the rate of absorption of water by Tadpoles (after Davenport).

Days after hatching.	Percentage of water.
1	56
2	59
5	77
7	89
9	93
14	96
41	90
84	88

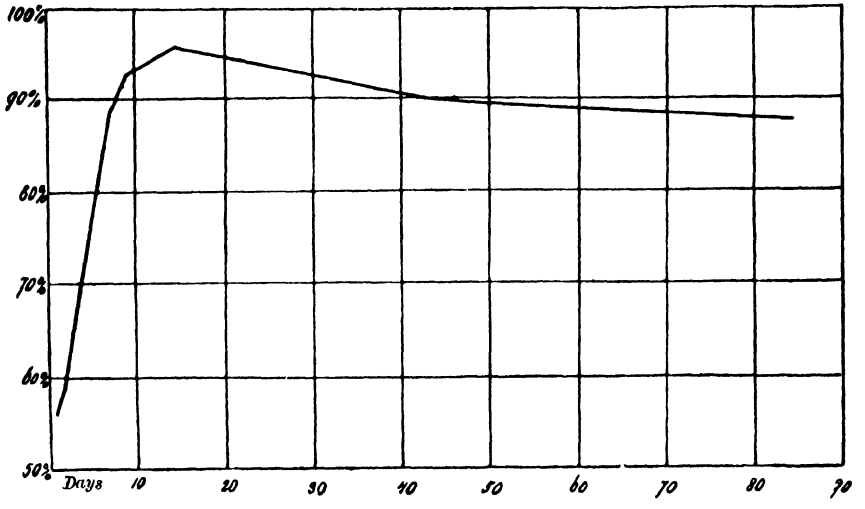


FIG. 59.—Curve showing change in percentage of water in Frog tadpoles from the first to the eighty-fourth day after hatching. Abscissae, days; ordinates, percentages. (After Davenport, from Korschelt and Heider.)

A different, and a less satisfactory, method has been employed by Loeb, hypertonic solutions being used to prevent the absorption of water. While the newly fertilized eggs of *Fundulus*

developed as normally in fresh water as in sea-water, only a blastoderm with occasionally a dwarf embryo was formed in a 5 % solution of sodium chloride in sea-water, and segmentation was arrested in the thirty-two-celled stage when the concentration of the salt was raised to 10 %. Older eggs were, however, far less sensitive, and after three or four days the embryos could be placed directly in a 27·5 % solution without arresting their development, though the heart beat more slowly and differentiation was less rapid.

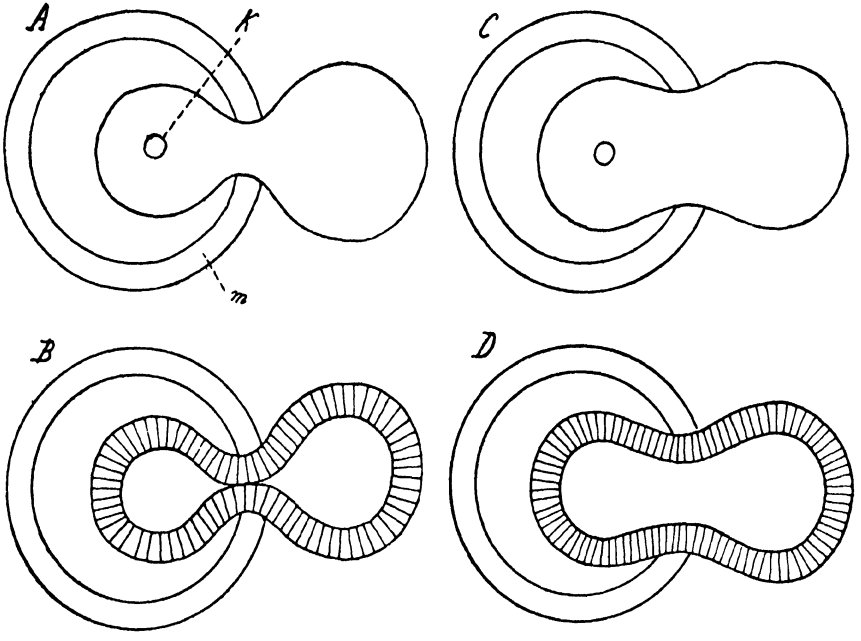


FIG. 60.—*A* and *C*, formation of ex-ovates in the egg of *Arbacia* by dilution of the sea-water; *k*, nucleus; *m*, egg-membrane; *B* and *D*, blastulae formed from *A* and *C*; *B* becomes constricted into two blastulae, each of which gives rise to a Pluteus; *D* produces a single Pluteus. (After Loeb, from Korschelt and Heider.)

The eggs can nevertheless be acclimatized to the salt. Removed from the 10 % solution after the thirty-two cells had been formed to ordinary sea-water for eighteen hours, they were capable, when once more replaced in the strong solution, of giving rise to embryos which lived for a considerable time.

Similar experiments made on *Arbacia* showed that though cell-division is suppressed in the hypertonic solution (2 % sodium

chloride) nuclear division continues all the same, for when returned to sea-water the eggs divided at once into as many cells as had in the meantime been formed in the controls, a result confirmed by Morgan.

That the normal egg is in a condition of osmotic equilibrium with the sea-water is further shown by its behaviour in sea-water diluted to twice its volume; in this experiment the egg

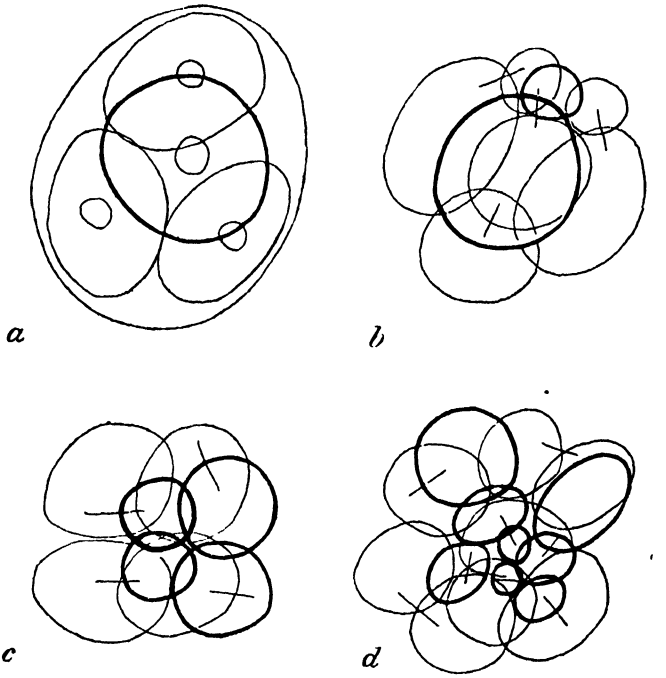


FIG. 61.—Variations in the segmentation of *Echinus microtuberculatus* produced by dilution of the sea-water. *a*, tetrahedral four-cell stage; *b*, eight cells, three premature micromeres; *c*, eight cells, two precocious micromeres; *d*, the same egg after the next division, the precocious micromeres have divided unequally, two normal micromeres have been formed. (After Driesch, 1895.)

(of *Arbacia*) absorbs water, swells and bursts its membrane and so produces a large ex-ovate which may develop independently of the rest of the ovum (Loeb) when replaced under ordinary conditions (Fig. 60). Driesch has produced irregularities of segmentation by the same means (Fig. 61).

Although, therefore, it seems reasonable to suppose that in the cases just quoted the observed effects really are due to the

increased osmotic pressure of the medium and consequent withdrawal of water from, or prevention of imbibition of water by, the eggs, the weak point of the experiment, and of all such experiments, is our ignorance of the extent to which the ova or embryos are permeable to the substance employed, since the osmotic effect, or withdrawal of water, will obviously vary inversely with the permeability. The neglect of this possibly disturbing factor has indeed led in some cases to quite unwarrantable conclusions.

In 1895 O. Hertwig showed that certain abnormalities could be produced by growing the eggs of the Frog (*R. fusca* and

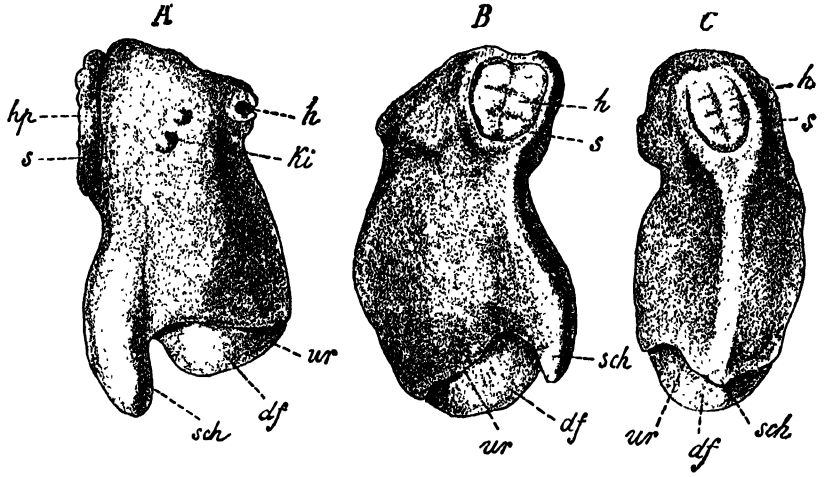


FIG. 62.—Three sodium-chloride embryos of *Rana fusca*. *df*, yolk-plug; *hp*, brain; *ki*, gills; *s*, margin of epidermic layer of ectoderm; *sch*, tail; *ur*, lip of blastopore. (After O. Hertwig, from Korschelt and Heider.)

esculenta) and of the Axolotl in a solution of common salt. In stronger solutions (1% to 0·8%) segmentation was confined to the animal hemisphere, though nuclear division went on in the yolk. Weaker solutions (0·6%) allowed of further, but distorted, development; the yolk-cells were unable to move beneath the lip of the blastopore, so that the latter remained open with a persistent yolk-plug, and the medullary folds failed to close in the region of the brain, a condition recalling the abnormalities known in Human and Comparative Teratology as Hemicrania and Anencephaly (Figs. 62, 65). The exposed region of the brain underwent a grey degeneration with dis-

integration of the epithelium. Other organs were, however, normally formed, the front end of the gut by invagination, the notochord and mesoderm, protovertebrae, heart, pronephros, auditory vesicles, optic vesicles, infundibulum, and liver, until the embryo died. The persistence of the yolk-plug has also been induced by Gurwitsch by means of halogen salts (sodium bromide and lithium chloride) and weak solutions of alkaloids (strychnine, caffein, nicotine) (Fig. 63), by C. B. Wilson in *Rana*, *Chorophilus*, and *Amblystoma* by means of sodium chloride and Ringer's solution, and by Morgan with various lithium salts; and Bataillon, who has used isotonic solutions of cane-sugar, sodium chloride, and a large number of other salts for the purpose, claims that in this case the results produced depend upon the osmotic pressure alone, and are therefore due to a withdrawal of water from the developing embryo.

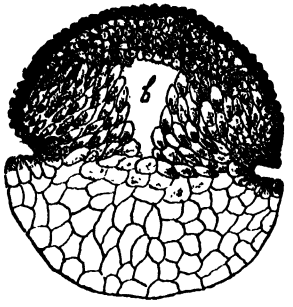


FIG. 63.—Meridional section through a lithium gastrula of *Bufo vulgaris*. *b*, blastocoel. (After Gurwitsch, from Korschelt and Heider.)

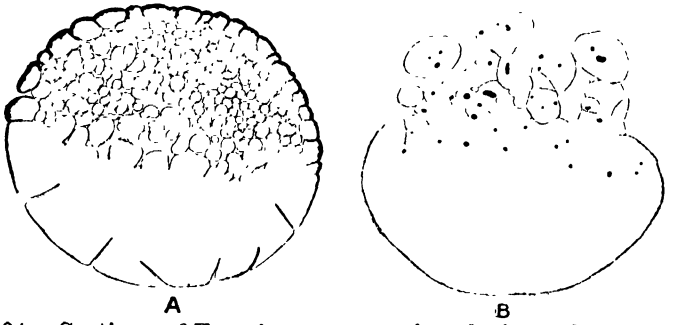


FIG. 64.—Sections of Frogs' eggs grown in solutions of, A, ammonium iodide (1·5%), and, B, urea (2·3%). In both cases segmentation is meroblastic, although in A there are a few large divisions in the yolk. In B the multinucleate cell masses of the animal hemisphere protrude above the surface. The nuclei are large, lobed, and homogeneously chromatic in both cases. (Ammonia is probably present in the solution of urea.)

Recent experiments made by the author do not, however, bear out this conclusion. In the first place, it is to be observed that isotonic solutions (isotonic with a 0·625 % NaCl solution) do not

produce the same, but markedly different effects. Some solutions arrest development at an early stage (during segmentation (Fig. 64), gastrulation, or the formation of the medullary folds); in others development proceeds but is distorted, the medullary folds remaining open in whole or in part, and the yolk-plug uncovered, or either of these malformations may occur without the other; in one case (dextrose) development is quite normal in form but very considerably retarded, while finally in urea development is normal both in form and rate (Figs. 66, 67). No legitimate deductions can be made from these experiments, how-

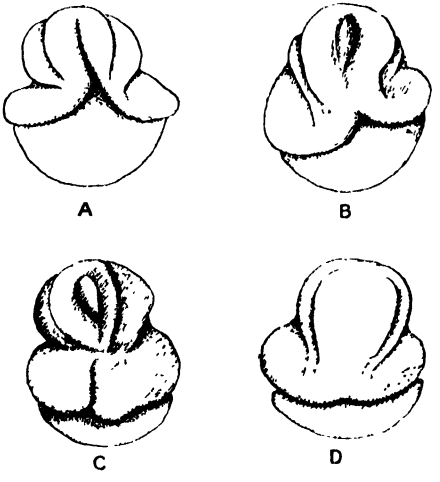


FIG. 65.—Frog embryos grown in a ·625% solution of sodium chloride. A and B after five days, C and D after six days. In all the yolk-plug is fully exposed. In A the medullary groove is wholly open, in B and C it is closed behind, in D it is closed throughout.

ever, until the permeabilities of the tissues to these solutions are ascertained. The tadpole requires water (Davenport), and the degree of shrinkage of the tadpoles in these solutions affords a means of determining the question; it appears that they are perfectly permeable to urea, more or less impermeable to cane-sugar, dextrose, and sodium chloride, the shrinkage being rather greater in the first than in the other two. On the assumption that the permeabilities of the embryo are the same as those of the tadpole, it follows that the greater effect produced on the former by sodium chloride than by cane-sugar, or, still more, than by dextrose, cannot be set down to the osmotic pressure of the solution alone,

a result which is further corroborated by the constancy in the relative toxicities of the bases and the acids in the case of the

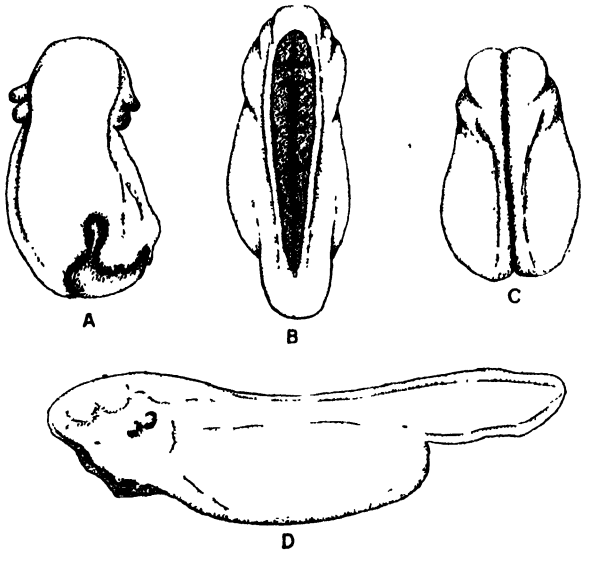


FIG. 66.—Frog embryos grown in isotonic solutions of, A, sodium chloride (·625%); B, cane-sugar (6·6%); C, dextrose (3·4%); and, D, urea (1·14%). In A the medullary folds are closed but the blastopore open; in B the medullary groove is open but the blastopore closed; in C development is normal, but retarded; in D development is normal, both in form and rate, though the embryos die soon after the stage shown in the figure.

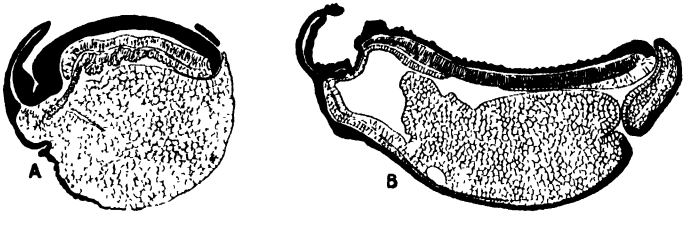


FIG. 67.—A. Longitudinal section of a Frog embryo grown in a ·45% solution of lithium chloride. The medullary groove is open, except in front and behind. The notochord is bent in several places and the gut roof much crumpled. B. Longitudinal section of a Frog embryo grown in a 6·6% solution of cane-sugar. The medullary groove is open, except in front, the cells in its floor degenerating. The gut roof is incomplete in part and there is an evident neurenteric canal.

monobasic salts. The observed deformities are therefore to be attributed to some other—chemical or physical—property of

the solutions, though what this is is not known.[1] It may be added that in Gurwitsch's experiments the concentrations of the alkaloids employed were certainly far below those which would be isotonic with a ·625 % solution of sodium chloride. It also follows that during the closure of the blastopore the Frog's egg does not need to absorb water from the outside; it may, in fact, be exposed to a very considerable degree of desiccation at this period without interfering in the least with the closure of the blastopore or of the medullary folds, a result which is all the more surprising in that the newly hatched tadpole imbibes water at so rapid a rate.

The experiments which have hitherto been considered relate to the need of water for normal development. There are, however, certain processes for which not the absorption, but, on the contrary, the abstraction, or at least the local abstraction, of water appears to be essential, the phenomena, namely, of fertilization. Cytologists have observed that the entrance cone and funnel, the mechanism by which the spermatozoon is swept into the interior of the egg, appear to be aggregations of a watery substance about the acrosome or apical body, and that the sperm sphere and aster are similarly due to the withdrawal of water by the centrosome in the middle-piece from the cytoplasm; in other words, that the stimulus whereby the spermatozoon restores to the egg its lost power of cell-division is essentially a process of local dehydration.

This inference is substantiated by the familiar experiments of Loeb, who has succeeded in rearing normal larvae from the unfertilized eggs of Echinoderms and certain worms by temporary immersion in certain solutions. In his earlier experiments he found that a mixture in equal parts of a $\frac{2.0}{8}$ n solution of magnesium chloride and sea-water produced more Plutei than any other solution tried, and hence believed the result to be specific and attributable to the magnesium ion. Later, however, this artificial parthenogenesis was successfully brought about by various isotonic solutions (chlorides of sodium, potassium and calcium, potassium bromide, nitrate and sulphate, cane-sugar

[1] In this view Stockard, as a result of experiments on *Fundulus*, concurs (*Arch. Ent. Mech.* xxiii, 1907, and *Journ. Exp. Zool.* iv, 1907).

and others). The increased osmotic pressure was, therefore, considered to be the cause of the phenomenon, and it was suggested that in ordinary fertilization the spermatozoon introduces a substance which has a higher osmotic pressure than, and is therefore able to withdraw water from, the egg.

Hunter has also shown that sea-water concentrated to 70% of its volume is sufficient to bring about the result. It must still be remembered that the permeabilities of the ova to the various solutions are not known; Sollmann, indeed, has proved the secondary swelling after the primary shrinkage of many eggs in hypertonic solutions, which must therefore enter and cause the dissociation of the cytoplasm.

Further, Delage has, as a matter of fact, denied that the increased osmotic pressure is solely responsible for the results. The French zoologist succeeded in making the ova develop in solutions hypertonic to sea-water, but found that isotonic solutions of different chlorides or mixtures of chlorides did not all give the same percentage of larvae. He holds, therefore, that other factors are involved. Other methods, as noticed elsewhere, are low temperatures and mechanical agitation.

Fischer has successfully demonstrated the phenomenon in the Chaetopods, *Nereis* and *Amphitrite*, Bullot in *Ophelia*, and Bataillon in Vertebrates (*Rana fusca* and *Petromyzon planeri*); but in this last case segmentation did not continue for very long and the processes of nuclear division were highly irregular. An attempt made by Gies to incite development (of Echinoids) by means of extracts of spermatozoa was unsuccessful.

Although in brilliancy of conception and completeness of execution Loeb's experiments are certainly pre-eminent over those of any other investigator, it should not be forgotten that about the same time Morgan had succeeded in inducing asters, and even the beginnings of segmentation, in the unfertilized ova of sea-urchins and some other forms by the use of salts and other substances, and that the way for all recent work was really paved by the original labours of O. and R. Hertwig, to be described in the next section.

Loeb did not undertake an examination of the cytological changes, but Wilson has shown that ordinary nuclear division occurs with asters and centrosomes: a primary radiation

centring in the nucleus first appears; this then fades away, and a definite aster with a centrosome is formed just to one side of the nucleus; this divides to form the first amphiaster (cleavage-spindle). Asters also arise independently of the nucleus in the cytoplasm (cytasters); these contain centrosomes, and may divide, and the cytoplasm divide round them. The part played by the cytasters in development is, however, insignificant; their activity soon comes to an end. The number of chromosomes is one-half the normal number. This latter statement is confirmed by Morgan, but denied by Delage, who asserts that, as in egg fragments enucleated and subsequently fertilized, the half number becomes doubled.

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8. THE CHEMICAL COMPOSITION OF THE MEDIUM

By means of solutions of alkaloids and other substances the brothers Hertwig have been able to incite very remarkable cytological changes in the eggs of sea-urchins (*Strongylocentrotus*).

The effects of nicotine are perhaps the most striking (Fig. 68, *a–e*). Various solutions—1% and less of a concentrated extract—were allowed to act upon the egg for different lengths of time (five to fifty minutes) before fertilization; the ova were then replaced in sea-water and fertilized. The cytoplasm is so paralysed by the

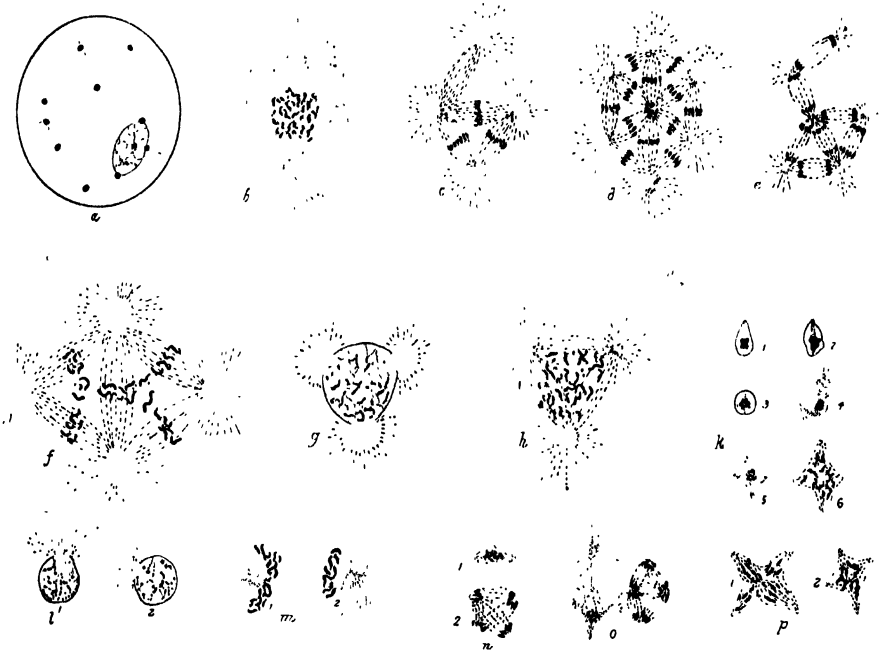


FIG. 68.—The effect of alkaloids and other poisons on the processes of fertilization and nuclear division in the egg of the sea-urchin, *Strongylocentrotus lividus*. (After R. and O. Hertwig, 1887.)

a. The egg was exposed to nicotine (one drop in 200 c.c. of sea-water) for ten minutes, and then fertilized; drawn fifteen minutes later.

b, *c*. The same for fifteen minutes; drawn after one and a half hours.

d. The same for ten minutes; drawn after three hours, ten minutes.

e. The same; drawn after three hours. Only part of the complex figure is shown; the remainder lies in another plane.

f, *g*, *h*. Exposed to a 0·05% solution of quinine for twenty minutes one and a half hours after fertilization; drawn from one to two hours later.

k. 1–5, male pronucleus, 6, female pronucleus. Exposed to chloral (0·5%) one minute after fertilization; fixed after 150 minutes.

l, *m*. Chloral 0·5% one minute after fertilization; fixed after six hours. Male and female pronuclei reconstructed and metamorphosing, in *m* the 'fan' form with commencing division.

n, *o*. Placed in chloral 0·5% five minutes after fertilization; preserved after ninety minutes.

n. Female pronucleus (four-rayed rosette), and male pronucleus (three-rayed rosette).

o. Fusion of pronuclei.

p. The same. Female pronucleus in the pseudo-tetraster forms.

poison that the normal vitelline membrane cannot be formed and consequently many spermatozoa enter. In such eggs segmentation does not occur in the ordinary fashion by successive binary divisions, but many small cells are simultaneously formed. The resulting blastulae are abnormal, the segmentation cavity being filled with a solid granular mass (Stereoblastulae), and very few reach the Pluteus stage. The irregularities of segmentation are due to the complex mitotic figures and divisions which polyspermy entails. One, two, three or more of the spermatozoa fuse with the female pronucleus; each has its own aster, which divides into two. Hence the most complex nuclear figures are formed.

In the case where two sperm-nuclei unite with the egg-nucleus a tetraster is formed, that is four asters united by spindles in a square or rhombus, or a triaster with an odd aster united to one angle of the system. The chromosomes are grouped in the equators of the four, or three, united spindles, as the case may be, and the egg divides simultaneously into four, or three.

The arrangement becomes still more involved when there are other sperms, whether these fuse with the female pronucleus or not. Each amphiaster is united by one pole to the tri-, tetra-, or polyaster developed round the combination nucleus, or to the poles of other amphiasters; in one case there were nineteen spindles in all, not, of course, all in one plane. Each centrosphere receives half the chromosomes of the spindle attached to it, and each cell, when division occurs, contains one or more nuclei.

Hydrochlorate of morphine will produce similar effects, but only with longer exposures—a 0·4 % solution for from two to five hours. Strychnine, however, is poisonous in very weak doses (·005 % to ·25 %), and quite short exposures are sufficient to call forth marked results. Other solutions successfully tried were chloral hydrate (from 0·2 % to 0·5 % for from one to four and a half hours), cocaine (from 0·025 % to 1 % for five minutes), and sulphate of quinine (·05 % for ten minutes). In quinine (·05 % for thirty minutes) and chloral (·5 %) the entrance cone was small and no asters were formed, from which the

Hertwigs argue that the contractility of the cytoplasm is impaired in these solutions. Chloroform dissolved in sea-water has the very interesting property of stimulating — without the addition of spermatozoa—the formation and separation of the vitelline membrane. The male generative cells are also sensitive to the action of these alkaloids, but not necessarily in the same measure. They can resist, for example, the influence of a solution of nicotine, which is ten times as strong as one necessary to evoke pathological changes in the ova. Though chloral hydrate (0·5 %) and quinine (0·05 %) are both temporarily fatal to the motility of the spermatozoa, sea-water restores the capacity for fertilization. Strychnine (0·01 %) and morphine (0·5 %) are without effect.

In the experiments just described the abnormalities seem to be directly due to the initial paralysis of the egg by the reagent and consequent polyspermy.

Should, however, the egg have been first normally fertilized, the irregularities produced by the subsequent action of the poison are, though well marked, not of the same kind, for in this case the vitelline membrane has already been formed and only one spermatozoon has gained admittance. Chloral hydrate (Fig. 68, *k–p*) was employed for ten minutes and at varying intervals after insemination (one, one and a half, five and fifteen minutes). Exposure to the solution very shortly after insemination first retards the progress of the sperm-head and the formation of its aster, and when later on the chromosomes are formed they lie heaped together in the centre of an achromatic figure described as a pseudo-tri- or pseudo-tetraster. This consists of three or four conical groups of fibres, the bases resting on, and the fibres connected to, the chromosomes, the apices outwardly directed and sometimes with, sometimes without, asters; in any case, however, they are not united by spindles, as is the case in the complex figures observed in polyspermy. Isolated asters are also to be seen in the cytoplasm, and, which is perhaps more remarkable, the female chromosomes are themselves the centre of a unipolar (fan-shaped) or multipolar apparatus of the same kind. The reader will not fail to notice the similarity to the phenomena occurring in artificial parthenogenesis.

Should the pronuclei unite—which is only possible before these pseudasters have been developed, if the eggs have been subjected to the action of the poison immediately (one minute) after fertilization—the conjugation nucleus itself becomes the focus of a similar system. In eggs poisoned after a longer interval (fifteen minutes) the male and female pseudasters may themselves unite.

The nucleus—or nuclei—divide irregularly, the chromosomes passing in unequal numbers to the poles of the figure. The several pseudasters and isolated asters, with which nuclei may possibly become secondarily associated, may be united by clear streaks of protoplasm, thus giving rise to a dendritic figure. Simultaneous and unequal division of the whole ovum follows.

Should the spermaster have already been developed—fifteen minutes after insemination—it degenerates. The subsequent changes comprise the formation of multipolar figures and irregular cell-division.

In later stages—when fertilization has been completed and segmentation is about to begin—the ova are almost or quite indifferent to nicotine, strychnine, and morphine; but chloral (0·5 %) destroys the asters which are already in existence and brings about a reconstitution of the combination nucleus with subsequent formation of a tetraster and quadruple division. In future mitoses, however, the spindles are bipolar. Cocaine and quinine (·05 %) (Fig. 68, *f–h*) have the same effect.

The importance of these experiments does not require to be emphasized. Not only do they throw a valuable light on the possible causes of those pathological mitoses that occur in malignant growths, they also contribute very greatly to the understanding of the normal processes of fertilization and karyokinesis.

Thus from the failure of the asters to appear in eggs treated with chloral before fertilization the brothers Hertwig argue that the contractility of the cytoplasm is diminished by this substance, and from the failure of the pronuclei to unite in eggs which have been immersed in the solution shortly after fertilization they suggest that it is the contractility of the ovum which normally brings about the union of the pronuclei. Since,

however, both male and female nuclei are able to divide, this division must be normally incited, not by their union with one another, but by the separate action of the cytoplasm on each, a view which is fully borne out by the phenomena of artificial parthenogenesis and merogony (the development of fertilized enucleate egg fragments), whatever interpretation may eventually be put on the 'contractility' of the cytoplasm.[1]

Another alkaloid which exerts an injurious influence on the ova of Echinoderms is atropine, the sulphate of which retards and dwarfs the development of *Asterias* and *Arbacia* (Mathews). Pilocarpine, on the contrary, has an accelerating effect, a result attributed by Mathews to its activity as an oxidizer, while atropine is regarded as a reducing agent, the property to which Loeb has also assigned the value of potassium cyanide in prolonging the life of unfertilized ova. The eggs of sea-urchins, when once laid, are only capable of fertilization and development within a certain definite limit of time, after the expiration of which they degenerate and die; after twenty-four hours, for example, they are only able, when fertilized, to reach the gastrula stage, and after thirty-two hours even fertilization is hardly possible. By treatment with an appropriate solution of potassium cyanide this limit may be considerably postponed. In the most successful series of experiments the ova were first placed in a solution of KCN $\frac{n}{750}$ in sea-water, and then removed successively every twenty-four hours to $\frac{n}{1400}$, $\frac{n}{2000}$, $\frac{n}{2500}$, $\frac{n}{3000}$. In the last solution they were kept for various lengths of time, then removed to pure sea-water and fertilized. As the table shows (Table XVII), segmentation was still possible after 168 hours' sojourn in the solution, but the greatest number of Plutei was obtained after only 66 hours' stay.

It was also shown that better results could be obtained with artificial parthenogenesis if the ova were first kept in the cyanide solution. Loeb points out that in the higher animals

[1] Strictly speaking, only the division of the male chromosomes can be regarded as being stimulated by the egg cytoplasm. What exactly it is which excites the female nucleus to divide is not at all clear.

the effects of this substance are due to its inhibition of oxidation; that this is the real cause of the prolongation of the life of the eggs is shown by the fact that when kept in an atmosphere of hydrogen for thirty-eight hours they were still capable of being fertilized and developing into swimming larvae.

TABLE XVII

Showing the effect of exposures of various length of Sea-urchin eggs to a solution of KCN $\frac{n}{3000}$. (After Loeb.)

Length of exposure in hours.	Result.
66	80 % Plutei, vitelline membrane formed
90	30 % Plutei, no vitelline membrane formed
99½	20 % Plutei, „ „ „ „
112	Less than 20 % Plutei
120	Gastrulae, but no Plutei
139	A few blastulae
140	Blastulae, not swimming
161	Eight-celled stage only
168	„ „ „

Simultaneous lowering of the temperature to the freezing-point enhanced the value of the cyanide treatment.

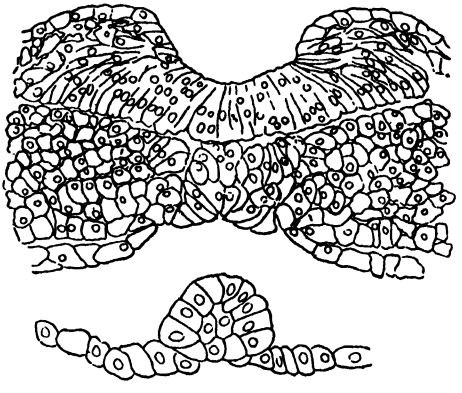
In later stages, however, immediately after fertilization and subsequently, the action of potassium cyanide is by no means beneficial; at this time, as we know, oxygen is a necessity (see above, p. 112); and Lyon has shown that the moment at which the ova are particularly sensitive to both KCN and the lack of oxygen is the same, about fifteen minutes after insemination.

Chemical agents are also able to incite irregularities of growth and abnormalities in later stages of development.

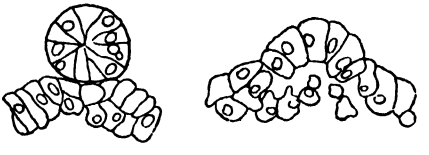
In a long series of experiments Féré has shown that monstrosities can be produced by exposing the Hen's egg to the unfavourable influence of a large variety of substances. Vapours of ether, alcohol, essential oils, nicotine, mercury, and phosphorus, injections of alkaloids such as morphine, nicotine, strychnine, and others, of bacterial toxines (those of tubercle, diphtheria), of peptones, dextrose, glycerine, several alcohols, certain salts (KBr,

KI, $SrBr_2$), are all baneful, retarding and distorting the embryo to a greater or less extent. Ammonia, it may be noted, is fatal at once.

It has already been shown (p. 123) that the malformations induced by sodium chloride in Amphibian embryos are to be set down to some other property than the osmotic pressure of the solution, and it is here only necessary to advert to



Cane Sugar.



Dextrose.

FIG. 69.—Cane-sugar (6·6 %). Two stages in the formation of the notochord from the whole thickness of the roof of the archenteron in the Frog. Dextrose (3·4 %). Secondary degeneration of the gut roof and ventral part of notochord.

some of the more interesting effects occurring in particular solutions.

Although the more poisonous salts (e.g. LiI, $CaCl_2$, $SrBr_2$, and others) inhibit altogether the formation of the blastoporic fold, a cause which normally assists in its production—the proliferation of small cells in the roof of the segmentation cavity—may continue to operate, with the result that that roof is thickened and thrown into puckers and folds.

Again, the notochord may be formed from the whole thickness of the archenteric roof (cane-sugar) recalling the mode of its development in Urodela and *Petromyzon* (Fig. 69); the solid medullary tube observed in potassium chloride and other salts reminds one of the rudiment of the nervous system in Teleostei and others, while the mode of closure of the medullary tube in, for example, some of the magnesium salts resembles that observed in *Amphioxus*; the formation of notochordal tissue from the wall of the neural

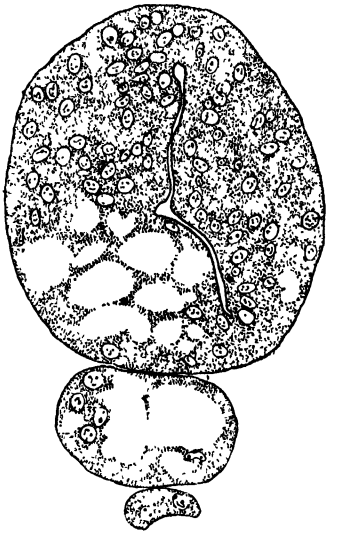


FIG. 70.—Formation of vacuolated notochordal tissue in the medullary tube of the Frog embryo under the influence of urea (1·6%). Underneath the notochord is the subnotochordal rod.

tube and the roof of the archenteron (Fig. 70) in strong solutions of urea (1·17 % to 1·56 %) shows that the prospective potentialities of these organs are not yet fixed, while the development of an optic cup without a lens in urea, sodium chloride, and sodium bromide demonstrates that the formation of the former is independent of that of the latter of these two parts of the eye.

The grey degeneration of the exposed part of the medullary plate (due to the distribution of the pigment throughout the cell-body), the protrusion of cells ('framboisia' of Roux), and disintegration of the epithelium which is so characteristic in

many of these solutions (cane-sugar, NaCl, LiCl, $MgCl_2$, $MgSO_4$), have been noticed by many observers (Roux, Hertwig, Morgan, Bataillon). All the more violent solutions attack the yolk-granules. In some cases the effect produced appears to be specific; thus in lithium salts the ectoderm is often pitted and wrinkled before any degeneration appears in the nervous system, and in ammonia salts, which are highly poisonous, the nuclei are much enlarged, lobed, highly chromatic, and homogeneous. The very similar appearance of the nuclei (Fig. 64) in those stronger solutions of urea which arrest development in an early stage suggests that the ammonia set free is the toxic agent in this case. In solution isotonic with ·625 % NaCl urea permits of normal development up to a certain point, when the embryos die.

In this connexion it is interesting to notice that Moore has found that sodium sulphate will act as an antidote to the poisonous effect of sodium chloride on tadpoles. Thus the average length of life of tadpoles in a $\frac{n}{8}$ NaCl solution was four and a quarter days, but was prolonged to twenty-one days by adding from 4 % to 8 % of Na_2SO_4. The poisonousness of sodium chloride, sodium nitrate, calcium nitrate, and magnesium chloride to *Fundulus* embryos and the value of other salts as antidotes has been shown by Loeb, while Lillie has noted that sodium is fatal but magnesium and calcium beneficial to the ciliary movement of *Arenicola* larvae, a result first obtained by Loeb for the Plutei of *Echinus*; the muscular contractions of the larva, on the other hand, are inhibited wholly by magnesium, partly by calcium, while sodium is necessary for their continuance. In an artificial solution which combines the three elements in the proper proportions normal development is possible. The nature of the part played by the ions—whether toxic or antitoxic—is, however, a very open question.

Arguing from the fact that the evil effects of such salts as sodium chloride and nitrate may be counteracted by calcium and magnesium salts, Loeb has suggested that toxicity and antitoxicity are functions of valency, and also of electrical charge, since it is further stated that toxicity increases with the valency

of the anion, antitoxicity with that of the cation. Ions of the same valency are not, however, necessarily equally antitoxic (Loeb and Gies, Lillie, Mathews), and sodium sulphate, as we have seen, may act as an antidote to the chloride (Moore). Mathews has accordingly sought for the cause of toxicity in another physical property, the decomposition tension of the salt, and has certainly succeeded in showing that the poisonousness of solutions to the eggs of *Fundulus* varies inversely with the decomposition tension, and that a similar relation holds good in certain other cases.

Lillie argues that a physiologically balanced solution is necessary, one in which the electrolytes are in a state of chemical equilibrium with the necessary ion-proteid compounds in the tissues. Solutions which only contain some of these substances, or solutions (for example, non-electrolytes) which contain none, are poisonous, because they permit of the outward diffusion of the needful ions.

It must be pointed out, however, that this explanation will not fit the cases where the embryo develops perfectly well in fresh water (*Fundulus*) or in distilled water (the Frog), and that some other reason must be found for the poisonous effect of cane-sugar upon the latter. The whole question, however, is one which belongs more properly to the province of pharmacology.

Poisonous although these salts are, the embryo can still be acclimatized to them. C. B. Wilson placed the unsegmented eggs of *Amblystoma*, *Rana*, and *Chorophilus* in a 0·05 % solution of sodium chloride; after twenty-four hours they were removed to 0·1 %, and then successively to stronger solutions by increments of 0·1 % until 1·0 % was reached, a concentration which quickly causes death under ordinary circumstances. In this case, however, development was normal, and the larvae hatched out and lived for some time.

The distortions of development which solutions of salts and other substances call forth in Amphibian embryos find a parallel in the malformations which Herbst has produced in Echinoderm larvae (*Echinus*, *Sphaerechinus*) by similar means; as in the former case, the results were at first assigned to the increased osmotic pressure of the media.

When potassium salts are added to the sea-water—for example, a 7 % solution in sea-water of a 3·7 % solution of KCl in tap-water—the egg gives rise to a Pluteus in which, though the gut is, as normally, tripartite, the skeleton is rudimentary and the arms suppressed (Fig. 71). Herbst suggests that the suppression of the arms is due to the absence of a stimulus normally exerted by the skeletal spicules. These abnormal forms may fuse together to form double monsters.

Such 'potassium' larvae are developed in sodium salts, but lithium has a more pronounced effect (Figs. 72, 73). In this case

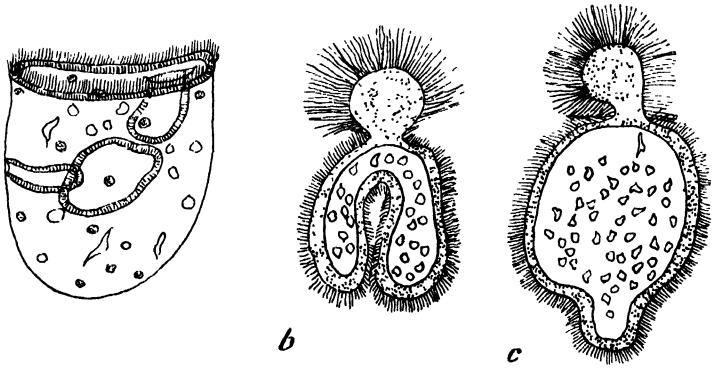


FIG. 71.—Potassium larvae of Echinoids. *a*. Potassium larva of *Sphaerechinus* (1860 c.c. sea-water + 140 c.c. 3·7% KNO_3). There is no skeleton. The gut is tripartite, and the mouth surrounded by the ciliated ring. *b*, *c*. Potassium larvae of *Echinus* (20% of 3% KCl). Note the button-shaped apical tuft of cilia, and, in *c*, the secondarily evaginated archenteron. (After Herbst, 1893.)

the blastula becomes constricted into two portions, a thin-walled gastrula wall provided with long cilia, and a thick-walled archenteron, which may be muscular and mobile, and is thickly covered with short cilia. The archenteron has, in fact, failed to invaginate, and the larva is an 'Exogastrula'. Occasionally there is an attempt at invagination at the end of the archenteric portion, and, after temporary exposure, the invaginated part may be divided into three, and a mouth formed. All the parts of the gut, however, remain in the same straight line. A middle section may be formed by further constriction of the archenteron (*Echinus*) or of the gastrula wall (*Sphaerechinus*). Double

monsters sometimes arise by fusion of these larvae by their archentera.

A skeleton is not usually developed; if present it is abnormal—in position, the spicules being placed near the animal pole and

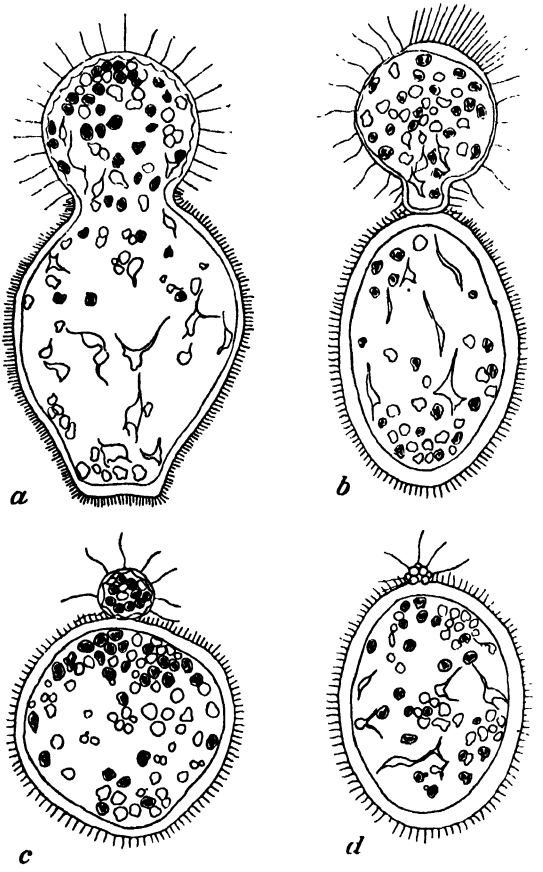


FIG. 72.—Lithium larvae of *Sphaerechinus granularis*. *a*. Larva partially constricted into gastrula wall and archenteric portions, the former with long, the latter with short cilia (980 c.c. sea-water + 20 c.c. 3·7% LiCl). *b*. Similar larva to the last, but a neck or connecting piece has been formed from the ectodermal portion. *c*, *d*. Progressive diminution of the ectodermal gastrula wall portion with increase in the quantity of Li.

the arms of the Pluteus formed under their influence near the mouth instead of by the side of the anus, in the number of the spicules, and consequently the number of arms (three, four, or five, instead of two), and in the number of their radii (four or five, instead of three).

The gastrula wall is often smaller than the archenteron, and, as the strength of the solution is increased, becomes still further reduced, until nothing of it is left but a small button at the

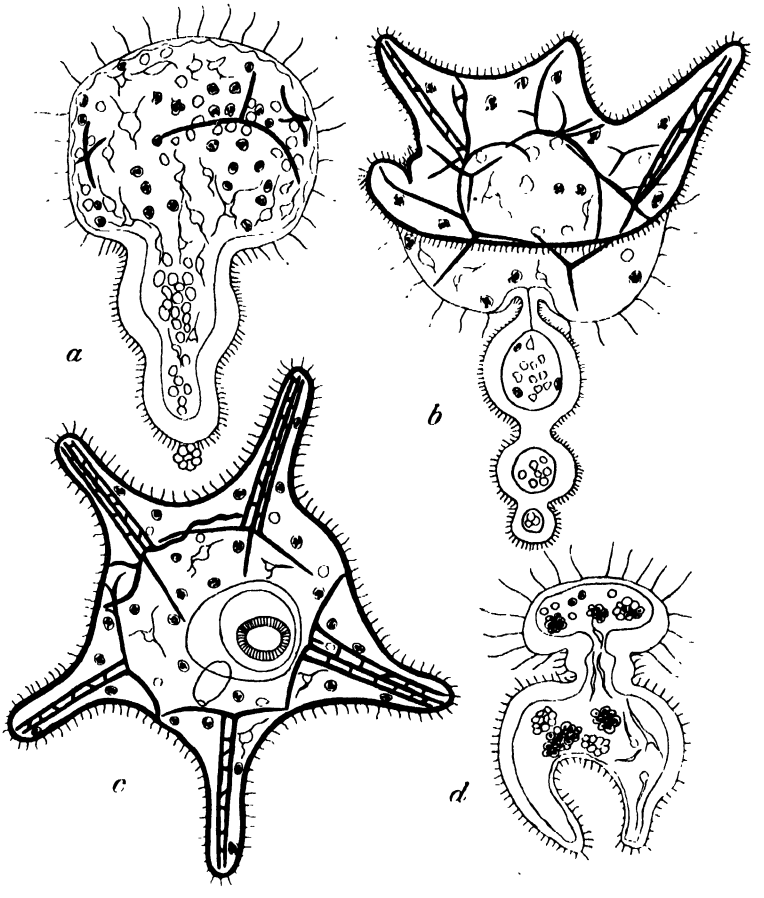


FIG. 73.—*a*. Larva with three skeletal spicules, and a 'cell-rosette' at the end of the archenteron. *b*. Larva with skeleton—more than three spicules—and arms developed. The neck is invaginated into the ectodermal portion, the gut tripartite. *c*. Five-armed Pluteus with five skeletal rods. The gut is normally invaginated and tripartite. *d*. Larva of *Echinus microtuberculatus*. There is a neck, and the gut is partly invaginated. In the blastocoel are aggregations of mesenchyme and pigment cells. (After Herbst, 1895.)

animal pole, which only indicates its real character by the long cilia which it carries. Such larvae Herbst terms 'Holoentoblastia'. This nearly complete suppression of the ectodermal

region can, however, only be realized when the salt is allowed to act at a stage in the blastula when the differentiation into the two primary layers is already beginning. Should the embryos be removed before this stage is reached, after twenty-four hours' exposure to the solution, only 'Exogastrulae', not 'Holoentoblastia,' can be obtained. Should, on the other hand, older blastulae, or gastrulae, or Plutei be placed in the solutions, they die without showing any signs of the characteristic abnormal development. From the fact that equimolecular solutions of monobasic lithium salts produced like effects (such solutions, it must be observed, are also chemically equivalent), Herbst concluded at first that the osmotic pressure was responsible for the abnormalities; but the permanent after-effects of temporary immersion just referred to subsequently convinced him that the ova were permeable to the lithium ions to which he now attributes the specific nature of the monstrosity. He suggests further that they act upon the endoderm cells by increasing their absorptive activity and their power of cell-division, while at the same time they inhibit the functions of those mesenchyme cells which are devoted to the formation of the skeleton.

As in other monstrosities, there is an alteration in the prospective potentialities of cells, elements which would normally be ectodermal becoming converted into endoderm, and additional mesenchyme cells being involved in the secretion of skeletal spicules.

It is only by lithium salts that the typical 'Holoentoblastia' can be produced; but Exogastrulae can be reared in others, in sodium butyrate, for example; in this solution a stomodaeum is formed, but is, like the archenteron, everted. Even lithium, however, is powerless to cause the 'holoentoblastic' reduction of the gastrula wall in the larvae of *Asterias*, although exogastrulation may, but need not, occur. A characteristic deformity is the absence of the pre-oral region, and the elevation of the mouth on a sort of hypostome. In *Amphioxus* and Ascidians it is impossible to obtain even exogastrulae by these methods. It is evident, therefore, that the specific morphological reaction depends not only on the nature of the substance employed, but also on the constitution of the reacting organism.

Herbst has not omitted to point out the significance of these—and indeed of all—monstrosities for the theory of the origin of those larger, discontinuous variations known as 'sports', or, in more modern phraseology, 'mutations'; and Vernon has been able to show statistically that the degree of continuous variation may also be altered by changes in the chemical environment.

In all the foregoing experiments the effect is observed of the addition of some chemical substance to the medium in which the embryo is placed. We have now to consider a very remarkable series of investigations, for whose planning and execution we are indebted to the genius of Curt Herbst, investigations in which substances which are present in the normal environment of the larva are omitted, and an insight thus gained into the part they play, if any, in the normal development of the organism. Herbst has indeed succeeded in demonstrating in the most conclusive manner the necessity to the sea-urchin egg for the normal performance of this or that phase of developmental function of a large number of the elements present in sea-water.

The sea-water at Naples, where Herbst carried out his work, has the following composition:—

$NaCl$	3 %
KCl	·07 %
$MgCl_2$	·32 %
$MgSO_4$	·26 %
$CaSO_4$	·1 %
$CaHPO_4$, $Ca_3P_2O_8$, $CaCO_3$, Fe_2CO_3, Si, Br, I	in small quantities.

It may be said at once that silicon, bromine, and iodine are unnecessary, and that, though earlier experiments led Herbst to believe that phosphorus and iron were essential, he has since assured himself that phosphorus is certainly, and iron probably,

not. All the other elements, however, can only be omitted under penalty of retardation, abnormality, or death (Figs. 74 A and B).

