

Chapter 3

The Origins of *Entwicklungsmechanik*

JANE MAIENSCHIEIN

1. Introduction

Leading developmental biology textbooks tell their readers that a new field of experimental embryology emerged somewhere around the end of the nineteenth or the beginning of the twentieth century. This field began, the texts generally explain, with the work of Wilhelm Roux on *Entwicklungsmechanik* (or developmental mechanics). In particular, Roux's set of experiments on isolated blastomeres led the way. As Scott Gilbert very clearly puts it in his developmental biology textbook (1), "With this series of experiments, Roux inaugurated his program of developmental mechanics (*Entwicklungsmechanik*), the physiological approach to embryology. No longer, insisted Roux, would embryology merely be the servant of evolutionary studies. Rather, embryology would assume its role as an independent experimental science."

The story is, of course, more complicated than the textbooks indicate. Roux did not single-handedly launch a new discipline, nor did any one set of experiments define the field. Rather, *Entwicklungsmechanik* emerged against a background of growing interest in both problems of individual development and experimental methods. This chapter explores the context, the content, the goals, and the methods of the *Entwicklungsmechanik* program more generally, showing how Roux's work fit into the larger picture.

2. Foundations of *Entwicklungsmechanik*

2.1. Wilhelm His (1831–1904)

The story of the foundations of experimental embryology and *Entwicklungsmechanik* begins in Germany. Anatomist Wilhelm His stimulated the move to study embryology for its own sake and devised new experimental approaches and techniques. In the first case, his improvement of the microtome to allow successful serial sectioning made possible the study of whole organisms, slice by slice, rather than the relatively chunky pieces that had resulted

before. His stimulated the rise of experimentation through his vehement polemical attacks on what he saw as the phylogenetic excesses of the leading popular German morphologist Ernst Haeckel.

Haeckel (1834–1919) had himself favored the study of embryos, but for the purpose of establishing past phylogenetic relationships, or the patterns of evolutionary lineages, and not for their own sake. Embryos held important keys to these phylogenetic relationships, Haeckel felt, because ontogeny very nearly parallels phylogeny (2). Immortalized as the "biogenetic law," this view that "ontogeny (for all practical purposes) recapitulates phylogeny" held a place at the center of morphology for Haeckel.

According to this view, all organisms are initially essentially alike. That is, they all correspond to an "Urform," or the original ancestor of us all. Each developmental stage then brings differentiation from that original type, but all organisms follow the same unbranching path of change until each organism stops developing and differentiating at the appropriate stage corresponding to its adult form. Although the developmental pattern could change in various ways if the environment acted to bring adaptations, Haeckel was adamant in maintaining that the earliest developmental stages remain quite primitive and unimportant for later development.

Although Haeckel was not the only morphologist by any means, he and Carl Gegenbaur achieved a popularity which gave their views an exceptionally wide hearing (3). Both sought publicity, and from the 1850s these two were together in Jena, where they lectured widely and attracted followers. Students joined their morphological programs because of their strong endorsement of Darwinian evolution and their emphasis on the way that embryological and morphological work could support Darwinism. As the German public became increasingly intrigued with Darwinism, and as Haeckel continued to write popular books for the general public, he and Gegenbaur remained the center of attention in morphology (see Ref. 4 for further discussion). But others, including His, began to question whether Haeckel's interpretations and his emphases were appropriate.

In particular, His was distressed that Haeckel's popular discussions of evolution gave his ideas more credibility than they would have attracted on the basis of the scientific contributions alone. As Lynn Nyhart has explained in her excellent study of German morphology in the late nineteenth century (5), much more was going on in the field than Haeckel's or even Gegenbaur's work. Morphology had found a home within medical schools, anatomical institutes, and zoology programs, for example. Yet Haeckel and Gegenbaur both insisted on the centrality of evolutionary questions and on the value of using embryos in particular to construct phylogenetic trees. This emphasis provided a focus for attack by those who disagreed, and His was one of the leading detractors.

In an explicit and ardent attack, His insisted (6) that individual embryonic development must be explained in mechanical terms directly affecting the individual itself. There was no need to appeal to the historical past to achieve a proper causal explanation. Instead, His called for a physiological approach to development, which would base embryology strictly on a study of the developmental processes taking place within the individual and not on evolutionary patterns of structural change.

Basic to this interpretation was His's assumption that the egg is already from its earliest stages differentiated in some way. This clearly contrasted with

Haeckel's insistence on the virtual identity of early developmental stages in all organisms (7) and brought His directly into conflict with Haeckel. His made it clear that he found Haeckel's views laughable and naïve about physiological causation in particular. He ridiculed what he saw as Haeckel's absurdly inaccurate, uninformed physical discussions, including his appeals to nonexistent or misunderstood concepts of material continuity, monism, and various mechanical formulae. Such tricks without proper understanding, His said, must remain only meaningless word games. He pointed out that, with Haeckel (8), "all these words, which are capable of strengthening a heart thirsty for knowledge, came into use: parental material, molecular movements, life characteristics, protein, form and protoplasm. 'Misce, fiat explicatio!' so runs the enlightening formula of our clever Doktor, and with this stroke he opens his eyes to all secrets of generation and life."

