FOUNDATIONS of EXPERIMENTAL EMBRYOLOGY

BENJAMIN H. WILLIER • JANE M. OPPENHEIMER

Foundations of Experimental Embryology

Edited by

Benjamin H. Willier
Professor Emeritus of Biology
The Johns Hopkins University

and

Jane M. Oppenheimer
Professor of Biology
Bryn Mawr College

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Preface

It is the purpose of this collection of articles, already recorded elsewhere in the literature, to answer a need of those who are interested in the early history of experimental embryology, and to encourage respect for those investigators who blazed the trails we now follow in contemporary embryology. In achieving these aims we tried to abide by two basic criteria in choosing the selections. Foremost was our desire to include only articles of unusual excellence and of both pioneering and enduring quality, namely those that have had a definite influence on the rise of experimental embryology. Secondly, we wished to maintain a balance of areas among the selections. We hope this small volume reflects something of the variety of the ideas, discoveries, and method of some of the beginning experimentalists in embryology and developmental physiology.

Most readers who have delved at all into the older literature of experimental embryology will, in all likelihood, find old favorites absent from this work. Some articles were too long for inclusion in a volume of this size. Such works give place to what it is hoped will be happy new discoveries for some readers. Embryology has not moved forward in a straight line, and as in all anthologies, some of the choices have been arbitrary. If the readers feel, however, that the basic aims of the editors have been achieved on the whole, the time and work invested in the preparation of this book will have been well spent. The editors will be content if others find the reading of the book as rewarding and intellectually stimulating as they did during its assemblage.

All of the articles, save that of Driesch, are reproduced in full without abridgment in any way. The editors feel strongly that it is important for the student to see how various workers have attacked key problems in the past—how ideas led to technical ways of testing their validity—how the results were analyzed and interpreted.

We terminate this preface by an expression of our deep indebtedness to all of those devoted friends of the embryo who have translated or aided

in the translation of the articles written in the German language. We are also indebted to John Spurbeck for his care and skill in preparing the photographic reproductions of the original illustrations. To these we have a special sense of gratitude since without their help this work would have been difficult if not impossible. The editors, however, are solely responsible for the text of *Editors' Comments* preceding each of the eleven articles reproduced.

Benjamin H. Willier
Jane M. Oppenheimer

Table of Contents

Foundations of Experimental Embryology

PART ONE

1888

Contributions to the Developmental Mechanics of the Embryo. On the Artificial Production of Half-Embryos by Destruction of One of the First Two Blastomeres, and the Later Development (Postgeneration) of the Missing Half of the Body

by W. ROUX

from the Anatomical Institute of Breslau

Roux, W. 1888. Beiträge zur Entwickelungsmechanik des Embryo. Ueber die künstliche Hervorbringung halber Embryonen durch Zerstörung einer der beiden ersten Furchungskugeln, sowie über die Nachentwickelung (Postgeneration) der fehlenden Körperhälfte.* Virchows Arch. path. Anat. u. Physiol. u. kl. Med. 114: 113-153; Resultate 289-291. Tafel II und III. Translated by Hans Laufer and printed by permission of Springer-Verlag.

* The portion of the article dealing with postgeneration is omitted in this translation.

During the second half of the ninteenth century it became gradually apparent that descriptive and comparative approaches to the study of how an embryo develops were inadequate for explaining the role of causal factors in the developmental process. The chief and most influential advocate of a new approach by experiment was Wilhelm Roux (1850-1924), a German anatomist. He founded a new discipline, causal analytical embryology, which he called developmental mechanics (Entwicklungsmechanik *in German). He drew up a program for procedure, and established the* Archiv für Entwicklungsmechanik der Organismen, *the first volume of which appeared in 1894-95. It was the first and for several decades the leading international journal for causal analytical embryology and still is important today.*

Roux defined the over-all program of Entwicklungsmechanik *as the resolution of developmental processes into simpler, but still complex, functional processes, and the analysis of these functional processes into really simple ones, which may be identical with those which underlie inorganic or physico-chemical processes.*