The method employed was a simple one. A series of artificial sea-waters was made up, from which, one by one, each of the elements was omitted, another being substituted in its place. Care was taken to make these artificial media approximately

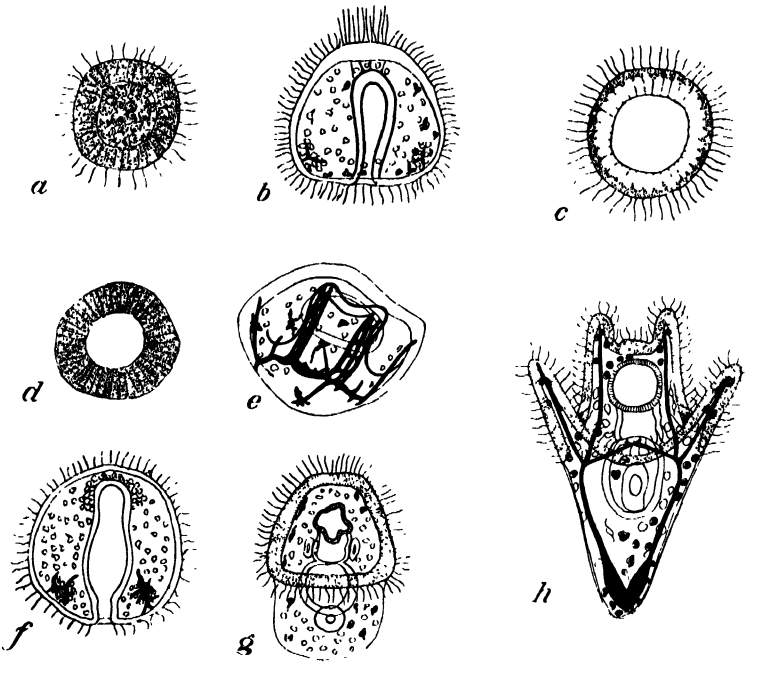


FIG. 74 A.

FIG. 74.—The necessity of substances contained in sea-water for the normal development of the larvae of sea-urchins.

a. Without OH. Ciliated stereoblastula of *Sphaerechinus*. *b*. KOH has been added. *c*. Normal blastula of *Sphaerechinus*. *d*. Blastula in a K-free medium. *e*. Reared in K-free and replaced in sea-water (*Sphaerechinus*). *f*. Larva from a medium devoid of Mg (*Sphaerechinus*). *g*. *Echinus* Pluteus with tripartite gut, mouth and coelom sacs, but neither skeleton nor arms; reared without $CaCO_3$ or $CaSO_4$. *h*. Normal Pluteus of *Echinus*.

isotonic with sea-water, and so exclude a possibly disturbing factor, the alteration of the osmotic pressure. The rôle of each of these necessary elements—or ions—will be considered separately and in some detail. *Sphaerechinus* and *Echinus* were the forms principally employed.

i. SO_4.

This is ordinarily provided by $MgSO_4$ and $CaSO_4$; when the fertilized ova are placed in a solution in which $MgCl_2$ is substituted for it (as, for example, in 3 % NaCl + ·07 % KCl + 5 % $MgCl_2$ + $CaHPO_4$ + $CaCO_3$) then their development is retarded from the blastula stage onwards, the embryos are small and degenerate without reaching the Pluteus stage (Fig. 74 B). The gut is straight instead of bent, and not divided into the

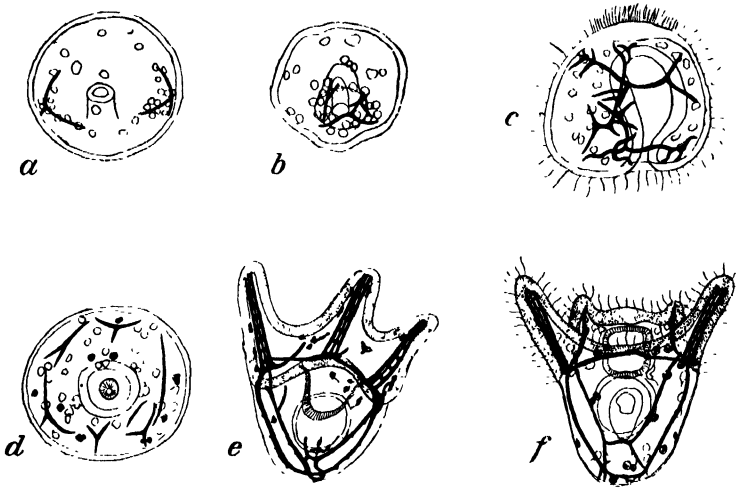


FIG. 74 B.

a. Normal position of skeletal spicules in *Sphaerechinus*. *b*, *d*. Abnormal position and number after treatment with SO_4-free medium. *c*. Larva of *Echinus* from a S-free solution. *e*. Pluteus of *Sphaerechinus* with three fenestrated skeletal arms, instead of two. Treated with a SO_4-free medium and replaced in sea-water. *f*. Normal Pluteus of *Sphaerechinus*. (After Herbst, 1897 and 1904.)

usual three parts; in *Sphaerechinus* no mouth is formed, the gut is evaginated (Exogastrula). The endoderm is very thick, the cells dark and dense.

The sulphuric acid radicle (sulph-ion) is thus necessary for the proper development of the gut, and necessary from the very beginning, for in embryos which have been kept in SO_4-free water up to the mesenchyme-blastula stage and then replaced in sea-water the alimentary tract is still abnormal.

Deprived of SO_4, in fact, the gut remains radially symmetrical,

and the same must be said of the skeleton. Normally there are two tri-radiate spicules, one to the right, the other to the left of the gut and some little way from it. Without the needful sulphate the spicules become placed near the gut, and may with the growth of the latter be pushed towards the animal pole. The number of spicules may also be diminished or increased to one, three, or four, arranged in a circle round the gut. On timely removal to sea-water, however, a secondary bilateral symmetry may arise by two of these outgrowing the rest and stimulating the development of the typical arms of the Pluteus. It seems that a sulphate is present in the calcareous skeleton of the Pluteus, as there is in that of the adult urchin.

A ciliated circum-oral ring is formed, but is abnormal in its position, at right angles instead of parallel to the long axis of the body. The pigment which should be secreted by the secondary mesenchyme cells (separated off from the inner end of the archenteron) remains in abeyance, and the apical tuft of cilia is hypertrophied. Other processes, however—fertilization, segmentation, and ciliary motion—are independent of SO_4.

The development of eggs which are allowed to remain in ordinary sea-water until the blastula stage is no better, whence Herbst concludes that no SO_4 is taken up during segmentation. During the early stages of gastrulation, however, they appear to absorb a store of it for future needs, for gastrulae reared in sea-water develop further in the SO_4-free solution than do those embryos which have been kept in it since fertilization. SO_4 is equally necessary for the continued life of the Pluteus and of the Bipinnaria larva of *Asterias*, and without it the rate of regeneration of the head of *Tubularia* is retarded and the number of tentacles reduced, until eventually a completely tentacleless head is evolved.

The necessary sulphate can be, to a certain extent, replaced by a thio-sulphate. The addition, for instance, of ·35% $Na_2S_2O_3$ to the SO_4-free solution renders it possible for the larvae to reach the Pluteus stage, though the arms are short, the skeleton small, and the pigment reduced. The larvae die.

Selenium and tellurium are both poisonous in an early stage.

ii. Cl.

A solution was made up in which the sodium chloride was replaced by sodium formate, the magnesium chloride by magnesium sulphate, the potassium chloride by potassium sulphate; thus, NaCOOH 3·5 % + $MgSO_4$ ·26 % + $MgSO_4$ ·4 % + K_2SO_4 ·12 % + $CaSO_4$ ·1 % + $CaHPO_4$ + $CaCO_3$.

The eggs did not segment, and even when KCl and $MgCl_2$ were used in their ordinary proportions, segmentation did not progress very far. Nor did the substitution of Na_2SO_4 for NaCOOH give any better results. A considerable amount of chlorine appears therefore to be absolutely necessary for the earliest developmental processes, its function being, Herbst suggests, to transport certain necessary cations, the tissues being possibly more permeable to NaCl than to Na_2SO_4. Later stages —blastulae, gastrulae, Plutei—all die in the Cl-free mixture.

Chlorine can be replaced in some measure by bromine. Plutei are formed, though with a distorted skeleton, *Tubularia* regenerates its head and the eggs of the fish *Labrax* develop as well as in sea-water. Iodine is, however, poisonous; so also are chlorates.

iii. Na.

2·96 % of $MgCl_2$ was added to a solution containing the usual amounts of KCl, $MgSO_4$, $CaSO_4$, $CaHPO_4$, and $CaCO_3$. In this the ova indeed segmented, but abnormally, the blastomeres being of unequal size. Death followed; nor was the addition of a certain small amount (·84 %) of NaCl sufficient to save them, though segmentation was normal and traces of an archenteron could be detected; with more NaCl (1·34 %) the gastrula stage was reached. The sodium which is thus necessary in the earliest period is also required later on; without it gastrulation is impossible to eggs which have been reared in sea-water even as far as the mesenchyme-blastula stage.

The part played by sodium is not clearly understood. It is known that it counteracts the evil effects of calcium and is necessary for the continuance of muscular contractions. Since calcium is necessary for the cohesion of cells (see below) Herbst opines that sodium may pull them apart; its action in that case is capillary.

Sodium cannot possibly be replaced by lithium, potassium, rubidium or caesium, all of which would at the necessary concentrations inevitably be poisonous.

iv. K.

In the artificial solution employed the small quantity (·07 %) of potassium present in sea-water is simply omitted.

Without it segmentation—except the first few phases—is impossible in *Echinus*. *Sphaerechinus*, however, segments, but the blastocoel is reduced, the cells are opaque and not vacuolated, and the ova, though ciliated, are motionless and die (Fig. 74 A, *d*, *e*).

Later stages are also sensitive to the want of potassium. Blastulae gastrulate, but are shrunken, with short archenteron, and in gastrulae the gut does not divide into three. Plutei, like all the others, die when deprived of it.

In the K-free medium spermatozoa temporarily lose their motility, and such spermatozoa cannot effect fertilization. The fertilization, however, of eggs which have been kept without potassium is possible; in fact, at this earliest stage, no potassium is absorbed, for eggs fertilized in sea-water develop no further in the K-free solution than do those fertilized and kept continuously in it.

The absence of potassium in segmentation leaves its effect upon later stages. Two days' exposure is not too long to prevent normal development on removal to sea-water, but five days' exposure causes abnormalities of the skeleton (asymmetrical with several triradiate spicules round the gut) and alimentary canal (no mouth).

De Vries has shown the importance of potassium for the turgor of young plant-cells, and its function here is probably similar, to promote growth, as the subjoined table of measurements shows. Its absence also affects the rate of development (Table XVIII).

Potassium can in a measure be replaced by rubidium and caesium. The use of lithium either has no effect or, in larger quantities, produces lithium larvae.

Other forms to which the lack of potassium was found to be fatal were the ova of *Asterias* and *Cotylorhiza*, and the adult

Amphioxus. Potassium is also necessary for the contractions of muscles (umbrella and tentacles of *Obelia*).

TABLE XVIII

Showing the effect of potassium upon the growth of Sea-urchin blastulae. (After Herbst.)

After	Ratio (unit = $\frac{1}{100}$ mm.) of cross-diameter to long diameter. Without K.	With K.
18 hours	$\frac{18{\cdot}5}{18{\cdot}7}$	$\frac{20{\cdot}4}{20{\cdot}7}$
24 hours	$\frac{18{\cdot}4}{19{\cdot}1}$	$\frac{21{\cdot}9}{22{\cdot}7}$
45 hours	$\frac{19{\cdot}7}{19{\cdot}9}$	$\frac{27{\cdot}5}{27{\cdot}8}$

Showing the effect of potassium on the rate of development of Sea-urchin larvae. (After Herbst.)

After	Artificial sea-water with ·008 % KCl.	·016 % KCl.	·024 % KCl.
36 hours	Small gastrulae.	Nearly Plutei.	Plutei.
60 hours	No mouth; gut not constricted.	Small Plutei.	Fully formed Plutei.

v. Mg.

The fertilized ova were placed in a solution which did not include the ·32 % $MgCl_2$ and the ·26 % $MgSO_4$ present in sea-water.

Segmentation proceeds normally, but the blastula is slightly smaller than when magnesium is present (the ratio of the diameters is $\frac{29}{35}$). The skeleton, however, though bilaterally laid down, is retarded and deformed (magnesium is present in the skeleton of the sea-urchin and possibly in that of the Pluteus) and the gut is not properly differentiated, not tripartite and without a mouth (Fig. 74 A, *f*).

When the $MgSO_4$ is replaced by an isotonic quantity of Na_2SO_4 the results are the same.

In the Mg-free solution cilia cease beating, development is retarded (Table XIX), and, though the spermatozoa retain their motility, the ova are so injured that fertilization is impossible unless they are restored to sea-water. The ova, in fact, seem to have a store of Mg which they lose in the Mg-free mixture. Fertilization can, however, take place without magnesium if the

eggs have been kept in sea-water, and such eggs develop, in Mg-free water, to precisely the same extent as those which have been fertilized in sea-water; at this period, therefore, the egg needs no external magnesium. The original store of this element apparently suffices also for the first steps in the formation of the gut; for the lack of it is felt equally in the later stages of its differentiation and in the first moments of its development; the alimentary canal is as abnormal in those larvae which have been kept without magnesium only up to the time when the mesenchyme and archenteric plate arise as in those which remain in the solution throughout.

TABLE XIX

Showing the retardation of development of Sea-urchin larvae deprived of magnesium. (After Herbst.)

Two solutions were employed, mixed in various proportions; one (1) has no Mg; the other (2) contains Mg.

Solution.	Result after 24 hours.
1	Nearly motionless; archenteron formed.
1 + 10 % of 2	Larger.
1 + 20 % of 2	Larger still.
1 + 30 % of 2	Larger; gut constricted; mouth formed.
1 + 40 % of 2	,, ,, ,, ,, ,,
1 + 50 % of 2	,, ,, ,, ,, ,,
2	,, ,, ,, ,, ,,

It is, in fact, only after the gastrula stage that magnesium is absorbed. Eggs, blastulae and young gastrulae, reared in sea-water develop far worse when placed in the Mg-free liquid than do gastrulae.

The formation of pigment and the contractility of muscles remain unaffected by the absence of Mg.

In Mg-free media the blastomeres of *Asterias* fall apart and the adult medusae of *Obelia* degenerate.

vi. Ca.

The calcium salts present in normal sea-water are $CaCO_3$, Ca_2SO_4, $CaHPO_4$, and $Ca_3(PO_4)_2$.

When the carbonate only is absent the blastulae are crumpled and opaque; a few gastrulate but are markedly abnormal, the ciliated ring being crumpled, the gut flattened on the oral side and the skeleton absent (Fig. 74 A, *g*). Should the skeleton have already been formed when the larvae are exposed it

becomes dissolved. When the sulphate only is omitted (magnesium sulphate being present) development is still inferior to the normal, inferior even to development in the presence of $CaSO_4$ but in the absence of $MgSO_4$; $CaSO_4$ may be replaced by $CaCl_2$. It seems, therefore, that the sulphate is necessary as a calcium salt.

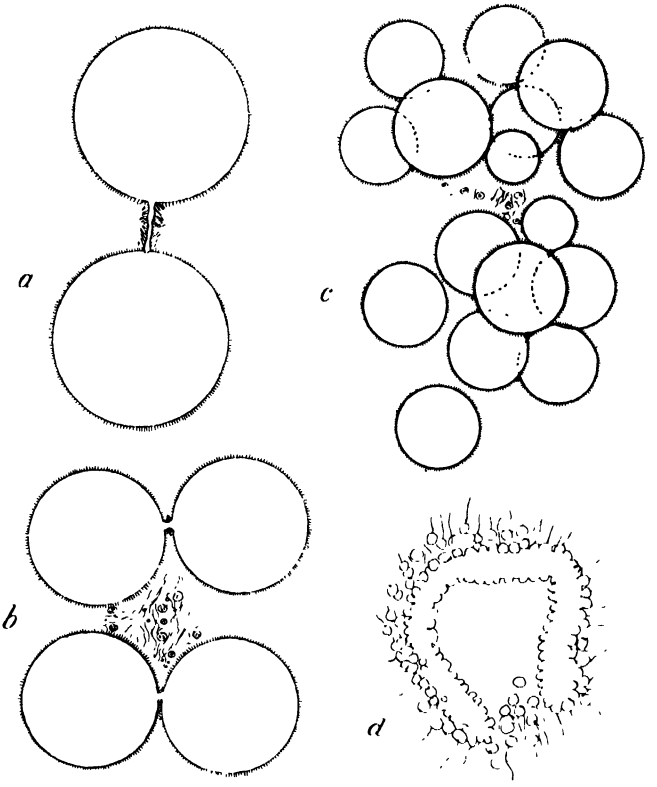


FIG. 75.—*a–c*. Separation of the blastomeres of *Echinus microtuberculatus* in a medium containing NaCl, 3·07%, KCl, 0·08%, $MgSo_4$, 0·66%, $MgHPO_4$ and $FeCO_3$, but no Ca. Note the radially striate border, which is the altered uniting membrane. *d*. Blastula disintegrating in the same medium. (After Herbst, 1900.)

If only the phosphate ($Ca_3(PO_4)_2$) is present the egg dies during segmentation, though, if the other phosphorus compound is substituted for it, the effect is the same as when the carbonate alone is omitted.

Should, however, all calcium salts be removed the result is more serious still (Fig. 75). The blastomeres are unable to

cohere, and separate as fast as division takes place, swimming about independently for a time, and then dying. The same phenomenon is witnessed when later stages are placed in such a calcium-free mixture.

On removal to sea-water division continues without separation, and should the egg membrane still be intact all the cells unite and a whole larva is formed. Even should the egg membrane be lost, a reunion of the cells is always possible so long as they remain in contact with one another. The separation is due to a change in the surface-tension of the cells; a visible change takes place, in fact, in the superficial layer which covers and unites the blastomeres; it becomes ill-defined and radially striated.

The lack of calcium also affects the rate of development, and causes shrinkage, but leaves karyokinesis, ciliary motion, and pigment formation unaltered.

Calcium is not replaceable by magnesium, strontium, or barium.

vii. CO_3.

As has just been pointed out, calcium carbonate is necessary for the due formation of the skeleton, although a beginning may be made without it.[1]

Whether the crumpling of the larva, due to diminution of internal osmotic pressure, which is observed in the absence of calcium carbonate is attributable to the lack of CO_3 or the lack of the hydroxyl ion is difficult to determine, since, as Herbst points out, a carbonate necessarily introduces OH, while the latter can convert into carbonates the CO_2 of the atmosphere and of respiration.

viii. OH.

The alkalinity of the sea-water—reckoned by the number of free hydroxyl ions—is provided by the calcium carbonate and calcium hydrogenphosphate. By the omission of these a solution —neutral to litmus—may be obtained in which the ova give rise to thick-walled, opaque blastulae with granular contents, ciliated

[1] $CaCO_3$ is necessary for the formation of the skeleton of the larva of the sponge *Sycandra setosa* (O. Maas, *S.-B. Ges. Morph. Phys. München*, xx, 1905). In a medium devoid of all calcium salts the Amphiblastulae fall to pieces.

but motionless, and doomed to eventual degeneration and death (Fig. 74 A, *a*, *b*). Very occasionally a gastrula with a short gut is formed.

When the blastulae are immersed in the solution they give rise to small, opaque gastrulae.

A certain degree of alkalinity is necessary for fertilization. The spermatozoa are less sensitive to a want of alkalinity, more sensitive to excessive alkalinity than the ova.

By the addition of a small amount of sodium hydrate to the neutral medium development is accelerated, but an increase of the alkalinity of ordinary sea-water is unfavourable. Loeb, on the other hand, has found that the addition of from ·006 % to ·008 % sodium hydrate to sea-water accelerates the development of *Arbacia*.

The formation of pigment and the vibration of the cilia are other processes which depend on the presence of the hydroxyl ion. Plutei die without it and their skeleton is dissolved.

The function of the ion does not appear to be to neutralize any acids produced by the tissues, for these give a neutral reaction even in OH-free media.

Since aeration improves the development of eggs in these media, and the more so if the air is deprived of its carbon dioxide, Herbst has concluded that one function of the OH ion is to neutralize the CO_2 and allow of the formation of the necessary carbonates. Another function is possibly, as Loeb suggested, the acceleration of processes of oxidation.

The experiments which we have been considering are unique of their kind, and it is impossible to exaggerate their importance. For, whatever may be the ultimate explanation of the facts, there can be no doubt whatever that the most complete demonstration has been given of the absolute necessity of many of the elements occurring in ordinary sea-water, its normal environment, for the proper growth and differentiation of the larva of the sea-urchin. Nor is this all. Some of the substances are necessary for one part or phase of development, some for another, some from the very beginning, others only later on. Thus potassium, magnesium, and a certain degree of alkalinity are essential for

fertilization, chlorine and sodium for segmentation, calcium for the adequate cohesion of the blastomeres, potassium, calcium and the hydroxyl ion for securing the internal osmotic pressure necessary for growth, while without the sulph-ion and magnesium the due differentiation of the alimentary tract and the proper formation of the skeleton cannot occur; the secretion of pigment depends on the presence of some sulphate and alkalinity, the skeleton requires calcium carbonate, cilia will only beat in an alkaline medium containing potassium and magnesium, and muscles will only contract when potassium and calcium are there.

The part played by each substance is therefore specific; for some particular part of the morphogenetic process it is indispensable. Not, of course, independently of internal factors, but in co-operation with them, it does, in fact, determine the production of organic form; and the relation between the embryo and the environment in which it develops is in this case, at any rate, of the closest and most intimate kind.

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9. SUMMARY

In the numerous experiments which we have been considering the effect is observed upon the development of the embryo of certain alterations in the constitution of that embryo's normal environment. Either some factor which is not usually present is added to the environment, or else some factor which is customarily found there is altered by increase or decrease, or removed altogether.

In some cases development remains undisturbed by this treatment, in others it may be merely generally retarded or accelerated, in others again it may be altered not merely in rate but in form, with the production of an abnormality or monstrosity, and if its effect is too intolerable the death of the embryo may ensue.

Throwing light as they do on the causes of the formation of natural monsters, such experiments are no doubt of the highest interest from a general teratological point of view. The mere possibility of the occurrence of such malformations is, however, itself a fact of the deepest morphological significance. A monster is an organism in which the development of some part or parts has either exceeded or fallen short of its normal limitations, and any such phenomenon points indubitably to a certain mutual

independence of the parts in the growth and differentiation of the organism; while some pursue their normal course, others deviate from it. It follows that when such deformations are due to changes in the external conditions the parts are not equally sensitive to the unusual influence to which they are exposed. Thus in the Frog embryos which exhibit persistent yolk-plugs and open brains when grown in solutions of various kinds, the yolk and the medullary folds are alone susceptible to the action of the poison, other parts are unaffected and continue their development as though under normal circumstances: or, again, a sea-urchin passes through the early stages of segmentation and gastrulation unchanged when placed in a sea-water from which magnesium has been removed, but the subsequent differentiation of the gut and the formation of the skeleton are abnormal; magnesium is necessary for these, though not for the earlier processes. A means is thus afforded of watching the behaviour of one or more parts independently of others, as, for example, of the animal cells in the gastrulation of the Frog's egg when the yolk-cells are injured, and the most valuable information contributed, often quite unexpectedly, to our understanding of the events of normal ontogeny.

Quite apart from this such experiments have already contributed, and will probably contribute still more in the future, to the study of variation. Between conspicuous monstrosities and those milder abnormalities which are termed 'sports' or 'mutations' there is every intermediate gradation, just as there is, on the other hand, no sharply defined limit between these discontinuous and those far smaller continuous variations to which the term has been often exclusively applied. The embryo is particularly sensitive to a change in its environment and reacts to such change by a variation in its form of greater or less degree. And not only that; as Vernon has shown, these changes can produce also an alteration in the variability of the species; and so provide greater opportunities for the operation of natural selection.

At the same time teratology is not the main inquiry with which the experimental embryologist is concerned. The problem that confronts him is to determine the part played by each factor of the external environment in the processes of

normal, specific growth and differentiation, and for the solution of this problem only those experiments, of course, are of avail in which such factors are either altered or removed.

By this means, as we have seen, it has been shown that a certain constitution of the physical environment, fixed within certain limits, is needful for the embryo; to these conditions it is closely adapted; those limits it can only transgress under pain of abnormality or death.

Every factor, or nearly every factor, is necessary for this or that phase or part of the process, some for the whole. Light of a certain wave-length will accelerate development, light of another kind, or in some instances darkness, will retard it, or stop it altogether; a certain degree of heat is indispensable; oxygen is required for respiration, water for growth; some eggs demand constant agitation, others comparative rest; fertilization, or segmentation, or gastrulation, or some one or other of the later phases of development may depend absolutely on the presence of some particular chemical element; remove the factor in question, whatever it may be, and that particular process will not occur, and the specific, typical end which is reached in normal development will not be attained. Nevertheless, the achievement of this end does not depend wholly upon extrinsic forces, for the ovum is no completely homogeneous 'isotropic' substance in which the complex circumstances of its environment conspire to produce heterogeneity and coherence. There is no evidence that any physical factor exerts a directive influence sufficient of itself to determine any part of the whole specific effect, although this may happen under extraordinary conditions, as when gravity impresses a bilateral symmetry upon the compulsorily upturned egg of the Frog, and so determines the median plane of the embryo.

Intimately bound up though these external conditions are with the proper conduct of the whole series of events whereby the organism comes gradually to resemble the parents that gave it birth, they can only operate in conjunction with internal factors which must be sought for not only in the initial structure and constitution of the germ-cells, but in the mutual interactions of the developing parts.

CHAPTER IV

INTERNAL FACTORS

1. THE INITIAL STRUCTURE OF THE GERM AS A CAUSE OF DIFFERENTIATION

§ 1. The Modern Form of the Preformationist Doctrine.

As has been already pointed out, it was in the hands of Roux that the principle of germinal localization first advanced by His assumed the rank and importance of a theory of development. On this 'Mosaik-Theorie' of self-differentiation the precise relation observable between the several parts of the embryo and certain definite regions of the undeveloped germ is not merely customary or normal, but necessary and causal. The germ-cell is endowed with a preformed structure corresponding to the structure of the organism which is to arise from it; each part of this structure is predetermined for the formation of some particular member of the embryo, and out of it no other member can, under ordinary circumstances, be made. The causes of its development, regarded as a specific activity of the organism, as leading to the production of a form which is like that of the parents which gave it birth, lie wholly within this pre-existing structure, and of each part within each part. Although the influence of the environment and, in later stages, of the parts on one another is not entirely excluded, the factors on which the differentiation of the whole and of each part depends are essentially internal, and all that happens is that by a continued process of cell-division the parts are separated from one another and the structure thus made palpable and manifest. Cell-division in the embryo is therefore qualitatively unlike.

The supposed proof, by Pflüger's experiment with forcibly inverted Frogs' eggs, of the complete 'isotropy' or equivalence of all parts of the cytoplasm, compelled Roux to locate the

self-differentiating substance, the Idioplasson, in the nûcleus, a view adopted and elaborated by Weismann, while the facts of regeneration necessitated the assumption of a reserve Idioplasson endowed with the potentialities of the whole, which is not qualitatively divided during development, but handed on intact to some or all of the tissues of the body.

The hypothesis then assumes the existence in the nucleus of the fertilized ovum of as many separate units as there are separately inheritable characters in the embryo, arranged there according to a plan which conforms to the structural arrangement of the parts which they represent, the separation of these units by continued 'unlike' nuclear division, and their distribution to the cytoplasm, where they determine the formation of the structures to which they are beforehand assigned.

For Roux the theory was no mere speculation, but the result of observation and experiment. The natural occurrence of half or partial monsters, the correspondence of defects in the embryo to injuries to the egg, and the coincidence of the first furrow with the sagittal plane in a large percentage (80 %) of, unfortunately, only a small number of eggs, suggested the self-differentiation of predetermined parts, while the last-mentioned observation led directly to the experiment in which one of the first two blastomeres of the Frog's egg was killed and a half-embryo produced from the survivor.

By means of a hot needle Roux succeeded in at least partially destroying one of the first two blastomeres. The other continued to segment, and passed through the ordinary stages; first the Hemimorula, with animal and vegetative cells and a segmentation cavity (though the last was sometimes absent), then by continued cell-division the Hemiblastula, followed by the Hemigastrula and Hemiembryo. In all cases the living tissues formed an exact half—either a right half or a left half—of a normal embryo, and ended abruptly at the (sagittal) plane of separation of the living and the dead. The segmentation cavity, however, was observed to extend in some cases across this plane, while in others it was confined wholly within the living cells.

In the Hemigastrula the greatest extent of the archenteron—

produced by the overgrowth of the blastoporic lip—was parallel to the plane of separation: the yolk-cells were pushed into the segmentation cavity. The Hemiembryo had half a medullary

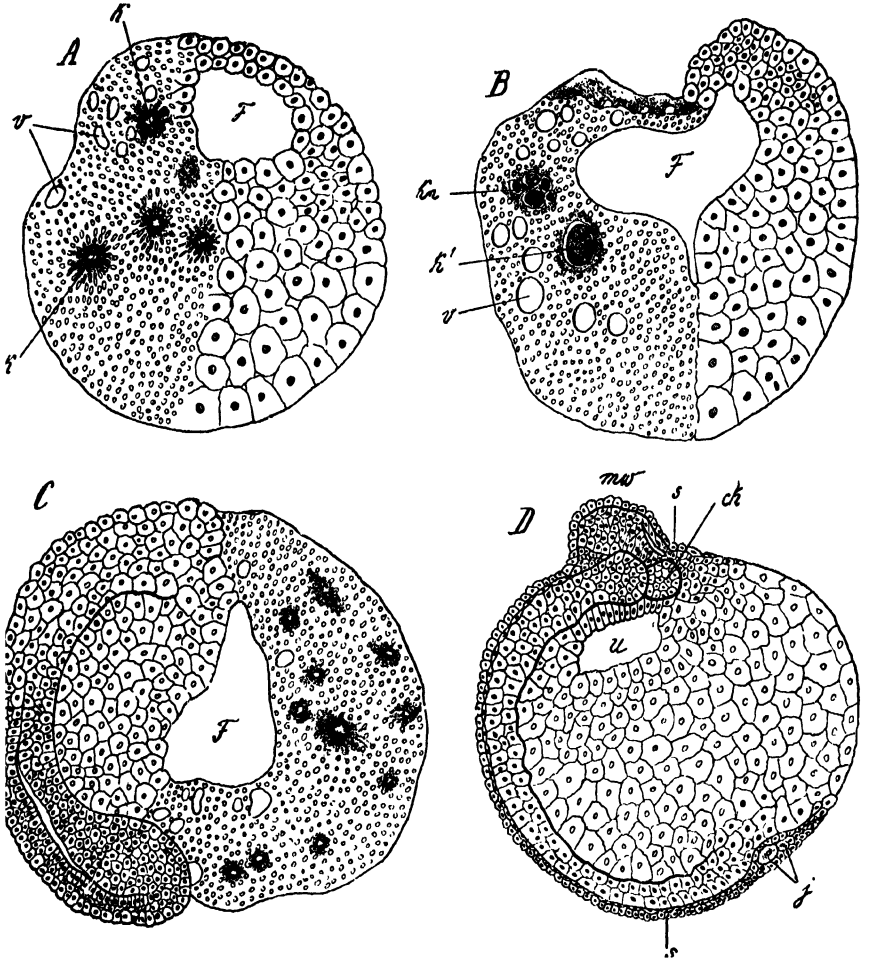


FIG. 76.—*A* and *B*. Meridional sections through Hemiblastulae of the Frog: *kn*, remains of nucleus; *k'*, large reticular nuclei; *k*, nuclei in the yolk; *F*, blastocoel; *r*, vacuoles. *C*. Hemigastrula lateralis. Oblique longitudinal section. *D*. Hemiembryo sinister, transverse section; *ss*, median plane; the right half of the egg is already completely cellulated, post-generation of the germ-layers has begun; *ch*, notochord, of *whole* size; *mw*, medullary fold; *u*, gut; *j*, two yolk-cells that have remained embryonic. (From Korschelt and Heider, after Roux.)

tube, mesoderm on one side, half a gut cavity—open towards the dead blastomere—but a whole notochord (attributed by Roux to premature regeneration).

Anterior half-embryos—occurring when the second furrow by an 'anachronism' appears first—were also observed, but posterior never.

Quarter and three-quarter embryos were obtained by killing three or one of the first four cells, and Hemiblastulae superiores by killing the four yolk-cells in the eight-celled stage.

The self-differentiation of the Idioplasson is thus demonstrated. Subsequently, however, the reserve Idioplasson comes into play, and the missing half is formed by a peculiar process, to which

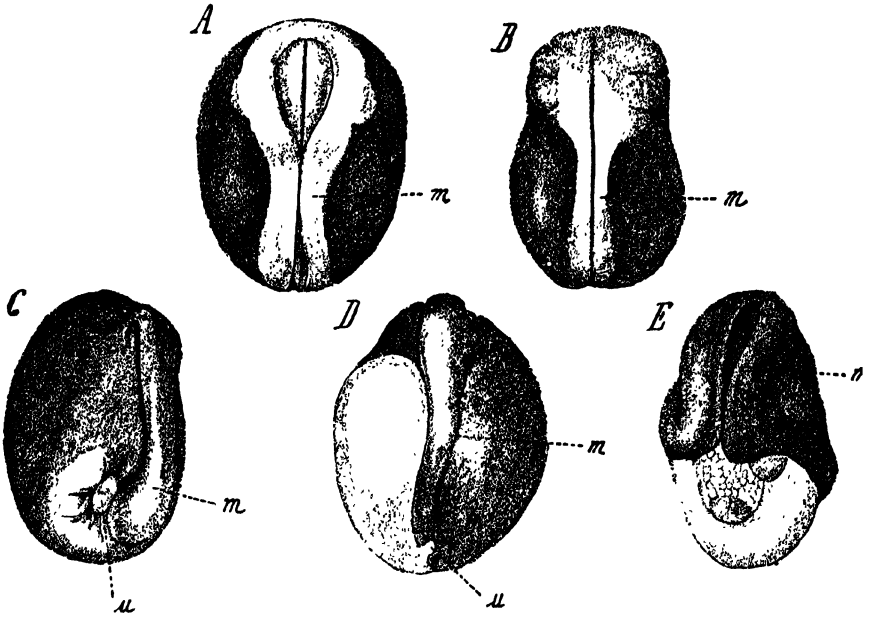


FIG. 77.—*A* and *B*. Normal Frog embryos with medullary folds (*m*), open (*A*) and closed (*B*). *C*. Hemiembryo dexter with almost complete post-generation of the ectoderm; *u*, yolk-plug. *D*. The same, older, but with less post-generation. *E*. Hemiembryo anterior (?) with beginning post-generation. (From Korschelt and Heider, after Roux.)

Roux has given the name of 'post-generation' to distinguish it from regeneration, in which lost parts are formed out of already differentiated tissue.

The injured blastomere first degenerates, is then reorganized, and finally post-generated.

In the first of these phases the cytoplasm and yolk are seen to be vacuolated, while the injured but not completely killed nucleus has given rise to small and large nuclei of normal structure

surrounded by masses of pigment and in addition to irregular chromatic masses – some pale, some deeply staining. Separating it from the uninjured half is a thin layer of yolkless protoplasm.

Reorganization is accomplished by any one of three methods. The first is nucleation followed by cellulation, that is to say, masses of cytoplasm arise round normal nuclei, some of which are derivatives of the injured nucleus, while others have migrated across from the living blastomere. The second method involves an abundant immigration of whole cells from the living embryo which resuscitate or feed on the yolk and nuclei of the injured half. In the third method overgrowths take place at various points of the tissues of the living half-embryo, the cells dipping into and feeding on the yolk as they pass across.

In post-generation, which only occurs after the first method of reorganization, each layer of the uninjured reforms the corresponding layer of the injured, the cells of the former exerting a directive stimulus upon the reorganized tissues of the latter, which thus pass through a process of 'dependent' differentiation. The ectoderm grows over from in front backwards and from below upwards, so that a structure superficially resembling a yolk-plug is formed at the hinder end; as it does so it becomes differentiated into the usual two layers. The medullary tube is formed in like manner. The mesoderm is formed from the ventral side and later divided into vertebral and lateral plates. The notochord was a whole from the beginning. The missing half of the gut is formed directly from the half-gut of the living embryo, not by any process of blastoporic invagination.

The conclusions drawn by Roux from this experiment have already been stated. Development is conceived of as a process governed essentially by a factor which is entirely internal, the preformed structure of the nucleus of the germ. Into the validity of this factor we have now to inquire by examining the evidence offered by the very numerous and similar experiments, performed on the eggs of animals of all kinds, which the 'Mosaik-Theorie' has evoked. We shall take these experiments in order, beginning with the form employed by Roux himself, the common Frog [1].

[1] For literature see following section.

§ 2. Amphibia.

It will be remembered that the Frog's egg possesses, when freshly laid, a symmetry which is radial about the axis, the line joining the centres (animal and vegetative poles) of the pigmented and unpigmented portions of the egg; the symmetry is marked internally by the arrangement of yolk and protoplasm, for the latter lies most abundantly in the smaller unpigmented, the former mainly in the larger pigmented portion. Since the yolk

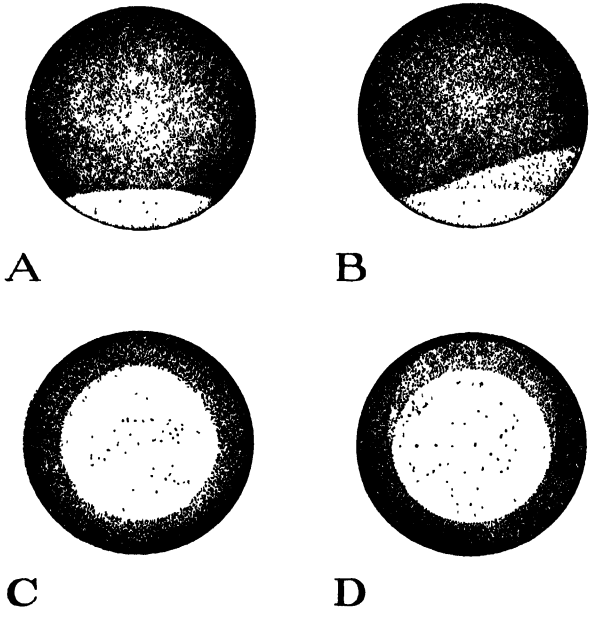


Fig. 78.—Formation of the grey crescent in the Frog's egg (*R. temporaria*). A, B from the side; C, D from the vegetative pole. In A, C there is no crescent, in B, D a part of the border of the pigmented area has become grey.

is heavier than the protoplasm the white pole is turned downwards, with the axis vertical, the egg being free to rotate inside its jelly membrane. Shortly after fertilization, however, the radial is replaced by a bilateral symmetry (in *Rana fusca* and *R. temporaria*), a grey crescent being formed on one side of the egg along the border of the pigmented area by the retreat of pigment into the interior (Fig. 78). The grey crescent eventually becomes white and added to the white area. In *Rana esculenta*

it is stated that the egg-axis becomes inclined to the vertical. This would seem to be an error, due to a confusion between the original white area and the white area enlarged by the addition

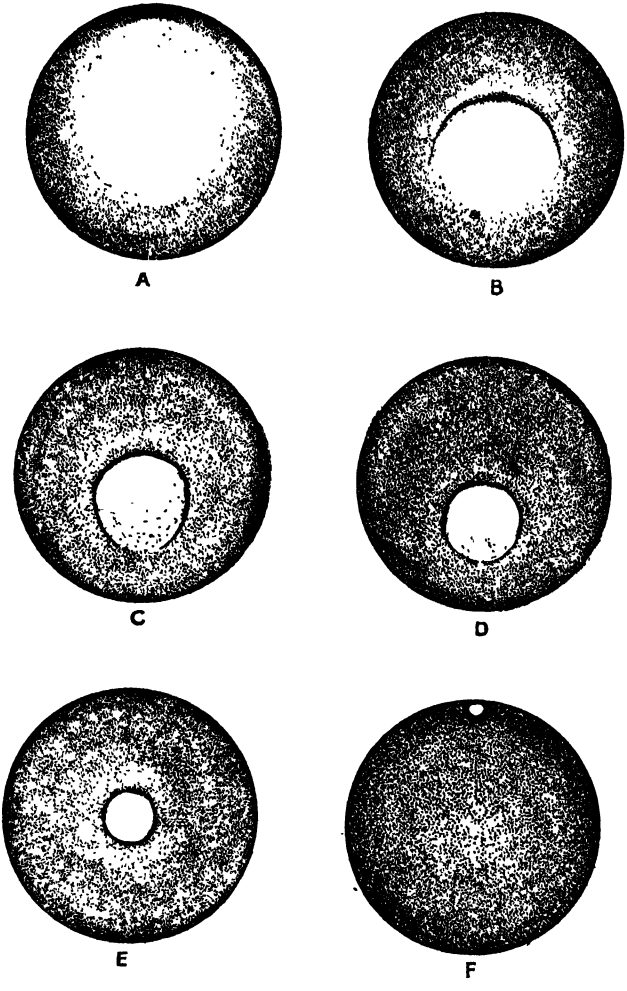


FIG. 79.—Diagrams of the closure of the blastopore in the egg of the common Frog (*R. temporaria*). In A–D the egg is viewed from the vegetative pole, in E, F from below. The dorsal lip is at the top of the figures. In D the ventral lip has just been formed and the blastopore is circular. In E the rotation of the whole egg has begun, and in F is complete.

of the grey crescent (compare Fig. 44 with Fig. 78). The egg is now bilaterally symmetrical about the plane which includes the egg-axis and the middle point of the crescent.

Since the crescent is formed on the side opposite to the entry of the spermatozoon, the latter is considered by Roux to determine the symmetry-plane of the egg. The first furrow is also stated to generally lie in this plane, and the dorsal lip of the blastopore, which marks, of course, the sagittal plane of the embryo, appears in the region of the grey crescent. The dorsal lip grows through 75°, starting 25° below the equator and passing beyond the vegetative pole; with the rotation of the whole egg and therefore also of its axis in the direction opposite to that through which the dorsal lip has travelled, the anterior end of the embryo, whose dorsal side is now uppermost, comes to lie a little above and behind the animal pole, while its posterior end is marked by the now fast-closing blastopore (Fig. 79).

According to Roux the first furrow and the sagittal plane coincide and the first two blastomeres are right and left, unless, by an anachronism, the second furrow, which separates anterior from posterior (this should be dorsal from ventral), occurs first. The third furrow, therefore, separates dorsal from ventral (correctly speaking, anterior from posterior). In the small number of eggs examined by Roux the percentage of coincidences was found to be high (80 %) and the deviations were attributed to experimental error.

Oscar Hertwig has, however, stated—on the strength of a small number of observations on eggs compressed between horizontal glass plates—that the angle between the two planes may have any value; while Schulze and Kopsch think it probable that they coincide in the majority of cases. The recent examination by the present author of a large number of cases has shown that the angle in question may indeed have any value from 0° to 90°, but that there is a decided tendency to small values, that is to coincidence, as may be seen from the annexed frequency polygon (Fig. 80), although at the same time there is no correlation, and therefore no causal connexion, between the two planes. Between the plane of symmetry and the sagittal plane, however, the correlation is considerable, and the tendency of the two to coincide much greater (Fig. 81), a result in conformity with the statements of other observers (Roux, Schulze, Morgan). Within certain limits, therefore, the right and left

halves, the dorsal and ventral sides (since the dorsal lip always appears in the region of the grey crescent) and the anterior and posterior ends (since the anterior end is always a little above the

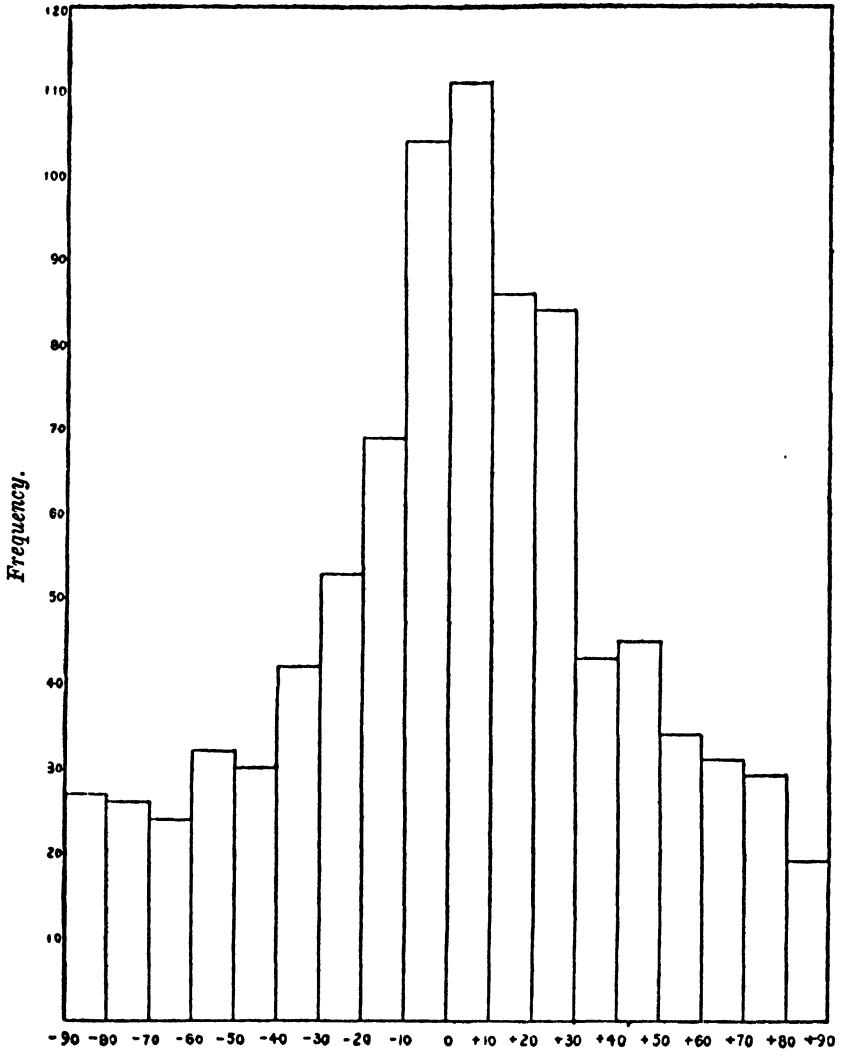


FIG. 80.—Frequency polygon of the angle between the first furrow of the Frog's egg and the sagittal plane of the embryo. $n = 889$, $M = 2{\cdot}12° \pm {\cdot}914$, $\sigma = 40{\cdot}39° \pm {\cdot}646$.

animal pole) are predetermined in the undivided egg. But the manner in which the material of the egg is cut up by the first two meridional divisions makes no difference to the result. In

these two divisions the qualitatively distinct parts of the cytoplasm may be separated from one another in any assignable manner, the nuclear material being distributed to these in any assignable order. The symmetry of segmentation has no relation

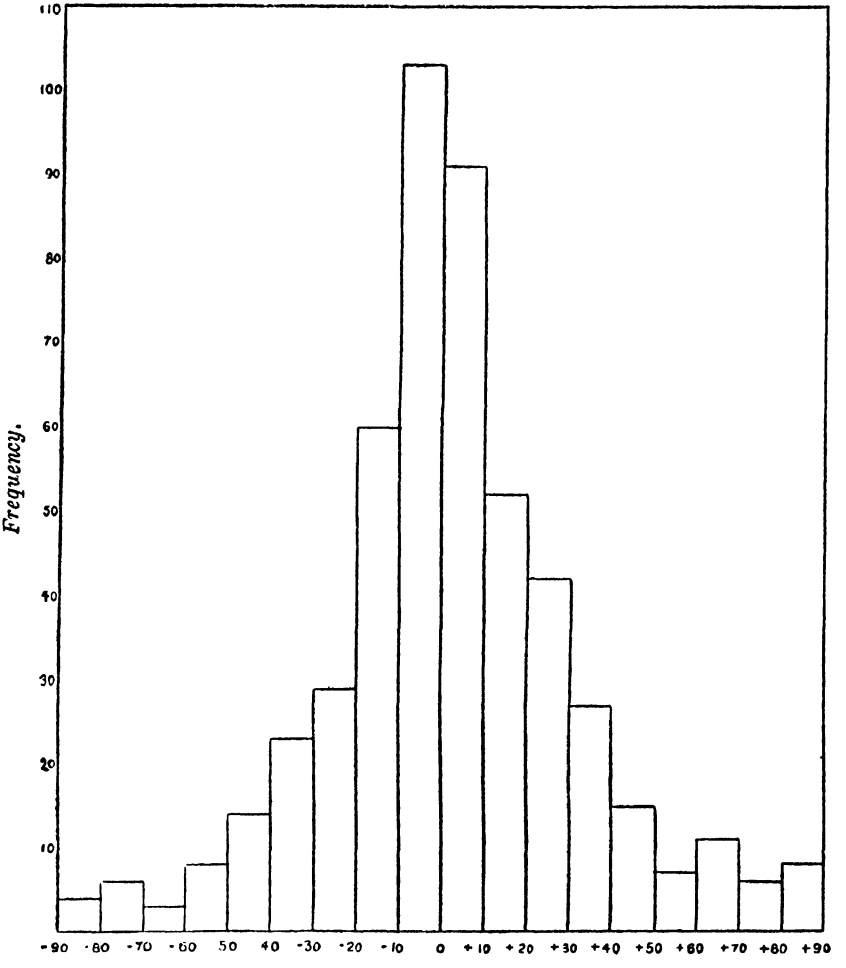


FIG. 81.—Frequency polygon of the angle between the plane of symmetry of the Frog's egg and the sagittal plane of the embryo. $n = 509$, $M = 2{\cdot}23 \pm {\cdot}889$, $\sigma = 29{\cdot}75 \pm {\cdot}629$.

either to the symmetry of the embryo or to the symmetry of the undivided egg, for the first furrow tends to lie either in or at right angles to the plane of symmetry and is not correlated with it to any great extent[1]. The third furrow, however, being always

[1] See, however, Appendix A.

at right angles to the axis, invariably separates anterior from posterior.

An observation of Morgan's has some bearing on this question; even when the first furrow divides the egg unequally a normal embryo results.

Even when the first two blastomeres are equal the cells produced by their subsequent division may migrate across the plane of separation (Kopsch). In *Necturus* there appears to be no relation between the first furrow and the sagittal plane (Eycleshymer).

The hypothesis of qualitative nuclear division has been tested by O. Hertwig in another way.

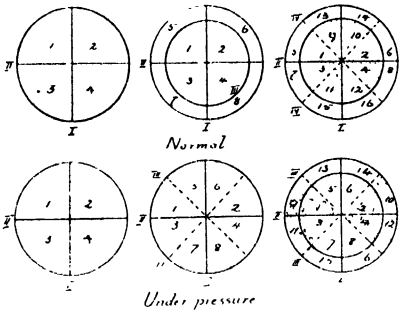
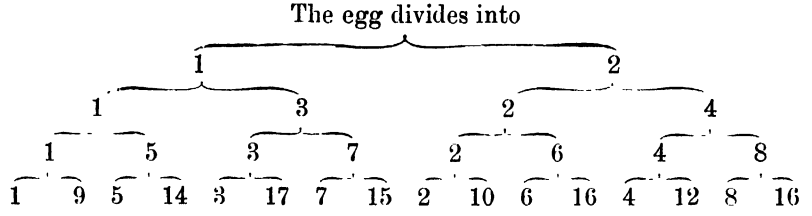


FIG. 82.—The first four phases of segmentation of the Frog's egg. Normally (the three upper figures) and under pressure between horizontal plates (the three lower figures) *I, II, III, IV*, the first, second, third, and fourth divisions. 1–16, the cells produced by successive divisions, numbered as follows:—



The animal cells in the normal egg and the corresponding cells in the compressed egg are stippled. (After O. Hertwig's account.)

The eggs are affixed to glass plates by their jelly, fertilized, and allowed to rest until the axis has become vertical. Pressure is then applied by a second plate, in the direction of the axis, the plates being horizontal, or at right angles to the axis, the plates being vertical or oblique. In such compressed eggs the direction of elongation of the nuclear spindles is distorted, and

consequently the 'qualitatively unlike' nuclei compelled to assume an abnormal arrangement. Between horizontal plates the first two furrows are, as normally, meridional and vertical (Fig. 82); the third, however, is parallel to the first (instead of latitudinal), and the fourth latitudinal (instead of meridional).