Instead, according to His, it was important to seek a mechanical explanation of development beginning with the egg, which is not an undifferentiated blob of matter, but a coordinated complex of "organ forming germ regions (*organbildende Keimbezirke*). The proper approach for the study of embryos would then begin with an understanding of "transmitted movement" as the original material undergoes mechanical foldings and rollings of the various elastic tubes and plates that make up the material. Analogous to processes known to occur in geology, these physiological processes of embryonic development would result in unequal growth of the various parts of the embryo and thus in differentiation of the parts. As differentiation progresses, the "principle of organ forming germ regions" translates the initial invisible internal chemical differences in the embryo into the visible complex differentiations of the adult body parts. Thus, His claimed that by establishing the nature of the initial differences in the egg and the subsequent physiological, mechanical processes, embryology could provide causal explanations of development. His had given embryology a new purpose and a suggested program of research.

Few followed His's peculiar interpretation of mechanical rollings and unfoldings very far. Yet sometimes inspired by His's general concern with embryological processes, others did take up embryological study, only with different emphases and assumptions. Here, too, His had an impact, this time by his concrete improvements to the microtome rather than by rhetorical appeals. Trained as a cytologist and anatomist, His had by 1866 developed a way to mount an object to be observed on a microscope stand, which held it steady. Other improvements by the 1880s allowed him to mount a very sharp knife so that it was also held steady. A knife could then move through the preserved, hardened object and make regular, very thin slices. It was even possible to make regular sequential serial slices of the whole organism. Floating the sections on warm water and then flattening them for observation provided much better and more complete specimens than had previously been available. Though His did not develop his microtome improvements commercially, others soon did (see Ref. 9). The evidence gathered with such techniques confirmed His's views that different organisms differ in their earliest stages. The resulting discussions helped to stimulate embryological study still further.

Though relatively few agreed with His's interpretations, others did follow his lead to embryology for its own sake. In addition, some also shared His's physiological emphasis on developmental processes within the individual. Whereas

His stressed the internal mechanics of the organism, however, some looked at the role of external factors in shaping developments. Questions about the relative importance of external and internal factors for development gained considerable attention in the next decades, inspired by His.

2.2. Eduard Pflüger (1829–1910)

Despite some contributions from a few French and English embryologists (10–12), the Germans retained dominance in the study of individual development. Especially the contributions of Eduard Pflüger, Gustav Born, Wilhelm Roux, Oscar Hertwig, Hans Driesch, and Curt Herbst (13) began to define a program in embryology, which became a starting point for modern experimental embryology.

Physiologist Eduard Pflüger held the position of Professor of Physiology at Breslau and worked on various problems related to sensory physiology. In the 1880s, he turned to the problem of what determines the sex of a frog embryo. Though it is not clear what motivated this research move, it was not as radical as it may now sound. The majority of biologists assumed that the production of one sex or the other in an individual was something that occurred in the course of development, stimulated at least partly by the factors external to the organism itself. Perhaps something in the environment provoked a sensory response that initiated sex determination, Pflüger may have thought. As was traditional in physiological research at the time, he then concentrated on manipulating and controlling the environment external to the developing individual frog. Given an external change, such as increased semen concentration, he asked (14,15) what effect it would have on the internal production of sex in the individual.

This was not the sort of question or the sort of approach that most morphologists would typically have adopted toward development. And physiologists had traditionally not asked questions about embryonic development. What Pflüger offered, then, was a new combination of the methods of physiology and the problems of morphological embryology; his research fell between the two types of older work in the two fields.

Why had he never seen any bicolored hybrid frogs, Pflüger asked next (16)? Since very differently pigmented species exist and since he had never seen any individual with two different pigmentation patterns, it must be that no hybridization occurs. But what prevents it: is it simply the mechanical fact that the species normally breed at different times? If that were so, then if he could obtain semen from one species and fertilize the other species artificially, he should be able to overcome the barrier and produce bicolored frogs after all. He tried the experiment but could not obtain any uniform results, even though he could consistently get results with artificial insemination within the same species. Something did seem to be preventing normal hybridization, but he could not determine what it was. Presumably it was some factor internal to the organisms themselves, he thought. This research suggested further lines for exploration and moved Pflüger on to other embryological questions using experimental approaches. He was certainly not convinced that internal factors direct all of development, and he raised questions to test the importance of external factors.