With causal analysis his guiding motive, Roux performed a simple type of experiment on the frog's egg at the two- and four-celled stages of cleavage. If he injured one of the two first-formed blastomeres, the surviving blastomere developed a half-embryo; this is the work that is presented here. With a strong predilection for philosophical speculations concerning development, Roux interpreted the facts as suggesting that differentiation might be one of two types, namely self-differentiation (*independent or mosaic development*) *or* correlative dependent differentiation (*interaction of cells or groups of cells*). *He felt that the fact that one cell at the two-cell stage develops a half-embryo suggests that each cell develops independently of its neighbor and thus that the total development represents the summation of partial mosaic developments.*

These conclusions of Roux were later shown to be erroneous for the frog's egg (A. Brachet, 1905; McClendon, 1910). However, Roux's work on the production of half-embryos is significant for several reasons. It initiated a new trend in method of attack on embryogenesis and marked a major turning point in its study by shifting emphasis from descriptive to experimental embryology. It pointed to a new direction for embryological thought and theoretical interpretation. It opened the way for a new and experimental attack on the significance of interrelationships between tissues and thus led to new insights into old problems of epigenesis. It is safe to say that all the analytical embryology of the late nineteenth and of the twentieth century has built upon foundations laid by Roux.

CONTRIBUTIONS TO THE DEVELOPMENTAL MECHANICS OF THE EMBRYO. ON THE ARTIFICIAL PRODUCTION OF HALF-EMBRYOS BY DESTRUCTION OF ONE OF THE FIRST TWO BLASTOMERES, AND THE LATER DEVELOPMENT (POST-GENERATION) OF THE MISSING HALF OF THE BODY

The investigations that will be recorded in this article are closely connected with my previous works on developmental mechanics and presuppose therefore a knowledge of their results, at least for full understanding. Since I have noticed that my previous works have remained almost unknown even to many specialists in the field, it appears proper to preface this treatise, meant for a larger circle of readers, with a brief review of the pertinent results.

The following investigation represents an effort to solve the problem of self-differentiation[1]—to determine whether, and if so how far, the fertilized egg is able to develop independently as a whole and in its individual parts. Or whether, on the contrary, normal development can take place only through direct formative influences of the environment on the fertilized egg or through the differentiating interactions of the parts of the egg separated from one another by cleavage.

For the egg as a whole I answered this question by rotating eggs in a perpendicular plane in such a way that, while the centrifugal force did not inhibit their development, the eggs continuously altered their orientation with respect to gravitational force, to the magnetic meridian and to the source of light and warmth. The result was that normal development was neither suspended, altered, nor even retarded by this process. We can conclude from this that the typical structures of the developing egg and embryo do not need any formative influence by such external agencies for their formation, and that in this sense the morphological development of the fertilized egg may be considered as self-differentiation. Nevertheless, several possibilities of external formative influence still remain that have not been tested by this experiment. These are of a very general character, for example His[2] made the hypothesis that many cells have a tendency to move toward the direction from which oxygen enters, thus enlarging the surface of the embryo. It is also conceivable that the blastomeres lying on the surface of the blastula and the gastrula gradually become flatter on their external sur-

1 Cf. W. Roux, Beiträge zur Entwickelungsmechanik des Embryo. No. 1. Zeitschr. f. Biologie. 1885. Bd. XXI.

2 W. His, Untersuchungen über die Bildung des Knochenfischembryo (Salmen). Arch. f. Anat. u. Physiol., anat. Abth. 1878, S. 220.

faces only because influences from outside cause their transformation into functional epithelia, thereby producing a mechanical tendency towards the densest concentration possible and toward the minimizing of the external surface, in contrast to the previous tendency towards the greatest possible sphericity for each individual cell. These speculations must still be checked against reality. The fact also must not be overlooked that the influence of external agencies may be a necessary condition for development, even though these influences may have no directly formative effect. For example, no development at all will take place without a certain amount of heat and also, later, of oxygen. But it cannot be deduced from this that such agencies determine which part of the egg produces the eyes, the blastopore, or the neural groove, or that they are the cause for the specific formation of the parts, despite the fact that abnormal formations result from an abnormal rise in temperature according to Panum, Dareste and Gerlach.

It has thus been shown that the development of the form of the fertilized egg, apart from that of several more general structures, occurs without external formative forces. We therefore have to look for the formative forces in the egg itself, which imposes a very pleasant limitation on further investigation.

As the result of this insight, it seems to me necessary to determine first of all whether all or many parts of the egg must collaborate if its structures are to form normally, or whether, on the contrary, the parts of the egg separated from one another by cleavage are able to develop independently of one another, and to show also, if possible, what share in the normal development each of the two principles has—that of differentiating interaction of the parts with one another, and that of self-differentiation of the parts.