Between vertical plates the first is meridional and at right angles to the plates, the second latitudinal (horizontal) and near the animal pole, instead of meridional, the third furrows are parallel to the first, and the fourth meridional and at right angles to the first in the four upper blastomeres.

Now if nuclear division is a qualitative process, if, further, the nuclei of the compressed egg are divided in the same way in successive mitoses as are the nuclei of the normal egg (that is to say if the first two nuclei are right and left, each of these then divided into dorsal and ventral, and each of these again into an anterior and a posterior), then as a result of compression their distribution and arrangement must be altered (as the figures show), parts which should be anterior will be lateral or ventral, and vice versa, and a monster will be formed. Such eggs give rise to normal embryos. Nuclear division is therefore not qualitative. From this conclusion there is only one escape; it may be argued that the orientation of the nuclei remains unaltered when the direction of the spindles is changed by the pressure, and that therefore the order of sequence of the cell-divisions may be varied ad libitum, may be as 'anachronistic' as is pleased, without affecting in the least the mode of distribution of the, qualitatively unlike, parts, a contention, surely, which would only be urged for the sake of supporting a thesis.

O. Hertwig has also repeated Roux's experiment, killing, or at least injuring, one of the first two blastomeres by a needle or by means of electricity. The uninjured half segments to form a mass of cells lying on the top of the dead blastomere, as a blastoderm lies on the yolk of a meroblastic egg, and separated from it by a segmentation cavity, although the latter may be wholly within the living cells. This is Roux's Hemiblastula, but in Hertwig's case the dead cell lies below, a point, as we shall see, of considerable importance. Later on a blastopore is formed, but always, according to Hertwig, within the bounds of

the living portion, not at the edge; the blastopore is usually symmetrical to the plane of separation of the two blastomeres. Beneath the lip of the blastopore an archenteron is formed, and notochord and mesoderm are differentiated. The closure of the blastopore is, however, prevented by the resistance offered by the dead yolk-mass, which lies ventrally and posteriorly, just as it is retarded by the yolk in a large-yolked Fish egg; in fact, were this dead yolk removed, the living portion, Hertwig maintains, would develop normally. A nearly normal embryo may, in fact, be formed, but more usually there are considerable abnormalities. The mass of dead yolk, though partially enclosed by the growing edge of the ectoderm, is sufficiently great to impede the ultimate closure of the blastopore, and sometimes of the medullary folds as well; the latter frequently diverge round the yolk-plug of dead and living tissue, the chorda being split as well; they may be symmetrical, or one side may be much less developed than the other, owing, it is asserted, to an asymmetry in the resistance offered by the dead cell, and in the more extreme cases this inequality may be so pronounced as to give rise to a condition which does not differ in any way from Roux's Hemiembryo lateralis; there is one medullary fold, a notochord, mesoderm on one side, and a gut cavity in the yolk-mass; to the bare side of the yolk-cells is attached the dead blastomere, only partially covered by extensions of the ectoderm of the living half. Such cases are, however, very rare; in the majority more, sometimes much more, than a half is produced from the living blastomere, for Hertwig denies that the missing parts are post-generated, as Roux maintains, though he admits the overgrowth and immigration of cells, as well as the persistence of living nuclei where the injury has been only partial; all these contribute to the formation of the embryo, which would be complete were its development not hindered by the presence of the inert mass of yolk.

The differences of interpretation put by Roux and Hertwig on the same phenomena appear to be radical. A reconciliation is, however, possible, for Morgan has observed that the whole or half development of the injured cell depends upon the position it takes up. If the original position—with the black pole

uppermost—is retained, then a half-embryo is formed, as is indeed the case, according to Hertwig's own evidence, when the egg is prevented from turning over by compression.

In such half-embryos Morgan finds no traces of Roux's post-generation, although a whole embryo may be eventually formed by regenerative processes, referred by the author to the retarded development of the living parts of the injured half. It should be noticed, however, that Roux's statements are confirmed by Endres and Walter.

Should, however, the white pole be uppermost, then a whole embryo of half-size results. In Hertwig's experiments, of course, the original position was usually not retained.



FIG. 83.—Double embryo of *Rana fusca*, from an egg compressed in the direction of the axis and inverted in the two-cell stage. (After Schultze, from Korschelt and Heider.)



A B

FIG. 84.—Double monsters of *Rana fusca*, obtained by the same method. (After Schultze, from Korschelt and Heider.)

This conclusion is still further strengthened by Schultze's observations on the development of eggs inverted in the two-celled stage and kept so. Each blastomere develops independently and a double monster is produced (Figs. 83, 84). The result is not due to the pressure, for controls similarly compressed developed normally.

The details of development of these eggs have been carefully worked out by Wetzel (Figs. 85, 86). In each inverted blasto-

mere the yolk sinks next to the plane of separation, while the protoplasm and pigment rises to the outer side. The two cells are now related as two whole eggs united by their vegetative poles, their axes in one and the same (horizontal) straight line; each has, in fact, acquired a totally new polarity of its own. When segmentation has been completed a groove appears, in the plane of separation, and gradually extends round the whole circumference; the ends of the groove are forked, but the branches of each fork unite as the groove grows round. The groove is, in fact, a blastoporic lip common to the two, the branches the individual lips, the material between them and finally covered over by them a common yolk-plug, the space between the two

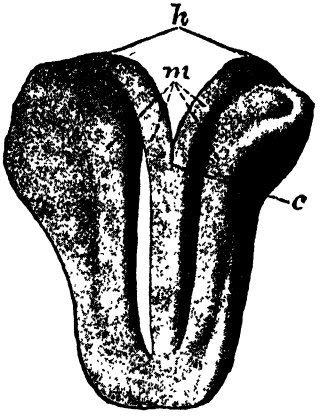


FIG. 85.—Double embryo obtained by the same method: *h*, heads; *m*, medullary grooves; *c*, line of union of the latter. (After Wetzel, from Korschelt and Heider.)

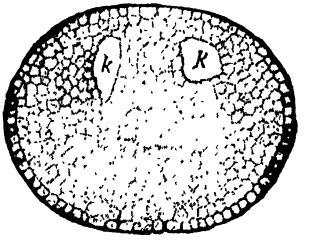


FIG. 86.—Section through a double blastula of the Frog (*Rana fusca*). *k*, blastocoels. (After Wetzel, from Korschelt and Heider.)

a common archenteron extending into an archenteric space in each individual. Subsequently medullary folds and notochord are developed in each below the groove, that is on the vegetative or postero-dorsal side of each, but anteriorly each grows out free of the other, and in this region medulla, notochord, and gut are single. The result is, therefore, two embryos placed back to back, and united by a common yolk-plug.

Experiments in which the four animal or the four vegetative cells of the eight-celled stage are killed are not very conclusive, as the development of the survivors does not go very far.

Samassa has, however, shown that a short archenteron with dorsal lip, together with traces of notochord and mesoderm, may be formed from the four animal cells alone, the dead yolk-cells making a floor to the archenteric cavity and protruding as a large yolk-plug; and Morgan has obtained a similar result with the four vegetative cells alone.

From all these investigations it seems reasonable to infer that each of the first two blastomeres of the Frog's egg may under certain circumstances acquire the polarity of a whole, and be capable of giving rise to an embryo whose complete development is only prevented by the impediment offered by the presence of the other, whether living or dead. Were it possible to completely separate the two blastomeres, we may surmise that each would become a perfect embryo: a surmise which is raised to a certainty by our knowledge of what happens in the newt. For in this form it is possible to separate the two cells by means of a noose of fine hair tied round the egg in the plane of the first furrow, as Herlitzka has shown, and in this case each half segments as a whole and develops into a whole larva of rather more than half-size (Fig. 87). Herlitzka has further investigated the dimensions of the organs in these embryos; those of the medulla and notochord he finds are the same as in embryos developed from a whole egg, those of myotomes and gut a little less. The size of the nuclei and cells in the medulla and myotomes is the same as in the whole ($\frac{1}{1}$) embryo[1]; the number of nuclei in the medulla is the same, in the myotomes one-half. He concludes that some organs need a certain minimum number of cells for their differentiation, while the cells must attain to certain dimensions.

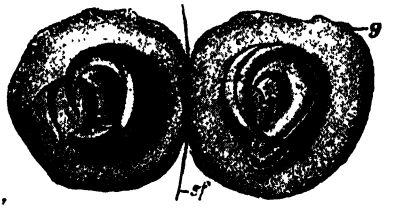


FIG. 87.—Egg of the newt (*Triton cristatus*), with two completely normal embryos, obtained by tying a thread (*sf*) round the egg in the first furrow. *g*, jelly membrane. (After Herlitzka, from Korschelt and Heider.)

[1] It will be convenient henceforward to designate the whole egg, or the embryo or larva developed from it, by the symbol $\frac{1}{1}$, each of the first two blastomeres and the embryo or larva, whether complete or incomplete, formed from it by $\frac{1}{2}$, and so on; thus a $\frac{3}{4}$ embryo means one which arises from three out of the first four blastomeres.

The discovery of Herlitzka has been followed up by the researches of Spemann, who has employed the same method of constriction, but with variations of degree, direction, and time. These differences have given the most interesting results.

The first furrow, according to this author, is usually at right angles to the sagittal plane and separates the material for the dorsal and ventral halves of the embryo; only rarely do sagittal plane and first furrow coincide. Both cases were, however, experimentally investigated.

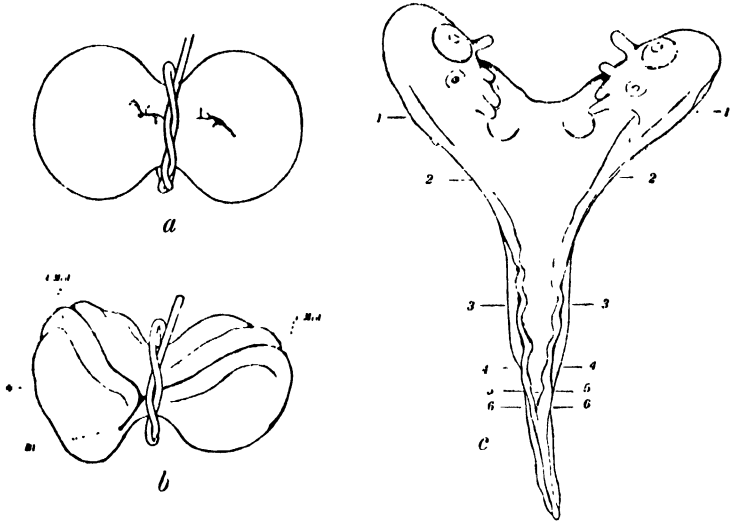


FIG. 88.—Three stages in the production of a double monster by strong median constriction of the Newt's egg. (After Spemann, 1903.) *a*. Beginning of gastrulation; there is a separate lip in each half. *b*. *l*. and *r*. *Med*., Medullary folds of left and right embryos; *, point where the medullary grooves separate; *Bl*, blastopore. *c*. The double-headed larva.

When the first furrow is in a horizontal plane, a slight constriction in the two-celled stage separates the dorsal lip of the blastopore in one portion from the ventral lip in the other. Medullary folds are developed in the first half only, the second forms a sort of yolk-sac appendage which is later absorbed by the single normal embryo. With tighter, but still incomplete, constriction the dorsal half alone becomes an embryo; it contains either the whole or only the dorsal portion of the blastopore. The ventral half contains either no portion of the blastopore or

only the ventral lip; it develops mesoderm but undergoes no further differentiation, and eventually drops off. Should the constriction be delayed until after the dorsal lip has appeared both halves may form an embryo, but the ventral embryo is usually imperfect. These differences are attributed by Spemann to differences in the time or place of constriction.

When a transverse constriction is made after the appearance of the medullary plate the anterior half develops as a whole, with complete nervous system and optic vesicles; the posterior forms a medullary plate, but no folds, and dies. After the appearance of the medullary folds, however, the anterior and posterior halves produced by transverse constriction develop as halves, although a case is described where each had a pair of auditory vesicles.

When the sagittal plane coincides with the first furrow, constriction in the two-celled stage gives rise to double-headed monsters; if the constriction is slight the most anterior organs only are involved (duplicitas anterior), the cerebral hemispheres, epiphysis, hypophysis, and paraphysis being doubled; there may be two complete pairs of eyes, or the median eyes may be more or less fused (Diprosopus triophthalmus). Tighter constriction brings about a reduplication of the chorda, auditory vesicles, and fore limbs (Dicephalus tetrabrachius). Similar effects are obtained by constriction in the blastopore stage, but not later; halves separated in the median plane when the medullary folds have arisen die as halves.

We shall now briefly consider another experiment by which the independence of one another of the parts is demonstrated. Schaper removed the brain, eyes, and, probably, the auditory vesicles from newly hatched tadpoles of *Rana esculenta*. The wound healed up. The mouth moved to the anterior end and the suckers up the sides of the tadpoles, which lived for nearly a week, in the course of which they grew 2 mm. They then showed signs of weakness and were preserved. It was found that the mouth had opened, that labial cartilages, pterygo-palatine bar, jaw-muscles, gill-bars, gills, heart and blood-vessels, trigeminus and vagus ganglia, oesophagus, pronephros and glomus, and dorsal muscles had all been differentiated.

There was, however, no operculum. The anterior end was occupied by a mass of mesenchyme. There was no sign of a regeneration of any of the lost organs except the anterior end of the notochord. The nerve ganglia were normal, but the spinal cord underwent degeneration. In spite of this the creatures could execute spontaneous and reflex movements.

What this experiment shows is, of course, that the organs of the trunk are not dependent for their development upon the presence of the brain; fresh researches would be necessary to determine how far in each case the parts are self-differentiating. In the second place the brain and eyes, when removed at this stage, cannot be remade by the tissues, but remain behind. So far, therefore, the body is at this stage an inequipotential system, as Driesch would call it. We know, however, that at an earlier stage the parts of the body are equipotential. We have, in fact, only another instance of that loss of totipotentiality, of that increase of independence and self-differentiation which takes place as development proceeds. Here, however, we are anticipating a conclusion which can only be completely stated after a discussion of the experiments performed on eggs of other types.

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§ 3. Pisces.

Morgan has shown that in the fishes *Ctenolabrus*, *Serranus*, and *Fundulus* there is no definite relation between the first (or second) furrow and the median plane of the embryo.

In *Fundulus* when one of the first two blastomeres is removed the other gives rise to a perfect embryo of more than half-size. In segmentation the first furrow is in the plane of what would be the second furrow of the whole egg, the second in that of the third, and the third more or less in that of the fourth; but as the successive furrows are at right angles to one another in both cases it is permissible to assert that the segmentation of the half blastomere is total.

Some of the protoplasm of the half that has been removed flows in underneath the other, is nucleated by it and added to its mesoderm. The size of the nuclei is the same in $\frac{1}{2}$ and $\frac{1}{1}$ embryos.

Morgan also found it possible to remove two-thirds of the yolk, but not more, without interfering with the normal development of the whole egg.

Injury to the germ-ring on one side of the dorsal lip resulted in a deficiency in the mesoderm of that side posteriorly; anteriorly both sides were equally well formed.

So Kastschenko has shown for Elasmobranchs and Kopsch for Teleostei and Elasmobranchs that injury to the lip of the blastopore will only interfere with the normal bilaterality of the embryo when effected quite close to the middle line.

A series of experiments similar to the last has been carried out by Sumner on the eggs of various fish (principally *Fundulus*, also *Exocoetus*, *Salvelinus*, and *Batrachus*).

By means of needles inserted into various parts of the blastoderm the exact mode in which the material of the latter is used in the formation of the embryo is first determined. It is shown that the material for the embryo is brought into position not by a concrescence of the lateral lips of the blastopore (sides of the germ-ring), but rather by an axial concentration of the cells originally situated in that ring at the posterior margin or dorsal lip, the cells so concentrated being continually pushed forwards in the middle line until the anterior end of the embryo comes to lie near the original centre of the blastoderm.

Injuries to various parts of the blastoderm and its margin (the germ-ring) prove that it possesses a certain degree of 'isotropy'.

Thus the entire embryonic region of the posterior margin was destroyed by electrocautery. The adjacent edges closed up and completed the germ-ring; at the posterior point of this a new embryonic shield was formed, in which ectoderm, endoderm, and notochord became differentiated. Or, again, a lateral piece of the germ-ring immediately adjacent to the embryo was removed without preventing the formation of a normal, bilaterally symmetrical embryo, although, as Morgan found, the mesoderm might be deficient posteriorly on the injured side.

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§ 4. AMPHIOXUS.

In this form the blastomeres may be separated by shaking. Their development has been observed by Wilson, whose account

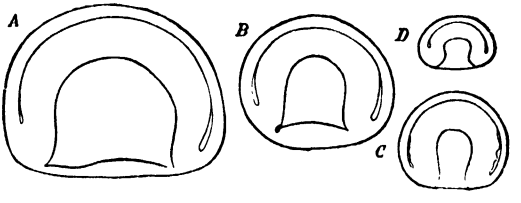


FIG. 89. *A*. Normal $\frac{1}{1}$ gastrula of *Amphioxus*. *B*. Gastrula from a $\frac{1}{2}$ blastomere. *C* Gastrula from a $\frac{1}{4}$ blastomere. *D*. Gastrula of $\frac{1}{8}$ normal size from an egg-fragment. (After Wilson, from Korschelt and Heider.)

has since been confirmed by Morgan. The isolated blastomeres soon become rounded; the first division is always transverse to the long axis.

A $\frac{1}{2}$ blastomere segments like a whole ovum, except that the second division may be unequal, and gives rise to a normal blastula, gastrula, and embryo of half the normal size; $\frac{2}{4}$ blastomeres behave in the same way (Fig. 89). Incomplete separation of the cells after the first or second division leads to double embryos in the first case, double, triple, or quadruple embryos in the second. The double embryos may make any angle with one another and continue to live for some time (Fig. 90).

A $\frac{1}{4}$ blastomere segments like a whole egg (the second division is, however, always unequal) and gives rise to a normal blastula and gastrula, more rarely to an embryo of one-quarter size.

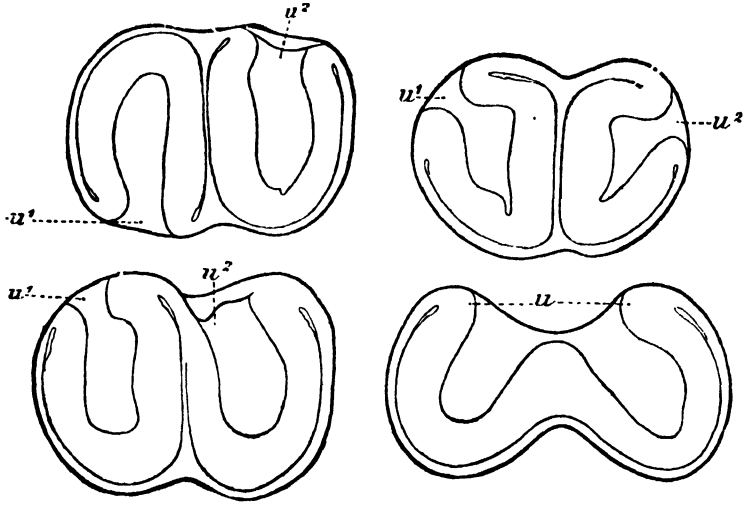


FIG. 90. Double gastrulae of *Amphioxus*, from incompletely separated $\frac{1}{2}$ blastomeres. u^1, u^2, separate blastopores; u, common blastopore. (After Wilson, from Korschelt and Heider.)

A $\frac{1}{8}$ blastomere will segment normally, or nearly so. A blastula is rarely formed, usually an open curved plate of cells, which are ciliated and swim about but do not gastrulate.

Lastly $\frac{1}{16}$ blastomeres divide but produce only an irregular heap of cells.

It is obvious that the potentiality of a blastomere to form a whole embryo diminishes with its germinal value, the ratio it bears to the whole ovum; but it is not so clear whether this diminished capacity is due to the lack of some necessary, specific, organ-forming substance or merely to the small size and lack of

undifferentiated material. Morgan has attempted to answer this question by counting the number of cells in the various organs of whole and partial larvae. It appears from his estimate that the number of cells in the archenteron, in the notochord and in the nerve-cord of $\frac{1}{1}$, $\frac{1}{2}$, and $\frac{1}{4}$ larvae is constant, and that though the size of the whole body is less, the dimensions of the notochord and nerve-cord are about the same in all three. It would seem, therefore, that there is a minimum size and a minimum number of cells necessary for the formation of these organs. Whether the failure of the $\frac{1}{8}$ blastomere to develop into a larva is, however, due to mere lack of material or the absence of some specific substance needful for the development of some particular organ is still undecided; for the $\frac{1}{2}$ and $\frac{1}{4}$ cells contain substance from both the animal and vegetative portions of the egg, while the $\frac{1}{8}$ are composed of one or the other substance exclusively, the furrows of the third phase being equatorial.

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§ 5. COELENTERATA.

Many years ago Metschnikoff observed a perfectly normal separation of the blastomeres at a certain period in the development of certain medusae (*Oceania*). If when the cells reunite in a later stage the order of their rearrangement is not constant, the egg-substance must be in some measure isotropic.[1]

More recently the equipotentiality of the blastomeres of these forms has been experimentally demonstrated by Zoja (Fig. 91).

The cells were separated by means of a needle. $\frac{1}{2}$ and $\frac{1}{4}$ blastomeres (in the case of *Liriope*, *Geryonia*, *Mitrocoma*, *Clytia*, and *Laodice*) and $\frac{1}{8}$ and $\frac{1}{16}$ blastomeres in the case of the two last mentioned will give rise by normal segmentation to blas-

[1] It should not be forgotten that in 1869 Haeckel had cut the blastulae of *Crystallodes* (a Siphonophor) in pieces and obtained from the larger fragments normal larvae. The development of small pieces was retarded and abnormal (*Zur Entwickelungsgeschichte der Siphonophoren*, Utrecht, 1869).

tulae with closed blastocoel and these to normal Planula larvae. In *Clytia* a hydroid was eventually reared and in *Liriope* a medusa with four primary tentacles from $\frac{1}{2}$ and $\frac{1}{4}$ cells. It is to be noticed that, since the third division in these eggs is equatorial, the $\frac{1}{8}$ and $\frac{1}{16}$ blastomeres must be either animal or vegetative.

Zoja also finds that in *Laodice* and *Clytia* the number of cells in partial larvae is proportional to the germinal value. In *Liriope* the number of cells in the endoderm is equal to that in the whole ($\frac{1}{1}$) larva.

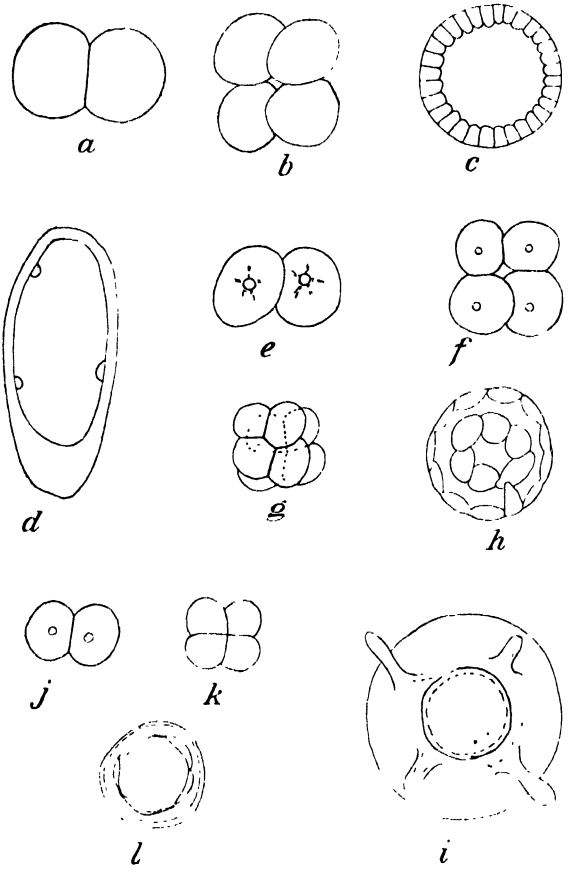


FIG. 91.
Partial development in Coelenterata. (After Zoja, 1896.)

a-d. Laodice cruciata: *a*, $\frac{2}{4}$ blastomeres: *b*, $\frac{4}{8}$ blastomeres; *c*, blastula from $\frac{1}{2}$ blastomere; *d*, $\frac{1}{2}$ larva during formation of endoderm. *e-l. Liriope mucronata*. *e-i*. Development of $\frac{1}{2}$ blastomere: *e*, division of $\frac{1}{2}$ blastomere into $\frac{2}{4}$; *f*, $\frac{4}{8}$; *g*, $\frac{8}{16}$; *h*, end of endoderm formation in $\frac{1}{2}$ embryo; *i*, medusa reared from $\frac{1}{2}$ blastomere. *j-l*. Development of $\frac{1}{4}$ blastomere: *j*, $\frac{2}{8}$; *k*, $\frac{4}{16}$; *l*, embryo with endoderm.

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§ 6. Echinodermata.

Easy to obtain in large numbers and eminently suitable for experiment, the eggs of the sea-urchins and starfishes have provided the analytical embryologists with abundance of material; and it is in this group that the possibility of rearing a perfect larva from a single blastomere was first demonstrated by the classical researches of Driesch.

The experiments which have been carried out, mainly by Driesch, but also subsequently by others, are very numerous and fall into two principal classes.

In the first the development of parts—whether isolated blastomeres or groups of blastomeres, fragments of unsegmented eggs fertilized or unfertilized, or pieces of blastulae and gastrulae—has been observed, the rate of their development recorded, and the relation of the number of cells and dimensions of the partial larvae to their germinal value determined. By the second series of investigations the effect of alteration of the type of nuclear or cell-division and consequent displacement of the blastomeres upon the subsequent development has been discovered.

Before discussing these experiments, however, a word must be said as to the normal behaviour of the whole egg.

The structure, segmentation and formation of the primary germinal layers have been very completely studied by Boveri in the sea-urchin, *Strongylocentrotus lividus* (Fig. 92). The axis of the ovarian egg is marked by the excentric position of the nucleus; at the point on the surface nearest which it lies the polar bodies are formed, and this point is the animal pole. At this point there is a fine canal (micropyle) in the jelly which surrounds the egg. After the extrusion of the polar bodies a brown pigment which had previously been uniformly distributed through the cytoplasm becomes aggregated in a dense but quite superficial subequatorial zone, the upper somewhat ill-defined border of which may, however, extend into the animal hemisphere. The pigment-free region left about the vegetative pole occupies from $\frac{1}{20}$ to $\frac{1}{10}$ the volume of the whole ovum. The spermatozoon may, but need not, approach the egg by the micropyle. The fertilization spindle lies in a plane parallel to the equator of the egg (the

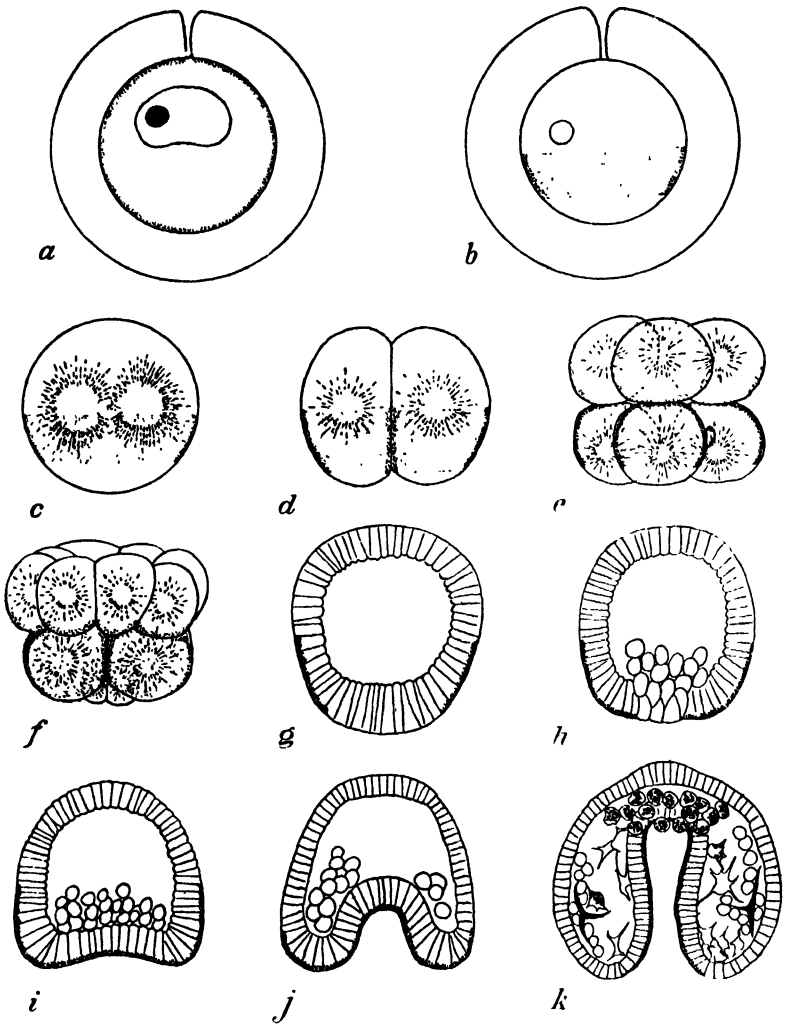


FIG. 92.—Normal development of the sea-urchin, *Strongylocentrotus lividus*. (After Boveri, 1901.)

The animal pole is uppermost in all cases, and in the first two figures the jelly with the canal (micropyle) is shown.

a, primary oocyte ; the pigment is uniformly peripheral.

b, ovum after extrusion of polar bodies: the pigment now forms a subequatorial band. The nucleus is ex-axial.

c, *d*, first division (meridional).

e, 8 cells, the pigment almost wholly in the vegetative blastomeres.

f, formation of mesomeres (animal cells) by meridional division : the vegetative cells have divided into macromeres and micromeres.

g, blastula. *h*, mesenchyme blastula.

i, *j*, *k*, invagination of the pigmented cells to form the archenteron of the gastrula. In *j* the primary mesenchyme is separated into two groups, in each of which, in *k*, a spicule has been secreted. In *k* the secondary, pigmented mesenchyme is being budded off from the inner end of the archenteron.

'karyokinetic plane') and elongates at right angles to the sperm-path, which thus lies in the plane of the first, a meridional, furrow (a similar observation had been previously made by Wilson and Mathews on *Toxopneustes*).

The second furrow is likewise meridional and at right angles to the first, the third equatorial or a little nearer to one or other pole; the animal cells get only a very little of the pigment. In the next phase the animal cells divide meridionally and equally to form the eight mesomeres, while the vegetative cells divide latitudinally and very unequally into four large pigmented macromeres next the equator, and four small quite unpigmented micromeres grouped round the vegetative pole.

In the blastula stage all the cells are of the same size, vacuolated and ciliated, and the polarity of the egg is only determinable by the position of the pigment zone. In the next stage the clear vegetative cells derived (presumably) from the micro-

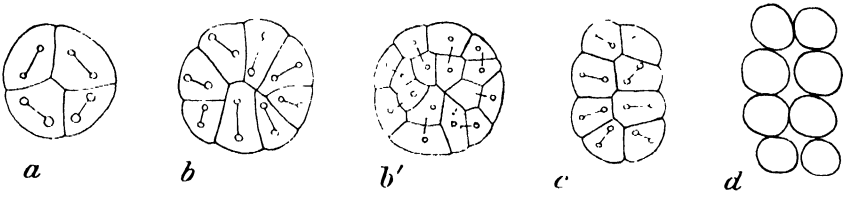


FIG. 93.—*Echinus*: segmentation under pressure.
a, preparation for third division (radial); *b*, preparation for fourth division (tangential); *b'*, after fourth division; *c*, another form of the 8-cell stage (third division parallel to first); *d*, the same after removal of the pressure. (After Driesch, 1893.)

meres wander in to form the primary mesenchyme—from which the first triradiate spicules are developed—and this is followed by the invagination of the pigmented cells to form the archenteron. Secondary pigmented mesenchyme cells are budded off from the inner end of the latter. The character and sequence of the divisions in segmentation are the same in other Echinid eggs, but the polarity is not marked by pigment, nor indeed recognizable till the micromeres have been formed, and again when the mesenchyme cells wander into the blastocoel.

By means of pressure—under a coverglass—Driesch succeeded (*Echinus*) in altering the direction of the spindles and consequently of the cell-divisions (Fig. 93). The first and second furrows were at

right angles to one another and to the coverglass, but whether meridional or not does not appear. The third furrows were again at right angles to the coverglass and at 45° to the first two; a flat plate of eight cells was thus formed. The next division resulted in the formation of sixteen equal cells still all lying in the same plane; the formation of micromeres is thus suppressed. Such eggs gave rise to normal larvae.

In the case just quoted the egg-membrane was intact; but similar results were obtained when it was broken. The second furrow was sometimes at right angles to, sometimes parallel to, the first, the third at right angles to both in the latter case, and the fourth

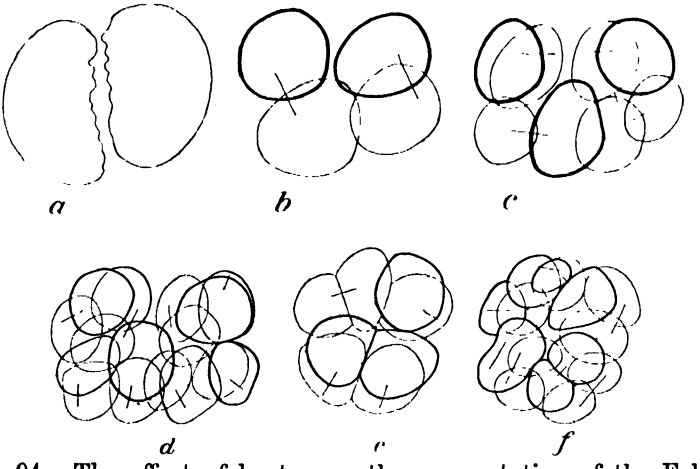


FIG. 94.—The effect of heat upon the segmentation of the Echinoid egg. *a*, *b*, *c*, *d*, four successive stages in the segmentation of the same egg of *Echinus*; *e*, *f*, two successive stages in the division of the same egg of *Sphaerechinus*. (After Driesch, 1893.)

parallel to the third; but if the pressure was released the blastomeres became rounded off and the sixteen-celled stage consisted of two plates of eight cells each; one or two micromeres were sometimes formed. The development of these eggs is also quite normal. These results have been confirmed by Ziegler.

In this, as in the similar experiment of Hertwig on the Frog's egg, the normal distribution and arrangement of the nuclei is interfered with without prejudice to the normality of subsequent development. Nuclear division cannot therefore, Driesch contends, be a qualitative process. Boveri has also shown in similar fashion that the order of segmentation may be disarranged and

the formation of the micromeres suppressed in *Strongylocentrotus* and a normal larva still result. The relation, however, of the egg-axis, as determined by the pigment-ring, to the symmetry of the larva remains the same as in the undisturbed egg.

Increased temperature, violent shaking, dilute sea-water, and calcium-free sea-water are all means by which Driesch has succeeded in disarranging the blastomeres of *Echinus* in various ways (Fig. 94). Thus when the temperature was raised from 19° C. (the

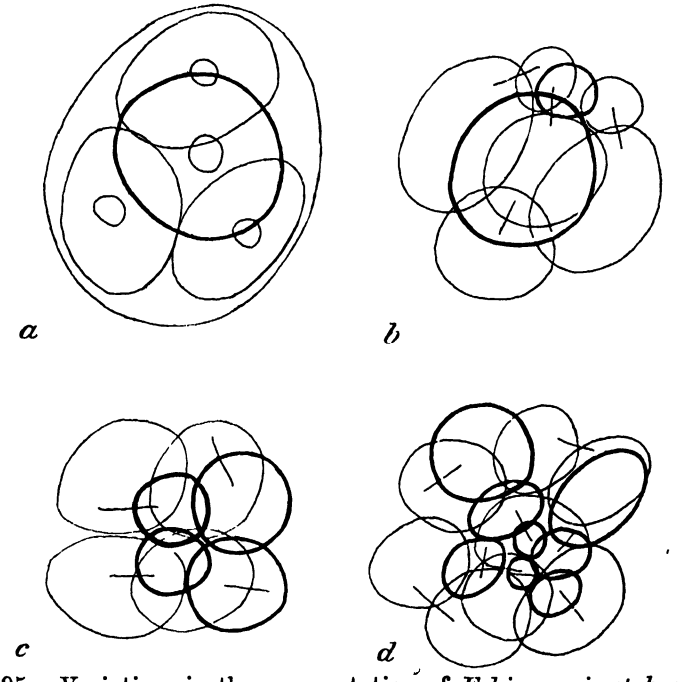


FIG. 95.—Variations in the segmentation of *Echinus microtuberculatus* produced by dilution of the sea-water. *a*, tetrahedral four-cell stage; *b*, eight cells, three premature micromeres; *c*, eight cells, two precocious micromeres; *d*, the same egg after the next division, the precocious micromeres have divided unequally, two normal micromeres have been formed. (After Driesch, 1895.)

normal) to 31° C. the first two (not subsequent) blastomeres separated, though they sometimes reunited. The next division was normal, but with the next phase one or two blastomeres divided in a direction perpendicular to that of the others, or the direction of division was different in each. In the following stage the micromeres were wholly or partly suppressed. So also in dilute sea-water (20% fresh water) the third division was unequal

in two or more blastomeres, and in the following an excessive number of small cells was formed (Fig. 95). In a less dilute mixture (16% fresh water) the blastulae divided to form each two Plutei. Again by shaking the eggs in the eight-celled stage the blastomeres were disarranged and made to lie almost in one plane; micromeres were, however, formed as usual from the vegetative cells, but not, of course, in the normal position (Fig. 96). In spite of these very considerable alterations in the size and positions of their constituent cells all these ova nevertheless gave rise to perfectly normal larvae.

By the use of calcium-free sea-water either the mesomeres or the macromeres and micromeres were separated into two groups. In the latter case the larva had two guts, in the former a normal larva resulted, so that, as Driesch points out, the ectoderm at the

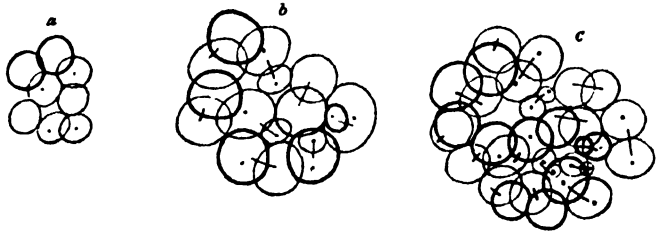


FIG. 96.—Disarrangement of the blastomeres of *Echinus* by shaking, *a*, eight cells; *b*, sixteen cells; notice that the micromeres are not close together. (After Driesch, 1896.)

antiblastoporic (animal) pole must in this case have been formed from the macromeres.

The conclusion to be drawn from these experiments seems obvious; not only the nuclei, but the parts of the cytoplasm too are equivalent; within at any rate fairly wide limits their normal arrangement may be disturbed without affecting in the least the normality of subsequent development; or, as Driesch phrased it, the destiny of a nucleus or blastomere is not determined by its original situation in the preformed structure of the egg, rather it is a function of its position in the whole embryo to which that egg gives rise. Its character is decided not by its origin, but by its final position.

Whether there really is a limit to the rearrangement is a question which must be reserved for future discussion. We are

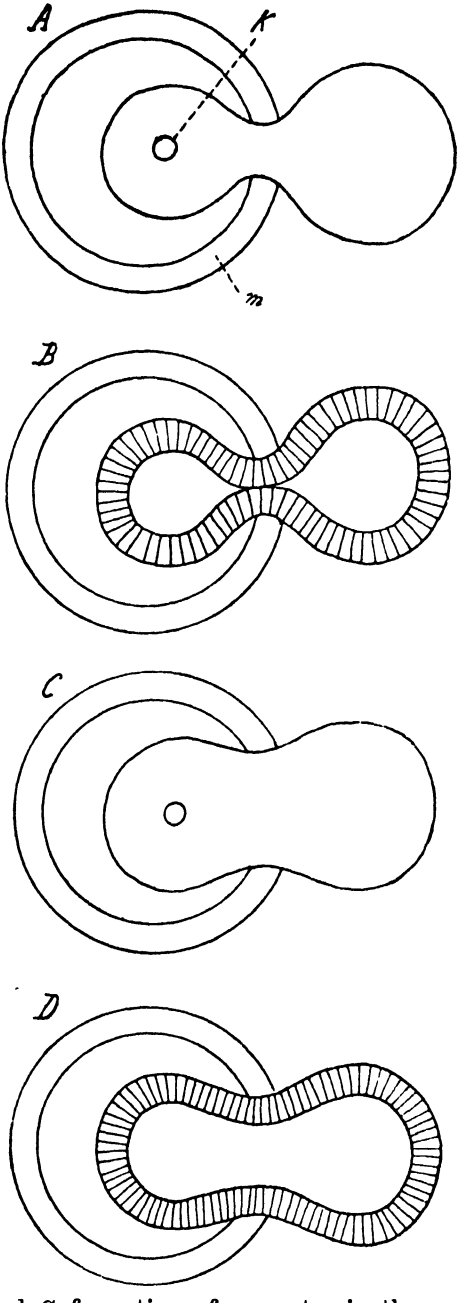


FIG. 97.—*A* and *C*, formation of ex-ovates in the egg of *Arbacia* by dilution of the sea-water; *K*, nucleus; *m*, egg-membrane. *B* and *D*, blastulae formed from *A* and *C*; *B* becomes constricted into two blastulae, each of which gives rise to a Pluteus; *D* produces a single Pluteus. (After Loeb, from Korschelt and Heider.)

also indebted to Driesch for a long series of experiments on the behaviour of isolated blastomeres and egg-fragments. In the forms which we have hitherto considered—the Vertebrates and Coelentera—an isolated cell segments as a whole and gives rise to a total larva. In these Echinoderms the isolated blastomere also gives rise to a total larva, but its segmentation is partial; only after segmentation has been completed does the open blastula close up and resume the polarity of a whole.

Various methods have been employed for separating the blastomeres. Loeb showed that in dilute sea-water (50 %) the egg

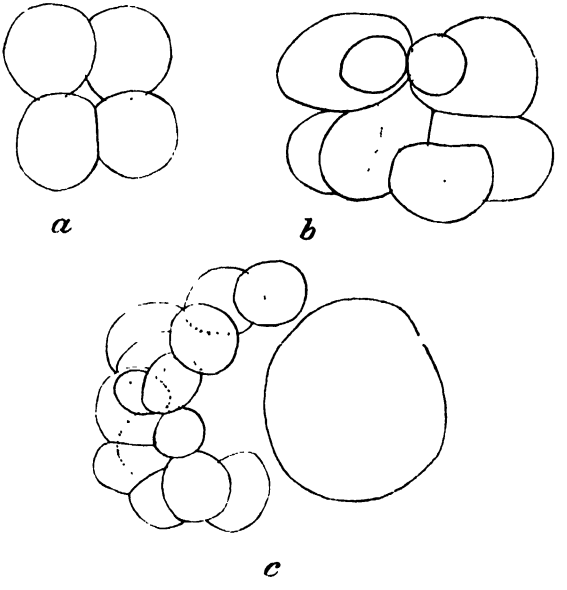


FIG. 98.—Development of the isolated $\frac{1}{2}$ blastomere of *Echinus microtuberculatus*. Two micromeres, two macromeres, four mesomeres: *a*, $\frac{4}{8}$; *b*, $\frac{8}{16}$; *c*, Hemiblastula: on the right is the remaining $\frac{1}{2}$ blastomere, dead. (After Driesch, 1892.)

swells, bursts its membrane and protrudes an ex-ovate which may be large and develop independently (Fig. 97). Driesch has used heat, pressure, violent shaking with fragments of cover-glasses, and the calcium-free sea-water introduced by Herbst.

The isolated $\frac{1}{2}$ blastomere in *Echinus* becomes rounded and segments as a half, as though the other blastomere were still present. It forms four mesomeres, two macromeres, and two micromeres,

and by division of these a hemispherical curved plate, a half-blastula (Fig. 98). When, as may happen, the other blastomere dies without being separated, it is embraced by the open side of the survivor. Only after the 32-celled stage is passed do the edges meet and close. A blastula of half-size is the result, which becomes later a gastrula and a Pluteus of perfectly normal form but of half the normal size.

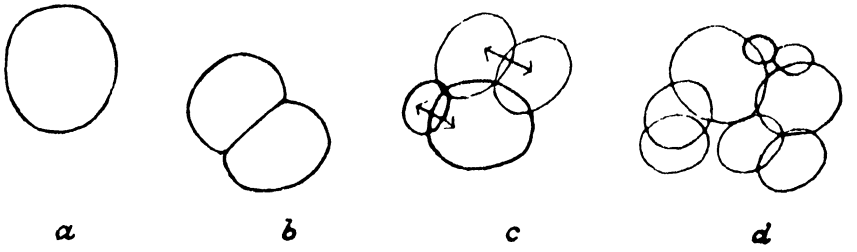


FIG. 99.—Segmentation of an isolated $\frac{1}{4}$ blastomere of *Echinus*; *a*, $\frac{1}{4}$, *b*, $\frac{2}{8}$, *c*, $\frac{4}{16}$, one micromere, one macromere, two mesomeres, *d*, $\frac{8}{32}$. (After Driesch, 1893.)

$\frac{3}{4}$ blastomeres behaved in the same way, segmenting as parts, developing ultimately into whole normal Plutei. Similarly $\frac{1}{4}$ blastomeres segmented partially (Fig. 99), with, in some cases, irregularities: two micromeres, for instance, were formed in the second division. The $\frac{1}{4}$ blastulae will gastrulate, but progress no further.

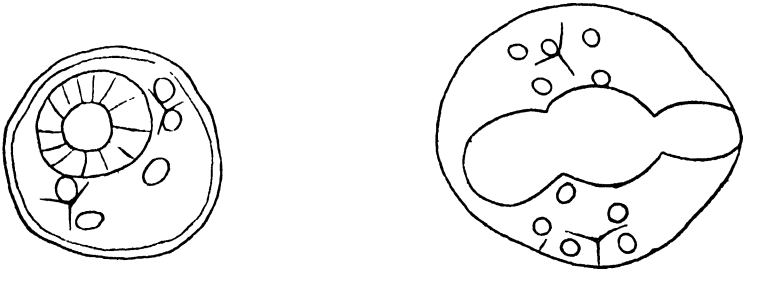


FIG. 100.—A, Gastrula with mesenchyme cells and triradiate spicules reared from a $\frac{1}{8}$ animal cell of *Sphaerechinus*. B, The same from a $\frac{1}{8}$ cell of *Echinus*: the gut is tripartite. (After Driesch, 1900.)

In the next stage—$\frac{1}{8}$ blastomeres—a difference becomes noticeable between the behaviour of the animal and vegetative cells. Both kinds of cells will gastrulate, secrete triradiate spicules and

develop a normally tripartite gut (Fig. 100). But this only occurs in a small proportion of cases, smaller for the animal than for the vegetative cells. Further, the former frequently give rise to blastulae provided with a row of long cilia (Fig. 101), while the latter are delicate, and many die.

Boveri has pointed out that the ability of animal cells to gastrulate depends, in *Strongylocentrotus*, on whether or not they contain a portion of the pigment-zone, and this on the position of the third furrow. Garbowski, however, denies that the pigment is itself an organ-forming substance. For in the first place there is a variety of *Strongylocentrotus lividus*, in which the pigment remains diffuse and never becomes concentrated to form a band at all; and secondly, even though, when present, it is usually in the subequatorial position described by Boveri, this is not always so; it may be oblique to the egg-axis, or wholly in the animal, or wholly in the vegetative hemisphere. It would appear, therefore, either that it is merely associated with some other substance which has up to the present remained invisible, or that in the cases described by Garbowski animal cells, if they were pigmented, would gastrulate as readily as ordinary vegetative blastomeres, in which case the manner in which the pre-determined material is cut up in segmentation would be a matter of indifference.

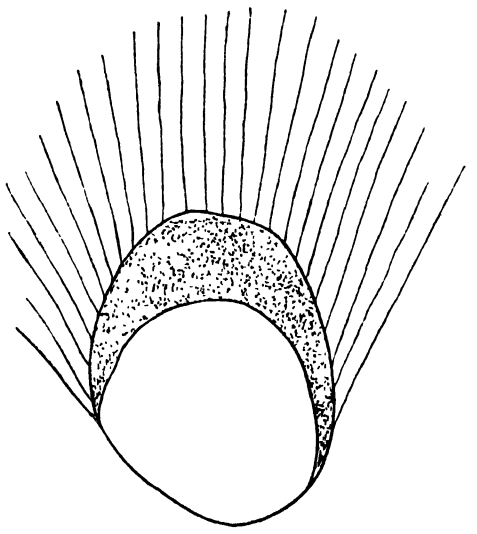


FIG. 101.—Long-ciliated blastula from a $\frac{1}{8}$ animal cell of *Echinus*. (After Driesch, 1900.)

The differences in the behaviour of the $\frac{1}{16}$ cells are still more marked. The micromeres will only divide to form a heap of about ten cells; the mesomeres give rise to either long-ciliated blastulae or imperfect gastrulae, with or without skeleton and

mesenchyme, and with an undivided archenteron (Fig. 102); the macromeres, on the other hand, become gastrulae provided with mesenchyme and spicules (Fig. 103).

Lastly, even $\frac{1}{32}$ blastomeres will occasionally gastrulate, probably only if derived from a macromere. This gastrulation was

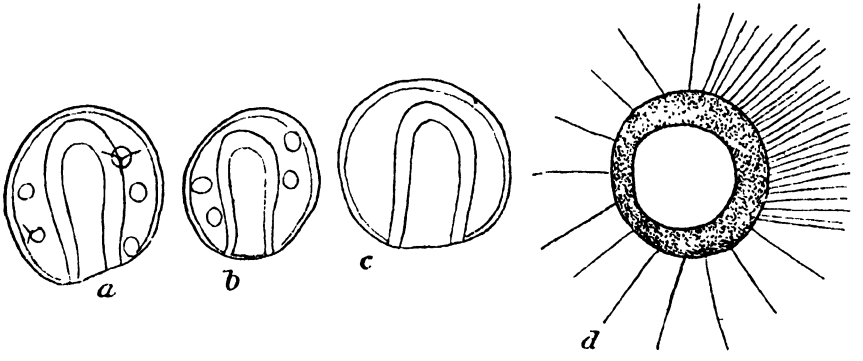


FIG. 102.—*Echinus*. Larvae reared from mesomeres (animal cells) of the 16-celled stage. *a* and *b*, mesenchyme gastrulae, *a* has spicules; *c*, gastrula without mesenchyme; *d*, a long-ciliated blastula. (After Driesch, 1900.)

not, however, observed directly, but the germinal value of the smallest gastrulae found was calculated by a method which will be described below. $\frac{1}{64}$ cells will not reach the gastrula stage.

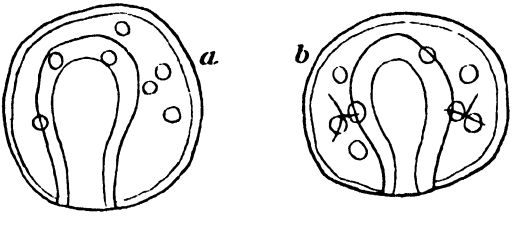


FIG. 103.—Two gastrulae of *Echinus* reared from $\frac{1}{16}$ macromeres; both have mesenchyme cells, one has triradiate spicules. (After Driesch, 1900.)

The differences between the developmental capacities of animal and vegetative cells may be studied in another way. The four micromeres were removed by Driesch in the 16-celled stage; the remaining twelve meso- and macromeres produced a normal Pluteus (an experiment also performed by Zoja on *Strongylocentrotus*). The eight (or four in the previous stage) animal cells alone, the eight vegetative cells alone also formed, in some cases, a perfect larva. Segmentation was in all these cases partial.