Pflüger next carried out a series of experiments to test the effects of orientation within the gravitational field on development (17,18). He placed the frog embryo firmly between two glass plates and rotated the whole arrangement in a variety of ways with respect to gravity. As a result, the cleavage plane that divided the first two cells appeared in a different place than it normally would have. He could tell this because the plane lay differently with respect to the very visibly different light and dark areas in the egg. He concluded that gravity determines the direction of the cleavage plane and that, since the initial cleavage plane persists and defines later cleavages and ultimately the body orientation, this experimentally altered external variation altered the internal orientation of the embryo. Thus, he concluded that external conditions can indeed direct development.

This, in turn, meant that the embryo could not already be lying within the egg, as some had suggested. But neither is the egg a mass of undifferentiated material driven by ancestral heredity, as Haeckel maintained. Rather, the egg experiences a "relative isotropy" and is differentiated partly by internal and partly by external factors. Perhaps some sort of molecular polarizations effect the internal organization, he suggested, but he did not know how. At least, some sort of mechanical cause must be operative.

Pflüger's work stimulated a flurry of enthusiastic experiments on frogs by a number of researchers, especially Gustav Born and Wilhelm Roux, who were also at Breslau in the Anatomical Institute. Pflüger had shown the promise of using manipulative experimental approaches to control external environmental influences on the embryo, which provided a way to get at the relative importance of factors internal or external to the developing organism. He had also raised physiological questions about causal explanations and functional processes in embryonic development. This suggestion that an experimental attack on embryological problems might be productive is what inspired others. Yet not all pursued Pflüger's particular set of questions or his interpretations. Other parallel lines of research also emerged.

2.3. Gustav Born (1851–1900)

Like Pflüger, Gustav Born studied amphibian development and sex differentiation. After a series of explorations into hybridization and sex production, Born began to investigate other differentiation processes as well. Also following Pflüger's work on the effects of gravity on frogs, Born (19–21) carried out similar experiments. He disagreed with his senior colleague's interpretations, however, and concluded instead that internal nuclear divisions decide the direction of the cleavage plane. Actually, he felt, the gravitational effect that Pflüger had regarded as so important is only "indirect, caused by the eccentric position of the nucleus and the presumed least specific gravity in the special case of the fertilized frog's egg" (22). Born did not question the importance of experimenting on embryos. Indeed, he endorsed that approach. What he doubted was Pflüger's particular interpretation which placed so much importance on the efficacy of external factors.

Another line of Born's work involved transplanting pieces of tissue from one organism to another to determine the respective contributions of each of the two parts to the hybrid developing embryo. This proved extremely influential on later research and ultimately inspired Ross Harrison and Hans Spemann in their own successful work on tissue culture and embryonic transplantation (23,24).

2.4. Wilhelm Roux (1850–1924)

Also at Breslau, Wilhelm Roux saw the promise of working with experimentation and with embryonic development. Roux agreed with Born that the direction of the cleavage plane is fixed by internal factors at an early stage and cannot be changed by altering the external conditions, as Pflüger insisted. In fact, Roux concluded from his early experiments (25) that the cleavage plane and the axis of the resulting embryonic body are both set by the second cell division. After that point, development follows a rigid pattern determined by internal conditions of some sort. Cell divisions simply do not cause differentiation, nor do external factors. The question was, "What does?"

Like Pflüger, Roux also rotated eggs within their gravitational field to discover the effects of such experimental manipulation. Yet he found that the embryos develop quite normally even under altered conditions. Based on his experimental evidence, Roux concluded that the eggs are self-differentiating rather than being driven by external conditions. By 1885, Roux was generating a general theory about the causes of embryonic development based on this idea of self-differentiation (26,27). He could, he insisted, provide a causal analytical account of development in this way. First, he felt, the individual passes through a stage of "independent" development of organs directed by the internal makeup of inherited structural patterns. This is followed by a stage of "dependent" development in which functional connections are made and which depends on a complex of factors internal and external to the organism itself. The cell divisions then cause the subsequent differentiations, he believed.

Roux felt that in stressing the direction of cell cleavage generally, the others had missed the importance of the nuclear division in particular. He suggested that the nucleus actually holds all the qualities for individual formation. He offered a theory of qualitative cell division, according to which each division actually separates off differential nuclear materials into the different daughter cells. The process is rather like producing a mosaic, he said, in which each resulting piece is different in that it has different bits of nuclear material from the others though it maintains its individuality and also remains part of a larger picture (28–30). Whereas Roux had at first followed his teacher Haeckel's emphasis on evolution and competition to stress the competition of hereditary units, in a "struggle of parts" ("der Kampf der Theile"), by 1883 he had moved beyond Haeckel (31). Instead he saw a more passive embryological process as taking place without the importance of such struggle.