As an argument for a certain independence in the development of the individual blastomeres, one could utilize, although not with certainty, the following fact about the egg of the frog, found by myself and shortly thereafter by Pflüger. This is that the first plane of cleavage of the egg represents the median plane of the future embryo, thus separating the material of the right and of the left half of the body, a fact which has been determined independently by van Beneden and Julin[3] for the ascidians. M.v.Kowalewski[4] later made observations that indicate similar conditions in the case of a teleost (*Carassius auratus*). At the same time I found a fact that we will use later on, that the cleavage plane perpendicular to the median plane of the future animal can be formed first

[3] Ed. van Beneden et Ch. Julin, La segmentation chez les Ascidiens et ses rapports avec l'organisation de la larve. Arch. de Biologie. T.V. 1884.

[4] Miecz. v. Kowalewski, Ueber die ersten Entwickelungsprozesse der Knochenfische. Zeitschr. für wissenschaftl. Zool. 1886.

although it normally appears second—and I later succeeded in producing this anachronism artificially.

In addition, it was already known to previous authors that the upper, black hemisphere of the frog's egg always corresponds to a definite side of the embryo, the dorsal side, according to these authors. This interpretation could no longer be considered correct, however, after my investigations and those of Pflüger. I recently have shown by means of certain localized defects on the cleaved egg that the middle portion of the black hemisphere of the frog's egg provides the material for the ventral surface of the embryo, in contrast to the previous view.[5]

I found moreover that the cephalic and caudal ends of the embryo are already determined at the stage of the first cleavage of the frog's egg and that in the case of *Rana esculenta,* the green or water frog, they are already recognizable by an oblique position of the axis of the egg, a condition which had already been determined independently by Van Beneden and Julin for the ascidians and later by M.v.Kowalewski for *Carassius* (although the latter author did not take the occasion to mention his predecessors as having observed this fundamental behavior in relatively closely related classes of animals). It is worth mentioning that observations pertinent to this matter had already been recorded in the posthumous papers of G. Newport, published in 1854. These aroused no notice at the time and were not discovered again until later. I showed furthermore[6] that the position of the cephalic and the caudal side of the embryo in the egg is normally determined by the union of the nucleus of the sperm and that of the ovum, the half of the egg penetrated by the male nucleus becoming the caudal half of the embryo, while the opposite half of the egg produces the cephalic half. It was possible to recognize the direct causal connection because I succeeded in fertilizing each egg from an arbitrarily chosen meridian and thereby determined the caudal side of the embryo of the egg at will. In the case of other animals where the side of the egg fertilized does indeed coincide with a definite side of the embryo, but where spermatozoa penetrate into the egg at a typical point, such a conclusion cannot be drawn with certainty but can at most be expressed as a conjecture.[7]

5 Anatom. Anzeiger. 1888, No. 25. Ueber die Lagerung des Materiales des Medullarrohres im gefurchten Froschei.

6 Beiträge zur Entwickelungsmechanik des Embryo. No. 4. Arch. für mikrosk. Anat. 1887. Bd. 29.

7 According to my previous investigations the location of the following form changes are normally determined by the arbitrarily selected location of the region of fertilization.

(1) The spermatozoon takes a typically curved course in the vertical meridional plane which passes through the point of sperm entry: in the fertilization plane.

(2) The union of the two sexual nuclei takes place in the fertilization plane.

(3) In *Rana fusca,* on the side of the egg opposite the side of fertilization, the dark

Such is the case with the hen's egg, where it has long been known that the location of the embryo is determined with regard to the axis of the whole egg, even though the exact relation of the median plane to the first cleavage and the exact relation of this to the direction of the nuclear fusion has not been discovered. V. Kölliker[8] had already surmised that that portion of the blastodisc of the hen's egg that divides most rapidly develops later into the posterior part of the blastoderm, in which the first traces of the embryo originate; and His[9] has shown further that in the blastodisc of the hen's egg after it has been laid every region of the external germ layer corresponds to a definite part of the future animal. For the further development of these parts, however, His assumes—in contrast to possible self-differentiation of the individual regions—mechanical interactions of the region of origin with adjacent or more distant regions. For two of these structures, the neural tube and the intestinal tube, I was able to demonstrate,[10] by separation of their primordia from the parts lateral to them, that such interactions are not necessary, since in spite of their isolation the development of the primordia was completed, and even faster than normally. According to this we should look for the formative causes effective in the development of these tubes in the parts which compose the tube itself, while the neighboring regions even offer a resistance to the development of the tubes, which must gradually be overcome. But from these results we must not deduce that all organs acquire their form by self-differentiation of the complex of