From the fact that the four animal cells together may form a Pluteus while each individually fails to pass beyond the gastrula stage, Driesch has argued that this failure depends solely on lack of sufficient material, not on the want of any specific gut-forming substance, and has supported his contention by other evidence. By placing the eggs of *Echinus* in dilute sea-water the third division was made unequal; the cells were then isolated in calcium-free water. The large cells thus formed must contain either more of Boveri's pigment-ring (or rather of the substance of the vegetative hemisphere) or else more of the animal hemisphere, probably the former, since a larger percentage gastrulate than is usual with animal blastomeres. According to Boveri, however, all ought to gastrulate if all contain the specific substance for the archenteron. Further, these large cells are able to form mesenchyme even when the gut is lacking although they possess the middle region of the egg in any case and may lack the micromere area.

Driesch also points out that a $\frac{1}{4}$ blastomere and a $\frac{1}{8}$ vegetative blastomere have both the same amount of Boveri's pigment-ring and mesenchyme area, the latter, however, only half as big a gut and half as many mesenchyme cells as the former, and lastly that $\frac{1}{8}$ and $\frac{1}{16}$ animal blastomeres will not only gastrulate but form mesenchyme as well.

The limitation of the potentialities of ectoderm and endoderm in later stages has been investigated also by Driesch—by observing the development of pieces or fragments of the blastulae and gastrulae. These pieces are obtained by cutting or shaking. Loeb has employed dilute sea-water to make the blastula swell, protrude through its membrane and become constricted into two.

In *Echinus*, *Sphaerechinus*, and *Asterias*, any piece of a blastula when first cut out is crumpled, but soon becomes rounded, and swims about and eventually gastrulates.

Monsters exhibiting a certain degree of duplicity have been produced by Driesch by shaking the egg of *Asterias* in the two-celled stage (this produces apparently a partial separation of the blastomeres) and by placing the blastulae of *Echinus* in diluted sea-water. In the latter case the gut is single, the skeleton double; in the former the gut is doubled, though the two may subsequently fuse, or even trebled, or may be

merely forked. The two guts may be similarly oriented or turned in opposite directions, and it is interesting to observe that Driesch, who believes the first furrow to coincide with the plane separating the two guts, accounts for the latter case by supposing that one blastomere had been rotated upon the other, so that their vegetative ends—the *locus* of the gut-forming substance—faced in opposite directions.

Neither the ectoderm of the early gastrula alone nor the endoderm is capable of giving rise to a larva, though the former can develope a stomodaeum, a statement confirmed by Morgan.

The vegetative half of a gastrula of *Sphaerechinus* will give rise to a normal small Pluteus whether the ectoderm only, or together with it the tip of the archenteron, has been removed (Fig. 104); the animal half will not. When the spicules, one or

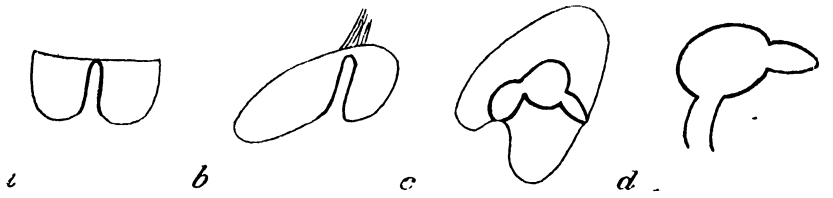


FIG. 104.—The potentialities of the cells of embryonic organs; *a*, *b*. The vegetative and animal portions of a gastrula of *Sphaerechinus granularis*, cut equatorially in two; *c*. Pluteus reared from a fragment of a gastrula; *d*. Normal tripartite pluteus gut. (After Driesch, 1896.)

both, are removed the skeleton is one-sided and the Pluteus consequently one-armed.

In *Asterias* the archenteron can form a new terminal vesicle and mesenchyme cells afresh when these have been removed (Fig. 105); but should the coelom sacs be cut off at a later stage, the (secondary) archenteron can form no more, though it divides into the usual three portions.

Parallel to the behaviour of isolated blastomeres is the segmentation and development of egg-fragments, obtained by shaking, cutting, or (Loeb) dilute sea-water. The fragments may be taken from already fertilized eggs, or from unfertilized: in the latter case each, whether nucleate or enucleate, must be subsequently fecundated.

In segmentation there are a great many differences, which appear to depend on the nature of the fragment, that is to say,

on the part of the egg from which it has been removed. In the case of the colourless egg of *Echinus* it is only possible to guess at the nature of the piece by observing the mode of segmentation.

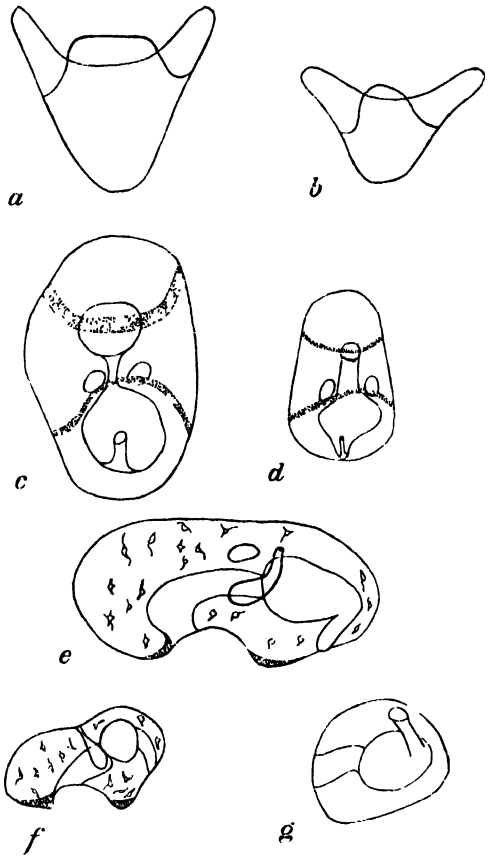


FIG. 105.—The potentialities of the cells of embryonic organs; *a*. Normal Pluteus of *Sphaerechinus*; *b*. Pluteus reared from a fragment of a gastrula; *c*, *e*. Normal Bipinnaria of *Asterias glacialis*; *d*, *f*. Bipinnaria from the vegetative half of a gastrula; *g*. Larva of *Asterias* with typical tripartite gut, but no coelom, from the vegetative half of a gastrula removed *after* development of the coelom sacs. (After Driesch, 1896.)

The first and second divisions may both be equal, as also the third and fourth; in this case (Driesch supposes) a large fragment is involved. Again, the first, second, and third may be normal, but the fourth and fifth equal: no micromeres, therefore, are formed, and the fragment is probably derived from the animal hemisphere. Or while the first and second are equal, one of the four cells will divide unequally; in the next division a large or small number of micromeres is formed, according, presumably, as a larger or smaller portion of the micromere region has been included in the fragment (Fig. 106).

In another case what appears to be a meridional half divides equally twice, and then two of the four equally (mesomeres), two unequally (macro- and micromeres) (Fig. 107), while a supposed vegetative half is segmented to form four large and four small cells.

Thus the type of segmentation is determined by the initial

structure, including the physical constitution, of the ovum alone, a conclusion in which Boveri concurs.

All these fragments will give rise to normal larvae, provided they are not too small. The least egg-fragment that will gastrulate has the same germinal value as the least blastomere, namely,

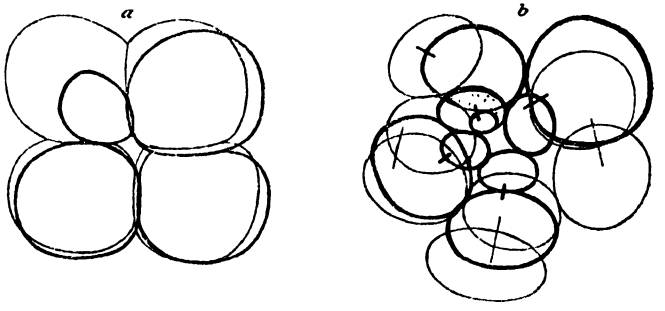


FIG. 106.—Segmentation of an egg-fragment of *Echinus* supposed to contain the whole of the micromere area of the egg, and some of the animal hemisphere. *a*. Eight cells, three equal pairs and one unequal pair; *b*. sixteen cells, four of the eight having formed micromeres, the other four divided equally. (After Driesch, 1896.)

$\frac{1}{32}$ (calculated from the volume of the smallest gastrulae found). Morgan, however, estimates the smallest egg-fragment capable of giving rise to a normal larva at from $\frac{1}{40}$ to $\frac{1}{60}$, the least blastomere that will gastrulate only $\frac{1}{8}$.

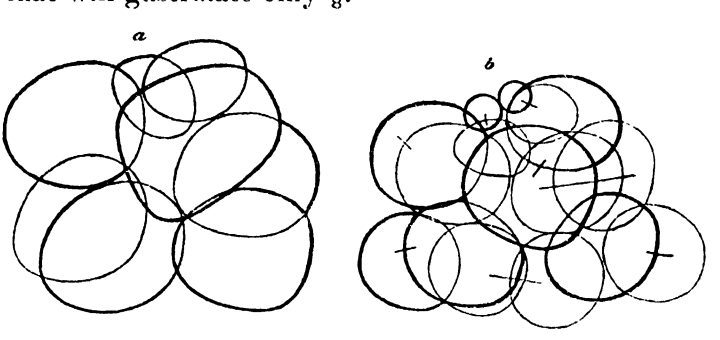


FIG. 107. - Segmentation of an egg-fragment (*Echinus*) supposed to be an exact meridional half. *a*. Eight cells, four being mesomeres, two macromeres and two micromeres; *b*. sixteen cells. (After Driesch, 1896.)

In partial blastulae, gastrulae, or larvae the number of cells is proportional to the germinal value; and this is true not merely of the whole embryo or larva, but of each of its organs, the

ectoderm, the mesenchyme, the archenteron. This assertion of Driesch's is corroborated by Morgan for the whole larvae, but not for the organs; according to him the number of cells invaginated to form the archenteron in a partial tends to approach that found in a whole larva, about fifty.

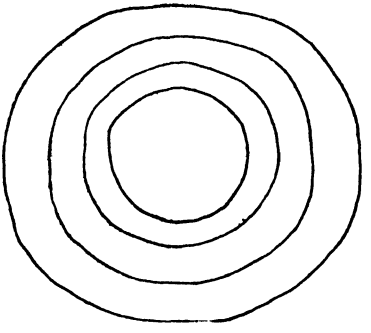


FIG. 108.—Outlines of $\frac{1}{1}$, $\frac{1}{2}$, $\frac{1}{4}$, and $\frac{1}{8}$ blastulae of *Sphaerechinus*. (After Driesch, 1900.)

Driesch has also determined the relation to their germinal value of the dimensions of partial blastulae, gastrulae, and Plutei and their organs (Figs. 108–112). As the table (Table XX) shows, the surfaces are very nearly as the germinal values, the ratios of the radii of the partial and total larvae consequently greater, of the volumes less than these values.

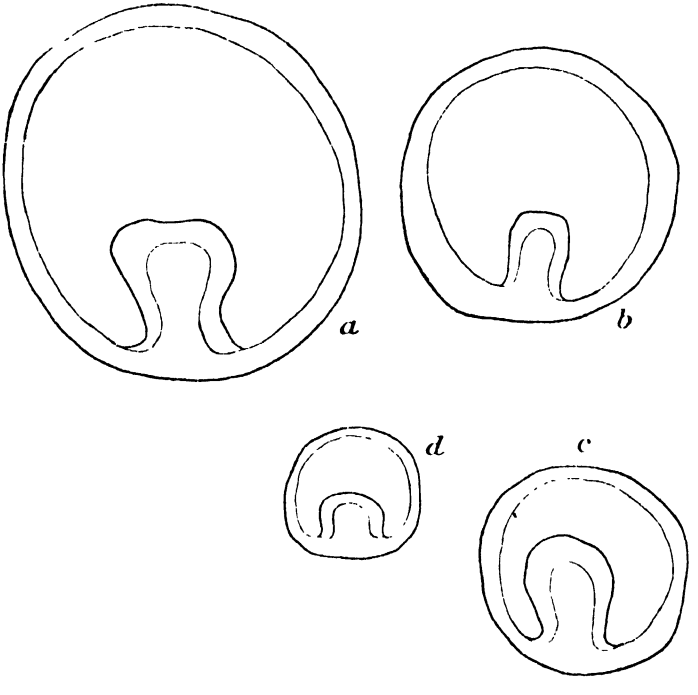


FIG. 109.—Early gastrulae of *Echinus*: *a*. $\frac{1}{1}$, *b*. $\frac{1}{2}$, *c*. $\frac{1}{4}$, *d*. $\frac{1}{8}$. (After Driesch, 1900.)

TABLE XX

Relation of dimensions of partial blastulae to their germinal value.

	Germinal values.			
	$\frac{1}{1}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$
Radii . . .	2	1·2	1	·75
Surfaces . .	4	2·25	1	·5625
Volumes . .	8	3·375	1	·4

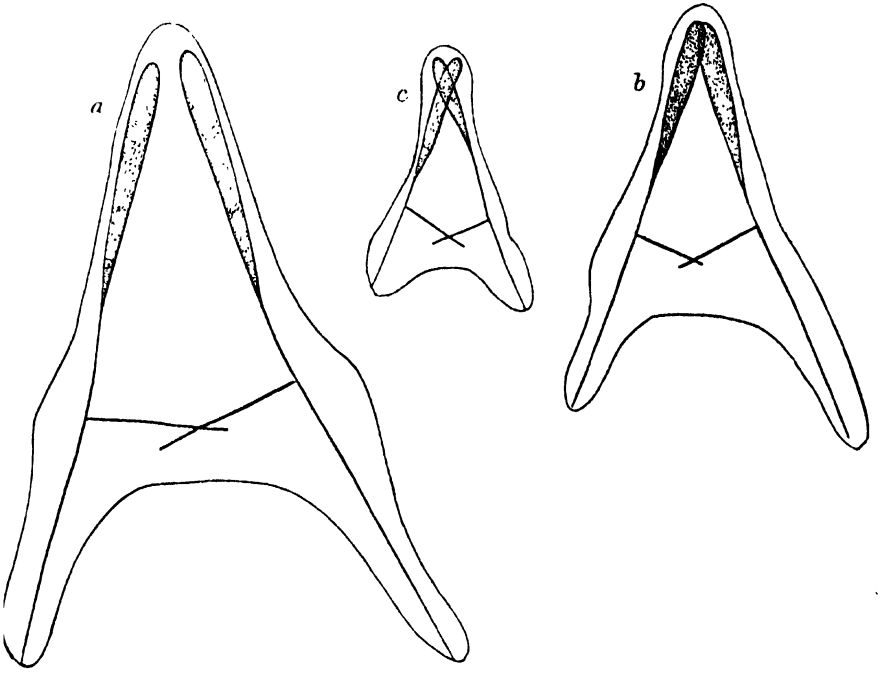


FIG. 110.—Plutei of *Echinus*: *a*. $\frac{1}{1}$, *b*. $\frac{1}{2}$, *c*. $\frac{1}{4}$. The skeleton is shown. (After Driesch, 1900.)

From the proportionality of the number of cells to the germinal value it follows that the cells of the whole and of the partial blastulae are of the same size.

If R, S, and V are the radius, surface, and volume of the whole egg, and r_n, s_n, v_n the corresponding magnitudes in a $\frac{1}{n}$ larva, then $s_n = \frac{S}{n}$, whence $r_n = \frac{R}{\sqrt{n}}$, and therefore $v_n = \frac{V}{\sqrt{n_3}}$. By this formula the germinal value of a larva of unknown origin may be calculated.

Finally, although segmentation takes place at the normal rate

in the isolated blastomeres, their subsequent development is progressively retarded as the germinal value decreases (Table XXI).

TABLE XXI

Showing the rate of development in partial larvae.

Day	Germinal value. $\frac{1}{1}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$
1	Gastrula	Early Gastrula	Still earlier Gastrula	Blastula
2	Pluteus	Early Pluteus	Gastrula with Skeleton	Blastula and Gastrula
3	Pluteus	Pluteus	Early Pluteus	Gastrula
4	Pluteus	Pluteus	Pluteus	Gastrula

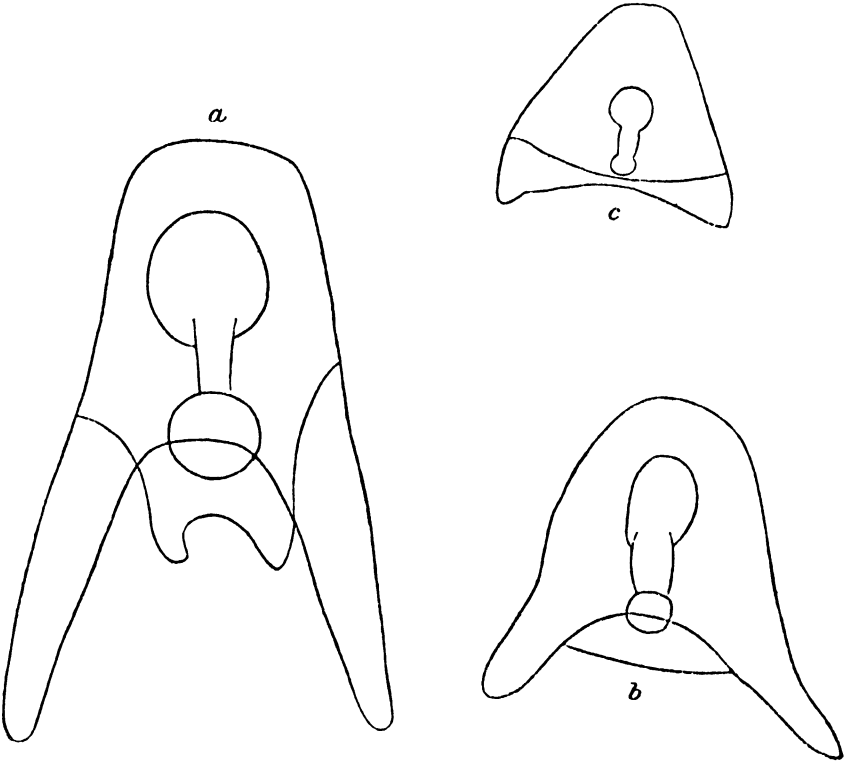


FIG. 111.—*Sphaerechinus* Plutei, showing the gut. *a* $\frac{1}{1}$, *b*. $\frac{1}{2}$, *c*. $\frac{1}{4}$. (After Driesch, 1900.)

It is evident from these experiments that, in the Echinoderms, while the isolated parts of the egg, whether blastomeres or egg-fragments, are only able to segment as they would have done had they remained in connexion with the whole, they are never-

theless capable of total development in a very marked degree. This capacity is not, however, unlimited, but diminishes as development proceeds, and it appears highly probable, and is indeed often admitted by Driesch, that the chief cause of this restriction is the lack of some specific substance and not the deficiency of mere material, though this may be a subsidiary factor. The progressive loss of potentialities is exhibited also by the embryonic organs as they are formed later on.

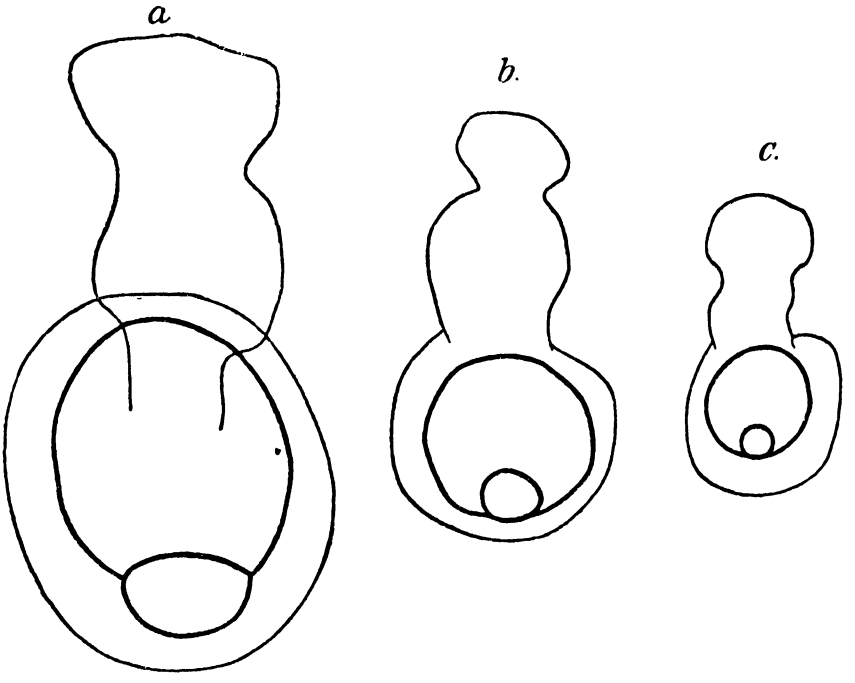


FIG. 112.—*Echinus*, the tripartite gut of the complete Pluteus: *a*. $\frac{1}{1}$, *b*. $\frac{1}{2}$, *c*. $\frac{1}{4}$. (After Driesch, 1900.)

Before concluding this section we must notice an experiment which is the converse of those considered above, namely, the production of one embryo from two eggs.

An unsuccessful attempt was made by Morgan to obtain this result. This author only succeeded in showing that the eggs of *Sphaerechinus*, when deprived of their membranes by being shaken, tend to fuse together, and that from such pairs double monsters may be produced with two guts and two skeletons. One skeleton may, however, very largely predominate over the other, which indeed remains rudimentary, and the guts may coalesce.

Driesch, however, who has employed the same method as Morgan and has corroborated his results, has been able to produce, from the fusion of two ova, a larva single in all its parts—mesenchyme, skeleton, and gut. The organs are in correct proportion but larger than the normal (Fig. 113). The number of mesenchyme cells is about twice the normal.

From this experiment it may of course be concluded that the egg substance possesses a degree of isotropy, but not that that isotropy is absolute. Nothing, it should be remarked, is known of the way in which the eggs which fuse to form one embryo are oriented upon one another. Garbowski, however, has stated

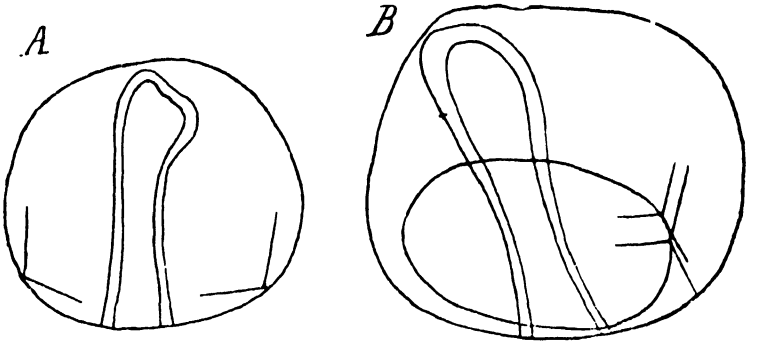


FIG. 113. *A*. Normal gastrula of *Sphaerechinus*. *B*. Single gastrula formed by the fusion of two blastulae. (After Driesch, from Korschelt and Heider.)

that fragments of different eggs of *Echinus* (in segmentation stages) may be grafted on one another, and that the product of their union will give rise to a normal embryo, whatever the relative positions of the fragments.

The fusion of blastulae to form giant Planulae has been noticed by Metschnikoff in the medusa, *Mitrocoma annae*, and in *Ophryotrocha* Korschelt has observed a similar fusion of distinct eggs in the body cavity of the parent when the latter is hermaphrodite. Lastly, Sala and Zur Strassen have observed the fusion in twos, threes, or more of the eggs of *Ascaris*, a result experimentally produced by the first author by exposure to a low temperature, while Zur Strassen has found that such double eggs, provided they had united before fertilization and then been fertilized by a single sperm, will produce perfectly normal embryos.

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§ 7. NEMERTINEA.

In the behaviour of its isolated blastomeres the Nemertine egg resembles the Echinoderm very closely; the cells segment as parts but ultimately give rise to wholes. The limit of this capacity is, however, sooner reached, for there is a sharper distinction between the potentialities of animal and vegetative blastomeres.

Experiments have been made on *Cerebratulus lacteus* by Wilson, on *Cerebratulus marginatus* by Zeleny; Zeleny and Yatsu have further investigated the development of fragments removed from various regions of the egg.

The axis of the egg is marked by the excentricity of the nucleus and the point of extrusion of the polar bodies. Maturation precedes fertilization. The first two furrows are meridional and divide the egg into four equal cells (*A*, *B*, *C*, and *D*). Subsequent cleavage is, however, on the spiral plan seen in Turbellarian, Annelidan, and holoblastic Molluscan eggs.

In the Nemertine the egg-axis becomes the axis of the Pilidium larva, the animal being the aboral, the vegetative the oral, pole.

In both the $\frac{1}{2}$ and the $\frac{1}{4}$ blastomeres cleavage is partial and an open blastula is formed, or sometimes a flat plate of cells merely, sometimes a closed sphere. The $\frac{1}{2}$ later becomes a larva with an apical organ, mesenchyme and archenteron, but no lappets; the $\frac{1}{4}$ larva has a solid archenteron but neither apical organ nor lappets. Development is slightly retarded; $\frac{2}{4}$ blastomeres give rise to a

larva with mesenchyme and apical organ, but the archenteron is solid. After the next phase—eight cells—the upper quartette

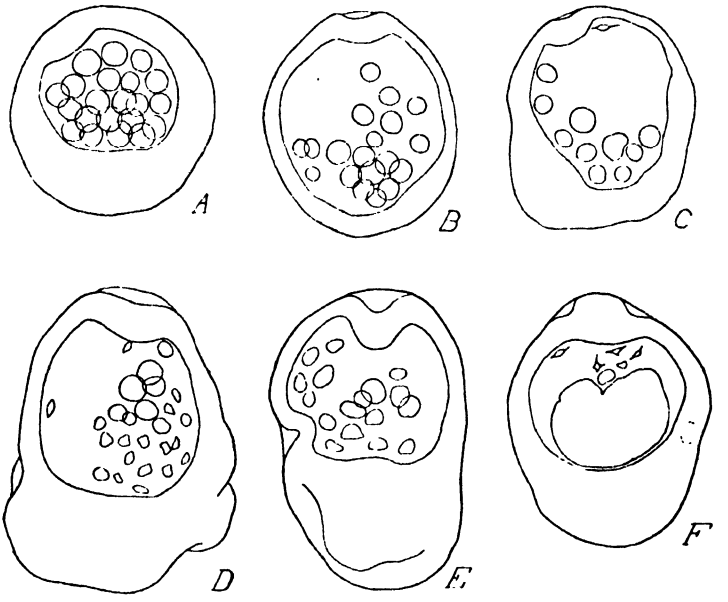


FIG. 114.—*Cerebratulus.* Larvae from upper quartettes of the 8-cell stage. *A.* Larva from whole quartette. *B, C.* The same, but one cell was injured. *D, E, F.* Slightly older, from complete quartette. (After Zeleny, 1904.)

(1 *a*–1 *d*) produces a larva with an apical organ and mesenchyme but devoid of an archenteron ; the larva developed from the lower quartette (*A*—*D*) has an archenteron but no apical organ, while a

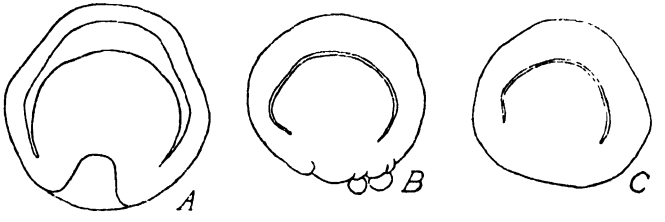


FIG. 115.—*Cerebratulus.* Larvae from lower (vegetative) quartettes of 8-cell stage. Note absence of apical organ, presence of large, hollow or solid, archenteron. (After Zeleny, 1904.)

meridional half, comprising two macromeres and two micromeres, develops both organs, though it is usually asymmetrical.

In the 16-celled stage the isolated apical cells (1 *a* 1–1 *d* 1) will give rise to a ciliated blastula with mesenchyme and an apical

organ, but without an archenteron, while from the remaining twelve is derived a ciliated embryo with a large solid archenteron, but destitute of an apical organ and lappets.

Wilson asserts that the size of cells in partial is the same as in total larvae; their number appears to be proportional to their germinal value.

From fragments of blastulae normal, or nearly normal, dwarf Pilidia may arise, but the degree of development again depends on the origin of the fragment. While the lower third becomes

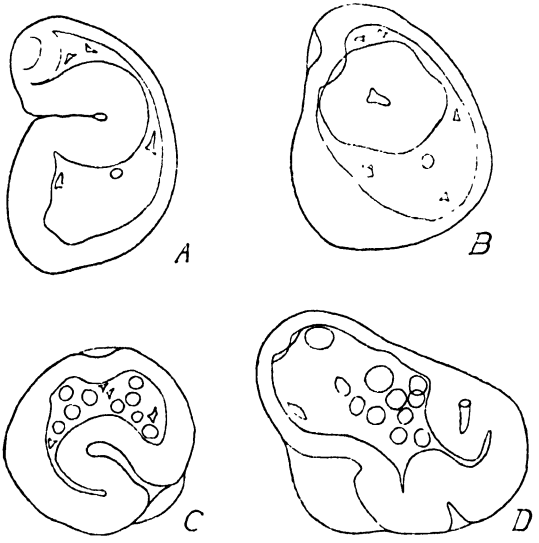


FIG. 116.—*Cerebratulus.* Larvae from portions of the 8-cell stage. *A*, *B*. Larva from lateral 4-cell group. Note presence of both apical organ and archenteron. *C*. Similar, but younger. *D*. Larva from upper quartette plus two cells of lower quartette. Note that there are three apical organs, a large blastocoel, a small archenteron, and two invaginations of ectoderm at the sides of the archenteron. (After Zeleny, 1904.)

a ciliated embryo without an apical organ, a piece of the upper two-thirds developed (in one case) into a larva with a gut, with two apical organs, but no lappets (Zeleny). Wilson states that in *Cerebratulus lacteus* the archenteron is small in animal fragments, the apical organ frequently, though not apparently always, absent in pieces of the vegetative hemisphere.

Animal fragments of the blastula have a greater total potentiality, a greater regulative capacity, than the animal blastomeres

of the eight-celled stage. This is attributed by Zeleny to the greater time and opportunity afforded them of regaining the polarity of the whole.

The behaviour of egg-fragments depends not merely on the part of the egg from which they are taken but also on the time at which they are removed.

Pieces taken from the ovum, no matter in what way, before the disappearance of the germinal vesicle segment as wholes and give rise to perfect Pilidia. Only in a small percentage (15 %) of cases is their development defective, the lappets being absent, or the gut abnormal, or the apical organ absent or multiplied (Yatsu). The proportion of perfect Pilidia obtained from fragments removed a little later, at the time of formation of the first polar spindle, is, however, less, only 52 %.

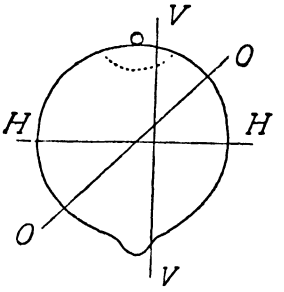


FIG. 117. — *Cerebratulus.* Diagram of egg with one polar body, and basal protuberance, to show directions of horizontal (*H*), vertical (*V*), and oblique (*O*) cuts. (After Zeleny, 1904.)

It is during the conjugation of the pronuclei that the definite localization of specific substances seems to begin, for now the fate of the fragment depends upon the direction in which the cut is made. Eggs from which the animal portion—about one-third of the whole —has been taken away become perfect Pilidia; when, however, by removal of the vegetative third, or by an oblique cut, the fragment does not contain the whole of the lower two-thirds, the lappets are defective and the apical organ sometimes absent as well. If, therefore, there is present at this stage a special material for this organ it cannot as yet be located in its ultimate position.

The cleavage of these fragments is always partial (Zeleny). After the first furrow the material for the apical organ has still not reached the animal pole: the animal region may be removed from both blastomeres without inhibiting its formation. Perfect Pilidia may also be produced from eggs in the two-celled stage when part of one blastomere has been obliquely cut away. Should, however, the blastomeres be cut apart before their complete

separation, each develops into a Pilidium with one lappet but no apical organ; it seems, therefore, that the material for this structure is at this stage placed in the bridge of plasma connecting the two cells, and is fatally injured by the operation.

Taken together these experiments make it, to say the least, highly probable that there are in the Nemertine egg definite substances connected necessarily, that is causally, with the formation of certain organs.

These specific organ-forming substances may be said to be preformed, but it is equally clear that they are not prelocalized, but only reach their ultimate destination in the course of development.

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§ 8. Ctenophora.

With the Ctenophora we come to a group of animals in which the development of a total larva from a single blastomere is no longer possible, though the missing parts may eventually be regenerated.

The egg consists of a central yolk-mass surrounded by a superficial layer of granular protoplasm. After fertilization this protoplasm passes wholly to one side—the animal hemisphere—and the egg divides meridionally into equal parts. The protoplasm then spreads itself over the outer surface of each cell, but returns to the animal side prior to the second cleavage, which is again meridional and at right angles to the first. Once more the protoplasm is distributed over the whole outer surface of each blastomere, only to be again concentrated before the third division, an unequal one, but nearly meridional. There are now four large cells lying together in a square and four smaller cells lying above them in two groups of two each, one at each end of the plane of

the second furrow. This becomes the transverse, while the first furrow marks the sagittal plane of the future organism. Each of these cells contains both granular protoplasm and yolk, but at the succeeding division nearly all of the former material passes into the eight small cells, which are nipped off and lie in an oval ring on the vegetative side of the egg[1] (Fig. 118). These small cells or micromeres will give rise to the ectoderm, the large cells or macromeres to the endoderm and mesoderm, the mesoderm

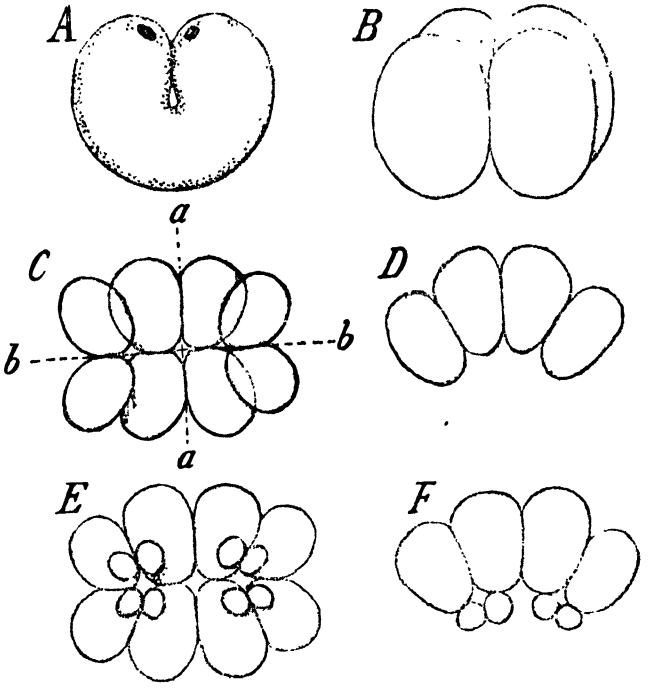


FIG. 118.—Normal segmentation of the Ctenophore egg. *a a*, Stomach or sagittal plane; *b b*, funnel or transverse plane. *C*, *E*, from micromere pole; *D*, *F*, from the side. (After Ziegler, from Korschelt and Heider.)

being separated off later on at the vegetative pole, which becomes the oral pole of the embryo. In normal development, therefore, the embryonic axes are marked out at quite an early stage.

Chun was the first to isolate the $\frac{1}{2}$ and $\frac{2}{4}$ blastomeres of

[1] Ziegler's description is that in this (the fourth) division the furrow first appears near the animal pole, but yolk then streams from the larger vegetative into the smaller animal portion, until the latter becomes the macromere, the former the micromere. Division is then completed and the micromeres are at the vegetative pole. This explains the divergences in the accounts of earlier observers (Agassiz, Kowalewsky, Chun, Fol, and Metschnikoff).

Eucharis by shaking. These gave rise to half larvae, provided with four costae, four meridional canals communicating with two subsagittal and two subtransverse canals in the ordinary way, one subgastric canal, one tentacle, but a whole funnel and a whole stomach, the latter formed by an oblique invagination; the side turned towards the missing blastomere was covered over by ectoderm.

Later on the missing half was regenerated, first the subgastric vessel, then the meridional canals, over these the costae, and finally a tentacle.

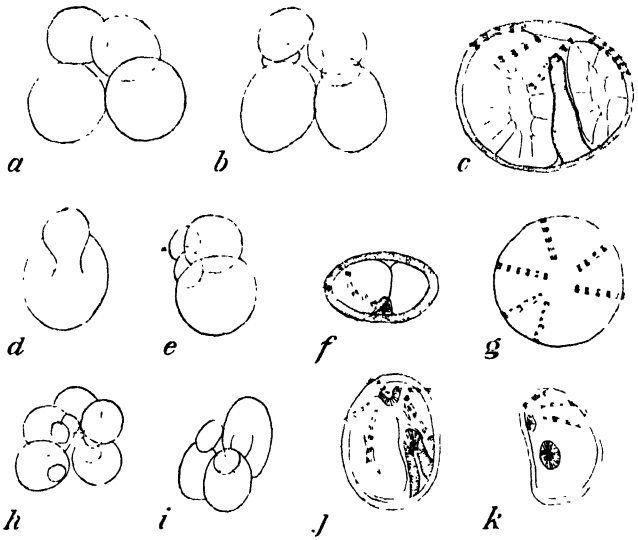


FIG. 119.—Development of isolated blastomeres in Ctenophora. *a*, *b*. Segmentation of $\frac{1}{2}$ blastomere of *Beröe ovata*: *a*, two large, two small cells; *b*, each gives off a micromere; *c* Larva from a $\frac{1}{2}$ blastomere with four costae; *d*, *e*. Segmentation of $\frac{1}{4}$ blastomere; *f*. The resulting larva with two costae; *g*. Larva with six costae from $\frac{3}{4}$ blastomeres. (After Driesch and Morgan, 1896.) *h*. Isolated $\frac{8}{16}$ blastomeres of *Beröe ovata*; *j*. The resulting larva with four costae, one sense-organ, and one stomodaeum; *i*. Isolated $\frac{6}{16}$ blastomeres; *k*. The resulting larva with three costae, one sense-organ, and one stomodaeum. (After Fischel, 1898.)

Such half larvae are found in the tow-net after a storm and may become (*Bolina*) sexually mature.

These experiments have been repeated and confirmed on *Beröe* by Driesch and Morgan. The authors add that the segmentation of these isolated blastomeres is partial. The $\frac{1}{4}$ larva has two costae only and a large and a small canal, the former passing to the costae, the latter to the opposite side and representing apparently

the other half of the funnel (Fig. 119 *a–g*). Fischel has carried the analysis of the potentialities of the blastomeres a step further (Fig. 119 *h–k*). Though cleavage is partial, as described by Driesch and Morgan, there are slight irregularities in the position of the blastomeres. A $\frac{1}{8}$ blastomere produces one costa only, $\frac{8}{16}$ blastomeres (four macro- and four micromeres) four costae, $\frac{10}{16}$ (five macro- and five micromeres) five, $\frac{6}{16}$, $\frac{12}{16}$, and $\frac{4}{16}$, when separated in the same way, respectively three, six, and two costae. Similar results were obtained by meridional division of the egg in later stages. Fischel confirms the statements of the other

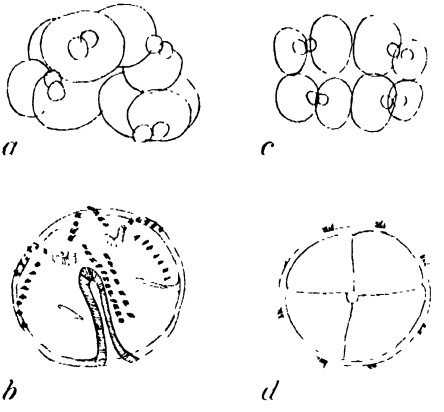


FIG. 120.—*a*. *Beröe orata*: egg in the 16-cell stage. The micromeres disarranged by pressure; *b*. The resulting larva with two sense-organs, and four costae radiating from each: one stomodaeum. (After Fischel, 1898.) *c*. Normal segmentation of the egg of *Beröe* from which a small portion of the vegetative hemisphere has been removed; *d*. Larva produced from the same, with eight costae, four endodermal canals, and one (central) stomodaeum. (After Driesch and Morgan, 1896.)

investigators as to the structure of the half larvae. In $\frac{3}{4}$ larvae, however, not three, but four, canals are formed, and the stomodaeum is vertical. The sense-organ is not found in embryos developed from small fragments. Removal of the macromeres left the number of costae unaffected, but displacement of the micromeres by pressure led to the formation of an abnormal larva with two sense-organs and eight costae arranged in two groups of four each round each sense-organ (Fig. 120 *a*, *b*), or, if the micromeres were separated into two lots, a group of four round one organ, a group of three round the other, and some scattered combs. With greater pressure three sense-organs were produced and the

costae were very irregular. In these eggs there was, however, only one stomodaeum, and only the normal number of canals.

It appears, therefore, that the individual costae are definitely related to the meridional divisions of the segmented egg, and further that the material which is concerned in their formation becomes located in the micromeres of the 16-celled stage.

Driesch and Morgan have also shown that if the vegetative hemisphere be taken away from the undivided egg, the animal portion, though it segments normally, is able to produce neither costae nor stomodaeum; it has, however, endodermal canals. Loss of a small portion of the vegetative hemisphere, however, does not interfere with subsequent normal development (Fig. 120 *c*, *d*).

On the other hand an egg which has been deprived of a lateral portion divides, by three unequal divisions, into eight cells, all of which form micromeres. Nevertheless, the larva has only four, five, or six costae, and some of these rudimentary, only three or four canals, and the stomodaeum, as in half larvae, is oblique.

As Driesch points out, the whole of the nucleus is here, and the embryo is still defective. Differentiation thus depends on the cytoplasm, but is quite independent of segmentation, which may be perfectly normal without determining the formation of a normal embryo. Driesch further argues that the observed defects are due to lack of sufficient material merely, not to lack of any preformed specific substance. Whether such substances are already present in the unsegmented as they undoubtedly appear to be in the segmented egg, it is perhaps impossible, on the evidence, to decide, but it may be observed that the development of perfect larvae from those eggs from which a portion of the vegetative hemisphere has been detached does not necessarily support the view advanced by Driesch.

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§ 9. Chaetopoda and Mollusca.

In the Chaetopoda and Mollusca (except the Cephalopods) it is possible to trace back almost every organ of the body to some particular cell, or group of cells, of the segmenting ovum, to describe, in fact, its lineage in terms of individual cells. Specific materials for the formation of the various parts seem to be sundered from one another by the process of cleavage, which thus presents the characters of a 'Mosaic' work in an exceptional degree. To what extent such a preformed structure does in reality exist experiment alone can decide, and the answer given by experiment, so far, at least, as the nucleus is concerned, is in the negative.

By means of pressure Wilson succeeded in preventing in *Nereis* the normal formation of the first quartette of micromeres; instead, the egg divided into a flat plate of eight equal cells. The pressure was then removed. All eight cells formed micromeres, and later on eight instead of the usual four macromeres were found in the Trochophore larva. Four of these necessarily contained nuclei which would normally be placed in cells of the prototroch, and yet the larva was normal. In this case, then, the causes of differentiation cannot lie in the nucleus; they must be sought for, if anywhere, in the cytoplasm.

In the eggs of these forms segmentation is holoblastic and of the spiral type, with the cleavages alternately dexiotropic and laeotropic, and it is easy not only to determine the lineage of each organ, but to compare the origin and the destiny of individual cells in a large series of forms. The first, second, and third quartettes of micromeres are usually destined for ectoderm formation, the second quartette cell in the D quadrant, 2 *d*, being the first somatoblast from which the ectodermal structures of the trunk are derived. The ciliated ring or prototroch is formed, in most cases, from certain derivatives of the first quartette, namely 1 *a* 2—1 *d* 2, the primary trochoblasts, each of which divides into a group of four; but it may be reinforced by secondary trochoblasts from the same or the second quartette. The second and third quartettes may provide larval mesoderm or mesenchyme. The trunk mesoderm is usually derived from 4 *d*. The remaining

cells of this quartette (4 *a*—4 *c*) become with the residual macromeres (A—D) the endoderm (or mesendoderm).

A peculiar structure seen in some forms (*Ilyanassa*, *Dentalium*,

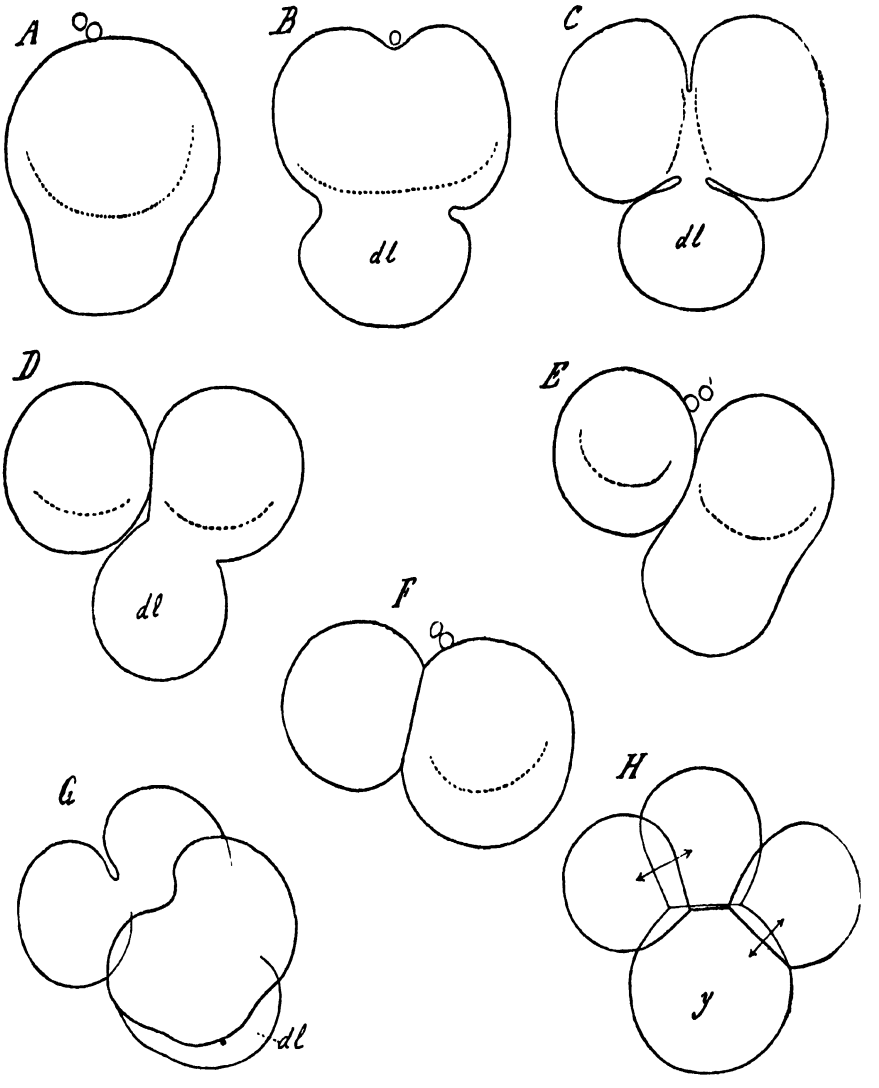


FIG. 121.--Normal cleavage of *Ilyanassa*. *A*, *B*. Formation of polar-lobe or yolk-lobe (*dl*); *C*. 'Trefoil' stage; *D*, *E*, *F*. The polar-lobe passes into CD; *G*. The second polar-lobe protruded in H; it passes into D. (After Crampton, from Korschelt and Heider.)

Chaetopterus, *Myzostoma*) is the yolk-lobe, or polar lobe, as it should properly be called. This is a mass of cytoplasm protruded at the

vegetative pole after fertilization. It becomes attached entirely to one of the first two blastomeres, namely to CD, into which it is withdrawn; prior to the next division it is again protruded, attached to D only, and then once more withdrawn: at the time of the next division it is protruded again, for the last time (Figs. 121, 122).

Crampton has isolated the blastomeres of *Ilyanassa* and watched their development (Figs. 123–6). $\frac{1}{2}$, $\frac{2}{4}$, $\frac{3}{4}$ (A, B, and C), $\frac{1}{4}$, $\frac{2}{8}$

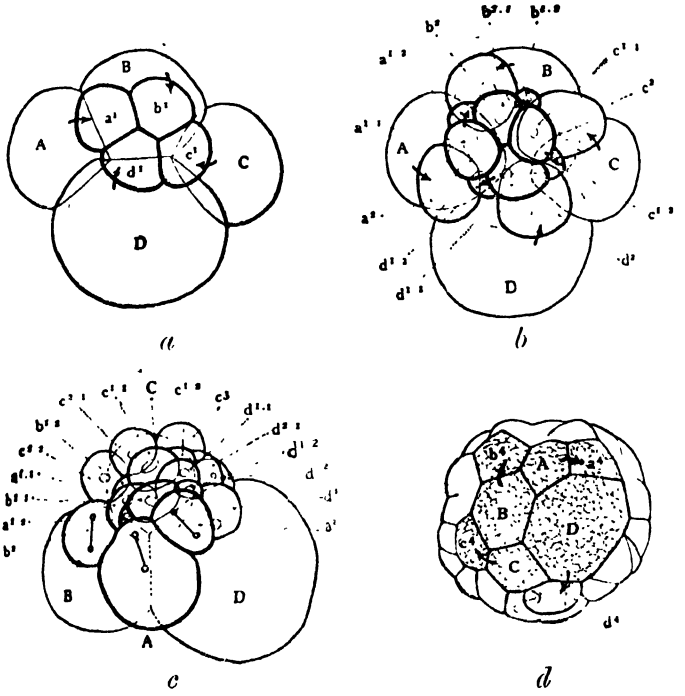


FIG. 122.—Normal cleavage of *Ilyanassa*. (After Crampton, 1896.) *a*, 8-celled stage from above; *b*, 16-celled stage from above; *c*, 24-celled stage, $\frac{3}{4}$ from above; *d*, optical section, slightly oblique, of a later embryo, from below. Division of mesoblast pole-cell (d^4).

(a macromere and a micromere), $\frac{3}{8}$, $\frac{1}{8}$ (a micromere), $\frac{5}{16}$, $\frac{7}{16}$ and $\frac{9}{16}$ all continue to segment as though the missing blastomeres were present, except for certain small irregularities in the positions of the cells; the A, B, and C blastomeres, separately or together, bud off the usual four generations of micromeres, but D only the first three; the division of the micromeres continues in nearly normal fashion. The $\frac{1}{8}$ micromere divides twice only. All die, however,

without giving rise to larvae. A $\frac{1}{8}$ macromere, on the other hand, or a $\frac{1}{16}$ or later blastomere, will not segment at all.

By lowering the temperature the cleavage may be made to resemble that of whole ova.

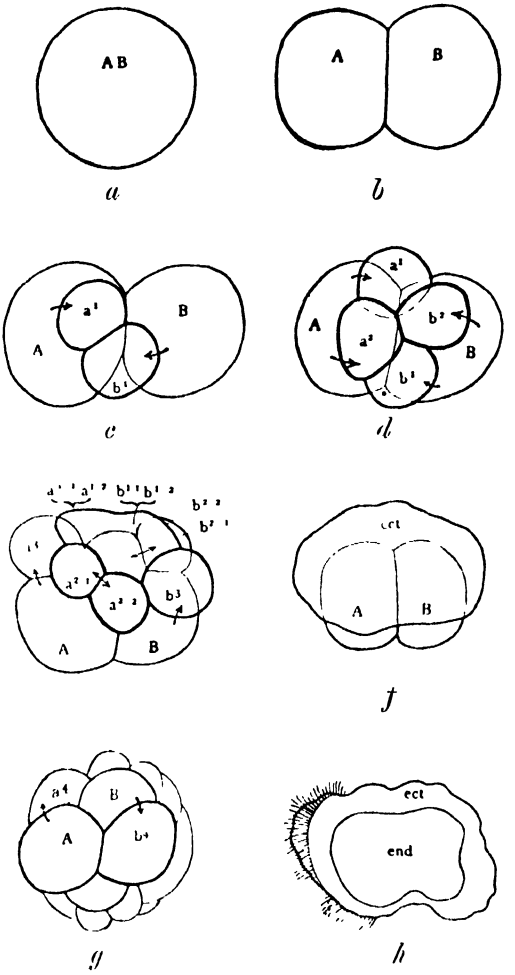


FIG. 123.—*Ilyanassa*: cleavage of smaller $\frac{1}{2}$ blastomere (AB). *a*, Undivided; *b*, $\frac{3}{4}$ embryo, from side; *c*, $\frac{4}{8}$ embryo, from above; *d*, $\frac{6}{12}$ embryo, from above: the dot indicates the former centre of the egg; *e*, $\frac{12}{24}$ embryo, from side; *f*, growth of ectoderm over the macromeres; *g*, formation of the micromeres of the fourth generation, from below; *h*, ciliated partial embryo of 46 hours. (After Crampton.)

The yolk-lobe influences the character of segmentation, for if it be removed CD divides like AB. More important than this is

the relation of the yolk-lobe to the mesoderm. When the former is cut off the latter is not formed at all. There are four generations of micromeres and four equal macromeres, and the larvae, though ciliated and able to swim, never reach the veliger stage (Fig. 127).

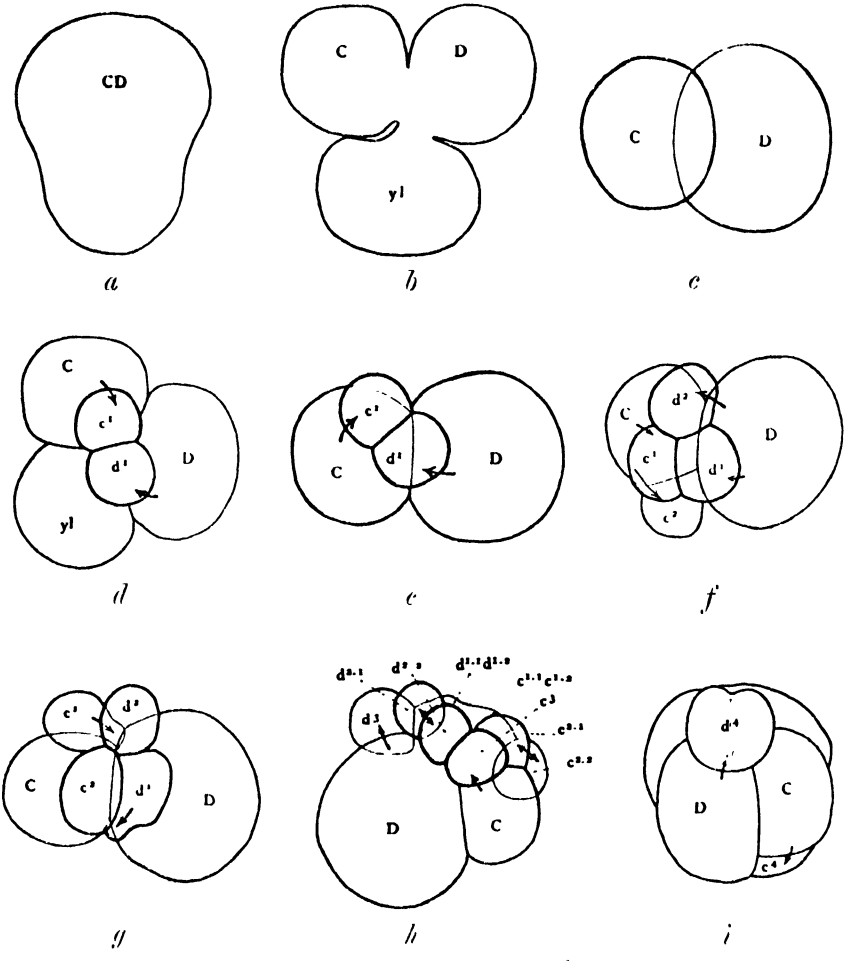


FIG. 124.—*Ilyanassa*: cleavage of CD. *a*, Formation of yolk-lobe (yl); *b*, 'trefoil'; *c*, $\frac{3}{4}$ embryo from above; *d*, early $\frac{1}{8}$ embryo, from above: secondary yolk-lobe (yl); *e*, resting $\frac{1}{8}$ embryo from above; *f*, $\frac{6}{12}$ embryo from above; *g*, passage to $\frac{8}{16}$ stage, $\frac{3}{4}$ from above; *h*, $\frac{12}{24}$ embryo, from side; *i*, division of mesoblast pole-cell (d^4), from below. (After Crampton.)

The potentialities of the blastomeres are thus strictly limited both in segmentation and differentiation. This result is amply confirmed by Wilson's researches on *Dentalium* and *Patella*.

In the latter genus the development of the isolated blastomeres, especially of those resulting from later divisions, has been minutely followed. Each separate first quartette cell (1 *a*—1 *d*) forms (Fig. 128) a closed ectoblastic structure with an apical organ at

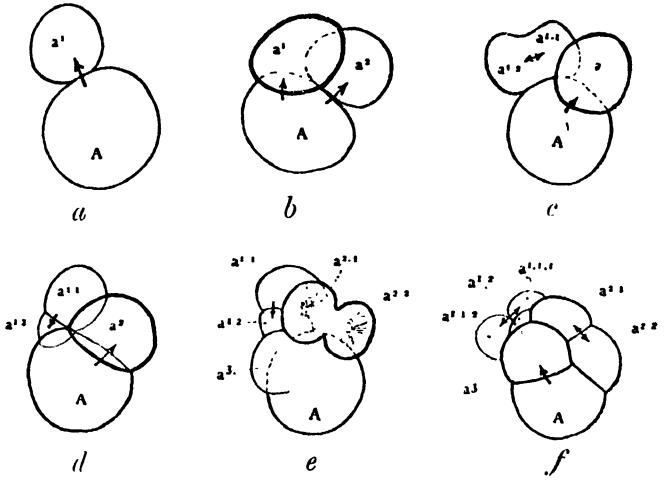


FIG. 125.—*Ilyanassa*: cleavage of $\frac{1}{4}$ blastomere (A). *a*, $\frac{2}{8}$, from side; *b*, $\frac{3}{12}$, from side; *c*, passage to $\frac{4}{16}$, first form, from side; *d*, resting $\frac{4}{16}$, second form, from side; *e*, passage to $\frac{6}{24}$, from side; *f*, $\frac{7}{28}$, from side. (After Crampton.)

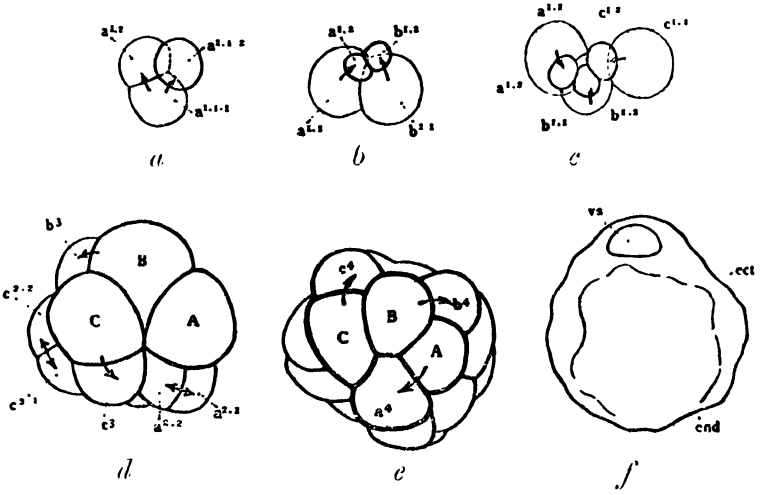


FIG. 126.—*Ilyanassa*: *a*–*c*, cleavage of $\frac{1}{8}$ blastomeres; *a*, $\frac{3}{28}$ stage, from one micromere; *b*, $\frac{4}{16}$ stage, from two micromeres; *c*, $\frac{6}{16}$ stage, from three micromeres. *d*–*f*, cleavage of $\frac{3}{4}$ blastomeres; *d*, $\frac{8}{24}$ embryo, from below: *e*, origin of fourth quartette, from below; *f*, embryo of 48 hours. (After Crampton)

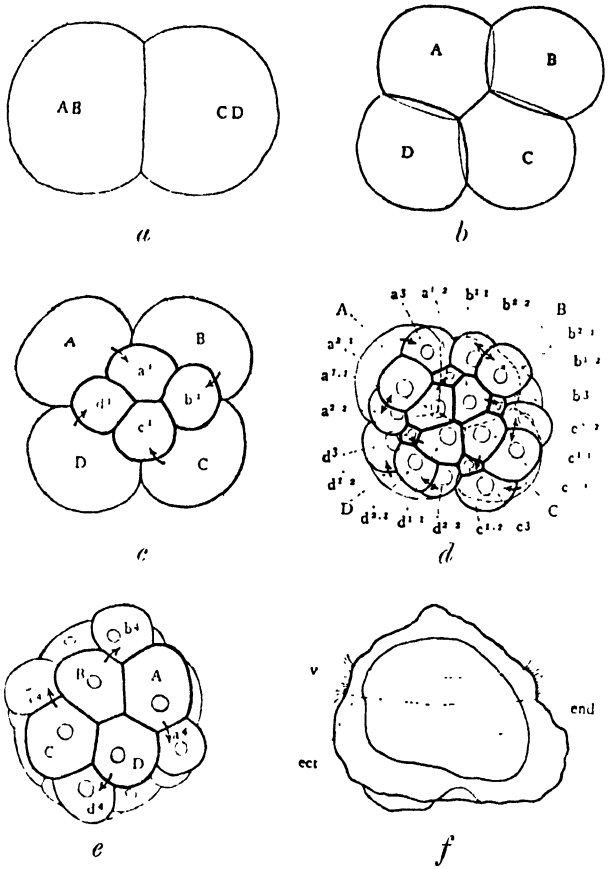


FIG. 127.—*Ilyanassa*: cleavage of the egg after removal of the yolk-lobe. *a*, $\frac{2}{2}$; *b*, $\frac{4}{4}$; *c*, first quartette formed; *d*, second quartette formed, first quartette divided; *a-d*, from above; *e*, from below: fourth quartette formed; *f*, ciliated embryo, from side. (After Crampton.)

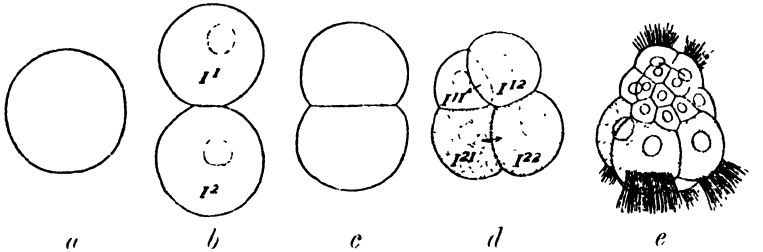


FIG. 128.—*Patella*: development of isolated $\frac{1}{8}$ micromeres. *a*, $\frac{1}{8}$ micromere; *b*, *c*, first division; *d*, $\frac{8}{8}$ stage, with two trochoblasts (stippled), one rosette-cell, and one primary cross-cell; *e*, larva of 24 hours, from the side, showing trochoblasts below, apical cells above. (After Wilson.)

one end, and four cells (derived from a primary trochoblast) provided with long cilia at the other. When the primary trochoblasts (1 *a* 2—1 *d* 2) are isolated each divides into four ciliated cells, the cilia arranged as normally in a single row, but no further (Fig. 129 *a–e*). If the daughter-cells of these trochoblasts (1 *a* 2. 1—1 *d* 2. 1 and 1 *a* 2. 2—1 *d* 2. 2) are separated they divide once only (Fig. 129 *f*), while their daughter-cells do not divide at all (Fig. 129 *g*, *h*). The sister-cells to these (1 *a* 1—1 *d* 1) behave in the same way, forming a closed larva with an apical organ at

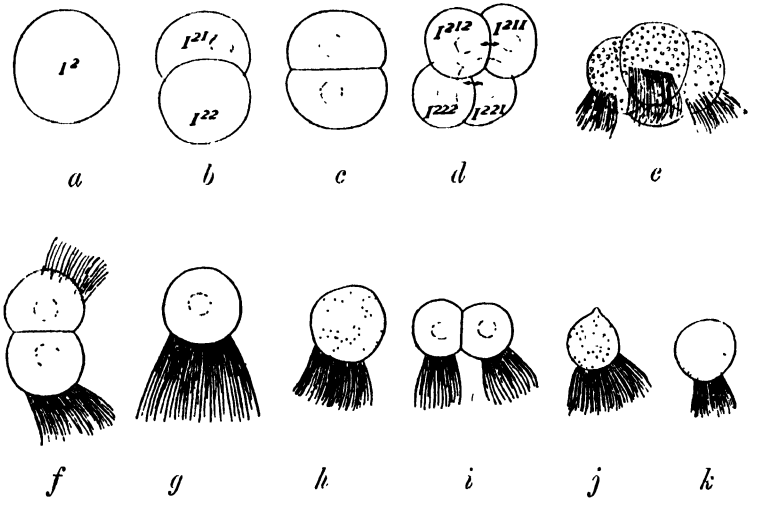


FIG. 129.—*Patella*. Development of isolated primary and secondary trochoblasts. *a*, Primary trochoblast; *b*, *c*, first division; *d*, second division; *e*, after 24 hours; *f*, pair of primary trochoblasts, the products of division of either 1^{21} or 1^{22}, after isolation; *g*, *h*, single primary trochoblasts, either 1^{212}, 1^{211}, 1^{221}, or 1^{222}; *i*, a pair of secondary trochoblasts, the products of 1^{12}; *j*, *k*, single secondary trochoblasts. (After Wilson.)

one end and secondary trochoblasts at the other, while the isolated apical cells and secondary trochoblasts divide as often as they would in the whole embryo and put out their cilia (Fig. 129 *i–k*, Fig. 130 *a–e*). Finally, the whole first quartette becomes an ectoblastic larva with an apical organ at one end and a prototroch at the other, but with no archenteron (Fig. 130 *f*, *g*). The cells of the second quartette (2 *a*—2 *d*) likewise form each a hollow ectoblastic vesicle containing larval mesenchyme, ciliated secondary trochoblasts, and a few (preanal) ciliated cells (Fig. 131).

The cells of the third and fourth quartettes do not develop at all.

A $\frac{1}{8}$ macromere (1 A—1 D), however, segments partially, to form a micromere of the second quartette, from which secondary

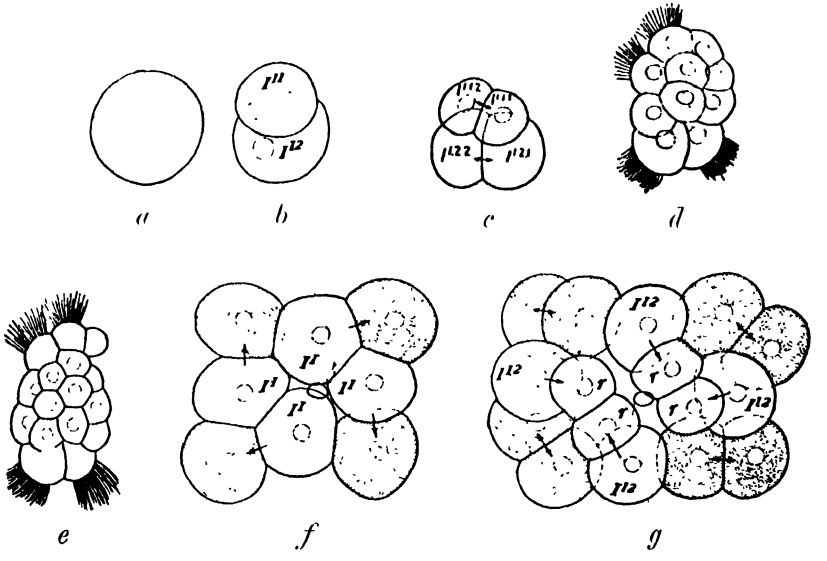


FIG. 130.—*Patella*. Development of isolated cells of first quartette and of the whole quartette. *a*, Isolated 1^1; *b*, its first division; *c*, its second division; *d*, after 12 hours; *e*, after 22 hours, showing apical cells and secondary trochoblasts; *f*, first division of isolated first quartette; *g*, second division. (After Wilson.)

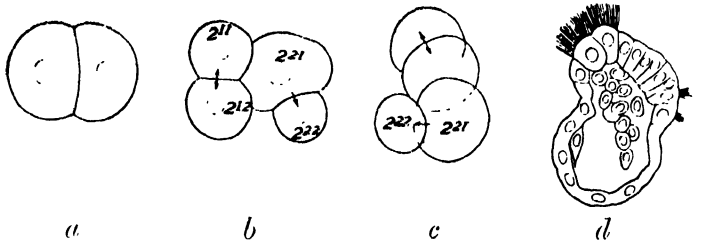


FIG. 131.—*Patella*. Isolated cells of second quartette. *a*–*c*, Cleavage; *d*, after 24 hours, showing secondary trochoblasts and ? preanal cells. (After Wilson.)

trochoblasts are later developed, and one of the third. Subsequently an archenteron is invaginated (Fig. 132). So the $\frac{1}{16}$ macromere (2 A—2 D) forms a micromere (3 *a*—3 *d*) and gives rise to a larva with entomesoblast (D) or entoblast only (A—C).

Isolated $\frac{1}{4}$ (A—D) or $\frac{1}{2}$ (AB or CD) larvae have an apical organ and a prototroch complete or semicircular; they may remain open or close up; sometimes they gastrulate.

It is evident that each cell has exactly the same potentialities for division and differentiation when isolated as when in connexion with its fellows. Only in the shifting of the cells and closing up of the whole cell-mass is there anything which approaches to total development.

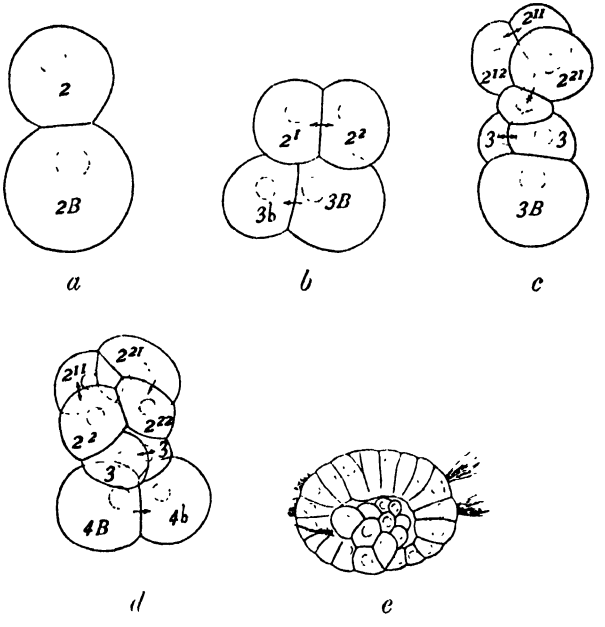


FIG. 132.—*Patella*. Development of isolated $\frac{1}{8}$ basal (macromere). *a*, First; *b*, second division; *c*, *d*, 56- and 64-cell stage. In *c* the position of the 2-group is normal, not in *d*; *e*, after 24 hours, showing two secondary trochoblasts (products of 2^{11}) and two feebly ciliated cells (preanal cells ?). (After Wilson.)

The dependence of this specification of the blastomeres upon definite substances preformed—though not prelocalized—in the unsegmented ovum is demonstrated by Wilson's experiments on *Dentalium*. The newly laid egg of *Dentalium* contains a brick-red yolk-mass surrounded by a thin hyaline ectoplasm; the latter is thickened a little to form an animal polar area and very considerably to form another large area at the vegetative pole. The nucleus is in the centre (Fig. 133). When the sperm enters the nucleus breaks down, and its substance becomes confluent with both

areas. This continuity is soon lost and the egg becomes divided into three regions, a clear layer spread over the animal hemisphere, below this the yolk-mass with the fertilization spindle, and a large,

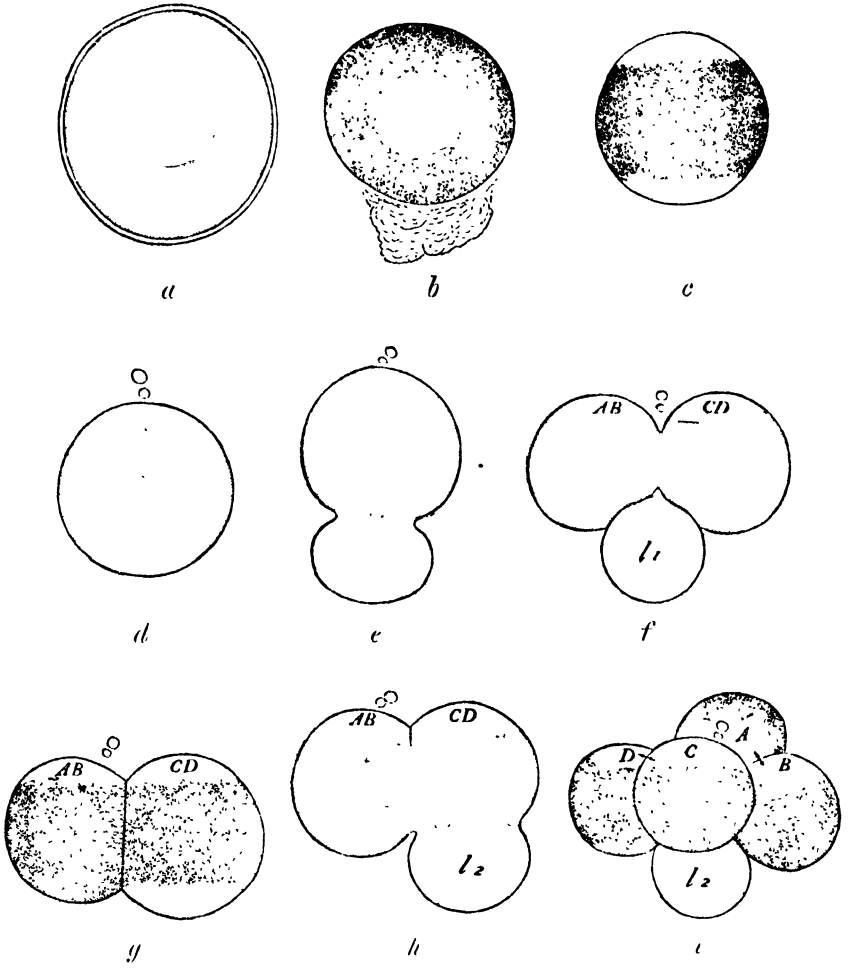


FIG. 133.– *Dentalium*. Normal cleavage. The red pigmented part is represented by stippling. *a*, Freshly laid egg, polar view; *b*, the same 20 minutes later, after shedding the membrane; *c*, the same from the side; *d*, one hour after fertilization, showing polar bodies; *e*, first division and protrusion of first polar lobe; *f*, 'trefoil' stage; *g*, the polar lobe has passed into CD; *h*, protrusion of second polar lobe (*l*2); *i*, second cleavage. (After Wilson.)

nearly yolk-free area at the vegetative pole, which is now protruded as the polar lobe. This becomes attached, as described above for *Ilyanassa*, to the D blastomere. The first quartette of

micromeres is formed from the clear animal area; after this division the polar lobe of D moves up to meet the upper white area, and so it comes about that a large part of this lobe passes into the second micromere, 2 *d*, which is the first somatoblast. In the other quadrants the second micromeres are formed from the upper white area alone. All the cells of the third quartette and (it is not known certainly whether this is the mesoblast) 4 *d* in the fourth are quite white. The larva is spindle-shaped with

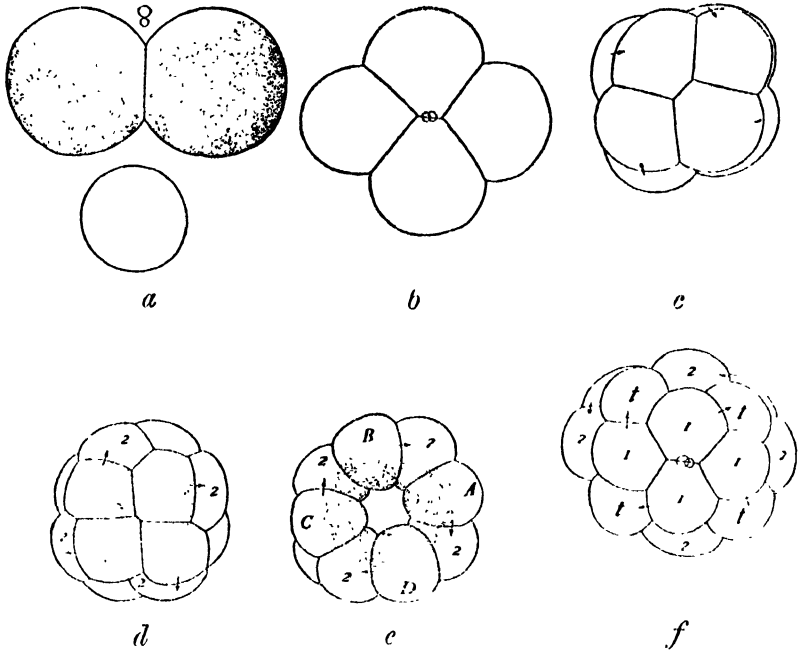


FIG. 134.—*Dentalium*. Cleavage after removal of the first polar lobe; *a*, first division: the isolated polar lobe is seen below; *b*, 4-cell stage, from animal pole; *c*, 8-cell stage, from animal pole; *d*, formation of second quartette, from vegetative pole; *e*, same stage as last, open type; *f*, same stage, from animal pole. (After Wilson.)

a broad, triple prototroch. There is an apical organ of long cilia and a tuft of sensory hairs at the opposite end (Fig. 135 *a*).

When the polar lobe is removed in the 'trefoil' (2-celled) stage, segmentation continues, but no polar lobe is formed in any stage, the D cells are not larger than those in the other quadrants, the embryo has no lower white area, the segmentation cavity may be open below, and the larvae are deficient and die (Figs. 134, 135 *b*). Post-trochal region and apical organ are both absent and

these missing parts are never regenerated; the pretrochal region is, however, increased and the three rows of cilia in the prototroch are usually developed.

The polar lobe is thus clearly connected with the formation of the trunk and the preoral sense-organ.

The segmentation of the isolated blastomeres is partial, as in the types already examined, but their subsequent behaviour depends on the presence or absence of the polar lobe. AB larvae or A, B, or C larvae closely resemble the deficient trochophore just described; CD or D larvae are normal, though the structures derived from the polar lobe are out of proportion to the rest

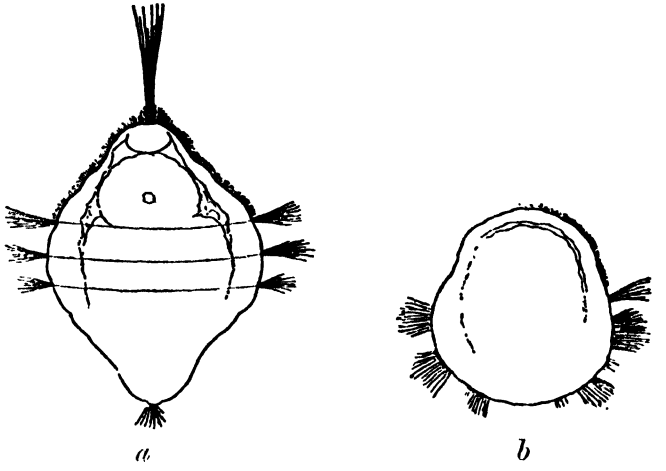


FIG. 135.—*Dentalium*. *a*, Normal trochophore of 24 hours; *b*, larva of 24 hours after removal of the first polar lobe. (After Wilson, 1904.)

(Fig. 136 *a–d*, *g*). The failure of the egg to develop normally is not due to insufficiency of material, for the volumes of CD and D are less than that of a whole but lobeless egg.

The micromeres 1 *a*, 1 *b*, 1 *c*, when isolated, become actively swimming, ectoblastic embryos provided with a prototroch, but with no apical organ, gut, or post-trochal region; the other micromere, 1 *d*, however, has an apical organ, though it is still without the hinder region and incapable of gastrulation (Fig. 136 *e*, *f*). It appears, therefore, that the material for the apical organ is now placed in this cell, and this is proved by experiment; for if the polar lobe is cut away during the second cleavage, or

from the isolated CD blastomere, then the larva has an apical organ, but no post-trochal region. Hence between the first and second divisions the stuff which determines the formation of this organ must migrate out of the polar lobe into the animal hemisphere of the D cell.

In the unsegmented egg the specific material for both apical organ and post-trochal region is situated in the vegetative

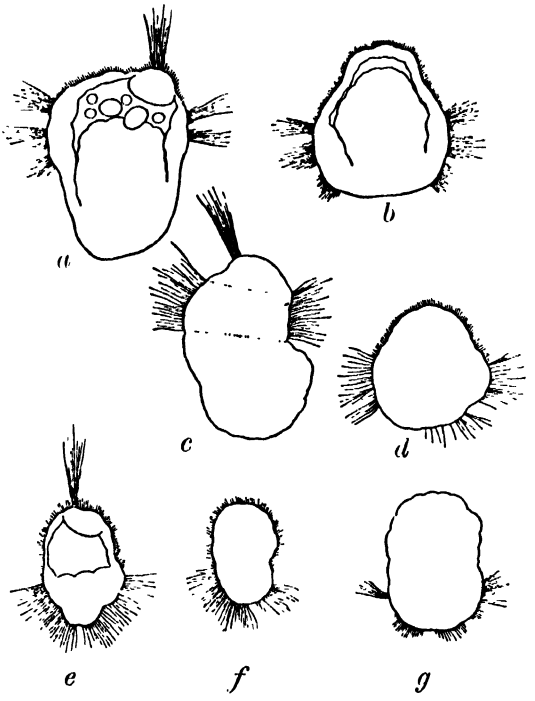


FIG. 136.—*Dentalium*. Larvae from isolated blastomeres. *a*, *b*, Twin larvae from the isolated CD (*a*) and AB (*b*) halves of the same egg, 24 hours; *c*, *d*, twin larvae from the isolated D (*c*) and C (*d*) quadrants of the same egg, 24 hours; *e*, *f*, twin larvae from the isolated posterior micromere 1 *d* (*e*) and the 1 *c* micromere (*f*) of the same egg, 24 hours; *g*, ¼ larva from one of the A, B, or C quadrants, 72 hours. (After Wilson.)

hemisphere. The animal half of the egg, whether removed after fertilization, or before and then fertilized, segments like an egg from which the polar lobe has been removed (Fig. 137 *b*), and develops neither apical organ nor post-trochal region (rarely an apical organ if the fragment is large). The vegetative hemisphere, when cut off before the entry of the sperm and afterwards

fertilized, segments as a whole with the polar lobe in correct proportion and gives rise to a dwarf larva with all its parts complete (Fig. 137 *a*, *c*). Many, however, die.

A meridional half-egg is able to develop normally, or nearly so.

The behaviour of the enucleate vegetative fragment of the fertilized egg is interesting. Though deprived of nucleus and centrosome it forms its polar lobe synchronously with the divisions of the animal portion; three times is the lobe protruded and three times withdrawn: the fourth time it is not taken back; this is the moment when the first somatoblast is given off. In this enucleate fragment the polar lobe is not in proportion but of the same size as in the whole egg.

Even the isolated polar lobe, or pieces of it, exhibits periodic movements, amoeboid, or of elongation, or protruding a smaller lobe.

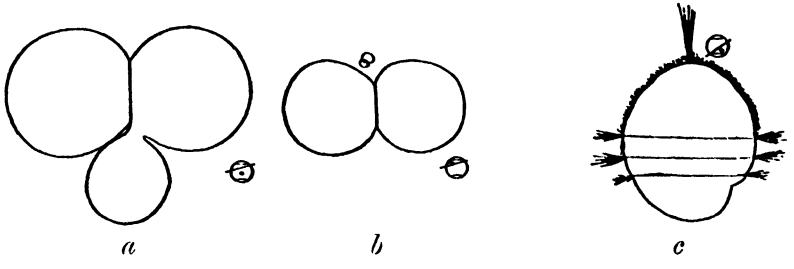


FIG. 137.—*Dentalium*. Development of egg-fragments, the plane of section being indicated in the small figures. *a*, *b*, Twins, after horizontal or oblique section near animal pole; *c*, trochophore developed from a fragment resembling that shown in *a*. (After Wilson.)

The existence in the egg of preformed substances specified for the production of particular organs is thus incontrovertibly established, and as long as an egg-fragment or blastomere contains all these it will give rise to an embryo which is, in form, perfect, though lack of enough material (or the shock of the experiment) may bring its life to an untimely end.

At the same time these substances, though preformed, are not necessarily prelocalized; their original is not the same as their ultimate situation, and in their redistribution we have to deal with a process which is, as Wilson remarks, truly epigenetic.

Before bringing this section to a conclusion reference must be made to another case in which it seems probable that definite

organ-forming substances exist, though experimental proof is at present lacking.

In the immature egg of the aberrant parasitic Polychaet *Myzostoma* there is, according to Driesch, at the vegetative pole a mass of greenish cytoplasm, the surface of which is convex towards the reddish brown mass of the rest of the egg. When the polar bodies are formed the red substance retreats to the animal pole, leaving a clear equatorial zone.

Before segmentation a polar lobe is formed—as in *Ilyanassa*

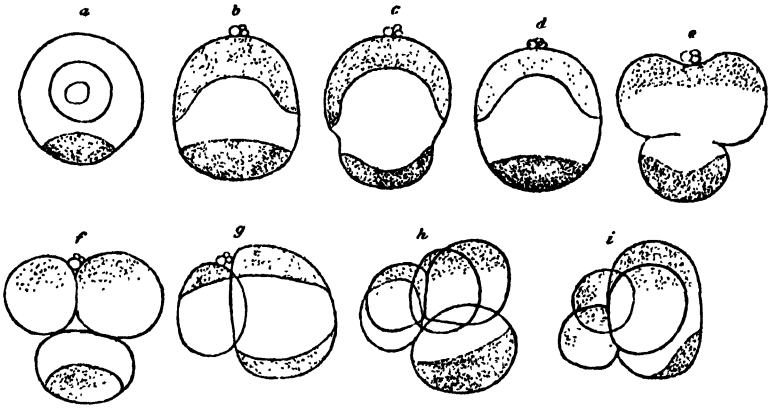


FIG. 138.—Development of the egg of *Myzostoma*. The green substance on the vegetative side is shaded finely; this passes into the polar lobe (*e*), and into the D quadrant (*i*). The red substance of the animal hemisphere is coarsely dotted. (After Driesch.)

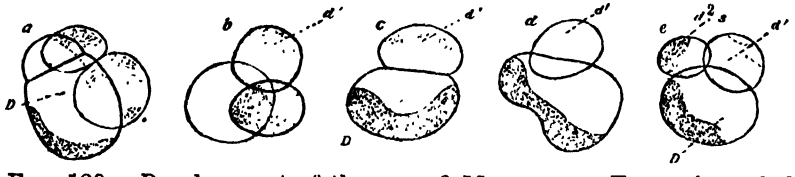


FIG. 139.—Development of the egg of *Myzostoma*. Formation of the cells 1 *d* (d^1 in *c*) and 2 *d* or *X* (d^2 in *e*). The latter cell takes a portion of the substance of the polar lobe. (After Driesch.)

and *Dentalium*—containing the green and part of the clear substance: it passes first to CD and then to D. When the latter divides it is again protruded and withdrawn. The cell 1 *d*, like 1 *a*, 1 *b*, 1 *c*, contains the reddish protoplasm. The second micromere in the D quadrant—2 *d*—takes part of the green substance, and Driesch suggests that the rest of the latter would eventually pass into the second somatoblast (4 *d* = M) (Figs. 138, 139).

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§ 10. ASCIDIA.

As has already been shown, there is in the Vertebrata and *Amphioxus* no constant relation between the first furrow and the sagittal plane of the embryo, although the symmetry of the embryo does stand in a very definite relation to that of the unsegmented ovum. Further, experiment has proved that the isolated blastomeres, up to a certain point at any rate, are capable of giving rise to normal total larvae. It is, perhaps, all the more remarkable that in another group of the Chordata, the Ascidians, the symmetry of the embryo is not only predetermined in the symmetry of the egg but also by the planes of segmentation, and that the potentialities of isolated blastomeres remain strictly what they would have been in the whole ovum.

The earliest experiments are due to Chabry (Figs. 140, 141). According to this author the first furrow in the egg of *Ascidiella aspersa* is meridional and coincides with the sagittal plane; the second, likewise meridional, coincides with the transverse plane; the third, equatorial, separates oral from aboral regions, and is therefore horizontal.

Isolated $\frac{1}{2}$ blastomeres or $\frac{2}{4}$ right or left blastomeres give rise to imperfect larvae with only one fixing papilla, only one atrial invagination and one pigment-spot, the eye; the last is absent in the $\frac{2}{4}$ left larva. $\frac{2}{4}$ anterior larvae have one fixing papilla, one sense-spot, a gut, a cerebral vesicle and a posterior notochord;

in $\frac{2}{4}$ posterior larvae the chorda and brain are absent, though the three germ-layers are all present.

In the segmentation of the isolated blastomeres the direction of division is altered, in that a furrow (the fourth) which in the whole egg is meridional and at an angle of 45° to the first two becomes parallel to the plane of separation, and so resembles the third furrow of the whole egg.

A right or left $\frac{1}{2}$ blastomere or $\frac{2}{4}$, anterior or posterior, right

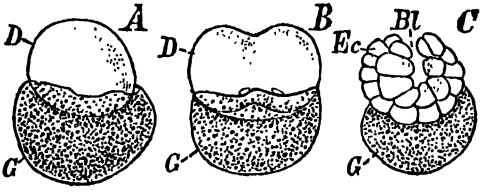


FIG. 140.—*A*. Egg of *Ascidiella aspersa* in the two-cell stage. One $\frac{1}{2}$ blastomere has been killed (*G*). *B*. The survivor (*D*) divides to form, in *C*, a small normal gastrula. (After Chabry, from Korschelt and Heider.)

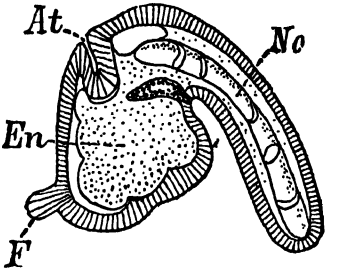


FIG. 141.—Larva of *Ascidiella aspersa* produced from $\frac{2}{4}$ blastomeres. *F*, sucker; *At*, atrium; *No*, notochord; *En*, endoderm. (After Chabry, from Korschelt and Heider.)

or left, blastomeres consist after this division of two tiers of four cells each; the cleavage of these cells resembles, therefore, that of a whole egg.

It should be noticed that the blastomeres cannot be actually separated; it is only possible to kill one, by a needle, and note the development of the survivor.

The more recent researches of Conklin on *Cynthia* have confirmed and extended Chabry's results.

The immature egg of *Cynthia* (Fig. 142) comprises a central grey yolk surrounded by a peripheral layer of yellow pigmented

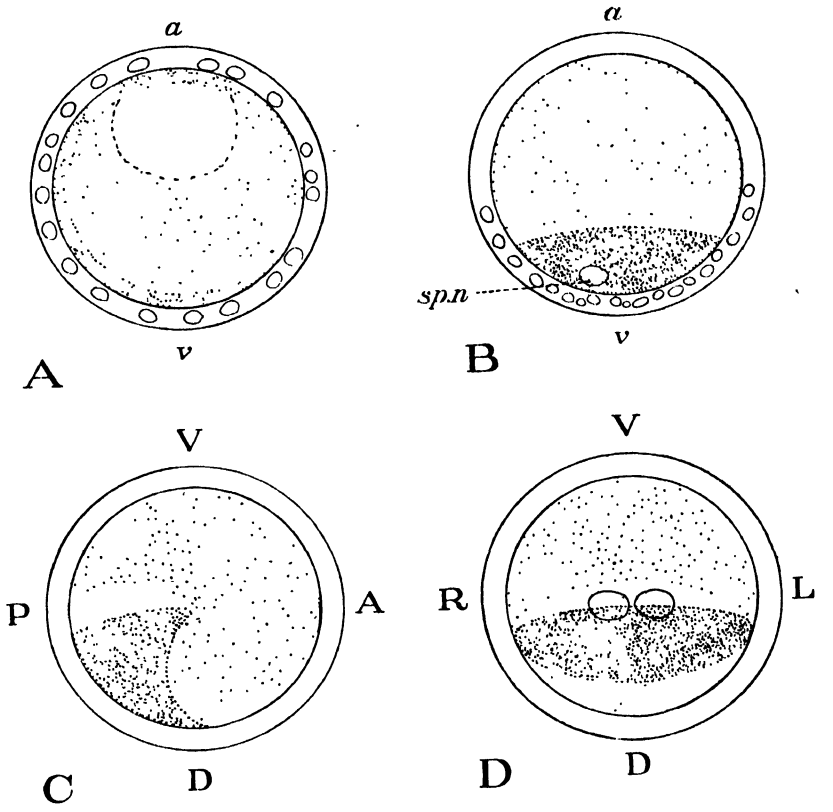


FIG. 142.—Normal development of the egg of *Cynthia partita*. Maturation and fertilization. (After Conklin.)

A. Unfertilized egg before the fading of the germinal vesicle (clear), showing central mass of grey yolk (lightly dotted), peripheral layer of yellow protoplasm (thickly dotted), test-cells and chorion.

B. After the entrance of the spermatozoon the yellow protoplasm has streamed to the vegetative pole (*v*); in it, excentrically, is the sperm nucleus (*sp.n*). The clear protoplasm derived from the germinal vesicle partly forms a layer over the yellow protoplasm, in part remains at the animal pole (*a*). The grey yolk occupies the animal part of the egg.

C. The yellow and clear protoplasmic substances have both streamed to what will be the posterior side (P). A, anterior, V, ventral (animal pole), D, dorsal (vegetative pole).

D. View of the same egg from behind. The two pronuclei are seen side by side in the clear area. R, right; L, left.

protoplasm; near one pole (the animal), and so determining the egg-axis, is the large germinal vesicle. Prior to the extrusion of the polar bodies the latter breaks down and provides a third substance, a clear cytoplasm. On the entrance of the spermatozoon near the vegetative pole a remarkable change takes place in the arrangement of these materials. The yellow protoplasm flows down to the vegetative pole and arranges itself symmetrically to the axis; above it is a zone of clear protoplasm derived from the germinal vesicle; these two areas together occupy about one-third of the egg. The remaining, the animal two-thirds, are occupied by the grey yolk with only a small quantity of clear cytoplasm at the animal pole in which, after the formation of the polar bodies, the female pronucleus is embedded.

The egg is still radially symmetrical, but by further shiftings of these substances it becomes bilateral.

The spermatozoon moves in the clear protoplasm to the equator on one side, the posterior side as it will be, and the yellow protoplasm follows suit; the first is now disposed in a broad equatorial band encircling half the circumference of the egg, the second forms a wide crescentic mass below it and reaches nearly to the vegetative pole. The female pronucleus, with its clear area, now joins the male.

The egg is now bilaterally symmetrical about a plane which includes the axis, and passes through the middle of the clear and yellow substances and between the two closely apposed pronuclei.

It seems doubtful whether the movement of the clear and yellow material to the posterior side is immediately due to the agency of the sperm, for the latter does not always take the shortest route to the equator; it may enter on what becomes ultimately the anterior side; and further, when two sperms enter they both travel to the same spot, appearing to be passively carried along by the independent streaming of the egg cytoplasm.

The two pronuclei next move into the axis of the egg in the animal hemisphere, together with their clear protoplasm. The grey yolk is thus largely displaced and shifted into the vegetative region. The egg, therefore, now consists of, an animal

hemisphere composed almost entirely of the clear substance, and a vegetative hemisphere, the anterior portion of which includes grey yolk and nothing else, the posterior portion the yellow protoplasm together with a little yolk near the vegetative pole.

The cleavage furrows separate these substances from one another in a perfectly definite way (Fig. 143). The first is

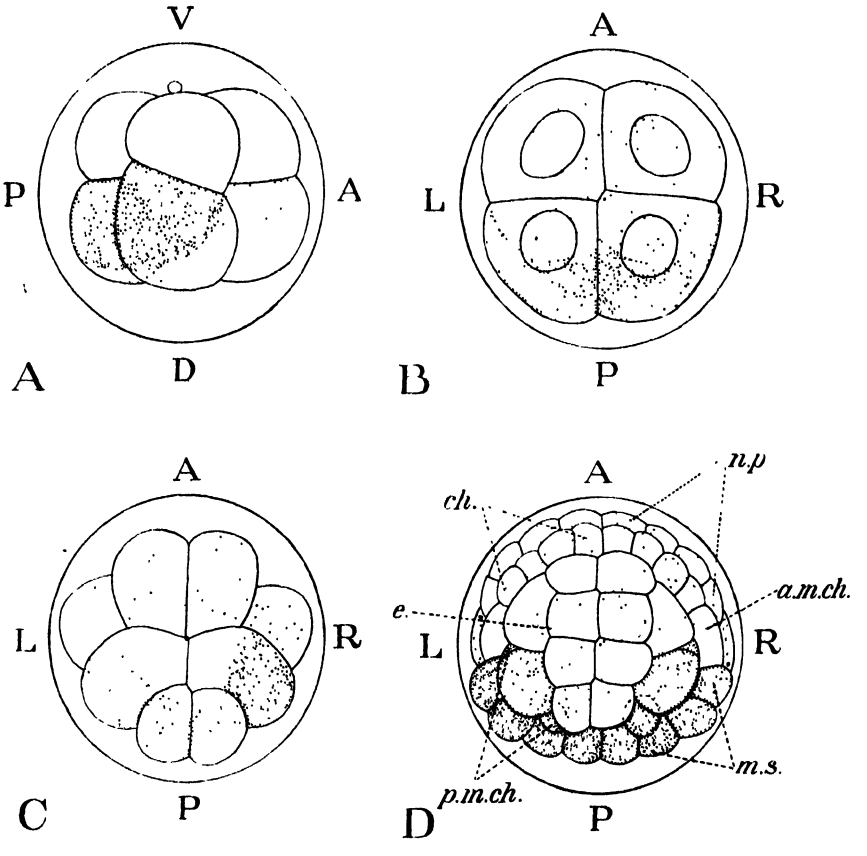


FIG. 143.—Cleavage of the egg of *Cynthia partita*. (After Conklin.)

A. 8 cells, from the left side.

B. 8 cells, from above. The yellow crescent (thickly dotted) is limited to the posterior dorsal cells: the animal cells are clear, the grey yolk (sparsely dotted) is found in the dorsal cells, both anterior and posterior.

C. 16 cells, from the dorsal side.

D. 74 cells, dorsal view, showing the division of the 4 neural plate-cells (*n.p*), and 4 chorda cells (*ch.*). There are 10 endoderm cells (*e*), 6 muscle-cells (*m.s.*), 8 posterior (*p.m.ch.*) and 2 anterior (*a.m.ch.*) mesenchyme cells.

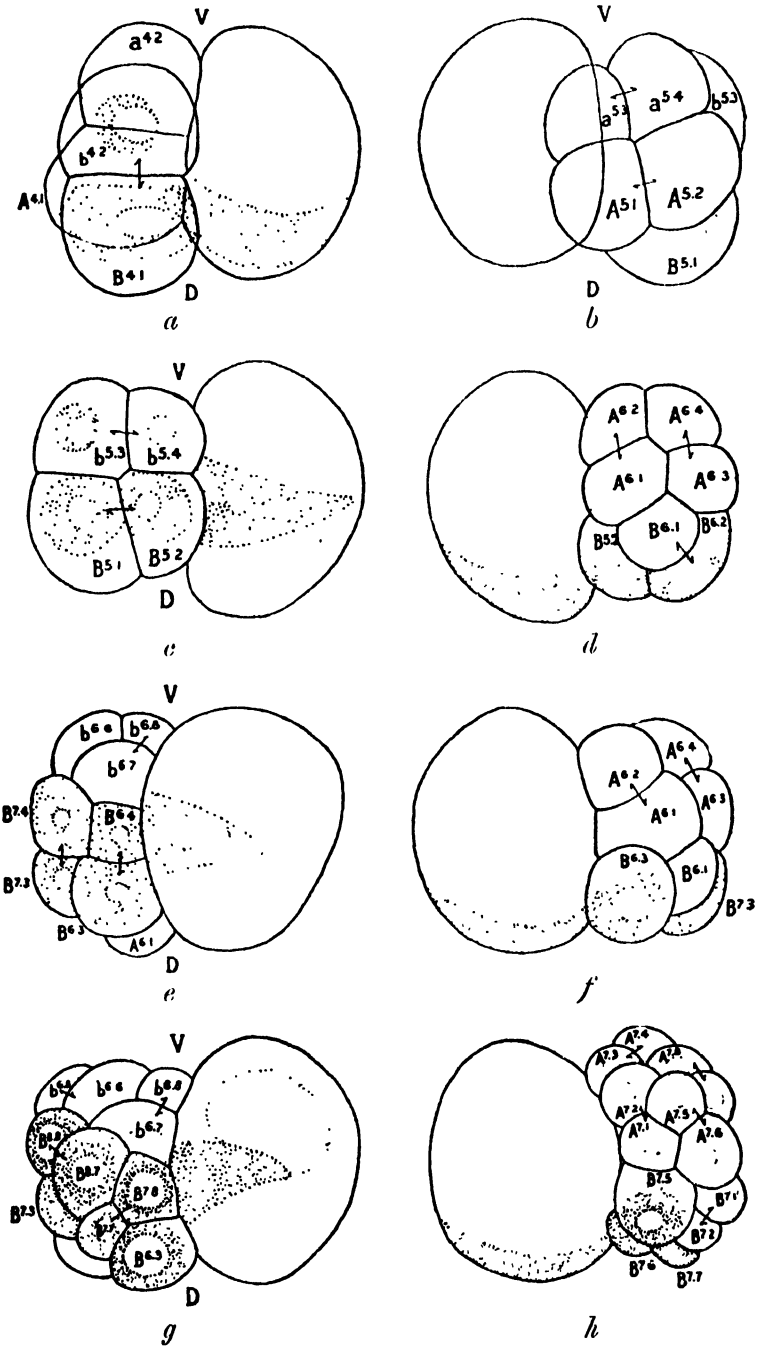


FIG. 144.—*Cynthia partita.* Successive stages in the development of the same right half embryo, the left blastomere having been injured in

meridional and sagittal, the second meridional and transverse, the third equatorial and horizontal. Since the animal pole will be ventral, the vegetative dorsal, the right and left sides of the future embryo are simultaneously determined.

The four ventral animal cells consist almost entirely of clear protoplasm, the two anterior dorsal entirely of grey yolk, the two posterior dorsal almost exclusively of the yellow substance.

The next furrows are, roughly, meridional and at angles of 45° to the first two; they emphasize the bilateral symmetry, for in the anterior half they meet the first furrow above, the second below, and conversely in the posterior half.

The direction of the divisions in the next phase may be most easily gathered from the figures. In the vegetative hemisphere four anterior chorda-neural cells are separated off; the six posterior cells include all the yellow substance and will give rise to the posterior mesenchyme and the muscles of the tail; six endoderm yolk-cells remain, four anterior, two posterior. The next division sees the separation of the chorda from the neural cells in front, of an outside ring of muscle-cells from an inside ring of mesenchyme cells behind, and of an anterior mesenchyme cell on each side from the anterior endoderm.

A few anterior animal cells become associated with the neural cells in the formation of the nervous system; the remainder of this hemisphere is ectodermal.

In gastrulation the endoderm cells first sink inside, followed by the chorda in front and the anterior and posterior mesenchyme cells and the muscle-cells at the sides and behind. Overgrowth is greatest at the anterior dorsal lip, the blastopore thus becoming posterior.

In normal development, therefore, the axes of the embryo are

the 2-cell stage. The yellow protoplasm is indicated by coarse stippling. (After Conklin.)

a, 8-cell stage, posterior view. V, ventral; D, dorsal. *b*, 16-cell stage, anterior view. Normally, A 5.1 and *a* 5.3 lie in front of A 5.2 and *a* 5.4. *c*, 16-cell stage, posterior view. In normal eggs the cells B 5.2 and *b* 5.4 lie behind B 5.1 and *b* 5.3. *d*, 30-cell stage, dorsal view. *e*, *f*, 34-cell stage; *e*, posterior, *f*, dorsal view. In normal eggs the cell B 6.4 lies laterally to B 6.3, and the cell B 6.1 lies between B 6.3 and A 6.1. *g*, 46 cells: posterior view. *h*, 48 cells: dorsal view. The cells B 7.1 and B 7.2 should lie next the middle line.

predetermined by the arrangement of certain substances in the egg, and segmentation exhibits the character of a 'Mosaic' work, segregating these specific materials into the rudiments of definite organs. The causal connexion between these materials and the organs to which they are beforehand assigned is demonstrated by experiment.