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hemisphere becomes lighter and takes the form of a gray crescent adjacent to the white hemisphere. This crescent is symmetrically oriented with respect to the meridian of fertilization. In the case of the green frog the pigment is likewise displaced, although perhaps in a somewhat different manner, so that the white portion reaches farther up on the same side.

(4) The first plane of division lies in the plane of the meridian of fertilization.

(5) The first appearance of the blastopore occurs in the meridian of fertilization, namely:

(6) On the half of the egg lying opposite to the side of fertilization, approximately at the border of the dark hemisphere and at the margin which subsequently becomes lighter (see No. 3).

(7) The lateral blastopore lips develop symmetrically with respect to this meridian.

(8) Both the neural ridges and the whole later embryo are located symmetrically with respect to the meridian of fertilization, that is to say, the plane of the meridian of fertilization becomes the median plane of the animal.

(9) The side of the egg that is fertilized becomes the caudal side of the animal.

In order to gain insight into the causal relationships upon which these multiple correlations are based, I have made an effort to produce artificial separations of these correlations and have frequently been successful. A further report on this matter will be forthcoming.

8 A. Kölliker, Entwickelungsgeschichte des Menschen und der höheren Thiere. Leipzig. 1879.

9 His, Unsere Körperform und das physiologische Problem ihrer Entstehung. 1874.

10 Beitrag 1 zur Entwickelungsmechanik des Embryo. Zeitschrift für Biologie 1885.

parts of which they are composed. On the contrary, each case must be investigated individually, and for many structures it is beyond all doubt that they are produced by mechanical interactions with neighboring parts, for example the shape of the liver, the lungs (His, Braune), bones (A. Fick), paths of many vessels (G. Schwalbe), etc. In connection with this I have shown, by producing an artificial rhomboid fossa on the neural tube which survived the deforming effect, that the embryo possesses vital adaptability to passive deformation to a very high degree; thus the theoretical possibility of such an origin has been demonstrated for the normal rhomboid fossa, which would agree with His' assumption.

In addition, numerous facts of pathology also argue for the self-differentiation of the parts of the egg, for example dermoid encysted tumors, etc., facts which I have collected in the article last referred to. Yet only direct experimentation with the egg can clarify for us with perfect certainty the actual participation of self-differentiation of the parts of the egg in normal development. Years ago[11] I worked along these lines and verified, in general, that operations that produce an extrusion of material from the cleaving and cleaved egg do not prevent development or cause general malformation. The resulting embryos develop rather normally and have only a localized defect or a localized malformation.

In order to acquire more specialized knowledge, I used the portion of the spawning period in the spring of 1887 that remained, after the conclusion of time-consuming experiments, for pertinent investigations on which I will report in the present article.

Although, as will be seen, the results were very extensive, many important questions had to be left temporarily unanswered, questions that could have been easily answered by continuation and a slight variation of the experiments. This present study is therefore only one installment, as it were, of the theme treated, that of self-differentiation.

The plan of the experiments was as follows:

In the first experiment the eggs of the green frog, *Rana esculenta,* were placed individually in glass dishes, and the oblique position of the black hemisphere and the direction of cleavage were sketched during the formation of the first cleavage. Then one of the first two blastomeres was pricked once or more with a fine needle. The present position of the egg was then compared with the drawing. A new sketch was made if there was a difference, and the location of the puncture points was

11 "Vorlaüfige Mittheilung über causal-autogenetische Experimente," Vortrag gehalten am 15. Febr. 1884. in der Schlesischen Gesellschaft für vaterländische Cultur. (Lecture delivered on February 15th, 1884 to the Silesian Society for Native Culture.) My neglect in sending in a review resulted in there being no notice of that lecture in the corresponding annual report of the Silesian Society. The report was first published in Beitrag 1 zur Entwickelungsmechanik Zeitschrift für Biologie 1885.