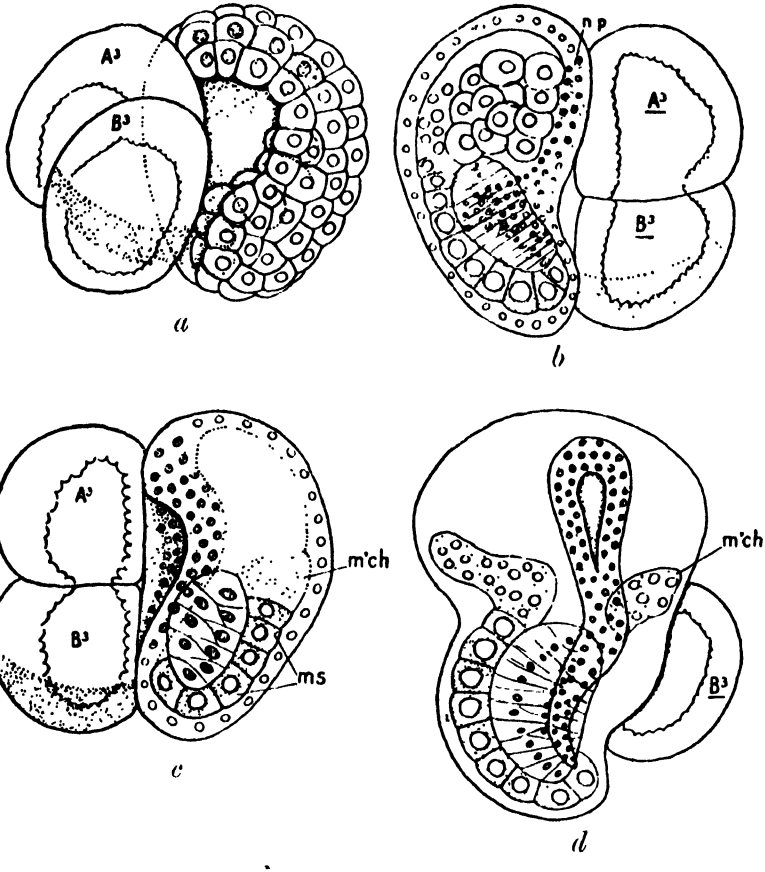


FIG. 145.— *Cynthia partita.* Half and three-quarter embryos. (After Conklin.) *a*, right half gastrula, dorsal view. The neural plate, chorda, and mesoderm cells are present only on the right side and in their normal position and numbers. *b*, left half of young tadpole, dorsal view. The notochord is normal except for size and number of cells: the muscle and mesenchyme cells are present only on one side: the neural plate (*n.p.*) is abnormal in form but not in position. *c*, right half of young tadpole, dorsal view: slightly younger than *b*. *m'ch.*, mesenchyme, *ms.*, muscle-cells. *d*, left anterior three-quarter embryo, dorsal view. The anterior half is entirely normal with anterior mesenchyme (*m'ch.*) on both sides. Posterior mesenchyme and muscle-cells upon the left side only.

The segmentation of a $\frac{1}{2}$ or of $\frac{2}{4}$ right or left blastomeres is partial except for the alteration in the direction of the furrows of the fourth phase already described by Chabry. The blastomeres divide as though the other half of the egg had not been killed (Figs. 144, 146, 147). Eventually a half gastrula is formed with exactly half the normal number of neural, chorda, endoderm, mesenchyme and muscle-cells; the gastrula cavity is open on the side of the dead blastomere. Many are, however, abnormal in that the endoderm and muscle- and mesenchyme cells do not invaginate (exogastrulae) or show invaginations of the ectoderm (pseudogastrulae).

The half gastrula may develop into a half larva, with the notochord, muscles, and mesenchyme lying on one side of the medullary folds. The only signs of the regeneration of the missing half are the overgrowth of the ectoderm on the naked side, and the passing over of a few muscle-cells; the full number of the latter is, however, never found on both sides (Fig. 145 *a–c*).

The $\frac{3}{4}$ larva is especially interesting, particularly when a posterior blastomere has been killed, for here the anterior end is complete with anterior mesenchyme on both sides; the posterior mesenchyme and muscles are, however, missing on either the right or left (Fig. 145 *d*). The chorda and neural plate are stated to be smaller than the normal in these larvae, and there is no sense-vesicle.

An embryo developed from the two anterior blastomeres of the 4-celled stage alone possesses the full number of neural, chorda, anterior endoderm and anterior mesenchyme cells; its hind end is covered by ectoderm but shows no trace of muscles or posterior endoderm or mesenchyme (Fig. 146 *c*, *d*).

The two posterior blastomeres produce an embryo devoid of neural plate and notochord, of anterior mesenchyme and endoderm. By overgrowth of the ectoderm a sort of solid gastrula is formed with a central mass of endoderm flanked by muscle-cells and posterior mesenchyme. No tail appears (Fig. 147).

$\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}$ blastomeres also segment and develop partially. An anterior $\frac{1}{4}$ has half the proper number of neural, notochordal, anterior endoderm and mesenchyme cells, but the neural plate does not become folded and the chorda cells protrude irregularly behind. A posterior quarter may gastrulate in the way described for the posterior half, but exogastrulae and pseudogastrulae occur.

Conklin has also divided the gastrula into anterior and posterior halves ; neither can regenerate the missing parts, though the cut surface of each becomes covered by ectoderm.

Driesch, however, states that in another form, *Phallusia mammillata,* anterior and posterior half gastrulae will, if

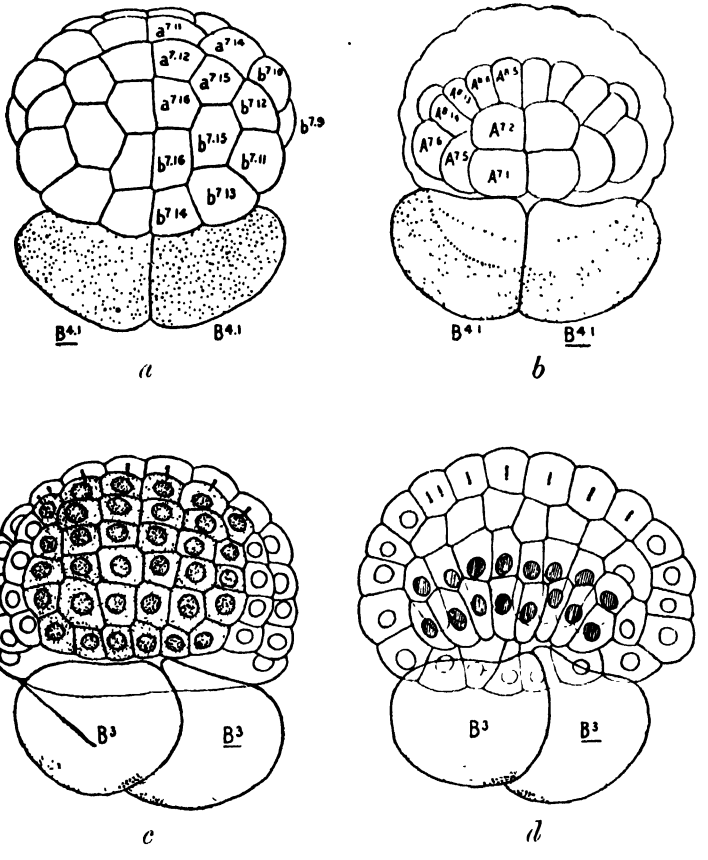


FIG. 146.—*Cynthia partita.* Anterior half and three-quarter embryos. (After Conklin.) *a*, *b*, anterior ventral three-quarter embryo, the posterior dorsal cells (B 4.1, B 4.1) containing all the yellow pigment having been killed in the 8-cell stage. *a*, ventral view; *b*, dorsal view. *c*, *d*, anterior half embryo: *c*, dorsal view, showing the neural plate (stippled); *d*, deeper focus showing two rows of chorda cells (the nuclei are shaded), and ectoderm and endoderm.

separated before complete closure of the blastopore, each develop into a larva lacking only the otolith and the eye. In a later stage of gastrulation this becomes impossible, although the

posterior portion of a young Ascidian will regenerate a new head with pharynx and eyes; the anterior half dies.

Driesch has also maintained that the isolated blastomeres ($\frac{2}{4}$, $\frac{3}{4}$, $\frac{1}{4}$) of the same species give rise ultimately to total gastrulae and these to larvae. The larvae are, however, unable to hatch out and are defective—with rudimentary eyes, otolith and suckers—or devoid of these organs altogether.

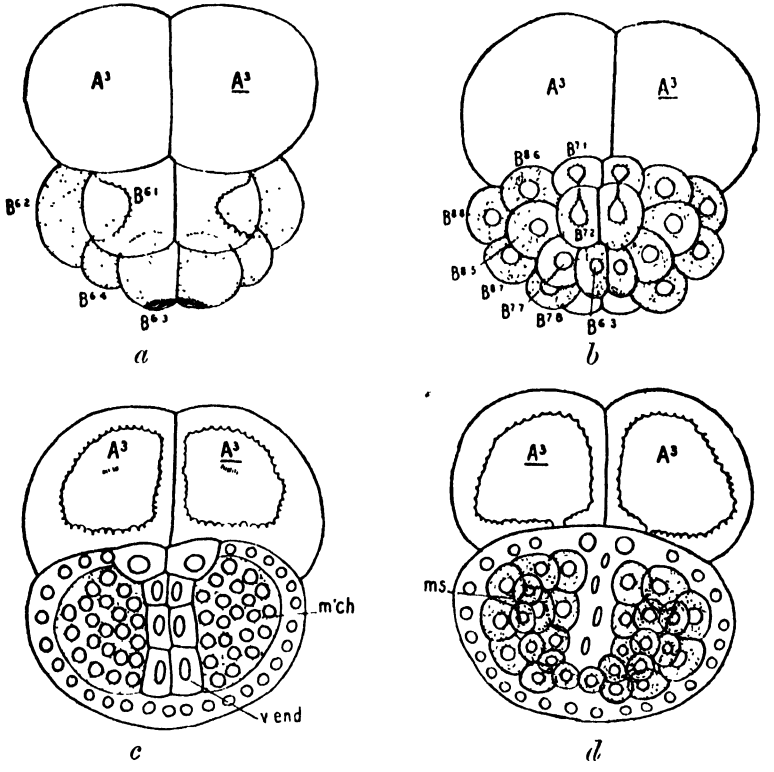


FIG. 147.—*Cynthia partita.* Posterior half embryos. (After Conklin.) *a*, 32-cell stage, dorsal view. Cleavage is quite normal. *b*, 76-cell stage, dorsal view. Muscle-cells (B 8.8, B 8.7 and B 7.8) and posterior mesenchyme cells (B 8.6, B 8.5, B 7.7, and B 6.3) and posterior endoderm cells (B 7.1, B 7.2) are seen. *c*, later stage, deep focus, showing ventral endoderm (*v end.*) with a mesenchyme (*m'ch.*) on each side. *d*, same stage as last, showing muscle-cells (*ms.*).

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§ 11. General Considerations and Conclusions to be Drawn from the Foregoing Experiments.

From the foregoing experiments certain general conclusions may now be drawn.

(1) First, the division of the nucleus during segmentation at least is not the qualitative process imagined by Roux and Weismann. The pressure experiments of Hertwig, Driesch, and Wilson have clearly demonstrated that the nuclei of the segmenting ovum may be disarranged without prejudice to the subsequent normal development of the embryo; and this result is amply confirmed by the normal behaviour of eggs (of sea-urchins) in which the mutual positions of the blastomeres, and therefore also of the nuclei, are altered, as well as by the very numerous cases in which it has been shown to be possible to rear a perfect embryo or larva from an isolated blastomere or blastomeres.

It is evident that during segmentation at least the nuclei are equipotential, and the hypothesis of self-differentiation in the form originally propounded by Roux can no longer be upheld. It has, in fact, been now abandoned by its author.

(2) But though in this direction its labours have ended negatively, modern experimental research is yet able to point to a positive achievement of the greatest value and significance. For the same series of investigations has shown that the cytoplasm of the undeveloped ovum is not the homogeneous and isotropic substance which the experiments of Pflüger led Roux to consider it, but heterogeneous, containing various specific organ-forming stuffs which are definitely and necessarily connected with the production of certain parts or organs of the developing animal. If the polar lobe of the mollusc *Ilyanassa* be removed the larva has no mesoderm; if the polar lobe of *Dentalium* be taken away the larva has no trunk and no apical organ; the egg of a Ctenophor

is capable of segmenting when deprived of its vegetative hemisphere, but the larva to which it gives rise has no combs and no sense-organ. The egg of the Ascidian *Cynthia* exhibits in its cytoplasm various substances, easily distinguishable by their colour; in segmentation these are distributed to the various blastomeres in a perfectly definite way. Remove one or more of these substances and the embryo will lack the organ or organs normally produced from them. Again, a Nemertine egg from which, at a certain stage, the vegetative portion has been cut away, gives rise to a Pilidium without lappets and without a sense-organ.

In these cases, then, at least, the existence of specific organ-forming stuffs in the cytoplasm of the egg has been proved; the removal of the stuffs inevitably entails the absence of the corresponding organ later on. But though it may therefore be justly said that such stuffs are preformed, it is not invariably true that they are prelocalized, present, that is to say, *ab initio* in what will be their eventual situation. For example, the stuff which determines the formation of an apical organ in the larva of *Dentalium* is originally in the polar lobe of the vegetative hemisphere: but between the first and the second divisions it migrates into the animal portion of the egg; and the same may be said of the formation of the sense-organ in *Cerebratulus* and the Ctenophora.

It seems highly probable, though it cannot be said to have been demonstrated, that such specific organ-forming substances are of universal occurrence.

The behaviour of the isolated blastomeres of the eggs of different forms, as well as of the blastomeres of the same form at different stages, is markedly different. The $\frac{1}{2}$ or $\frac{1}{4}$ blastomere of an Ascidian egg can never produce more than $\frac{1}{2}$ or $\frac{1}{4}$ larva; the isolated blastomeres of *Patella* produce just so much, dividing just so many times, as they would have produced had they remained in connexion with their fellows; a radial group of blastomeres (one or more $\frac{1}{8}$ blastomeres, or two or four or more $\frac{1}{16}$ blastomeres) of a Ctenophore egg gives rise to a larva with one, two or more costae and meridional canals, as the case may be. On the other hand, the isolated $\frac{1}{2}$ or $\frac{1}{4}$ blastomeres of a Nemertine ovum will produce a complete larva, while, after the next division, the totipotentiality is lost; for an isolated

animal cell gives rise to a larva without an archenteron, an isolated vegetative cell to one in which the apical organ is lacking. So in *Amphioxus* $\frac{1}{2}$ and $\frac{1}{4}$ blastomeres develop into whole embryos, but $\frac{1}{8}$ blastomeres, whether of animal or vegetative origin, will not gastrulate. In other cases, however, the potentialities do not become restricted so soon. Either the four animal or the four vegetative cells of a Frog's egg will give rise to an embryo in which the blastoporic lip and archenteron are developed, while in Echinoderms the least blastomere that will gastrulate is $\frac{1}{32}$. It is not, nevertheless, every cell of the 32-celled stage that is capable of developing an archenteron, but only those, as Driesch has conceded, of vegetative origin.

But whatever differences there may be in the potentialities of the cells of different ova, or of the same ova at different stages or in different regions, it is clear that sooner or later the capacity of a part to become a whole is lost; and it seems only reasonable to conclude that this loss of totipotentiality is due to the loss of one or more necessary cytoplasmic substances, to the lack of specific material, and not merely of material. In the egg of *Amphioxus*, which is telolecithal, there is an obvious difference, in the amount of yolk and size of the granules, between the animal and vegetative hemispheres; a difference in the capacities of these two regions has been experimentally demonstrated in the Nemertine egg; and, although it may often appear homogeneous, the ovum of the sea-urchin, in all probability, possesses that 'stratification' of potentialities, that is of organ-forming stuffs, at right angles to its axis, on which Boveri has so strongly insisted, for Driesch has admitted that vegetative cells 'gastrulate' more readily than animal cells; it would appear, then, that a gut-forming substance is present, and a mesenchyme-forming substance too, but that the concentration of these substances steadily diminishes from the vegetative to the animal pole. Radially about the axis the distribution of these substances may be taken to be uniform, since meridional fractions of the egg, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{2}{4}$ or $\frac{2}{8}$ or $\frac{3}{8}$ blastomeres, are invariably totipotential.

The same explanation may be applied to other cases. Hence, speaking generally, the limitation of the potentialities of the parts will depend simply on the original distribution of the

substances in the unsegmented egg. Should they be already segregated (wholly or in part) in distinct regions, as in Ascidians, Molluscs, and Ctenophors, then even the first blastomeres will to that extent be unable, when isolated, to give rise to more than they would have done in the uninjured egg; should they be uniformly distributed about the axis, but unequally along the axis (Nemertines, Echinoderms, *Amphioxus*), then a limitation of potentialities will appear when the third division separates the animal from the vegetative cells[1]; should the arrangement be

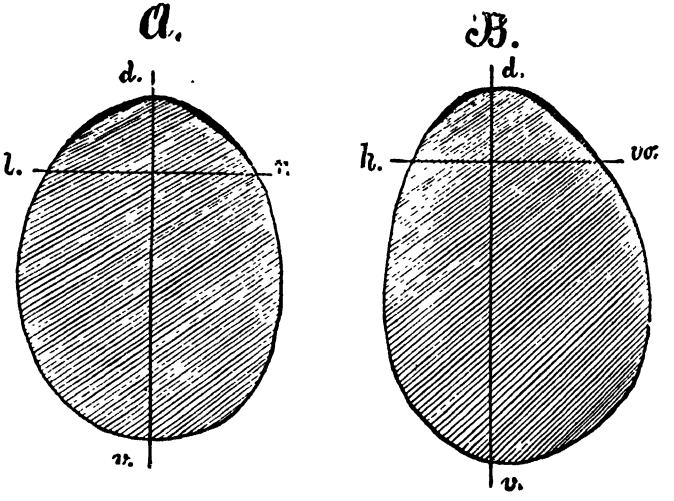


FIG. 148.—Diagrammatic sections through the unsegmented egg of *Loligo pealii*. (From Korschelt and Heider, after Watase.) A is in the transverse, B in the sagittal plane of the future embryo. In B the anterior side (*vo.*) is more convex than the posterior (*h.*). *d*, dorsal (animal pole); *v.*, ventral (vegetative pole); *l.*, left; *r.*, right. The protoplasm is black, the yolk shaded.

uniform about the egg-centre, then animal and vegetative blastomeres will be alike totipotential (Coelenterata).

At the same time it would appear to be true that the capacity of total development may be lost for mere lack of material, for Driesch found that while all four (or all eight in the next stage) animal cells of the sea-urchin egg might give rise to a Pluteus larva, the isolated cells would not. The isolated cells may, how-

[1] We may notice that O. Maas (*Arch. Ent. Mech.* xxii, 1906) has found that the isolated anterior flagellated and posterior granular cells of the Amphiblastula larva of *Sycon* are not equipotential. The latter can fix and give rise to a young sponge, the former cannot.

ever, gastrulate ; when once the gastrula stage has been reached, therefore, further development may be simply a matter of sufficiency of substance. So again the octants of the Ctenophore egg are alike, but each can produce one costa, and one only.

There are then certain preformed organ-producing substances, and the arrangement which they either possess before, or acquire

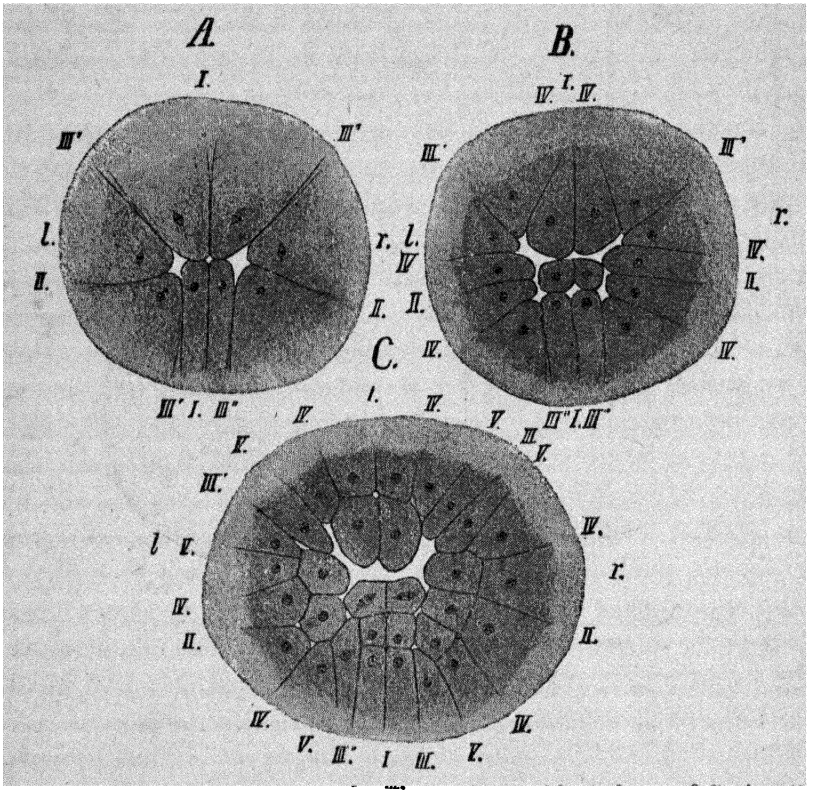


FIG. 149.—Three segmentation stages in the blastoderm of *Sepia officinalis*; the segmentation is of the bilateral type. *l*, left; *r*, right; *I–V*, first to fifth cleavages. The top sides of the figures are anterior. (After Vialleton, from Korschelt and Heider.)

during, segmentation determines the fixed relation which can be observed in almost all cases between the structure of the egg and the axes of the embryo. Thus in Amphibia the head of the embryo is formed at or near the animal pole, and the plane of egg symmetry becomes the sagittal plane. In Mollusca (not Cephalopoda) and Annelida the apical sense-organ is developed at the animal pole, while the D quadrant is posterior. A blasto-

pore is in very numerous cases formed at the vegetative pole, the bilateral symmetry of the eggs of Cephalopoda (Figs. 148, 149), Insecta, some Crustacea, and Ascidia becomes that of the embryo; in *Ascaris* the first four cells lie in one plane, which becomes the median plane, the germ-cell is posterior and the animal pole dorsal; and so on.

(3) The instances in which the segregation of the organ-forming substances into the blastomeres takes place at once are especially interesting, as they appear to fulfil exactly the requirements of the 'Mosaik-theorie', if that doctrine be transferred from the nucleus to the cytoplasm, for here, undoubtedly, segmentation is a qualitative process, a sundering of potentialities. It must be pointed out, however, that even in these cases the factors which determine segmentation and those which determine differentiation may be as distinct as they are elsewhere.

In the Frog there is a much greater correlation between the symmetry of the egg and the median plane of the embryo than between the former and the plane of the first furrow.

A $\frac{1}{2}$ or $\frac{1}{4}$ blastomere of a Nemertine or a sea-urchin segments as a part, and yet it eventually develops into a whole larva: and the same is true of egg-fragments. All the isolated blastomeres of a Mollusc segment as parts, yet in *Dentalium*, that one which contains all the necessary stuffs, CD or D, can give rise to a complete embryo, while its fellows cannot. The converse case is to be found in Ctenophors; here the egg which has been deprived of its vegetative portion segments as a whole and yet produces an embryo devoid of costae and sense-organs.

There are also certain forms (*Amphioxus* and Vertebrates, Coelenterata) where the isolated cell segments as a whole, and this appears to depend on the rapidity with which the part can rearrange its material or resume the polarity of the whole (Wetzel). It is further to be noticed that the capacity of a part for total segmentation, like its power of total differentiation, may differ in the same egg at different times and under different conditions; the fragment of an immature egg of *Cerebratulus* divides like a whole egg, an isolated blastomere like a part; again, in *Ilyanassa* the cleavage of a part may be made total by lowering the temperature.

When, further, it is remembered how closely similar the

geometrical pattern of segmentation may be in the eggs of different animals, whether of the same or of different groups (spiral segmentation of the eggs of Molluscs, Chaetopods and Turbellaria, similar segmentation of the eggs of, for example, some Echinoderms, Polyzoa, and Vertebrates), without necessarily involving a similarity in the fate of cells which are identical in origin, it will, I believe, be conceded that the factors which are responsible for cleavage and those which determine differentiation are distinct, though the two may, as in the so-called 'Mosaik' segmentations, coincide: and where, as in Ascidians, the Ctenophora, the Cephalopoda, this coincidence is complete, the symmetry of the egg, the symmetry of segmentation, and the symmetry of the embryo are one.

The former factors must be sought for in the arrangement of yolk and protoplasm, possibly in the relative strengths of the centrosomes, in surface-tensions, in the pressure exerted by egg-membranes and so forth; the latter in definite cytoplasmic organ-forming stuffs.

(4) Like the parts of the egg the parts of the elementary organs of the embryo are at first equipotential, but exhibit a gradual limitation of potentialities as their development proceeds. As Minot puts it, there is a 'genetic restriction' of capacities. Thus the archenteron of *Asterias* can form a new terminal vesicle when this has been removed, but only before the outgrowth of the coelom sacs. The anterior half of the newt embryo will develop into a whole embryo when cut off before the medullary folds have arisen, but not after. When one vitelline vein of a chick is destroyed its fellow will form a whole, not a half heart; and Sumner's experiments quoted above have shown that the margin of the Teleostean blastoderm is isotropic.

(5) There still remains to be noticed a point of great importance. It certainly has not been demonstrated, and it cannot be pretended that there are in the developed egg as many specific substances as there are separately inheritable qualities in the body. On the contrary, the evidence of experiment speaks against such a supposition; for, as Driesch has urged, were every separately inheritable quality to be represented in the germ by a distinct morphological unit, the equipotentiality of

the isolated parts would be inconceivable. Although the formation of the larval and elementary organs (germ-layers) may depend upon such stuffs, yet within each such elementary organ the parts are at first and remain for a time equipotential.

Differentiation is progressive. The origin of the primary differentiations has been found; it remains for the experiments of the future to discover how fresh distinctions arise until the organization of the whole is complete. Suggestions as to the importance, in this respect, of certain other internal factors have been made, notably of the structure of the nucleus, and the reactions of the parts on one another. These suggestions, for they are hardly more than that at present, we shall have to discuss. First, however, we must briefly consider the part played by the spermatozoon in determining the structure of the egg and the symmetry of the embryo.

§ 12.

It is of the greatest interest to observe that the definitive distribution of these specific substances in the unsegmented egg, and so the determination of the embryonic axes, may be brought about, partly at least, by the spermatozoon.

In the Frog (*Rana fusca*, *R. temporaria*, and *R. palustris*, probably also *R. esculenta*) the egg soon after fertilization becomes bilaterally symmetrical. As first Roux and later Schulze have shown, this is due to the appearance on one side of the border of the pigmented area of a grey crescent, caused by the retreat of the pigment into the interior. The grey crescent subsequently becomes white and added to the white area. The side on which it appears is opposite to that on which the spermatozoon had entered, and later becomes the dorsal side of the embryo; there is also a considerable correlation between the plane of symmetry thus bestowed upon the egg and the future sagittal plane, since the latter lies in a large majority of cases in or close to the middle point of the crescent.

This relation between the point of entry of the sperm and the egg- and embryonic symmetry has also been experimentally demonstrated by Roux, who, by applying the sperm to any arbitrarily selected meridian of the egg (by means of a fine cannulus, a brush, or a silk thread), was able to show that

fertilization can take place from any meridian, and that the point of entry so selected becomes the ventral side later on; in other words, the fertilization meridian becomes the sagittal plane. This, as we have seen, is true, or approximately true. Roux, however, believed that the plane of the first furrow also coincided with the other two, in fact that it was the first furrow which determined the sagittal plane, and he brought forward evidence to show that the first furrow, if it did not pass through the point of entry of the sperm, at least either included, or was parallel to, the inner end of the crooked path of the spermatozoon within the egg, the so-called 'copulation' path (Fig. 150). As we now know there is very little correlation between the first furrow and the sagittal plane. Nevertheless Roux's work remains of

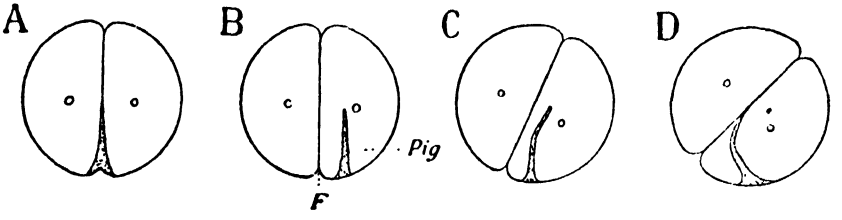


FIG. 150.—Roux's diagrams to show the relation of the sperm-path (*Pig.*) to the first furrow in the Frog's egg. In A the furrow includes the sperm-path, in B it is parallel to it, in C it is parallel to the inner portion of the path (copulation path), in D it includes only the very last portion of the copulation path. (From Korschelt and Heider, after Roux.)

the greatest significance, for it seems extremely likely that, while it is the actual point of entrance of the sperm and the first, radially directed, part ('penetration path') of its track within the egg, which determines the position of the grey crescent, and so of the sagittal plane, it is the second part which determines the direction of the first furrow, since the centrosome will divide at right angles to this 'copulation' path, or line of junction of the two pronuclei; the axis of the fertilization spindle is of course given by the line of separation of the two centrosomes, its equator by the plane including the two pronuclei and the egg-axis, and this spindle-equator is the plane of the first furrow.[1]

In the egg of the sea-urchin *Toxopneustes* the definitive egg-axis would appear to be fixed not by the original position of the egg-

[1] See, however, Appendix A.

nucleus at all, but by the position of the excentric segmentation nucleus. Both male and female pronuclei move through the egg for a longer or shorter distance till they meet, and the combined nucleus then takes up its definitive excentric position (Fig. 151). The plane of the first furrow is given by the egg-axis and the point of entrance, approximately, of the spermatozoon, which may thus be said to determine the symmetry of segmentation, and so of the embryo, since this plane becomes, it is said (Selenka,

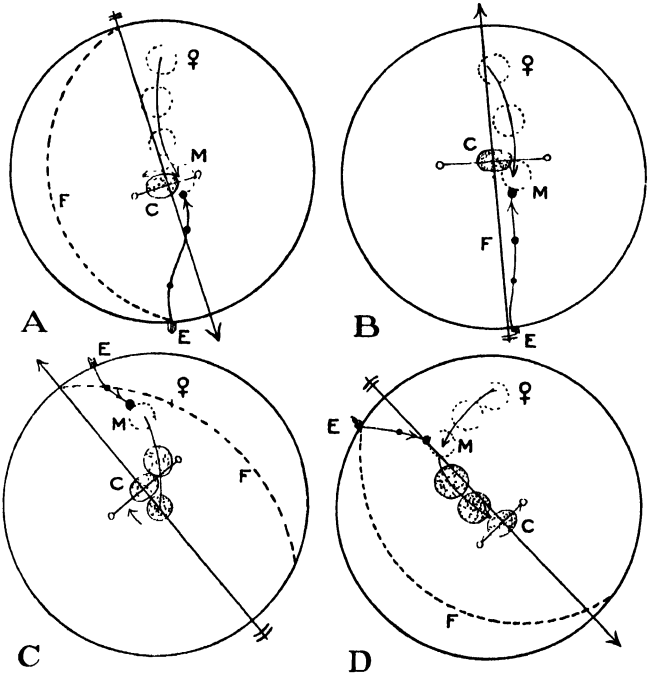


FIG. 151.—Diagrams from successive camera drawings of the living eggs of *Toxopneustes*, to show the determination of symmetry during fertilization.

The original position of the egg nucleus is shown by the position of the dotted circle marked ♀; its path by the succession of such circles.

E, the entrance-point of the spermatozoon, and cone of entrance. The male pronucleus is rendered in black, its path marked by the line uniting the black dots.

M is the meeting-point of the pronuclei. The cleavage nucleus, C, is shaded; its successive positions are indicated. The axis of the fertilization spindle is shown by the line with a small circle at each end passing through the cleavage nucleus; the arrow passing through this is the definitive egg-axis, its point the animal pole. The cleavage nucleus is slightly excentric, and nearer the animal than the vegetative pole.

F is the meridian of the first furrow.

A and B are opposed in polarity, similar in other respects. C and D differ in polarity, and otherwise as well. (After Wilson and Mathews.)

Garbowski, Driesch), the median plane of the Pluteus. In this case there is practically no distinction between 'penetration' and 'copulation' parts of the sperm-track, and, the centrosome dividing perpendicularly to this path, the point of entrance naturally lies in, or nearly in, the first furrow. The other alternative, as Wilson has pointed out, is to suppose that the definitive polarity of the egg existed before fertilization, but exerted no influence upon the egg-nucleus, although able subsequently to compel the fertilization nucleus to take up a position in its axis.[1] There are other Echinoderms, however (*Asterias*, *Strongylocentrotus*), in which the original and the definitive egg-axes coincide.

Another very interesting case is *Cynthia* (Fig. 142). In the immature egg of this Ascidian the cytoplasm is concentrically arranged, since the yellow pigmented protoplasm forms a peripheral investment for the central grey yolk: the egg-axis is determined only by the position of the germinal vesicle. Upon the entrance of the spermatozoon the yellow substance together with the clear plasma of the germinal vesicle, which has in the meantime broken down, streams towards the vegetative pole and becomes radially arrayed about the axis. By a further streaming movement these two substances are carried up with the male pronucleus—or pronuclei in cases of dispermy—to the equator, so marking the future posterior side of the now bilateral egg. These changes are evidently an effect of fertilization: it cannot, however, be asserted that the point of entrance of the sperm determines the posterior end, since it may enter on the opposite side and get carried across.

There are a few other instances in which an alteration of the egg-structure has been noticed to follow on fertilization. In Ctenophora the peripheral layer of granular cytoplasm becomes aggregated at the animal pole (Agassiz); in Polyclads (Lang) and Cirripedes (Groom) the egg becomes telolecithal; and so in *Physa*

[1] In *Diplogaster longicauda* also it appears to be the point of union of the two pronuclei which determines the definitive egg-axis and orientation of the embryo. The polar bodies are always extruded and the female pronucleus formed at that end of the ellipsoid egg which is turned towards the ovary, while the sperm enters at the opposite end. The pronuclei may meet and unite at either end; but it is at the end where their union takes place that the smaller of the first two blastomeres is found and, later, the hinder end of the embryo (H. E. Ziegler, *Zeitschr. wiss. Zool.* lx, 1890).

(Kostanecki and Wierzejski): the 'polar rings' appear in *Allolobophora*, *Clepsine*, and *Rhynchelmis* (K. Foot, Whitman, Vejdovský), while in Teleostei the periblastic hyaline layer becomes concentrated as the blastodisc at the animal pole. The case of *Dentalium* has been already referred to (p. 223, Fig. 133).

Although the cases in which the rôle played by the spermatozoon in determining egg and embryonic structure are, as has been observed, not very numerous, yet it is fully to be expected that renewed investigation will show that some such rearrangement of the materials of the egg is in many, perhaps in all, instances one of the first results of fertilization.

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§ 13. What Part does the Nucleus play in Differentiation?[1]

We have already criticized and rejected the hypothesis that the division of the nucleus during segmentation is a qualitative process. It may still be urged, however, that the nucleus is not insignificant in differentiation. In support of this contention the following arguments have been put forward.

1. It is urged that the nucleus is essential for the life of the cell,

[1] See also Appendix B.

and not only for its metabolism (Figs. 152, 153) but for the production of form (Fig. 154).

2. It is obvious that the germ-cells are the vehicles whereby the inheritable characters of the species are transmitted from one generation to the next; they are the material basis of inheritance. The germ-cells of the two sexes are, however, as unlike as possible in every character except their nuclei, in which, as the study of maturation has abundantly shown, they are exactly alike. In fertilization, the union of the two germ-cells, two distinct processes are involved. The first is the mutual stimulation whereby the lost power of cell-division is restored. This is a process independent of the nuclei (in Metazoa). The other is, however, the union of the nuclei (or their chromosomes); and since the offspring are held to inherit equally from the two parents, it has been supposed that with the union of the nuclei, the only parts of the cells that are exactly alike, the paternal and maternal contributions are intermingled, and that therefore it is in the chromatin of the nuclei that the vehicle of transmission of hereditary qualities must be looked for.

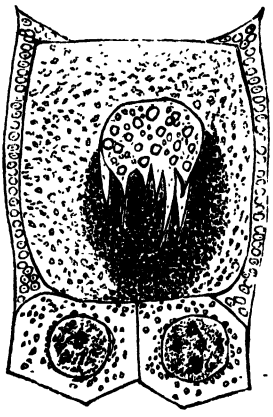


FIG. 152.—Egg-cell of *Dytiscus marginalis* in its follicle with two nurse cells. From the nurse cells nutritive material passes into the egg-cell, towards which the nucleus sends out pointed pseudopodia. (After Korschelt, from Korschelt and Heider.)

3. Thirdly, it has been pointed out (originally by Roux) that karyokinesis appears to be a mechanism expressly adapted to the simultaneous division of a large number of qualitatively different units. From the existence of karyokinesis it is argued that the nucleus is not homogeneous, and that in development the various units of which it is composed are directly concerned in the process of differentiation.

The doctrine of the individuality of the chromosomes has been brought forward in support of this belief, as well as the constancy of their number.

4. The diminution of the chromosomes. Boveri has observed in *Ascaris*, in the nuclei of purely somatic cells, a peculiar process

to which he has given the name of the diminution of the chromatin. The middle parts of the chromosomes in somatic cells become divided transversely into small granules, the ends remain rod-shaped. The granules of the middle parts are alone divided and pass to the daughter nuclei, while the ends are cast out into the cytoplasm and there degenerate. The chromosomes of the germ-cells (or of the parent-cells of the germ-cells) do not undergo this change, but remain intact (Fig. 155).

It might be argued that in such a case as this the determination of certain characters —somatic—is brought about by the expulsion of the chromatin into the cytoplasm.

5. A great deal of stress has been laid on the importance of the experiment, due to Boveri, in which the enucleate fragment of the egg of one species of sea-urchin gives rise, when fertilized with the sperm of another species, to a larva which exhibits the characters of the male parent alone.

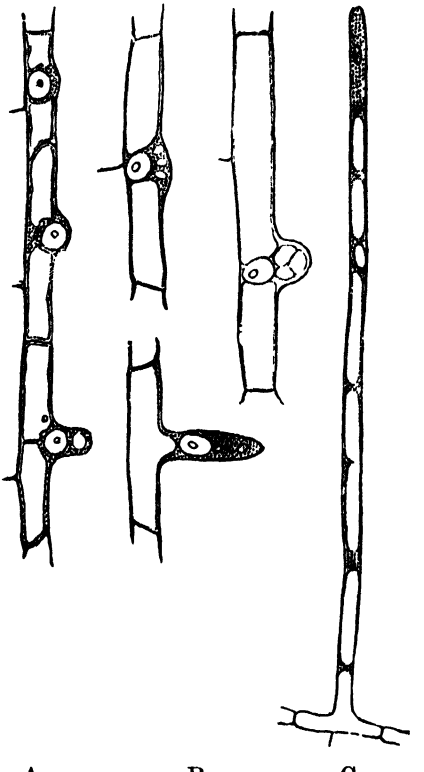


FIG. 153.—A, origin of a root-hair in *Pisum sativum*. The nucleus is placed at the point of origin. B, the same in *Cucurbita pepo*. C, root-hair of *Cannabis sativa*: the nucleus is at the growing point. (After Haberlandt, from Korschelt and Heider.)

6. Boveri has brought forward evidence to show that the abnormal development of Echinoderm eggs which follows on polyspermy is in reality due to the irregular distribution of the chromosomes to the daughter-cells. Hence it is argued that the chromosomes are qualitatively different.

These are the reasons which are, or have been, brought forward in support of the belief that the nucleus is not insignificant in differentiation. We may now discuss them in order,

premising only one thing, namely, that both maternal and paternal nuclei are certainly not necessary for the production of a new individual, however beneficial it may be for the elements of two parents to be commingled in the body of one offspring. In artificial parthenogenesis an egg is stimulated to development by a solution, and produces a normal larva; this creature possesses nuclei of maternal origin. The converse case is seen in the phenomenon of 'merogony'. An enucleate egg-fragment, fertilized with sperm of the same species, gives rise to a new organism, provided only with paternal nuclei.

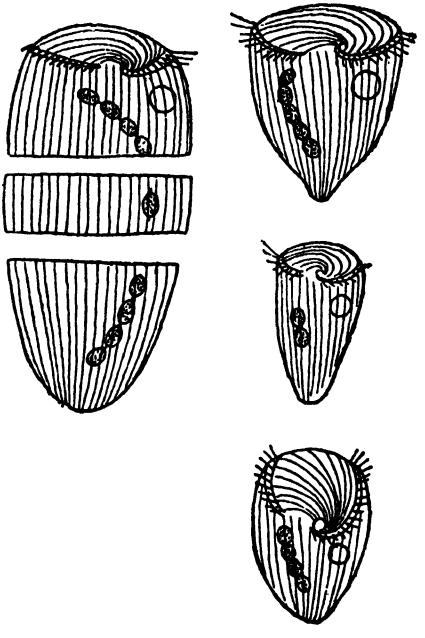


FIG. 154.—On the left the protozoon *Stentor*, cut into three pieces, each containing a piece of the nucleus. On the right, each piece has regenerated the missing parts and become a complete *Stentor*. (After Gruber, from Korschelt and Heider.)

The older view that the union of the pronuclei was the essential act in fertilization can no longer be held, for, certainly, both nuclei are not necessary. The most, therefore, that can be said will be that a complete set of the chromosomes (or smaller chromatin units) of the species is necessary for differentiation. Whether this is so or not we must now inquire.

1. It will of course be allowed that the nucleus is necessary for the life of the cell and to a certain extent for the assumption of form. The numerous and familiar experiments on Protozoa have sufficiently established this point.[1] A nucleate piece will live and regenerate lost parts, an enucleate piece will not (Fig. 154).

2. It will also be admitted that the maturation processes in the germ-cells of the two sexes are extraordinarily alike and end in the reception by each germ-cell of one-half the normal

[1] See especially M. Verworn, Die physiologische Bedeutung des Zellkerns, *Pflüger's Arch.* li, 1892; in this paper an account of the work of Gruber and others will also be found.

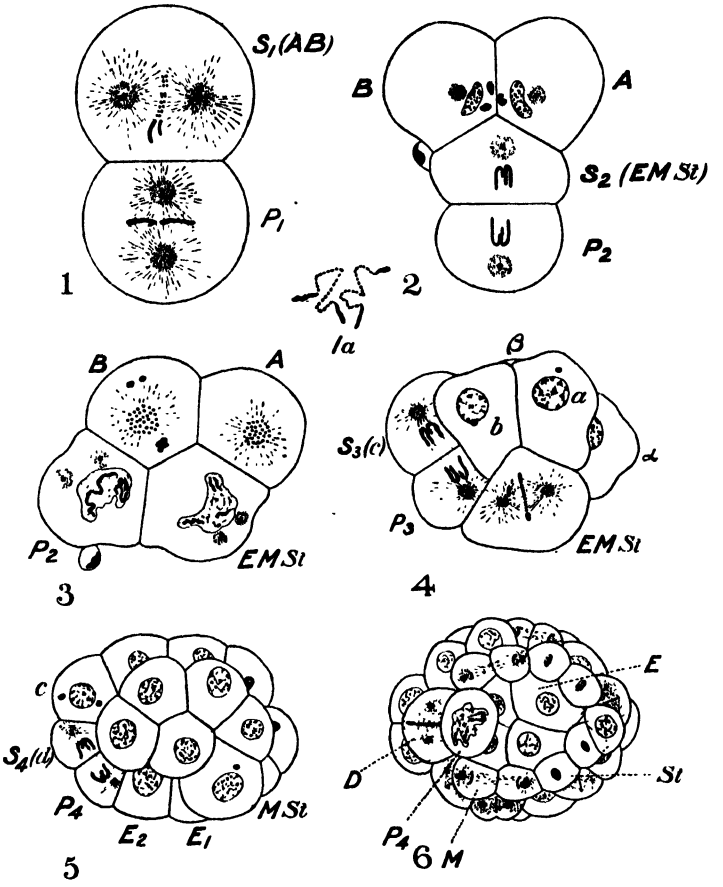


FIG. 155.—The process of chromatin diminution as seen in the somatic cells of *Ascaris megalocephala*. (After Boveri, 1899.)

1. Mitosis in the 2-celled stage. In the first somatic cell (S_1 or *AB*), the primary ectoderm, the chromatin undergoes diminution, not in the germ-cell (P_1). 1*a* chromosomes being 'diminished'.

2. 4-cell stage, T-shape. In *A* and *B* the discarded masses of chromatin are seen. S_2 (*E M St*), second somatoblast or endomesostomodaeal rudiment.

3. 4-cell stage, lozenge-shape. In *A* and *B* the next mitosis is beginning, in P_2 and *E M St* the nuclei are in the resting stage. *A* is anterior, *A* and *B* are dorsal, all four cells lie in one plane, the sagittal plane of the embryo.

4. In *E M St* the chromatin is being diminished. Division of P_2 into P_3 and S_3 (*C*), the secondary ectoderm. *a*, *b*, primary ectoderm of right, *a*, *β*, primary ectoderm of left side.

5. The endoderm (E_1, E_2) has now been separated from mesoderm and stomodaeum. P_3 has just divided into P_4 and S_4(*d*), tertiary ectoderm.

6. Diminution of chromatin in S_4 (*D*). The four endoderm cells (*E*) beginning to be invaginated: on each side two mesoderm cells (*M*) in which granular chromosomes may be seen, and two stomodaeal cells (*St*). Ventral view.

number of chromosomes of precisely the same shape and size. In fertilization the two sets of chromosomes—each in half the normal number—are united. It is of course difficult to believe that this extraordinary resemblance of the nuclei, while all the other characters of the cells are unlike, is without significance.

3. With regard to the third point a distinction must be made between two hypotheses. The first is the individuality of the chromatin, the second the individuality of the chromosomes.

For the first, as we shall see, independent evidence exists, and mitosis is certainly a mechanism admirably adapted to the simultaneous division (or separation of already divided halves) of a large number of qualitatively distinct bodies.

But the second hypothesis in no way follows from the first, for the grouping of the unlike units may obviously be different at each successive division without in the least impairing their individuality. There is, indeed, evidence for the persistent individuality of the chromosomes in only one or two cases. Boveri has noticed, in the segmenting egg of *Ascaris*, a constancy in the position of the pockets of the nuclear membranes which lodge the ends of the chromosomes, and a similarity in the arrangement of the chromosomes in the nucleus in successive nuclear divisions. Sutton again has observed that in *Brachystola* each chromosome forms a separate reticulum, situate in a separate pocket of the nucleus. There are other cases in which each chromosome forms its own vesicle (Echinoderm eggs). These instances are, however, few and far between. There are so many nuclei in which nothing can be observed of the chromosomes in the resting stage, often there is nothing but a fine granular mass, and a study of the early and end stages of mitosis seems to show that the chromosomes are precipitated from solution in the nuclear sap and redissolved when the division is over.

In such a solution the chromatin elements would retain their individuality, their qualitative differences, just as each one of a number of crystals retains its distinctive properties in a mixed solution and exhibits them when recrystallization occurs. In such a sense, and such only, is it possible to speak in general of the individuality of the chromatin.

4. With regard to the diminution of the chromosomes in

Ascaris, Boveri has pointed out that the extruded outer ends of the chromosomes are very irregularly distributed to the daughter-cells, whereas they should surely, if they are determinants, be equally divided between the two. More than that, however, Boveri has found evidence which shows that not only does the diminished chromatin not determine the characters assumed by the cytoplasm, but that on the contrary it is the cytoplasm which decides which chromosomes shall be diminished, and which remain intact.

Boveri has observed certain cases of dispermy in *Ascaris*, which are followed by simultaneous division of the ovum into four, consequent, presumably, on a quadripolar mitosis which is due, in turn, to the presence of an extra pair of centrosomes. The total number of chromosomes present in such eggs is $3n$, where n is the reduced number, since each spermatozoon introduces n. This number becomes doubled by division, and the number is then $6n$ or 12, since n in *Ascaris megalocephala v. bivalens* = 2. These twelve chromosomes have to be distributed over the four cells into which the ovum divides: their distribution is irregular. The next division is described as being tangential in two of the cells, at angles of 45° to the first divisions in the other two: it leads to the formation of two groups of four cells each, in each of which the cells are arranged in the T shape characteristic of the normal four-celled stage. The normal four-celled stage is reached by (1) an equatorial division, (2) followed by a meridional division in the animal cell (AB), and a latitudinal division in the vegetative cell, which separates a cell S_2, which like A and B is somatic, from a cell P_2 at the vegetative pole. The chromosomes in P_2 are intact, in A, B, and S_2 they are diminished (Fig. 155).

Boveri argues that if the diminution of the chromosomes were an intrinsic property of the chromatin, there should always be 6 ($3n$) chromosomes intact and the rest diminished, just as there are always 4 ($2n$) intact in the normal egg, no matter how the chromosomes had become distributed over the first four cells and their descendants. This, however, is not the case. Whole chromosomes are found only in those cells—one in each group of four—which correspond in position to the P_2 cell of the

normal egg; the chromosomes in the other cells are all diminished, and no cell contains chromosomes of both kinds. Further, the number of whole chromosomes may be 5 in one cell and 3 in the other, or 4 and 3, or 3 and 3, or 4 and 2, or 3 and 2.

Boveri concludes, it seems with perfect justice, that it is the (vegetative) cytoplasm which has determined that the chromosomes it contains shall remain whole, while, contrariwise, the animal cytoplasm of the other cells brings about the process of diminution.

In *Ascaris lumbricoides* there is a diminution of the chromosomes very similar to that seen in *A. megalocephala*, and, to judge from

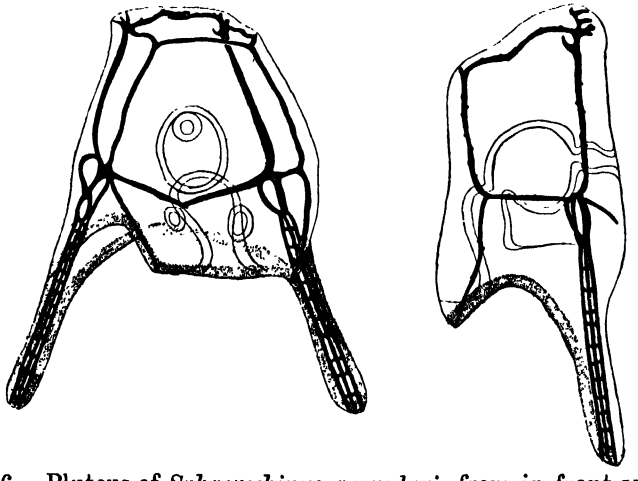


FIG. 156.—Pluteus of *Sphaerechinus granularis* from in front and from the side. (After Boveri, 1896.)

certain abnormalities similar to those just mentioned, brought about by the same means (Bonnevie). In *Dytiscus* there is something like a diminution of the chromatin in the nurse-cells, while the oocyte retains all the chromatic material intact (Giardina). The distinction seen here may, however, only be that observed in all ova between the nutritive and reproductive functions of the nucleus (trophochromatin and idiochromatin).

5. Boveri's hybridization experiment, on which rests the assertion that the nucleus alone conveys the inheritable characters of the species, was as follows.

The Plutei of *Sphaerechinus granularis* and of *Echinus microtuberculatus* were found (at Naples) to be markedly different.

The larva of the former (Fig. 156,) is short and squat, the oral lappet not divided into lobes, and the skeleton provided with a fenestrated anal arm—produced by three long parallel bars united by numerous cross-bars—an apical branch to the oral arm, and, at the apex, a square 'frame' formed by the union of twigs from the last mentioned and from the apical arms.

The larva of *Echinus*, on the other hand, is long and lank, the oral lappet is deeply cleft, and in the skeleton the anal arm is not fenestrated; there is no apical branch of the oral arm, and the ex-

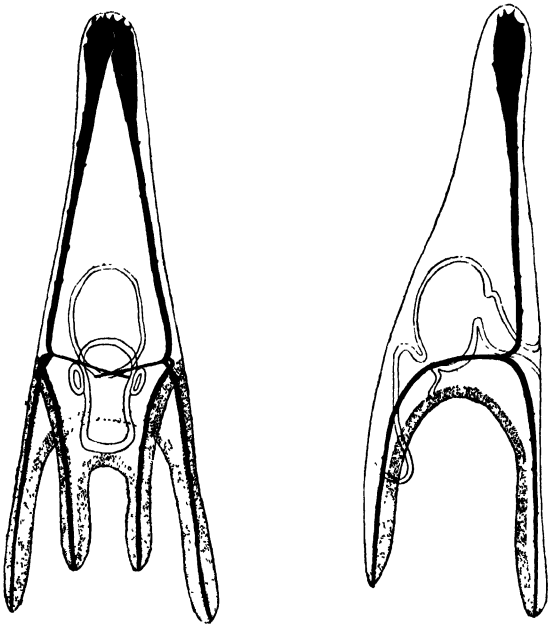


FIG. 157.—Pluteus of *Echinus microtuberculatus* from in front and from the side. (After Boveri, 1896.)

tremity of the apical arm is thickened and club-shaped (Fig. 157). Boveri first fertilized the ova of *Sphaerechinus* with the sperm of *Echinus*, and so produced a hybrid larva whose characters were intermediate between those of the two parents in the following respects—in form, being shorter and broader than that of *Echinus*, longer and narrower than that of *Sphaerechinus*, in having the oral lappet slightly divided, and in the skeleton, the extremity of the apical arm being swollen and branched, the anal arms being double but not fenestrated, and the oral arms being (sometimes) provided with an apical branch (Fig. 158).

A mass culture of egg-fragments of *Sphaerechinus*—containing presumably whole eggs, and nucleate and enucleate fragments—was then made, and this was fertilized with the sperm of *Echinus*. Amongst the larvae developed in such a culture Boveri found a small number (twelve) of dwarf individuals which possessed the paternal (*Echinus*) characters alone (Fig. 159 *b*), and he suggested that these had come from enucleate fragments of eggs. He was further strengthened in this opinion by the fact that the nuclei were small, which he attributed to their containing only one-half the normal number of chromosomes.

This conclusion has been adversely criticized by Seeliger. Seeliger, working at Trieste and subsequently at Naples, has

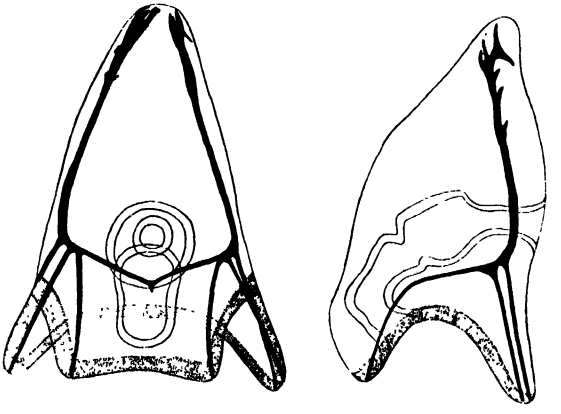


FIG. 158.—Pluteus of the cross *Sphaerechinus granularis* ♀ × *Echinus microtuberculatus* ♂, from in front and from the side. (After Boveri, 1896.)

pointed out that the differences between the Plutei of these two sea-urchins are not as great as Boveri asserted them to be—the extremity of the apical arm in *Echinus* is, for instance, not always club-shaped—and that therefore it cannot be so confidently asserted that the hybrid is intermediate. Secondly, he asserts that in an ordinary hybrid culture (whole eggs of *Sphaerechinus* fertilized by sperm of *Echinus*) there are to be found together with variable larvae of more or less an intermediate type, individuals with purely paternal characters, though the pure *Sphaerechinus* type never occurs, and that the nuclei are variable in size. This contention has been upheld by Morgan, and, after a good deal of controversy, has finally been admitted by Boveri himself. No conclusion can therefore be drawn from the original experiment.

An explanation of the discrepancies in the results of the different observers has been offered by Vernon. Vernon has found that *Strongylocentrotus lividus* (which has a Pluteus almost exactly like that of *Echinus*) is at a minimum of sexual maturity in the summer months, but that from that time onwards the power of the male to transmit its characters to the hybrid larva, when crossed with *Sphaerechinus* ♀, increases, and that it is possible to obtain a culture consisting entirely of larvae of the paternal type, in respect, that is to say, of the skeleton. Vernon was inclined to look upon this difference in maturity as a seasonal variation, but Doncaster has since attributed it to the effect of temperature alone.

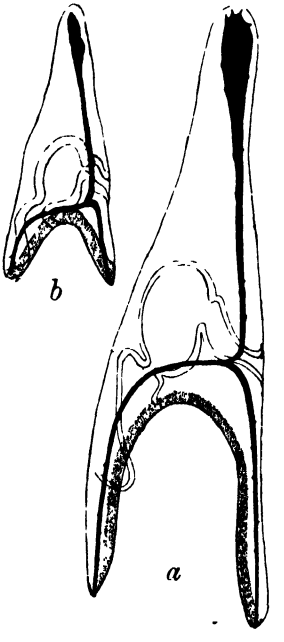


FIG. 159.—*a*, Fully formed Pluteus of *Echinus microtuberculatus*. *b*, Young dwarf larva of the pure *Echinus* type reared from an enucleate (?) egg-fragment of *Sphaerechinus* fertilized with the sperm of *Echinus*. (After Boveri, 1896.)

The transmission of the characters of the skeleton may therefore be the function of the nucleus, though, as just pointed out, there is no stringent proof of this. There are other larval characters, however, which, as Driesch has justly urged, must depend on the cytoplasm of the ovum, the colour, for example, if the egg is pigmented, and the size of the larva, so far as this depends on the size of the egg. From a series of hybridization experiments between *Echinus*, *Sphaerechinus*, *Strongylocentrotus*, and *Arbacia*, Driesch indeed concludes that the rate of cell-division up to the formation of the primary mesenchyme, the vacuolation of the ectoderm cells (in *Sphaerechinus*), the number of the primary mesenchyme cells, are all characters which depend on the ovum, and the ovum alone, and therefore on its cytoplasm. With regard to the skeleton Driesch admits that this, and hence, indirectly, the shape of the larva, may be influenced by the sperm, at least when the egg of *Sphaerechinus* is fertilized either by *Echinus* or *Strongylocentrotus*. Fischel has made similar experiments and arrived at a like conclusion.

Boveri has traversed some of these statements of Driesch's. The number of primary mesenchyme cells is, he maintains, a mean between the paternal and maternal number, and the form of the larva may be intermediate before the skeleton is developed. Between these contradictory opinions it is not easy to decide, but it may be mentioned that the number of mesenchyme cells is peculiarly liable to variation, as Driesch has himself found.

Before concluding this section we should mention some most interesting experiments on heterogeneous cross-fertilization.

Loeb was the first to show that by the addition of a small quantity of calcium chloride and sodium hydrate to sea-water, it was possible to fertilize the eggs of *Strongylocentrotus purpuratus* with the sperm of *Asterias ochracea*. The matter has since been more completely investigated by Godlewski, who has succeeded in rearing Plutei from the eggs of sea-urchins fertilized with the spermatozoa of the Crinoid *Antedon*. The necessary condition of success is the prior treatment of both eggs and sperm with an abnormally alkaline sea-water (about 1·75 c.c. $\frac{n}{10}$ NaOH per 100 c.c. of sea-water).

The sea-urchins employed were *Sphaerechinus*, *Strongylocentrotus*, and *Echinus*. The eggs of the first reached the gastrula stage, of the two last the Pluteus. Fertilization was found to be perfectly normal, with production of a vitelline membrane, rotation of the sperm-head, and so on. Cleavage was of the Echinoid type (maternal) with formation of micromeres at the fourth division; no such micromeres occur in *Antedon*, the fourth division being meridional in both hemispheres. Primary mesenchyme was formed (this again is absent in *Antedon*), but the number of cells was slightly in excess of the normal: and after gastrulation the skeleton of the Pluteus was developed (except in *Sphaerechinus*); the larva of *Antedon* of course has no skeleton.

The *Antedon* chromosomes persisted and the nuclei of the hybrids were intermediate in size between those of the parent forms.

When an enucleate fragment of the egg of *Echinus* was fertilized by *Antedon* all the processes mentioned above were normal, except that segmentation was irregular, and that development ceased after gastrulation. The death-rate of these larvae was high.

6. It remains for us now to consider the conclusions which Boveri has drawn from the behaviour of eggs in which owing to pathological mitosis the distribution of the chromatic elements was irregular.

The investigations of O. and R. Hertwig had previously shown that if the eggs of sea-urchins were weakened by treatment with poisons (strychnine, quinine, and others), then the vitelline membrane which normally prevents the entrance of more than one spermatozoon was not formed, that consequently several

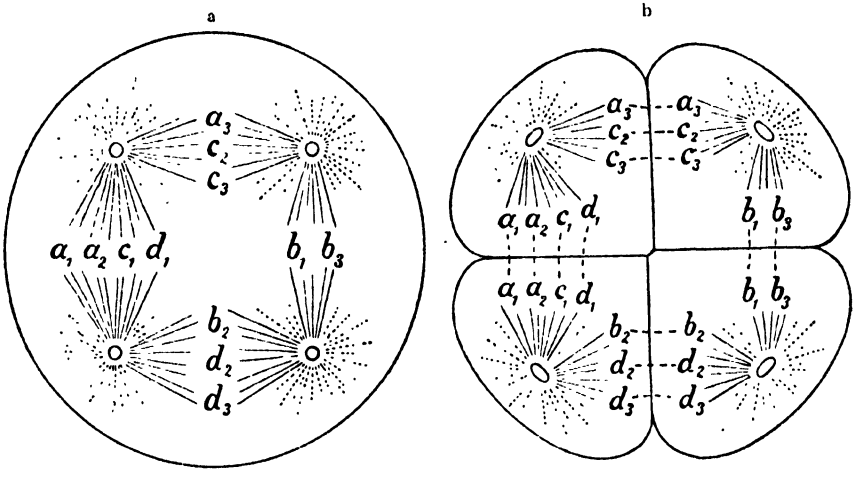


FIG. 160.—Diagram of one case of irregular chromosome distribution in a doubly fertilized egg; a_1, b_1, c_1, and d_1; a_2, b_2, c_2, and d_2, and a_3, b_3, c_3, and d_3 are the three complete, specific sets of unlike chromosomes. (After Boveri, 1904.)

spermatozoa entered, with the resulting formation of multipolar mitoses and irregular cleavage.

Boveri has succeeded in inducing dispermy in the eggs of sea-urchins without the use of the poisonous reagent, by simply adding an excess of sperm to the eggs. The number of abnormal larvae found in such a culture is greater than that observed when the quantity of spermatozoa used is less, and in proportion to the number of dispermic fertilizations.

In dispermic eggs there is an extra pair of centrosomes, and these two usually form with the other two a quadripolar figure with four spindles occupying the four sides of the square (Fig. 160). In the equators of these four spindles are placed the chromosomes,

the number of which is $3n \times 2$, where n is the reduced number (in *Echinus* 9), since there are two male and one female pronuclei and all the chromosomes have divided. Cell-division occurs across each spindle and the whole breaks up simultaneously into four. To these four cells the $3n \times 2$ chromosomes are distributed, and, as Boveri points out, the chance of each cell obtaining a complete set of the n chromosomes of the species (supposing these to be different from one another) is

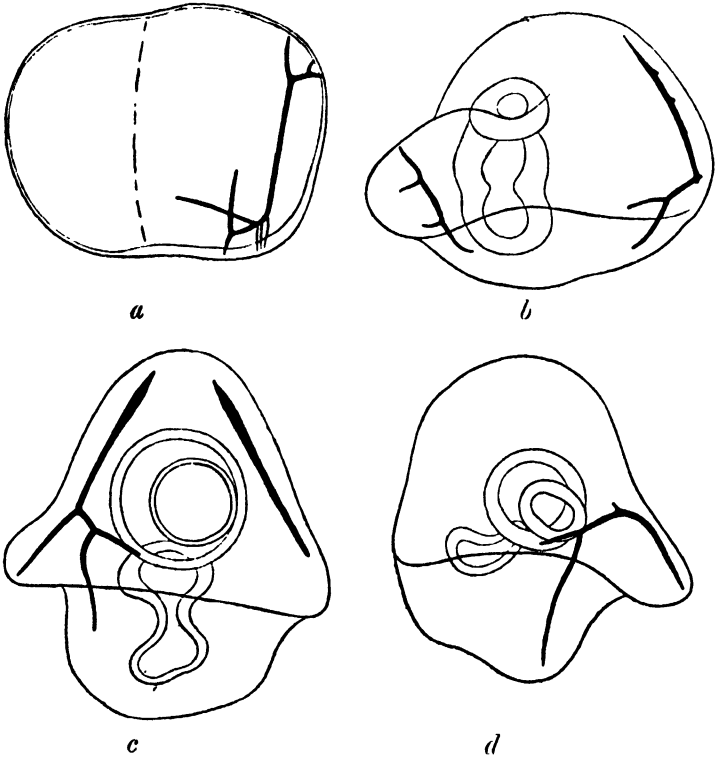


FIG. 161.—Larvae from doubly fertilized eggs; *a*, of *Sphaerechinus granularis*; *b–d*, of *Echinus microtuberculatus*; *a*, *c*, and *d* are from tripartite, *b* from a quadripartite egg. (After Boveri, 1904.)

small. Such eggs, as a matter of fact, practically always give rise to abnormal larvae. Out of 1,170 examined not a single one was normal.

The chance, however, that one cell of the four will receive a complete set of chromosomes is far greater, and when the $\frac{1}{4}$ blastomeres were isolated it was found that 25 % gastrulated, though none gave rise to Plutei. Moreover, all four developed differently.

Again, by shaking the eggs it is possible to prevent the division of one centre. A tripolar mitosis is the result and a simultaneous division into three. Out of 695 such eggs 58 became normal Plutei. This is also in accordance with the theoretical expectation, for the probability of each of three receiving a complete set of chromosomes when there are $3n \times 2$ to be distributed is much greater than when these chromosomes have to be distributed amongst four cells.

The abnormal larvae are of various types, some showing no trace of the Pluteus organization, some a small trace, such as a skeleton on one side. Others have no gut, or some of the pigment cells are missing (Fig. 161).

The distortion of development cannot be attributed to the deficiency in the number of chromosomes. Each of the cells of a quadripartite ovum may have $\frac{3n \times 2}{4} = \frac{3n}{2}$ chromosomes, for the larvae reared from them may have all their nuclei of the same size (containing the same number of chromosomes), and it is known (artificial parthenogenesis and merogony) that a larva will develop quite well with only n. Nor is it needful that all the parts should have the same number of chromosomes, for normal larvae may be experimentally produced, with n chromosomes in the nuclei of one region of the body and $2n$ in the rest (see below). At the same time dispermy does not necessarily involve abnormality: in such eggs the two pairs of centres may remain apart and no quadripolar figure be produced: these develop into ordinary Plutei (Fig. 162 B). Conversely, quadripolar mitoses may be found in monosperm eggs, but these produce pathological blastulae. No explanation of the abnormality, therefore, is possible, so Boveri concludes, except that it is due to the irregularity in the distribution of the chromosomes. For normal development it is essential that each cell should receive not a definite number but a definite combination of chromosomes, a complete set of the chromosomes of the species. It is the lack of this complete combination in one or more cells that is the direct cause of the derangement of development.

Hence the chromosomes are not alike, but qualitatively different.

Their division is, however, never (except in reduction) a

qualitative but always a quantitative process. The combination is handed on in its entirety to every cell of the body. The chromatin has in this sense an individuality, for however much the chromosomes may disappear while the nucleus is at rest, the set of qualitatively distinct units always re-emerges (though not necessarily in the same order) to be quantitatively divided between the two daughter-cells. We reach, therefore, a conception of the chromatin akin to Roux's idea of the 'Reserve-idioplasson', the germ-plasm which is passed on entire to all the tissues of the body.

And now Boveri proceeds to develop a theory already suggested by Driesch of the part played by the chromatin in differentiation. The cytoplasm is heterogeneous, anisotropic; and these differences in the cytoplasm are sufficient to account for the earliest differentiations, the pattern of segmentation, the determination of the embryonic axes and possibly some few others. But then the chromatic elements come into play—not the chromosomes, which are too large and too few to be regarded as determinants—but far smaller subdivisions of these. Under the influence of the cytoplasm, different in the various cells, some of the units become patent and active, while others remain latent, and by the reaction of the former upon the cytoplasm new differentiations arise which will in their turn call forth into activity new nuclear elements.

Whatever value may be attached to these speculations, there can be no doubt of the importance of the experiments on which they are founded. Provisionally we have good ground for believing in the dissimilarity of the chromatic elements and of their necessity for differentiation. It only remains to be seen whether further investigations on other forms will bear out the interpretation which Boveri has placed upon his observations.

There is one more point worthy of notice. An ordinary sexually produced individual possesses in its nuclei $2n$ chromosomes, that is, two complete sets. In maturation one division of the chromosomes is transverse, and Boveri suggests that in this transverse division homologous units are separated from one another, or, to use the expression made familiar by current Mendelian theory, members of pairs of allelomorphs.

Before bringing this section to a close a brief reference must

be made to the relation established by Boveri, as a result of the examination of sea-urchin larvae (*Echinus*), between the number of chromosomes on the one hand and the size of the nucleus and the size of the cell on the other.

The number of chromosomes in a larva, or part of a larva, may be varied in the following ways.

1. In merogony the number is half the normal number, i. e. *n*. (The larva is termed by Boveri hemikaryotic arrhenokaryotic.)

2. In artificially parthenogenetic larvae it is also *n*. (Hemikaryotic thelykaryotic.)

3. In 'monaster' eggs the number is twice the normal, i. e. 4 *n*. (Diplokaryotic.)

This condition is brought about by shaking the eggs, and so preventing the division of the centrosome. The pronuclei unite and enter a resting stage. From this the chromosomes emerge in the full number and split, but since the centrosome remains single, there is no cell-division, and the chromosomes once more form a resting nucleus, but now in twice the normal number.

Later the centrosome divides and the egg develops normally.

4. If the eggs are kept for twenty-four hours in sea-water and then fertilized with sperm which has been weakened in dilute potash the male pronucleus lags behind its centre. The latter divides about the female pronucleus, and thus in the two-celled stage one blastomere has a male and a female nucleus, and therefore 2 *n* chromosomes (amphikaryotic), while the other has only a female (thelykaryotic). In this stage, or subsequently, the male unites with the female pronucleus, and so one-half, or one-quarter, possibly only one-eighth, of the larva has nuclei with 2 *n*, the remainder nuclei with *n* chromosomes.

5. It sometimes happens in dispermy that the two pairs of centrosomes remain apart without forming a quadripolar figure (Fig. 162). Such eggs, as we have seen, develop normally. Sooner or later the cells become divided into two sets, one with nuclei containing 2 *n* chromosomes (amphikaryotic), derived from the female and one of the male pronuclei, the other having *n* chromosomes from the remaining male (arrhenokaryotic).

A superficial examination shows that the nuclei of hemikaryotic embryos and larvae are smaller but more numerous than in

normal (amphikaryotic) embryos or larvae of the same stage. In diplokaryotic larvae, on the other hand, they are larger and fewer; while in larvae in which one portion is amphikaryotic, the other hemikaryotic, the two regions are distinctly marked off by the difference in the size and number of their nuclei (Fig. 163).

The exact relation between the number of chromosomes originally entering into the composition of the nucleus and the dimensions of the nucleus and the cell has been ascertained by Boveri to be as follows :—

I. The surface-area of the nuclei is directly proportional to the number of chromosomes. The chromatin, it is worth noticing, is often placed beneath the nuclear membrane.

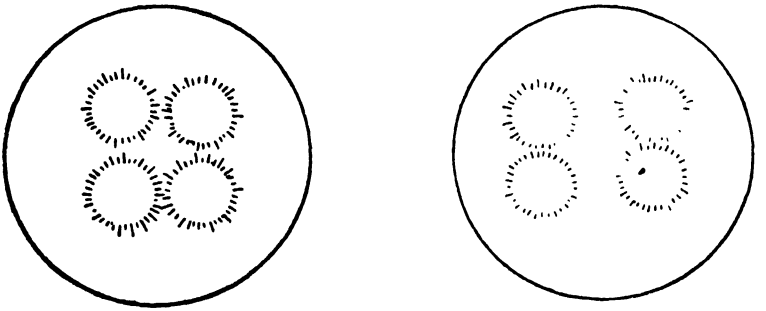


FIG. 162.—Dispermic eggs of *Strongylocentrotus lividus*. A, The four centres have united to form a tetraster. B, The two pairs of centres have remained apart to form two independent spindles, one of which contains the female and one of the male nuclei, the other the remaining male nucleus. (After Boveri, 1905.)

II. The number of nuclei is inversely proportional to the number of chromosomes; but

III. Cell-volume is inversely proportional to the number of cells in larvae of the same stage developed from whole eggs of the same size; hence

IV. Cell-volume varies directly with the number of chromosomes and with the surface area of the nucleus. This Boveri terms the fixed relation between nucleus and cytoplasm.

V. It follows from I and IV that nuclear-volume increases more rapidly thon cell-volume.

VI. Conversely, the law holds good of egg-fragments of different sizes but containing the same number of chromosomes.

Since, in the larvae developed from such fragments, the

thickness of the cell-layers is the same, the surface may be taken as an index of the volume and is proportional to the original volume of the fragments. Since the number of chromosomes is the same, the cell-volumes should be the same, and the number of cells ought to be proportional to the surface. This was, as a matter of fact, found to be true in the few instances examined.

This result is merely a restatement of Driesch's rule, that in larvae developed from isolated blastomeres the surface of the larvae and the number of cells are both proportional to the germinal value.

It appears, therefore, that the moment at which segmentation shall end may be determined by the moment at which this constant relation between nucleus and cytoplasm is reached.

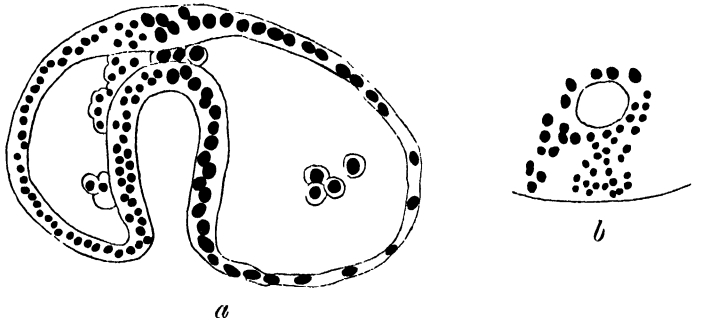


FIG. 163.— *a*, Gastrula reared from an egg of *Strongylocentrotus lividus*, in which the sperm nucleus had passed entirely into one of the first two blastomeres. Optical section of a stained preparation. (After Boveri, 1905.)

b, Ectoderm in the neighbourhood of the blastopore of a dispermic gastrula of *Strongylocentrotus lividus*. (After Boveri, 1905.)

Driesch has, however, recently reported certain exceptions to his rule, amongst the mesenchyme cells in particular. These may be more or less numerous than they should be, may differ in size and in the size of their nuclei. It is possible, as suggested, that the division of the centrosome has been suppressed (monaster) with consequent reduplication of the chromosomes. Such an explanation may account for the variability in the size of the nuclei observed by Seeliger in his hybrids.

In the Alga *Spirogyra* Gerassimow has observed a close relation between the size of the nucleus and that of the cell; here, however, it is the nuclear volume which is directly proportional to the cell-surface.

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2. ON THE ACTIONS OF THE PARTS OF THE DEVELOPING ORGANISM ON ONE ANOTHER

Up to the present we have discussed only the original structure of the fertilized ovum as an internal factor in differentiation. We have now to consider to what extent the development of each part may be due to influences exerted upon it by other portions of the embryo.

One of the first suggestions of such an interaction was put forward by Oscar Hertwig. 'We recognize', he says in his *Zeit- und Streitfrage*, 'apart from the nature of the egg-substance itself, the conditions of development, first in those perpetually changing mutual relations in which the cells of an organism are placed to one another, and secondly in the influences exerted upon the organism by the external world'; and again, 'physiologically expressed, the dissimilar differentiation of cells consists in the reaction of organic substance to dissimilar stimuli,' and

he proceeds to illustrate his meaning by referring to the influence of light in determining whether the sexual organs shall be formed on the upper or under side of a fern prothallus, to the dependence of the fate of indifferent buds of plants upon external conditions, to the formation of stems on the upper, roots on the lower side of a horizontal stolon of *Antennularia*, as demonstrated by Loeb, and to certain alterations in the character of epithelia and other tissues which are brought about by pathological conditions.

For a more detailed elaboration of this very fruitful idea we are indebted to Herbst. Herbst classifies organic reactions to stimuli as either directive or formative. The former are of two kinds, tactic when the response is some locomotion of a freer body, tropic when the movement exhibited in response is a movement of growth. The reactions may be further classified according to the nature of the stimulus, gravity, light, heat, electricity, chemical substances, currents of water, the contact of a solid surface. Thus we have positive and negative heliotropism, galvanotaxis, geotropism, galvanotropism, thigmotaxis, and so on, and many examples of all of these are cited. These are mostly familiar facts, but some, for their special interest, may be quoted here. Thus the hydroid *Sertularella* in one-sided illumination produces stolons instead of hydranths, the tubes of the Polychaet *Serpula* grow upwards towards the light (or against gravity), an inverted stock of *Sertularia* produces a polyp sympodium at the upper—originally lower—end, the stems of *Antennularia* are negatively, the stolons positively geotropic, spermatozoa swim to solid bodies, and stolons of Hydroids attach themselves to some foreign object.

Speaking generally, a specific reaction depends not merely on the nature of the stimulus, but on the structure of the reacting body itself. Further, the degree of sensitiveness to the stimulus is not constant. It varies at different periods of life, under the influence of external conditions such as temperature, and with the strength of an already existing stimulus (Weber's Law).

Now many of the events of ontogeny, as we have seen, resolve themselves into movements of the parts of the organism—movements of free parts, locomotion, and growth movements—

and the suggestion is made by Herbst that these movements are to be interpreted as so many responses to stimuli, which are then not merely directive but formative, the stimuli being either external or exerted by other parts of the body. Thus the spreading of the blastoderm over the yolk may be a case of oxygenotaxis, the movement of cells of the Frog's blastula towards one another described by Roux[1] as Cytotropismus may be due to a mutual chemotactic stimulus, the migration of cells to the surface of the Arthropod ovum is perhaps oxygenotactic, the vitellophags are chemotactic, the application of mesoderm cells to the outgrowing axis cylinders of nerve fibres, of connective tissue and muscle-cells to blood-vessels, of the choroidal cells to the optic cup (they are absent ventrally when the choroid fissure fails to close), the reunion of separated blastomeres in Triclads and others, may all be tactic phenomena of a positive kind, while the dispersal of the elements of a complex may be negative. Similarly, the outgrowth and anastomoses of nerves, glands, ducts, the concrescence of layers may be tropisms of various sorts.

The suggested stimuli in some cases proceed from the external environment, in others they are exerted by one part on another. The former have already been discussed, but it may be pointed out again that the effect called forth depends both on the stimulus and on the reacting organism or organ. Neither in these cases nor in any others is the production of specific form due to the action of an external agency upon a homogeneous material. For even in those instances where the fate of some apparently indifferent tissue seems to be decided by the action of such an agency—as in the production of stems from whichever side of an *Antennularia* stock happens to be uppermost (Loeb)—it is evident that all that the external agent can do is to decide between a limited number of definite alternatives in respect of each of which the parts of the organism are equipotential. Responses to stimuli of the second kind possibly play an important part in ontogeny. Unfortunately, the experimental evidence for this is at present somewhat meagre.

In the fish *Fundulus* the yolk-sac is deeply pigmented, the pigment-cells being especially closely aggregated round the

[1] See above, Ch. II. 1, p. 55.

blood-vessels. When the creature is deprived of oxygen the pigment disappears, and it is supposed that under normal conditions the pigment cells are chemotactically attracted by the oxygen in the blood (Loeb).

Two other cases come from Echinoid larvae. Driesch has been able to displace, by shaking, the two groups of primary mesenchyme cells, those destined to secrete the skeletal spicules of the

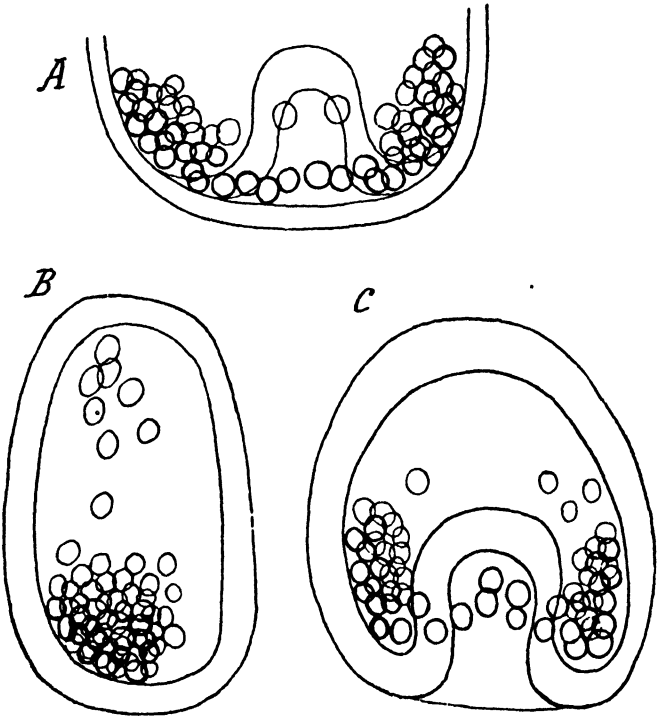


FIG. 164.—Diagrams of Driesch's experiment into the responsiveness to stimuli of mesenchyme cells in *Echinus*. *A*, Normal arrangement of mesenchyme cells. *B*, The cells deranged by shaking, returning in *C* to their original position. (From Korschelt and Heider, after Driesch.)

larva. The cells, displaced towards the animal pole, return to their original position after six hours, and subsequent development is normal (Fig. 164). Driesch has attributed this movement of the cells to a stimulus exerted by the endoderm.

Again Herbst has found that when the larvae of sea-urchins are placed in solutions of lithium salts or in sea-water deprived of the sulph-ion, or devoid of the carbon-ion, the skeleton of the

Pluteus is either distorted, in the first two cases, or absent, as in the last. The distortion takes the form of a multiplication of the triradiate spicules, and the arms are correspondingly multiplied. In water devoid of CO_2 there are no spicules and no arms.[1] Herbst has therefore urged that normally the outgrowth of the ciliated ring into the arms is due to a stimulus—thigmotropic, perhaps—exerted by the tip of the spicule. The converse of this is seen later on when the arms diminish in length as the calcium carbonate of the Pluteus skeleton is made use of by the developing urchin.

The development of the lens of the vertebrate eye has also been asserted to be due to a contact stimulus exerted by the optic cup upon the overlying ectoderm. Spemann was the first to show that when the medullary plate of the Frog (*Rana fusca*) was injured in front of the optic vesicle, the optic cup (if developed at all) may or may not come in contact with the ectoderm, the latter being, at this point, uninjured. In the first case a lens is formed, in the second not. Similar experiments have been carried out by Lewis on other species of Frog and on *Amblystoma*, with similar results. Lewis has also shown that a lens will be formed from any patch of ectoderm taken from some other part of the body and grafted over the optic cup. Schaper's evidence in support of this conclusion is doubtful. This author removed the whole of the nervous system from a 4 mm. tadpole by a horizontal cut with the exception alone of the downwardly growing fore-brain and optic vesicles. The wound healed and development continued. The optic cup was solid, being filled with a mass of degenerating cells. A lens was formed opposite the upper edge of the optic cup. It did not, however, separate from the ectoderm, but differentiated *in situ*, with development of lens fibres. Hence Schaper argues that the formation of the lens is a process of self-differentiation. The contact of the optic cup is, however, as he admits, in this case not excluded.

A more serious objection is raised by Miss King, who has found (in the Frog) that even when, owing to a prior injury, the optic cup is far away from the skin, or in some cases absent altogether,

[1] See above, Ch. III. 8, Figs. 73, 74.

the lens may still be formed. It may also be mentioned that Mencl has described a double-headed monster of *Salmo salar* in which one head has two well-formed lenses, though the other parts of the eye are absent. The lenses, however, lie quite close to the fore-brain, which may have exerted the stimulus, supposing a stimulus to be necessary.

It will be seen that the evidence is extremely contradictory, and forbids any definite conclusion.[1]

It is curious that in tadpoles grown in certain solutions (sodium chloride, bromide, and nitrate) the optic cup is often at some distance from the ectoderm, and that in these cases the lens is absent, though it may happen that the upper edge of the optic cup is sharply bent down in front of the aperture, as though to form a lens from the edge of the iris, as occurs in the regeneration of the lens, after extirpation, in the Newt.[2]

Spemann and Lewis have also found that in the absence of contact between the optic cup and the ectoderm the cornea is not developed, that is to say, the overlying ectoderm does not 'clear' (lose its pigment), it does not thin out, and Descemet's membrane is not formed. The more extensive series of experiments is due to Lewis, who turned back a flap of skin and wholly or partially removed the optic vesicle, the skin being then returned to its place. In the first case there was no eye, no lens, no cornea, though on the other side of the body all were present. In the second case the lens and cornea were both formed, provided that enough was left of the vesicle for the optic cup to come into contact with the ectoderm. As a control experiment the flap of skin was merely removed and replaced; the development of the eye was normal. If at a later stage, when corneal changes had already begun, both optic cup and lens were taken out, the cornea was not completed. The pigment indeed disappeared, but the ectoderm did not diminish in thickness and Descemet's membrane was not formed.

Removal of the lens alone did not interfere with the production

[1] Stockard has recently shown that when the egg of *Fundulus* is placed in $MgCl_2$ the two optic cups wholly or partly fuse while a single or double median lens is developed (*Arch. Ent. Mech.* xxiii, 1907). This would seem to support the view advanced by Lewis.

[2] See above, Ch. III. 8, p. 134.

of the cornea provided that the optic cup could touch the ectoderm, and if this same condition is fulfilled, the cornea will be differentiated in the ectoderm that grows in after the layer that originally lay over the eye had been destroyed, but if the cup and lens are taken away no new cornea is formed even after four weeks. The size of the cornea is in proportion to the area of contact. Finally, if, at a later stage still, the optic cup and lens are taken away, the already formed cornea degenerates.

Should the results of these extremely interesting investigations be confirmed, the development of some parts at least of the vertebrate eye would be processes of 'dependent differentiation'[1], dependent, that is, on causes outside themselves, on stimuli exerted by other parts, though not, of course, wholly dependent, since the structure of the reacting, as well as that of the stimulating, organ is contributory to the quality of the effect. How far such an action of the parts upon one another is of general occurrence as a factor in differentiation future research alone will show. It seems probable, however, that it will in any case be found to be limited to the first moments in the formation of the organs[2], for we know from other evidence that as development proceeds the system which was 'equipotential', to use Driesch's phrase, becomes 'inequipotential', that the parts lose their totipotence, that they gain independence and the power of self-differentiation, that the value of the correlations between them diminishes. This is what Minot has called the law of genetic restriction. But in early stages there are correlations between the organs, the differentiation of one does depend upon that of another, and it is this mutual stimulation of the parts that figures so prominently in the theory of development worked out by Driesch. This theory we shall have to examine in the following chapter.

[1] Although the expression 'dependent differentiation' is due to Roux, and although Roux expressed the opinion that certain developmental processes—the beginning of parthenogenetic development, the determination of the position of the grey crescent and first furrow by the entrance-point of the spermatozoon, the 'post-generation' of the dead blastomere by the living one in the Frog's egg, for instance—were of the nature of responses to stimuli, still the rôle assigned by the 'Mosaik-Theorie' to the influences of the parts upon one another in differentiation was subordinate, and restricted to the later stages of development.

[2] Except, presumably, in cases of 'composition' where elements of different origin unite in the formation of a single organ.

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CHAPTER V

DRIESCH'S THEORIES OF DEVELOPMENT

General Reflections and Conclusions

To the inquirer into the causes of development the central difficulty must always be the problem of differentiation. Growth and division of the nucleus and the cell, processes which always accompany differentiation, are, as we have seen, side issues; but the increase of structure, the production of form out of the relatively formless germ, and the gradual passing of this into a new individual which is like the parents that gave it birth, this is the marvel which has always excited the wonder of the observer, and demands all his wit to understand and to explain.

Experimental investigation, as far as experiment has at present gone, has shown, first, that a certain complexity of the physical and chemical environment is a necessary condition of normal development; that complexity may, it is true, vary within certain limits, but those limits can only be transgressed under pain of abnormality or death. In the second place it has been demonstrated that the initial structure of the germ, and the mutual interactions of its parts as they develop are both indispensable internal factors. It now only remains for us to discuss the value of those theories which are not only attempts to explain, to give the most general account of, the phenomena in causal terms, but also serve to provide a light to guide the investigations of the future. One such hypothesis we have already examined in some detail, Roux's hypothesis of self-differentiation. According to this belief not only is the development of each part determined by causes which reside in itself alone, but the parts—or rather their determinant representatives, which are all *ex hypothesi* necessarily present *ab initio* in the undeveloped germ—are located in the nucleus. The qualitative division of the nucleus sunders these units from one another, which then determine the characters assumed by the cytoplasm, and so the whole process happens.

As we have seen, the hypothesis, in this its original form, is

untenable. Quite apart from the fact that the complex architecture of the nucleus still demands an explanation—an explanation of the same kind, perhaps, which would at once involve us in an infinite regress—the facts which experiment has brought out show conclusively that nuclear division is never a qualitative process. In the other direction the hypothesis errs in attributing a homogeneity, an isotropy to the cytoplasm, for the same experiments have proved the existence in the ovum of definite substances, necessarily concerned in the production of the primary organs of the embryo.

There is, however, no evidence to show that—as imagined in Weismann's, and to a certain extent in Roux's hypothesis—there is a separate morphological unit for each separately inheritable character of the species; such an idea would indeed seem to be precluded by the ease with which, in some cases at least, the germ may be divided into parts, each of which is endowed with the potentialities of the whole.

And yet development is specific. How, then, is this mechanism of inheritance to be conceived of? It is to Hans Driesch that we are indebted for an exhaustive attempt to think out the whole problem. In his *Analytische Theorie der organischen Entwicklung* Driesch starts with the facts with which we are already acquainted, the similarity of the nuclei, the dissimilarity of the cytoplasm in the several regions of the developing germ.[1]

The arrangement of these dissimilar substances determines first of all an axis in the egg, an axis with unlike poles; around this axis the cytoplasm is radially symmetrical or isotropic, but in the direction of the axis it is not; or, as Boveri puts it, there is a 'stratification' of the substances of the egg at right angles to the axis, the concentration of the animal substance decreasing towards the vegetative pole, that of the vegetative substance in the contrary direction. There are cases (Coelenterates, Sponges, Ctenophora) in which this radial is the only symmetry; but in other types (Bilateralia) a third point may be established by the disposition of some special substance (the grey crescent in the Frog's egg or the yellow pigment in the

[1] It may be mentioned that in 1887 Platner had already denied the existence in development of any qualitative nuclear division.

Ascidian *Cynthia*, for example), or by the arrangement of the blastomeres (as by the large posterior cell of Annelids and Mollusca), and this point, together with the axis, determines a plane about which the ovum is bilaterally symmetrical. The axis and the plane of symmetry of the egg are definitely related to the axis and symmetry of the embryo, the substances to its primary organs.

Further, in very many, though not in all, instances the parts of the ovum—blastomeres or egg-fragments—are totipotent; and the same is true of the parts of elementary organs like the archenteron of Echinoderms or the optic vesicle of Amphibia. The totipotence is, however, sooner or later lost, and this limita-

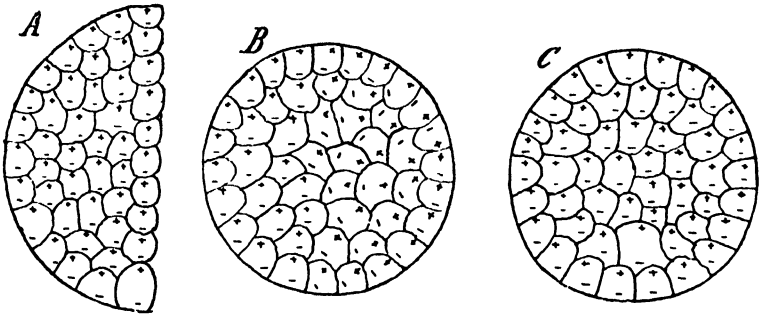


FIG. 165.—Diagram to illustrate Driesch's conception of the minute structure of the (Echinid) ovum. It is supposed to be composed of particles all similarly polarized and oriented to the whole. In *A* one—meridional—half is shown. In *B* this has become spherical and the parts have been disturbed. In *C* they have regained the original orientation. (From Korschelt and Heider.)

tion is apparently due to the way in which the substances are distributed in the ovum, an explanation which seems to be accepted by Driesch for most cases. But in accounting for the phenomena in the Echinoderm egg, the form with which he himself has chiefly experimented, he urges a different hypothesis. Here he conceives of the egg as composed of like particles, each of which is polarized and oriented in the same manner as the egg itself (Fig. 165), and consequently the only limitation to totipotence is due to size; any isolated part that is not too small can develop into a whole as soon as its polarized particles have reassumed a similar orientation. Again, it is stated that the blastomeres can be disarranged to any extent without interfering

with the normal development of the larva; they are all equivalent, without limit, at least until the ectoderm and endoderm have been differentiated; any part can contribute to the formation of any organ: 'jeder Theil kann jedes.'

This conception, of the absolute equipotentiality of the parts, as we have already had occasion to remark, is erroneous; but for Driesch it is of the first importance, for it dominates, as we shall see, all his theoretical speculations.

The position of the embryonic axes and primary organs being thus determined in the whole egg (or its isolated parts), it is still left to inquire into the causes which decide the destinies of the remainder. Driesch's answer to this question is twofold: there

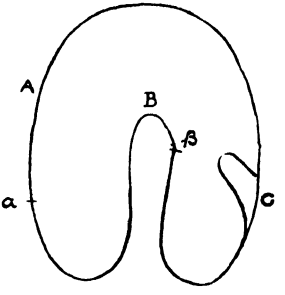


FIG. 166.—Diagram to illustrate the possible part played by stimuli ('inductions') in ontogeny, and by 'position'. *A*, *B*, and *C* are three larval organs (ectoderm, endoderm, stomodaeum). *C* may exert a stimulus on that part of *B* nearest it; this part, reacting to the stimulus, becomes *β*. Were the position of *C* altered the position of *β* in the equipotential system *B* might be altered too, so that the fate of any part of *B* would be a function of its position relative to *C*. So, under the influence of *B*, part of *A* may become *α*. (After Driesch, 1894.)

are two possible factors, one is 'position', the other 'induction'. In the case of the first, the destiny of a part is imagined to be determined by its distance from the system of points already established, 'its fate,' so runs the famous formula, 'is a function of its position in the whole.' It would, however, be absurd to suppose that the behaviour of any one of a number of precisely similar bodies could depend upon its mere geometrical position. The points already differentiated—the animal pole, for instance—must be supposed to exert an influence with a force which is some function of the distance upon the parts which are at present equivalent, and so to excite their differentiation (Fig. 166).

Properly speaking, therefore, this factor of 'position' belongs to the second category of 'induction'.

An 'induction' is simply an effect produced upon the parts that are developing by other parts, or possibly by some factor in the external environment. These inductions are, according to Driesch, of the nature of those events which are brought about by the addition of a single antecedent condition, an 'occasion', to an assemblage of antecedent conditions, as a spark fires a rocket or the movement of a lever sets a piece of machinery in operation; or, in the language of physiology, the causes of these inductions are stimuli, the effects, reactions or responses,

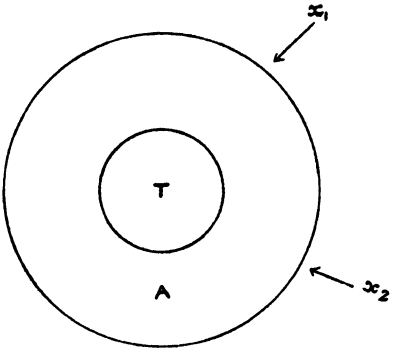


FIG. 167.—Diagram to illustrate Driesch's hypothesis of the part played by the nucleus in development. *A* is the cytoplasm, *T* its totipotent nucleus, x_1, x_2 are stimuli.

A is sensitive to x_1 and becomes A_1, *T* is sensitive to *A*, and is set in activity (*Ta*). A^1 being sensitive to *Ta* becomes A_1, and A_1 is sensitive to x_2, and so on. (After Driesch, 1894.)

the quality and the quantity of which are determined not only by the stimulus but also by the nature of the reacting body itself.

The analysis of these 'inductions' is not, however, yet complete. Occurrences of this kind imply the stimulus, the reception of the stimulus and the response. The first of these is some other organ or some external agent; the second and third are functions of the organ which is to be produced; and Driesch imagines that while it is the cytoplasm which is receptive of the stimulus, it is the nucleus which makes the response. Each totipotent nucleus is supposed to contain ferment-like bodies; under the influence of some stimulus received through the cytoplasm some

one of these is called into action; this works on and alters the cytoplasm; this then becomes receptive to a new and different stimulus, and so on, as shown in the accompanying diagram (Fig. 167). The cytoplasm thus becomes perpetually altered, while the nucleus retains the entirety of the potentialities of the organism, a supposition which is held in reserve to account for the phenomena of budding and regeneration.

The progressive limitation of potentialities, therefore, which we observe in ontogeny is a limitation of sensibilities to stimuli, equality of potentialities an equality of such sensibilities. In an 'equipotential system' (the blastula or the archenteron of *Echinus*) all (meridional) parts are equally susceptible, and what stimulus each will as a matter of fact receive is a function of its position in the whole, that is to say of its distance from the centres of force situate in the dissimilar points fixed *ab initio* in the system of symmetry of the egg (Fig. 166).

Development then proceeds from the comparatively simple organization given in the structure of the fertilized ovum by the creation of ever-increasing complexity by the action and reaction of the parts on one another. Each ontogenetic effect produced becomes in turn the cause of further effects, the possibility of fresh specific action, for it becomes the seat of a new specific stimulus and response, and so on until the complexity of the 'ultimate organs' of the adult is achieved.

In order, however, that differentiation may be normal it is clear that these stimuli and these responses must be accurately co-ordinated: the right stimulus must be ready at the right time and at the right place for the right organ to respond to. This indispensable temporal and spatial co-ordination is the 'Causal Harmony' of development; and it is given by the initial structure of the egg and the constitution of the external environment.

This is, however, not the only kind of harmony involved in development. Potentialities become restricted as differentiation progresses, and the development of the primary, secondary, tertiary and subsequent systems of organs successively produced is a process of 'self-differentiation', depending on factors residing in each system itself. Nevertheless these independent systems

frequently unite later on to form complex organs, and the necessary co-ordination of these is a 'harmony of composition'.

Such, in brief, is the idea worked out in the *Analytische Theorie*. On this hypothesis development is an epigenesis; it involves a *superadditio partium*, an increase of structure, a creation of fresh form out of the simple cytoplasmic organization of the egg, and the causes which operate in this process of morphogeny are just that initial structure and the stimuli which the parts respond to, stimuli which may proceed from the parts themselves or from the world outside.

These responses are primarily physico-chemical and only secondarily structural, and they happen in accordance with a pre-established harmony. Only in this sense can development be described as evolution; but this is not the evolution of the preformationists of the eighteenth century, nor even that of the school of Roux and Weismann, less gross, more subtle, but still morphological; rather it is the realization of a form which is physically and chemically predetermined, not structurally preformed, in the simple organization of the germ.

The hypothesis is worthy of the attention of every serious embryologist. It is scientific in the strict sense of the word, for in employing Herbst's suggestion to bring the events of ontogeny into the category of physiological responses to stimuli it gives meaning and precision to Hertwig's somewhat vague idea of the 'mutual relations of the parts', and makes thereby a genuine effort to think the particular under the universal, to bring the facts of embryology under wide general laws of causation.

Its chief weakness is at present lack of evidence; the development of every organ requires to be examined by the touchstone of experiment before the theory can rise beyond the rank of a working hypothesis. That, however, is the common lot of all working hypotheses, and this one has certainly some counterbalancing advantages. Its conception of the rôle of the nucleus has been to some extent borne out by Boveri's recent researches and adopted by their author, a conception which, as we have observed before, is not irreconcilable with the facts of budding and regeneration. Secondly, only the very simplest original

organization of the germ is demanded. This organization is not, however, to be looked for in the similar orientation of similarly polarized particles. On the contrary, it is to be found in the existence of definite cytoplasmic organ-forming substances whose arrangement must be simple enough to allow of the divisibility of the whole into totipotent parts, complex enough to account for that limitation of the potentialities of these parts which, sooner or later, inevitably ensues. And lastly, when modified in this important respect, Driesch's views are no longer hopelessly at variance with Roux's position, provided that we discard the vicious fallacy of qualitative nuclear and cell-division, and substitute for the idea of complete morphological preformation *ab origine* the successive preformation by the action of the parts on one another in the organs of each stage of the structures that are to be developed out of them in the next. To use Roux's own terms, development, in its early stages at least, is both a process of 'self-' and of 'dependent' differentiation; or, as Nägeli expressed it, it is by the continued combination and permutation of a few original elements that inheritance is brought about.

Driesch, however, is not merely a scientific thinker who is fully alive to the prime importance of pushing the causal analysis to its extreme limit. He is also a philosopher; and as a philosopher he realizes that when science has said all it has to say the account may still need to be completed from a new and distinct point of view. In the case of living organisms this new standpoint is the teleological. The harmony—causal harmony and harmony of composition—deduced from development, the functional harmony exhibited by the organs of the adult, all appear to be directed to an end, which is the reproduction or the preservation of the specific form, and it is only when this end is understood that the mere reference to beginnings which a knowledge of the mechanism gives acquires a genuine significance. Purposiveness, in a word, is a characteristic of all organic functions and cannot be ignored.

This principle is borrowed avowedly from Kant's *Kritik of the Teleological Judgement*. Like the scientists of to-day, Kant lays it down as a rule that the mechanical method, by which natural phenomena are brought under general laws of causation and

so explained, and without which 'there can be no proper knowledge of Nature at all,' should in all cases be pushed as far as it will go,[1] for this is the principle of the determinant judgement.[2]

There are cases, however, in which this alone does not suffice.[3] The possibility of the growth and nutrition, above all of the reproduction and regeneration of organisms is only fully intelligible to human reason through another quite distinct kind of causality, their purposiveness. Organisms are not mere machines, for those have merely moving power. Organisms possess in themselves formative power of a self-propagating kind which they communicate to their materials. They are, in fact, natural purposes, both cause and effect of themselves, in which the parts so combine that they are reciprocally both end and means, existing not only by means of one another but for the sake of one another and the whole. The whole is thus an end which determines the process, a final cause which 'brings together the required matter, modifies it, forms it, and puts it in its appropriate

[1] §§ 70, 78, 79, 80.

[2] It should be mentioned, perhaps, that Kant employs the terms 'determinant' and 'reflective' judgement in two senses. In the *Introduction* to the *Kritik of Judgement* judgement is defined as that faculty which thinks the particular as contained under the universal, and is stated to be of two kinds. It is 'determinant' when the universal is given (as in the mathematical sciences). When, however, only the particular is given, for which the universal has to be found (as in the inductive sciences), it is 'reflective', and Kant insists that the 'reflective' judgement requires a principle which it cannot borrow from experience, and this principle is, in brief, the ultimate intelligibility of Nature by us. Only so far as this holds good can we hope to gain a knowledge of general, empirical laws of causation.

The 'reflective' judgement is again of two kinds, for which Kant, however unfortunately, employs the same two terms, 'determinant' and 'reflective'. The duty of the former is, assuming that all phenomena are explicable in mechanical terms ('causal' terms in the usual sense of the word), to push the analysis of these efficient causes (*nexus effectivus*) to its extreme limit. The latter, on the other hand, is concerned with a kind of causality after the analogy of our own causality according to purposes, in order that it may have before it a rule according to which certain products of nature, namely organisms, must be investigated. The whole argument of the *Kritik* is directed to proving that these two uses of the judgement are mutually supplementary and both indispensable, though in the last resort the first has to be subordinated to the second.

In the text the terms 'determinant' and 'reflective' are used in the second sense. The 'determinant judgement' of the deductive sciences does not of course come into this discussion at all.

[3] §§ 64–66.

'place'.[1] Such purposiveness is internal, for the organism is at once its own cause and an end to itself, not merely a means to other ends, like a machine whose purposiveness is relative and whose cause is external.

Such is the principle of the teleological judgement. It is a 'heuristic principle'[2] rightly brought to bear, at least problematically, upon the investigation of organic nature, 'by a distant 'analogy with our own causality according to purposes generally,'[3] and indispensable to us, as anatomists, 'as a guiding thread if we 'wish to learn how to cognize the constitution of organisms 'without aspiring to an investigation into their first origin.'[4]

For 'we cannot adequately cognize, much less explain, 'organized beings and their internal possibility, according to 'mere mechanical principles of nature', and it is therefore absurd 'to hope that another Newton will arise in the future who shall 'make comprehensible by us the production of a blade of grass 'according to natural laws which no design has ordered'.[5]

Could our cognitive faculties rest content in this maxim of the reflective judgement it would be impossible for them to conceive of the production of these things in any other fashion than by attributing them to a cause working by design, to a Being which would be 'productive in a way analogous to the 'causality of an intelligence'.[6]

Natural science, however, needs not merely reflective but determinant principles which alone can inform us of the possibility of finding the ultimate explanation of the world of organisms in a causal combination for which an Understanding is not explicitly assumed, since the principle of purposes 'does 'not make the mode of origination' of organic beings 'any more 'comprehensible'.[7] And then, in a passage[8] remarkable for its prophetic insight, Kant proceeds to show how this might be. 'The agreement of so many genera of animals in a common 'scheme... allows a ray of hope, however faint, to penetrate into 'our minds, that here something may be accomplished by the aid 'of the principle of the mechanism of nature (without which 'there can be no natural science in general). This analogy of

[1] § 66. [2] § 78. [3] § 65. [4] § 72. [5] § 75. [6] § 75. [7] § 78. [8] § 79.

'forms,' he says, 'which with all their differences seem 'to have been produced according to a common original type, 'strengthens our suspicions of an actual relationship between 'them in their production from a common parent, through the 'gradual approximation of one animal-genus to another—from 'those in which the principle of purposes seems to be best authen- 'ticated, that is from man, down to the polype, and again from 'this down to mosses and lichens, and finally to the lowest 'stage of nature noticeable by us, namely, to crude matter. 'And so the whole Technic of nature, which is so incompre- 'hensible to us in organized beings that we believe ourselves 'compelled to think a different principle for it, seems to be 'derived from matter and its powers according to mechanical 'laws (like those by which it works in the formation of crystals).'

A purposiveness, however, must be attributed even to the crude matter, otherwise it would not be possible to think the purposive form of animals and plants.

Although there are doubtless in the *Kritik* many obscurities and apparent inconsistencies, to which we cannot allude now, the general meaning of Kant's reflections upon organisms is perfectly clear. He who would 'complete the perfect round' of his knowledge must think not only in beginnings but in ends. The end in the case of a living being is plain—it is the maintenance and reproduction of its form; the end in the case of the cosmic process, though perhaps not so plain, is to be sought in the ethical, or, in Kantian phraseology, the 'practical' concept of the freedom of the moral consciousness of man.

Such a position is quite intelligible, philosophically; and it can only be a matter of surprise that Driesch has not been able to abide by it. In his later writings he has indeed executed a complete change of front and repudiated the philosophical doctrine laid down in the earlier treatise; and the principal reason for this *volte-face* is that there are cases in which the localization of ontogenetic effects cannot be explained by any theory of formative stimuli. In the theory we have already considered the causal harmony which secures the due co-ordination in space and in time of the stimuli and responses into which the process of

differentiation is resolved is held to result from the initial structure of the germ and to be maintained by the constitution of the environment. Now, however, it is urged that no materia factor can possibly account either for this harmony or for the secondary harmony of composition or the functional harmony seen in the activities of the adult. When, for example, the gastrula of a sea-urchin is transversely divided into two, each develops into a diminished whole larva in which the gut becomes divided into the characteristic three regions, and all the other organs are formed in correct proportion. For each of these acts in the whole uninjured larva an explanation may conceivably be given in terms of stimuli or forces emanating from the originally distinct parts of the egg and producing effects which vary with the distance upon other parts, as suggested before. A mechanism may be thought of which, when set in motion, will achieve a certain end in accordance with its own pre-established harmony, but a mechanism which can be subdivided *ad libitum*, or almost *ad libitum*, and the parts of which will still achieve the same end, will still behave as wholes with their parts co-ordinated in the same ratio, temporally and spatially! Such a mechanism is an inconceivability, for to ensure the result which does happen the working distance of the forces imagined must be altered in each case according to the size of the fragment removed. Something is therefore required to superintend, to co-ordinate, to harmonize the causes of development in the case not only of the part but of the whole egg as well; and this something is not material. A corroborative proof of the inadequacy of the purely material explanation—the causal explanation in the ordinary sense of the word—may be derived from a consideration of certain other vital processes. The facts of acclimatization and immunity betray an extraordinary adaptability of the organism to a change in its environment; an organ will adapt itself structurally to an alteration, quantitative or qualitative, of function (Roux's 'functional adaptation'); lost parts can be regenerated; and then there is the physiology of the nervous system!

In all these cases of 'regulation'—and indeed in all other responses to stimuli—the same element, inexplicable in chemical

and physical terms, exists as must exist in development. This entity is not a form of energy but a vital constant, analogous to the constants or ultimate conceptions of mechanics and physics and chemistry and crystallography, but not reducible to these, just as these cannot be translated into one another. Driesch describes it as rudimentary feeling and willing, as a 'psychoid', as 'morphaesthetic', or perceptive of that form which is the desired end towards which it controls and directs all the material elements of differentiation. Its activities are thus *verae causae*—unconditional and invariable antecedents—psychical factors which can intervene in the purely physical series of causes and effects, and for it he revives the Aristotelian term 'Entelechy'. Such is the 'vitalism' introduced by Hans Driesch, a teleological theory clearly, but not the 'static' teleology of the *Analytische Theorie*; rather it is a 'dynamic' teleology which not only sees an end in every organic process but postulates an immaterial entity to guide the merely mechanical forces towards the realization of that end.

This theory would seem to be open to serious criticism, and from two sides, the scientific and the philosophical.

In the first place, we must remind Driesch that on his own showing a comparatively simple structure is all that is necessary to form the starting-point of a developmental process, however complex that may be, and that there is no reason why such a structure should not be divisible into portions, each of which will possess all the parts of the structure in correct proportions, and be therefore totipotent. But such division cannot continue indefinitely, for as we know, and as Driesch knows too, there is always, sooner or later, a restriction of potentialities, and this is due to the manner of distribution *ab origine* of the constituent parts of the whole. When Driesch asserts that this restriction is due to size alone, to mere lack of material, and not lack of specific material, when he tells us that the blastomeres can be dislocated indefinitely without prejudice to a normal development, when he exclaims that 'Jedes jedes kann', he is manifestly led away by the reaction against the theory of the preformation of as many units as there are inheritable characters on the one hand, and on the other by his own erroneous presuppositions as to the con-

struction of the egg out of like particles all similarly polarized and all oriented in the same way.[1]

This being so, the first argument based on the 'causal harmony' will fall to the ground; for this will be given in the initial structure of the egg, and if that may be divided, then the 'causal harmony' may be divided too. The correct proportionality of the organs of partial larvae, then, offers no peculiar difficulty. The corroborative argument is founded on a consideration of responses to stimuli. This is not a question for the embryologist, but it may be pointed out that there are still physiologists who maintain that even the complex phenomena presented to us in the activities of the nervous system are susceptible of a purely mechanical explanation.

The second series of objections to the new 'vitalism' is philosophical. Driesch has quoted the authority of Kant and Aristotle in support of his doctrine. The former is, however, rather a difficult witness, as Driesch is well aware. He complains, indeed, that Kant's teleology is descriptive or 'static' rather than 'dynamic', as is perfectly true, except in the case of man, a point of which Driesch naturally makes the most. There are no doubt passages where Kant speaks of 'a cause 'which brings together the required matter, modifies it, forms it 'and puts it into its appropriate place',[2] but against these must be set the explicit statement that if the body has an alien principle (the soul) in communion with it, 'the body must either 'be the instrument of the soul—which does not make the soul 'a whit more comprehensible'[3]— or be made by the soul, in which case it would not be corporeal at all. 'Vitalism' can glean small comfort from this.

Let us turn, then, to the second authority.

Aristotle's matured reflections on the soul (ψυχή), its nature, functions, and development, are to be found in the treatises *De Anima* and *De Generatione Animalium*.

Soul is defined in the most general way as an activity of

[1] C. M. Child (*Biol. Centralbl.* xxviii, 1908) has recently published a similar criticism of Driesch's absolutely equipotential systems. Child points out in particular that in the regeneration of the head of *Tubularia* the proportionality of the parts differs in different regions of the stem, and under different conditions, and cannot therefore always be exact. The equipotentiality of the system is therefore not absolute.

[2] § 66. [3] § 65.

a natural organic living body, life being autonomous nutrition and growth and decay. The activity (ἐντελέχεια) may, however, be latent or patent, passive or active, sleeping or waking, without losing its peculiar characters. This activity is substance (οὐσία), but substance as 'form', as opposed to the material substance of the body; the living body is therefore also a substance, in a double sense.

Soul is not, however, identical with the body, but as form, proportion (λόγος), activity (ἐνέργεια), essence (τὸ τί ἦν εἶναι), it is related to the body, mere matter (ὕλη) and potentiality (δύναμις), in just the same way as the seal is related to the wax, and the body is the instrument whereby it effects its purposes; though subsequent in time it is prior in thought to the body, as all activities are to the materials with which they operate.

At the same time neither it nor its parts are separable from the body, with the exception, possibly, of mind (νοῦς); it is indeed the actual or possible functioning of the body, like the seeing of the eye or the cutting of the axe, and with the disappearance of the capacity of this functioning the soul itself also perishes.[1] Lastly, it is a cause (ἀρχὴ καὶ αἰτία) in a triple sense: first, as the source of motion, secondly, as that for the sake of which the body exists, and thirdly, as its essence (οὐσία), or formal cause.[2]

The soul is of several kinds, which form together an ascending series, each member of which is necessarily involved in those above it.[3]

The lowest is the nutritive soul (θρεπτική), found in all living things, and the only soul possessed by plants. It is defined as motion in respect of nutrition, decay and growth, processes which involve alteration (ἀλλοίωσις) in the body, and its functions (ἔργα) are to utilize the food for the maintenance and reproduction of the form of the body, and to control and limit growth.

The second is the perceptive soul (αἰσθητική), the possession of which distinguishes animals from plants. Perception is a kind of alteration (ἀλλοίωσίς τις),[4] and consists in being moved and affected. The fundamental and indispensable perception is touch (ἀφή),

[1] *De An.* II. 1. [2] Ibid. II. 4. [3] Ibid. II. 3, 4. [4] Ibid. II. 5.

for it is concerned in the acquisition of the food. It is invariably present; the others may or may not, some or all, be present.

Some animals are also possessed of a capacity of locomotion, and the performance of this function requires again a special kind of soul.

Lastly, there is the reasoning soul (*διανοητική*), or mind (*νοῦς*). This is found in man alone, unless there be other beings similar to him, or even nobler than he. Mind alone is eternal and separable from the body.

In all reproduction (except in *generatio equivoca*) the starting-point of a new individual is what Aristotle calls a *σπέρμα*. In plants, in which he does not recognize the sexes, this is the seed; in sexually produced animals it is the result (*κύημα*) of the mingling of the male (*γονή*, or *σπέρμα* in a narrower sense) and female elements; the latter is an egg or, in Mammals, the catamenia.[1]

This *σπέρμα*, he holds, does not come from all the organs of the body by a kind of pangenesis, but is a tissue, homogeneous like bone and flesh, and separated out from the food in its final stage of digestion, when it is in the form of blood and ready for assimilation, and hereditary resemblance is explained by the fact that the food which is about to be assimilated by the organs is naturally like that set aside to form the *σπέρμα*.[2]

In the matter of the *σπέρμα* all the parts of the organism that is to be formed are indeed present potentially, but this means no more than that the material is there.[3] Actually (*ἐνεργείᾳ*), they cannot be present until the soul has been developed, and in particular the soul that is characteristic of animals, the perceptive.[4] Out of this matter the organs are differentiated successively, the heart first, not only as a matter of observation (on the chick), but as a theoretical necessity, since in it is the principle of growth, then the blood, the blood-vessels, the tissues gathering about these by a process of condensation and coagulation, the fore-parts of the body first, and then the hinder.[5] The *σπέρμα*, then, or *κύημα*, is a material cause of development, but it is also a cause in other senses; it is the efficient cause, since it must

[1] *De Gen.* I. 18-20. [2] Ibid. I. 18, 19; IV. 1. 39. [3] Ibid. II. 4.
[4] Ibid. I. 19; II. 3, 5. [5] Ibid. II. 6.

contain the source of motion, and it is further the final and the formal cause. These several causes are not, however, all contributed by both parents. The teaching of Aristotle is that the matter is provided by the female, and the female alone.[1] The egg (or catamenia) is described as being matter (ὕλη), body (σῶμα), potentiality (δύναμις), passive (παθητικόν), and merely quantitative, although it is true that a sort of soul, the nutritive, is somewhat grudgingly conceded to it, since unfertilized eggs appear in some sense to be alive.[2] The male element, on the other hand, provides the principle of motion (ἀρχὴ τῆς κινήσεως) and the form (εἶδος); it is qualitative, it is activity, it produces the perceptive soul, if it is not itself that soul, and it is responsible for the 'correct proportionality' (λόγος) of the organization.[3]

The male element contributes only motion; it acts upon the female element as rennet acts when it coagulates milk, except that the analogy is incomplete, since the γονή brings about a qualitative, and not merely a quantitative, change in the material on which it operates.[4] To this it imparts the same kind of motion which itself possesses, the motion which was present in the particles of the food in its final form from which it was itself derived.[5]

The communication of this motion is enough to set going the machinery (αὐτόματον); the rest then follows of itself in proper order.[6] To impart this necessary motion is the function of the nutritive soul, which is primarily associated with the male, only somewhat doubtfully with the female, element; the perceptive soul which is, and therefore presumably also imparts, motion of a kind (ἀλλοίωσις) is found in the former alone.[7] As to the third kind of soul, mind, Aristotle says little, but it is not introduced in the male element: it is separable and comes in from outside.

Lastly, the sperm of the male acts like a cunning workman who makes a work of art, using heat and cold as its implements as the workman uses his tools;[8] for this heat and this cold could never of themselves—by coagulations and condensations—produce the form of the body, as the older naturalists had supposed,

[1] *De Gen.* I. 20, 21; II. 1, 4. [2] Ibid. II. 5. [3] Ibid. I. 20, 21; II. 1, 4. [4] Ibid. I. 20; IV. 4. [5] Ibid. II. 3. [6] Ibid. II. 1, 5. [7] Ibid. II. 3; *De An.* II. 5. [8] *De Gen.* II. 4.

regarding only the material and efficient, and ignoring the formal and the final cause; for the organic body is not what it is because it is produced in such and such a fashion, rather it is because it is to be such and such that it must be developed as it is.[1]

And here lies the kernel of the whole matter. For while Aristotle has made it perfectly plain that according to his idea, the soul, at least its nutritive and perceptive faculties, is to be regarded as a function of matter and that this function may be ultimately expressed in terms of movement, and further that development is a mechanism which is set going by the communication of motion proceeding from the 'soul' of the male element, and derivable in the last resort from the 'motions' into which the 'functions' or 'soul' of the parent can be resolved, to the mere matter which the female provides, it is equally evident that he does not regard this mechanical explanation—in terms of material and efficient causes—as satisfactory or complete. But when we inquire why, he gives us no certain and consistent answer. On the one hand there are passages in which he tells us that there must be something which controls the material forces and imposes upon them a limit and proportionality of growth,[2] that the soul makes use of these forces as the artist makes use of his implements,[3] and such passages are naturally interpreted by Driesch in the sense of a 'dynamic' teleology; it is the ψυχή (not, of course, νοῦς, but the two lower kinds) which superintends and controls, and the ψυχή is 'Entelechy'. Elsewhere, however, we are informed that even the proportionality of the developing parts is simply the outcome of the motion imparted by the male, which is *actu* what the female material only is *potentiâ*.[4]

Moreover it may be questioned whether Aristotle ever intended to imply more than an 'analogy with the causality of purpose' when he uses the figure of the workman and his implements to illustrate his meaning of the formal cause. The formal cause of a work of art is an intelligible 'vera causa', it is the idea in the mind of the artist antecedent to the execution of the work, but the formal or final cause of an organism, the end which it apparently strives to attain, is only metaphorically prior in time to the existence of the organism itself. Prior in thought, how-

[1] *De Gen.* V. 1. [2] *De An.* II. 4. [3] *De Gen.* II. 4. [4] Ibid. II. 1. 44.

ever, it certainly is, for it is only the performance of its functions (ἐντελέχεια) by the organism complete in all its parts that makes the mere mechanism of development comprehensible to us; the process, therefore, exists for the sake of the end. Only as efficient cause is the soul prior in time; only so far as it is prior in thought can it be said to be a final cause.[1]

Such a teleology is, it is obvious, indistinguishable in principle from the position in which Kant leaves us. It is the position adopted by Driesch, as we have seen, in the *Analytische Theorie*, but abandoned in the *Vitalismus* in favour of a theory of 'pyschoids'.

Now, quite apart from the meaning which Aristotle may or may not have intended to convey, there appear to be grave objections to this belief.

This 'psychoid', to which the name 'Entelechy' is surely misapplied, this rudimentary feeling and willing, which is aware of the form it desires to produce, must be, psychically, at least as complex as the phenomena it is designed to account for, and stand, therefore, as much in need of explanation as they, which will involve us at once in an infinite series of such entities. In fact, to borrow the epithet which Driesch himself has bestowed on the nuclear architecture imagined in the Roux-Weismann hypothesis, it is only a photograph of the problem, and not a solution at all. Again, when we ask what the *modus operandi* of this cause is we get no reply either from Driesch or from any other neo-vitalist, though this is just the knowledge that we so urgently stand in need of. The objection that the intervention of a psychical cause in a physical process is unintelligible, an objection which would probably appeal to many, may be waived, for in the last resort the connexion between any—even simply mechanical—causes and effects is equally hard to understand. It may, however, be seriously doubted whether these entities are not being 'multiplied beyond necessity', and whether the progress of science would not be better served by an adherence to a simpler philosophy.

'Vere scire est per causas scire.' The maxim of the great founder of modern inductive science is the watchword of embryologists to-day. By exact observation and crucial experi-

[1] *De Part.* II. 1. 7.

ment, utilizing every canon of induction, the facts of development are to be brought under wide general laws of causation, which will be in the first instance physiological laws—of response to stimuli, of metabolism, and of growth: by means of these laws we can predict, and our predictions can be verified. The thought process cannot, however, rest here. Ultimately—as we believe—it may be possible, no more than that can yet be said, but it may be possible to state the widest generalizations of biology in chemical and physical, and these again in purely mechanical, terms. Thus evolution of form in the individual as well as the larger evolution of form in the race, become but the final terms in a far vaster cosmic progress, from 'homogeneity to heterogeneity'.

The idea, of course, is perfectly familiar; it is the analysis of purely physical causes carried to its extremest limit. Phenomena are thought out in terms not of origins merely but of one origin, and that one origin is the only mystery that remains. This unification of the sciences always has been, and must still remain, the dream and the faith and the inspiration of the scientific man, and could such an edifice of the intellect ever be realized the task of science would have been completed.

But where science leaves off there philosophy begins, and it is for philosophy to attempt the solution of this last mystery of all.

Philosophy cannot rest content in an endless regress of cause and effect, and a first supreme cause, first in time that is, is metaphysically out of the question. An original homogeneity is equally unthinkable, for out of a system all whose parts are absolutely alike, by no imaginable process could any heterogeneity ever be evolved.

That first simplicity must have contained *potentiâ* all that has since developed out of it, it must have possessed a structure, an arrangement of parts such that the end which it has realized, or is to realize, would be what it is or will be, and to regard the end as well as the beginning is the duty of philosophy, a duty which Aristotle and Kant have both impressed on us. The outlook of pure science, an outlook to which, *quâ* science, it cannot too rigidly confine itself, is thus supplemented and enlarged.

Knowledge through material and efficient causes is rounded into a whole through a knowledge of the final cause, which, in the last analysis, is just as much a *vera causa* as they are; for in our total ignorance of what constitutes the invariable connexion we observe between antecedent and consequent it is as true that the second causes the first as the converse. Only because of the inherent desire of the human mind to predict from the past, which is known, to the future, which is not, have we come in ordinary usage to restrict the term to the antecedent.

The ultimate end of the human or any other race we cannot tell, the ultimate end of the universe we cannot tell, any more than we can imagine its absolute beginning, unless we find these ends in the freedom of the moral consciousness of man. But the end of an organism, the production of specific form and the maintenance of that form which itself has produced, does seem self-evident and plain, though we must never forget that form is variable and subject to change, that species are not immortal any more than individuals, and that the effort to achieve that form does not invariably succeed.

Here, however, we touch on the fringe of a problem—the problem of evil—too large to be discussed in this place. Putting this aside, a purposiveness is an unmistakable characteristic of the functions of living things, of the production and preservation of form, a characteristic which still remains when those functions have been expressed in terms of the chemistry of the proteids. It is only, however, by a remote analogy with our own 'causality according to purposes' that we can speak of organic functions as purposive; it is only *as if* they were guided and controlled by an intelligence; their purposiveness is indeed only the expression of our inability to comprehend their beginnings except in terms of their ends; it is relative to us, though not, therefore, any the less real.

Biology, then, although built upon the ultimate conceptions of chemistry and physics, has yet peculiar features of its own. Its relation, indeed, to these lower sciences is just what their relation is to one another. A survey of the whole hierarchy displays to our view a series in ascending order of complexity; each member of this series has its own ultimate conceptions, the

most general expression for the facts with which it deals, but the ultimate conceptions of each, as in Aristotle's series of 'souls', are necessarily involved in the one next above, while conversely each endeavours to translate its own ultimates into those of the science below: a translation, however, which, be it never forgotten, leaves the reality of the original undestroyed.

Thus, mechanics expresses molar motions in terms of pure numbers, physics explains forms of energy—heat and light and electricity—as the motions of molecules; chemical affinity is to be reduced to the mutual attractions of intramolecular atoms; purposive responses to stimuli may be stated in terms of chemical reaction, and the psychical phenomena of mental and moral science—understanding and feeling and will—are a form of these.

The cosmic process thus takes place in a succession of stages, and the peculiar features which mark each individual stage are simply the outcome of an increase in complexity of the peculiarities characteristic of the stage below. To establish this is the final achievement of science.

Nevertheless, the facts with which each science starts, the facts which come first in the order in which knowledge is acquired, do not become wholly merged in those simpler facts into which they are at each stage translated; when the translation has been accomplished the original still remains.

The 'secondary qualities', as well as the other properties of those bodies whose behaviour the physicist investigates, are as real as, no more and no less relative to our intelligence than, those 'primary qualities' of impenetrability and extension—exhibited by those bodies whose behaviour forms the subject-matter of mechanical science—into which it is his endeavour to translate them, just as the primary qualities themselves obstinately refuse to be reduced to mere number. Chemical affinity remains as a phenomenon *sui generis* after it has been reduced to the operations of intramolecular forces, and the purposiveness of responses to stimuli is something over and above the chemical reactions to which they are rightly referred. Last of all, the final term in the series, the mental and moral consciousness, the 'other side' of certain purely physical functionings of the organism, is as real as any of those qualities of matter which

have been step by step involved in its evolution. In a word, the increase in the complexity of the phenomena which marks the transition from each of these stages to the next is itself a new phenomenon and cannot be ignored.

And herein we may perhaps discover the essence of the relation between 'mind' and 'matter', whether in ordinary function or in development. The mind is not matter, not even living matter; rather it is the new quality constituted by an increase in the complexity of living matter, immaterial and as distinct from that matter as is 'blueness' from vibration of a certain wave-length. Dependent on and inseparable from matter, however, it is; when that matter, whether in the individual or in the race, attains a certain degree of complexity, then and then only does mind appear; and with the disappearance of that complexity it perishes.

While, therefore, we have no reason for supposing that the mind 'comes in from outside', we are at the same time saved from that somewhat extravagant 'psycho-physical parallelism' which, to explain the evolution of consciousness, postulates a complete psychical, accompanying the complete physical series of causes and effects, and credits, of necessity, the merest matter with the rudiments of feeling, thought and will. On the other hand, if the mind cannot be said to intervene in the physical series, there seems to be no alternative but to suppose that the operations of the one, when they exist, are parallel to those of the other.

Such a system of philosophy as that which we have here ventured to suggest can give no countenance to a Vitalism which interpolates an unnecessary psychical element into the complete causal chain of physical events. But it is not for that reason to be condemned as materialistic; for the mind, developed out of and conditioned by matter, the last term and final cause of the whole process, is not itself matter but an accompaniment of certain material complexes, and still remains when they have been resolved into simple mechanical expressions. And in this mind, last in time but first in thought, a larger philosophy will perceive not only the end towards which, in time and space, matter strives, but the Understanding which, itself eternal, imposes the forms of space and time upon that Nature which it makes.

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APPENDIX A

FURTHER REMARKS ON THE RELATION BETWEEN THE SYMMETRY OF THE EGG, THE SYMMETRY OF SEGMENTATION, AND THE SYMMETRY OF THE EMBRYO IN THE FROG.

In the measurements, referred to above (pp. 165–8), of the angles between the plane of symmetry of the egg (as determined by the position of the grey crescent), the first furrow and the sagittal plane of the embryo, it was found (1) that there was a certain tendency for the first furrow and the sagittal plane to coincide, since in a large number of cases small angles preponderated over large ones, the standard deviation of this angle from the mean (which was practically = 0°) being $\sigma = 40{\cdot}39° \pm {\cdot}65$; (2) that there was a much greater tendency for the plane of symmetry and the sagittal plane to coincide, the standard deviation of the angle between these two planes being $\sigma = 29{\cdot}75° \pm {\cdot}63$; (3) that the first furrow tended either to coincide with or to lie at right angles to the plane of symmetry, the standard deviation about 0° being $18{\cdot}70° \pm {\cdot}60$, that about 90° being $23{\cdot}29° \pm {\cdot}86$, the value of σ for all the observations being $47{\cdot}90° \pm 1{\cdot}19$. The correlation between the first furrow and the sagittal plane was found to be $\rho = {\cdot}138 \pm {\cdot}031$, that between the plane of symmetry and the sagittal plane $\rho = {\cdot}372 \pm {\cdot}025$, that between the plane of symmetry and the first furrow $\rho = {\cdot}087 \pm {\cdot}032$.

These results may be tabulated as follows :—

	σ	ρ
First Furrow and Sagittal Plane.	$40{\cdot}39° \pm {\cdot}65$.	${\cdot}138 \pm {\cdot}031$.
Plane of Symmetry and Sagittal Plane.	$29{\cdot}75 \pm {\cdot}63$.	${\cdot}372 \pm {\cdot}025$.
Plane of Symmetry and First Furrow.	$47{\cdot}90 \pm 1{\cdot}19$.	${\cdot}087 \pm {\cdot}032$.

Full details of these results will be found in a paper in *Biometrika* V. 1906.

For the purpose of making these measurements the eggs were placed in rows parallel to the *length* of glass slides, and the angles measured between the various planes and lines ruled *across* the slide. Such eggs compress one another by their jelly

coats; further, the eggs taken from the uterus were placed haphazard on the slides with the axis making any direction with the vertical. The egg takes about half-an-hour to turn into its normal position with the axis vertical, and during this interval gravity may possibly act upon the yolk and protoplasm, of different specific gravities, and impress a plane of bilateral gravitation symmetry upon the egg, as occurs when the egg is permanently inverted (see above, pp. 82–87). This obliquity of the axis may possibly affect the relations between the planes, and the mutual compression may also be a disturbing factor, since it is known that in compressed eggs the nuclear spindle is perpendicular to the direction of the pressure (pp. 34–36).

These angles have therefore now been measured under four different conditions:

(*a*) The eggs are close to one another in the rows and the axis is horizontal. (Since the rows are parallel to the length of the slide the pressure, if any, must be in the same direction, while the surfaces of compression or contact are *across* the slide. The eggs were always so placed that the vegetative poles faced in one direction and the planes of 'gravitation symmetry' were at right angles to the length of the slide. This holds good of all the following experiments.)

(*β*) The eggs close, but the axis vertical with the white pole below. In these there can be no gravitation plane of symmetry.

(*γ*) The eggs spaced, but the axis horizontal. In these the jellies do not touch.

(*δ*) The eggs spaced and the axis vertical. In these, therefore, both the supposedly disturbing factors are removed. The results are given in the following table:—

	A First Furrow and Sagittal Plane.	B Plane of Symmetry and Sagittal Plane.	C Plane of Symmetry and First Furrow.
(*a*)	$\sigma = 38{\cdot}42 \pm {\cdot}70.$ $\rho = {\cdot}201 \pm {\cdot}028.$	$\sigma = 31{\cdot}86 \pm {\cdot}56.$ $\rho = {\cdot}263 \pm {\cdot}027.$	$\sigma = 41{\cdot}59 \pm {\cdot}84.$ $\rho = {\cdot}118 \pm {\cdot}029.$
(*β*)	$\sigma = 33{\cdot}44 \pm {\cdot}56.$ $\rho = {\cdot}352 \pm {\cdot}021.$	$\sigma = 30{\cdot}17 \pm {\cdot}51.$ $\rho = {\cdot}276 \pm {\cdot}022.$	$\sigma = 39{\cdot}71 \pm {\cdot}61.$ $\rho = {\cdot}023 \pm {\cdot}024.$
(*γ*)	$\sigma = 33{\cdot}49 \pm {\cdot}96.$ $\rho = {\cdot}292 \pm {\cdot}039.$	$\sigma = 27{\cdot}53 \pm {\cdot}84.$ $\rho = {\cdot}399 \pm {\cdot}036.$	$\sigma = 36{\cdot}60 \pm 1{\cdot}108.$ $\rho = {\cdot}075 \pm {\cdot}043.$
(*δ*)	$\sigma = 31{\cdot}45 \pm {\cdot}73.$ $\rho = {\cdot}364 \pm {\cdot}033.$	$\sigma = 26{\cdot}80 \pm {\cdot}82.$ $\rho = {\cdot}451 \pm {\cdot}035.$	$\sigma = 34{\cdot}46 \pm 1{\cdot}065.$ $\rho = {\cdot}186 \pm {\cdot}043.$

It is evident from this that gravity and 'mutual compression' (as I will for the moment term it, though it is doubtful whether the pressure has anything at all to do with the result) do affect the magnitude of the angles between these three planes, for in each

case the standard deviation falls, while the correlation coefficient rises, when they are both removed. It will be observed that, while gravitation (γ) has less effect than compression (β) upon the angles B and C, the reverse is the case with the angle A. We may be able to find a reason for this later on.

There is one point worth noticing. It is quite clear that gravity is not indispensable for the development of a grey crescent and plane of symmetry, though it is true that the position of this plane may be affected by gravity even in the short interval that elapses before the egg turns over.

The values for the compressed eggs with horizontal axes (α) compare fairly well with those previously obtained, except in the case of the plane of symmetry and the first furrow. In the former series the latter tended either to coincide with or to lie at right angles to the former. In the present series this is not the case. This difference is probably to be attributed to the fact that many of the eggs in the first series must have been placed on the slide with the white pole upwards: possibly also the 'compression' was greater then than now.

It is fortunate that the same data enable us to study exactly the relation between the first furrow and the plane of symmetry on the one hand, and the direction of 'compression' and of the gravitation symmetry plane on the other. It must be remembered that these two are at right angles to one another.

Consider first the first furrow.

(α) When the eggs are close but the axis horizontal the first furrow tends to lie at right angles to the slide, that is, *in* the direction of compression, but at right angles to the gravitation symmetry plane. ($\sigma = 38{\cdot}16 \pm {\cdot}69$.)

(β) When the eggs are close but the axis vertical this tendency is not quite so marked. ($\sigma = 46{\cdot}67 \pm {\cdot}71$.)

(γ) When the eggs are spaced and the axis horizontal it is still there, but slight. ($\sigma = 49{\cdot}32 \pm 1{\cdot}40$.)

(δ) When the eggs are spaced and the axis vertical the direction of the first furrow is random. ($\sigma = 52{\cdot}76 \pm 1{\cdot}17$.)

We may conclude, therefore, that the first furrow tends to lie in the direction of the 'compression' and at right angles to the plane of gravitation symmetry. The latter tendency, we know, exists in forcibly inverted eggs, together with a tendency to lie in the plane of symmetry and at 45° to it (above, p. 84). Pressure experiments also show that division is in the direction of pressure (p. 34 sqq.).

The direction taken up by the plane of symmetry under these different circumstances is quite distinct from that of the first furrow. It appears to be determined in the first instance by

gravitation, as it usually lies in the gravitation symmetry plane. It is not, however, only so determined, for if the eggs (compressed and with axis horizontal) be allowed to develop in the light the plane of symmetry lies either in the gravitation symmetry plane, or in the direction of the incident light (*parallel* to the length of the slide in the experiment), while in the dark it lies only *across* the slide. That this second effect is due to the light and not to the pressure is shown by the fact that it occurs when the eggs are spaced, and that it may be made to vary in position by varying the position of the slide with regard to the light. Light, therefore (ordinary daylight), as well as gravity, can help to determine the position of the plane of symmetry, and when the latter is excluded it appears that this plane is placed either in or at right angles to the source of light.

Light appears to exert no effect upon the first furrow.

It is now intelligible why, when all these factors are operative, the relation between the first furrow and the planes of symmetry of egg and embryo should be disturbed, since, in the conditions of the experiment, those factors which determine the position of the former are at right angles to those on which the direction of the latter depends.

It still remains for us to inquire into the internal causes of the direction of these planes in the egg. Roux, as has been pointed out, has asserted that the grey crescent appears on the opposite side of the egg to that on which the spermatozoon has entered (pp. 80, 165), and further that the point of entry of the sperm also determines the meridian of the first furrow, since this either includes the sperm-path, or is parallel to it, or, when it is crooked, includes or is parallel to the inner portion or 'copulation' path, which is taken to represent the line of approximation of the two pronuclei; the outer part being simply the 'penetration' path. Roux also arbitrarily selected a fertilization meridian (meridian of the sperm-entry), and showed that this became the ventral side (opposite the grey crescent) later on, as well as the meridian of the first furrow (p. 248).

I have been able to accurately investigate—by means of sections—the relation between the fertilization meridian, first furrow, and sperm-path in a number of eggs in which the direction of the symmetry plane had been previously determined, and the results of the measurements of these angles are given here. The eggs fall into two series, those which were compressed and had their axes horizontal (*a*), and those which were spaced and had their axes vertical, the white pole being below (*b*). In (*a*) the gravitation symmetry plane and the direction of compression were at right angles to one another, as before.

	δ	α
Meridian of sperm entry and first furrow.	$\sigma = 21{\cdot}02° \pm 1{\cdot}63.$ $\rho = {\cdot}435 \pm {\cdot}074.$	$\sigma = 31{\cdot}04° \pm 1{\cdot}34.$ $\rho = {\cdot}613 \pm {\cdot}038.$
Meridian of sperm entry and symmetry plane.	$\sigma = 25{\cdot}67° \pm 1{\cdot}35.$ $\rho = {\cdot}302 \pm {\cdot}083.$	$\sigma = 41{\cdot}01° \pm 1{\cdot}78.$ $\rho = {\cdot}006 \pm {\cdot}061.$
Sperm-path (penetration path) and first furrow.	$\sigma = 17{\cdot}94° \pm 1{\cdot}15.$	$\sigma = 21{\cdot}47° \pm {\cdot}93.$

From this it is clear that there is a very close relation indeed between the point of entry of the spermatozoon and the direction of the first furrow, especially when the disturbing effects of pressure and gravity are removed. There is, however, little relation between the sperm meridian and the plane of symmetry even under the most favourable circumstances, and when the conditions are not favourable the correlation is negligible. There is however (in the δ series) a considerable correlation ($\rho = {\cdot}479 \pm {\cdot}070$) between the *sperm-path* and the plane of symmetry. It should be remembered, however, that all these eggs were exposed to the light. From what we know of the effect of this agent upon the direction of the symmetry plane, it would not perhaps be too bold a hazard to surmise that in darkness there would be a correlation between the sperm entrance and the plane of symmetry.

Even after the removal of this disturbance there remain factors which interfere with the completeness of the correlation between these planes; these must probably be looked for in the incomplete radial symmetry of certain eggs—due possibly to pressure in the uterus—and to the slight squeezings and distortions the eggs may be subjected to when they are being taken from the Frog.

It will be seen that the relation between the sperm-path and first furrow is closer than that between the latter and the sperm entrance. This is because though the furrow may be placed to one side of the entrance point, it may still be parallel to the path, or, if not to the 'penetration' path then to the inner or 'copulation' path, as observed by Roux. This 'copulation' path is usually observed when the penetration path is turned *away from* the first furrow, that is, when it has not been directed towards the egg-axis.

The same data give the position of the point or of entrance with regard to the direction of 'pressure' and 'gravitation symmetry'. In the (α) series the sperm tends to enter in the direction of 'pressure', that is, on that side of the egg on which it is in contact with its neighbours. Hardly a single spermatozoon enters on that side of the egg on which the white pole had been turned up, and very few on the opposite side.

It is scarcely possible to suppose that either the compression of the egg or the gravitation plane brings the spermatozoa round to the side of compression, but it may be imagined that either by capillarity or by some chemotactic stimulus the spermatozoa are especially attracted to the point where the rapidly swelling coats of adjacent eggs come into contact, and that therefore fertilization is principally effected upon this side. This explains why the first furrow lies so often in this direction. The pressure may of course affect the position of the planes in the egg later on.

When the eggs are spaced the sperm enters on any side at random.

The deviation of the sperm entrance from the egg-axis (the angle between sperm-entrance radius and egg-axis) varies in the two series of observations. When the eggs are spaced and the axes vertical, the sperm enters mainly near the equator, never near the animal pole; when the eggs are compressed and the axis horizontal, usually at about 45° from the axis, though it may enter near the pole or near the equator. This difference obviously depends on the difference in the initial position of the eggs on the slide. The deviation has apparently very little effect on any of the planes we have been considering.

Finally, let us try and gain some conception of the mechanism by which the direction of the furrow depends on the point of sperm entry. It is apparently quite simple, for the sperm-path is directed usually towards the axis, the sperm nucleus travels along that path to meet the female nucleus, which is also in the axis, the centrosome of the sperm divides at right angles to that path, the fertilization spindle is developed between the diverging centrosomes and cell-division takes place in the equator of the spindle; the first furrow includes therefore the sperm-path. Should, however, the 'penetration' path not be exactly radial, for whatever reason, the sperm nucleus turns aside to meet the female pronucleus, there is a 'copulation', as distinct from a 'penetration' path, the centrosome divides at right angles to the former, and this, then, is included in or parallel to the plane of the furrow. In those cases in which the sperm-path is parallel to the furrow it is always quite close to it, and we may suppose perhaps that the first division has not been quite equal. (The division of the centrosomes has not, I believe, been observed in the Frog, and the foregoing description has been taken from the Axolotl. In this genus the definitive centrosome is formed from the sperm nucleus, when the latter has already penetrated some little way into the egg.)

The causes of the formation of the grey crescent which marks the symmetry plane are not so clear.

Roux describes it as being due to the immigration of superficial pigment. Now we have strong reason for believing that both the entrance-funnel—produced when the spermatozoon first touches the egg—and the sperm-sphere are local aggregations of watery substance. The accumulation of what appears to be a more watery substance about the middle piece which has been observed in the Axolotl, appears also to occur in the Frog: at least the same formation of large clear vacuoles in the sperm-sphere may be seen in the latter as in the former. Should this be actually so, we may suppose that the streaming movement centred in the entrance-funnel and sperm-sphere is responsible for drawing away the pigment from a certain region of the surface; hence the grey crescent. The sperm-sphere is on the inner side of the sperm nucleus: hence the grey crescent would appear on that side of the egg which is opposite to the entrance of the spermatozoon, should no disturbance of the streaming movement have taken place, and, since the sperm-path is radial, would be symmetrically disposed with regard to it. In this case, fertilization meridian, sperm-path, grey crescent and plane of symmetry, first furrow, and, later on, sagittal plane, would all coincide. There is, as we have seen, a very fair correlation between the sperm-entrance and the first furrow, and again between the sperm-path and the grey crescent. But should some other streaming movement of the cytoplasm be set up by the gravitation of the heavy yolk particles, or by pressure, or by light, then the relation between the two processes, the division of the centrosome which determines the direction of the first furrow, on the one hand, and on the other, the streaming movement towards the sperm-sphere which determines the position of the grey crescent, would be disturbed, and while the entrance point of the sperm might still continue to determine, though not so completely, the position of the furrow, it might come to be without relation to the symmetry of the egg and of the embryo; and this is what is actually observed.

Though it is difficult to assign the exact cause of each and every deviation from the rule, this much is certain, that however they may coincide in 'typical' development (I use Roux's expression), the factors which determine cell-division, and those which determine differentiation, may be influenced by different external causes in widely differing ways, and are therefore presumably distinct. Nor does this artificial separation of the two processes in any wise prejudice the complete normality of the development of the embryo.

Lillie has shown (*Journ. Exp. Zool.* iii. 1906) that in the egg of *Chaetopterus* there are granules of different kinds which pass, in segmentation, into definite cells. By means of the centrifuge some of these—the endoplasmic—may be driven to one side of the egg, but in whatever position these organ-forming granules may be thus artificially placed, the cleavage has the same relation to the egg axis (as determined by the polar bodies) as in the normal egg. The factors of cell-division are thus separable from those of differentiation.

To the cases quoted in the summary on pp. 245, 246 might be added the various instances in which an egg may be made, by heat or pressure or shaking, or in artificial parthenogenesis, to segment abnormally and yet give rise to a normal larva.

APPENDIX B

ON THE PART PLAYED BY THE NUCLEUS IN DIFFERENTIATION

(i) Boveri has more recently (*Zellen-Studien*, vi, Jena, 1907) published a very elaborate account of the irregularities produced by dispermy in Echinoid eggs, in which are brought forward still more facts in proof of the qualitative difference of the chromosomes.

As has been stated above, p. 263, dispermy is induced by the simple expedient of adding a large quantity of sperm to the eggs. The following types of dispermy are distinguished.

A. Tetracentric, i. e. each sperm centre divides.
   (i) Tetraster, with four spindles.
   (ii) Double spindle, i. e. the female and one male pronucleus lie in one spindle, the other male lies aside in its spindle.

B. Tricentric, one sperm centre remaining undivided.
   (i) Triaster, a tripolar figure with three spindles.
   (ii) Monaster-amphiaster, the undivided sperm centre remaining apart with one sperm nucleus.

C. Dicentric, neither sperm centre dividing.
   (i) Amphiaster, a spindle is formed between the two centres.
   (ii) Double monaster: the centres remain apart, one with one male, the other with the other male and the female pronucleus.

The segmentation of these eggs is as follows.

The tetraster divides simultaneously into four, which may either lie in one plane if the divisions are meridional, or be tetrahedrally arranged. In the first case another meridional division ensues, followed by an equatorial, then eight micromeres are formed, eight macromeres, and sixteen mesomeres. In the latter case not more than three cells can share in the micromere region and only four or six of these are produced. The triaster eggs, having divided simultaneously into three (meridionally), subsequently show six micromeres, six macromeres, and twelve mesomeres.

The segmentation of the double spindle eggs is interesting and important. Usually the egg divides across the two spindles

into two binucleate cells, but it may divide at once into four, or into three, one of which is binucleate. The interest lies in the binucleate cells, for they continue to produce uni-nucleate and binucleate cells until the latter divide simultaneously into four, and this simultaneous division may sometimes involve an irregular distribution of the chromosomes, with fatal consequences to the cell. Boveri had already produced evidence of the evil effects of an irregular distribution of the $3\ n \times 2$ chromosomes present in triasters and tetrasters. A more detailed account is now given.

Of the tripartite (triaster) ova about 8 % on an average produced Plutei. In these larvae three regions may be distinguished in the egg by the size of the nuclei (proportional to the number of chromosomes) and the boundaries between them may be shown to correspond to the divisions between the three blastomeres. The form is asymmetrical in skeleton and pigment, but Boveri shows that both sides are normal, as though the larva had been compounded of two types such as occur, as individual variations, in any culture. It is suggested therefore that the slight differences in the two sides are due to differences in the two sperms.

Some of the larvae have partial defects in skeleton or pigment, or the skeleton may be much reduced on one side, or one-third of the cells may be pathological, i.e. disintegrate in the segmentation cavity, while the remaining two-thirds are sound and sometimes symmetrical. In this case it is supposed that the degenerate cells had separated from the others at an early stage, and that the remainder had had time to recuperate. In others two-thirds are degenerate, one-third normal, or all three degenerate. When the three blastomeres are isolated and allowed to develop independently, segmentation is partial, with two micromeres, two macromeres, and four mesomeres, and often all three develop normally up to the blastula stage. After that only one or two, rarely all three, become Plutei, the rest giving rise to stereoblastulae or stereogastrulae, full of degenerating cells.

The isolated quarters of tetrasters also segment partially and normally, but few give rise to Plutei. The whole simultaneously quadripartite eggs only rarely give rise to what may be called a Pluteus (2 cases in 1500); but very degenerate larvae are found, with masses of disintegrating cells inside, which are assigned to one of the four blastomeres. Stereogastrulae—with nuclei of all the same size—are frequent.

As has been already mentioned, Boveri points out that the probability of each cell of a triaster receiving a complete set of the n chromosomes of the species when there are $3\ n \times 2$ to be distributed must be greater than that of each cell of tetraster obtaining a full complement, and the probability for one isolated

cell must be greater than that for the whole egg. What the mathematical values of these probabilities are Boveri does not know, though he makes an attempt to reckon them—not theoretically, but by means of a mechanical apparatus; the attempt is not quite successful. The fact, however, remains that eight per cent. of the triasters produce normal Plutei, only ·06 per cent. of the tetrasters. This does not depend on the cells receiving too much or too little chromatin (see p. 265), nor again on the fact that the ratio between size of nucleus and size of cytoplasm (see pp. 268, 269) can only be satisfied by certain definite numbers of chromosomes, and the only explanation remaining is that for normal development of each and every part the nucleus of each cell must contain a complete set of the specific chrosomomes; from which it follows that the chromosomes are qualitatively unlike.

A word may be said about the double-spindled eggs (Type A. i). The larvae from these sometimes show abnormal regions, and this is attributed to one or more of the binucleate cells having divided with a tetraster and irregular distribution of chromosomes. Of all such eggs 50 % gave rise to normal Plutei.

The degenerative changes undergone by the nuclei of these larvae are of several types, to be associated again with differences in the combinations of chromosomes.

(ii) Boveri's experimental proof of the qualitative difference of the chromosomes does not of course of itself involve a belief in the individuality of these bodies, for if the chromatin is concerned in inheritance, it is necessary to suppose that the number of qualitatively distinct bodies is far greater than the number of chromosomes, and these bodies may be differently grouped during each successive resting stage.

The hypothesis of the individuality of the chromosomes, i.e. of a constancy in the manner of grouping of these particles, rests in the first instance on such facts as those observed by Sutton in *Brachystola*, where in the spermatogonia the chromosomes are of different sizes, which may however be arranged in pairs, together with an odd one or accessory chromosome. In the resting stage the accessory chromosome remains apart in a separate vesicle, while the large chromosomes lie in separate pockets of the nuclear membrane, the small ones, each as a separate reticulum, in the main body of the nucleus. In the spermatocyte a number of bivalent spiremes appear, which show the same differences of sizes as the pairs of chromosomes previously, and the accessory chromosome.

The accessory chromosome passes into two only of the four spermatids and is supposed to be a sex-determinant.

Similar facts have been reported by Wilson for several Insects (see *Journ. Exp. Zool.* ii, iii, 1905, 1906).

Wilson finds constant size differences between pairs of chromosomes, and either an accessory odd chromosome (which passes into only one half of the germ cells) or a pair of idio-chromosomes of unequal size (one of which goes to one half, the other to the other half of the spermatozoa), or both the accessory and the idio-chromosomes (giving four kinds of spermatozoa). The idio-chromosomes are supposed, again, to play a part in sex-determination. Several other observers have found these accessory chromosomes, idio-chromosomes, and pairs of chromosomes of different sizes in various Insects (Boring, *Journ. Exp. Zool.* iv. 1907; Stevens, ibid. ii. 1905, v. 1908; McClung, *Biol. Bull.* iii. 1902, ix. 1905; Montgomery, *Biol. Bull.*, vi. 1904; Baumgartner, *Biol. Bull.* viii. 1904–5; Zweiger, *Zool. Anz.* xxx. 1906; Nowlin, *Journ. Exp. Zool.* iii. 1906); in Spiders (Wallace, *Biol. Bull.* viii. 1904–5; Berry, *Biol. Bull.* xi. 1906); and in Myriapods (Blackman, *Biol. Bull.* v. 1903; Medes, *Biol. Bull.* ix. 1905).

It is a noteworthy fact that the accessory chromosome retains its individuality in the resting stage (looking like a chromatin nucleolus), while the others break up. The belief in the individuality of these others rests therefore on the constancy of the relative sizes from generation to generation.

Further support for the hypothesis may be derived from theoretical speculations. We know that only *n* (one-half the normal number) chromosomes are necessary for normal development provided that they comprise a complete set. In sexual reproduction *n* maternal unite with *n* paternal. A study of the reducing division shows that *n* whole chromosomes first pair with and are then separated from *n* whole chromosomes, and that when they differ in size those of the same size pair together, and it looks as though paternal were here separated from maternal, though the distribution of paternal and maternal to the two cells will differ, almost certainly, in different cases.

If the particles of which the chromosomes are composed are also to be paired and separated, it would appear to be necessary that their grouping should be constant, in other words that the chromosomes should retain their individuality.

(iii) A case of heterogeneous fertilization between eggs of Sea-urchins and the sperm of *Antedon* has been described above (p. 262). Loeb has recently succeeded in rearing Plutei from the eggs of *Strongylocentrotus* fertilized by the sperm of a Mollusc (*Chlorostoma*). Cytological details are not given (*Arch. Ent. Mech.* xxvi. 1908).

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ADDENDA ET CORRIGENDA

P. 5, 5 lines from bottom, *for* unicellular *read* multicellular.

P. 28, line 10, *after* irregular, *insert* and in Triclads.

P. 57. To Literature *add* J. Sachs. Die Anordnung der Zellen in jüngsten Pflanzentheilen, *Arb. Bot. Inst. Würzburg*, ii, 1882.

P. 114. To Literature *add* G. Bunge. Weitere Untersuchungen über die Athmung der Würmer, *Zeitschr. physiol. Chem.* xiv, 1890.

P. 140, line 22, *for* prospective potentialities *read* prospective significances.

P. 225, 2 lines from bottom, *for* is now placed in *read* has now moved into.

P. 271. To Literature *add* W. S. Sutton. On the morphology of the chromosome group in *Brachystola magna*, *Biol. Bull.* iv, 1902.

P. 278. To Literature *add* J. W. Jenkinson. On the effect of certain solutions upon the development of the Frog's egg, *Arch. Ent. Mech.* xxi, 1906.

OXFORD
PRINTED AT THE CLARENDON PRESS
BY HORACE HART, M.A.
PRINTER TO THE UNIVERSITY