At the same time, another German researcher in Freiburg had come to similar conclusions. Like Roux, August Weismann drew on evidence from his observations of developing cells, but also went well beyond that immediate data to offer a larger theory of development (32–34). Despite improvements in both

equipment and microscopic and related techniques for preparing and observing specimens, no one could see very clearly what the nucleus was doing (especially Weismann, who was afflicted by visual problems). So any theory based on nuclear division must remain largely conjectural and grounded in indirect and circumstantial evidence. Weismann put forth a theory based on the assumption that physical hereditary units exist within the nucleus, and he postulated a mechanism for the separation of those units. His quite sophisticated theory offered three levels of units: biophores (which are arranged in packets called *ids*), *ids* (which actually determine each particular characteristic of the developing embryo), and *idants* (which correspond to the chromosomes, the smallest units actually visible).

At each cell division, the *idants* divide into parts which differ from each other and which then move into the daughter cells. This occurs because division is transverse (across its center) rather than longitudinal (along its length) and the *ids* are arranged as discrete units along its length. For each characteristic, the *id* contains a set of biophores which undergo a competition, or a sort of struggle for existence to decide which will prevail and what the resulting cell will become. So qualitative division of the chromosomes (or *idants*) decides which *ids* and biophores will exist in each cell, and competition among the biophores decides which of the remaining possible characteristics will obtain. By this time, Weismann emphasized competition among the parts more than Roux did, but their basic conceptions of the structure and functioning of the hereditary units were compatible. This idea, which underwent various modifications, provided a wide-ranging theory which covered many of the facts of heredity and development. Widely labeled as the Roux-Weismann mosaic theory, it provided a focal point for further research and for heated discussion.

While pursuing this general theory, Roux also continued his experimental studies. In 1888, he carried out what became his most famous work, the so-called half-embryo experiments. Very much committed to his own view of internally directed self-differentiation through nuclear division, Roux set out to test that theory. He recognized his view as the leading alternative to Pflüger's hypothesis that external conditions cause differentiation. As Roux put it (35),

The following investigation represents an effort to solve the problems of self-differentiation—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.

Roux explained that there was already considerable support for his own view, but that in the spirit of a proper "causal analytical experimental embryology," only direct experimentation could yield definite results and decide the issue. His experiments involved working with the two-cell stage, just after the first division. If he punctured one of the two cells with a hot needle, then he assumed it would die. As a result, it obviously could not continue to develop. But the other cell would continue to do something. The question was what, exactly, the still living cell would do. Would it continue to develop in its normal way, thereby strongly suggesting that the cause of its differentiation lay in some strongly predetermined way internally within the cell itself? Or could the re-

remaining cell compensate and develop as a whole organism or in some abnormal way, suggesting that it was responding to the altered conditions outside itself? In other words: independent or dependent cleavage?

Clearly Roux expected his own interpretation to be confirmed by the experimental results. But it is also noteworthy that he expected to find an answer through experimental results. Careful observation could not provide enough information in itself, nor were the interpretations sufficiently unambiguous. Only with the isolation of some factors out of the general confusion and only with the control that experimentation offered could a definite answer be achieved, Roux felt. He continued to stress this epistemological point more and more emphatically over the next decade (especially Refs. 36,37).

Despite its great promise, the experiment proved much more difficult to carry out effectively than Roux had imagined. First, puncturing the eggs so that only one of the two blastomeres was affected was difficult. Further, getting the resulting blastomeres in those few successful cases to survive and continue developing was even more difficult. In fact, he succeeded in getting only about 20% of the experimental eggs to survive. He also achieved a few cases in which he killed one of the first four blastomeres to test the effects of the second cell division as well. But for Roux the numbers of successful cases or failures were not important. Even a very few surviving blastomeres could provide information about what happens in development, for the results should be reasonably definitive. If self-differentiation could occur once, it should be able to occur regularly.

Unfortunately, from the point of view of posterity, Roux did not remove the punctured blastomere from its partner. Thus, the remaining living cell was not really completely independent as it had the now presumably dead blastomere still hanging around. Fortunately, for the sake of advancing debate at the time, Roux did not at the time realize the importance of that factor. For he found what seemed to him clear results. The living blastomere produced a partial embryo, which advanced to either the blastula or the gastrula stage and no further. There was no compensation or adjustment for the missing material, he found. This told him that "in general we can infer from these results that each of the two first blastomeres is able to develop independently of the other and therefore does develop independently under normal circumstances." Furthermore (38),

All this provides a new confirmation of the insight we had already achieved earlier that developmental processes may not be considered a result of the interaction of all parts, or indeed even of all the nuclear parts of the egg. We have, instead of such differentiating interactions, the self-differentiation of the first blastomeres and of the complex of their derivatives into a definite part of the embryo. . . . We can say cleavage divides qualitatively that part of the embryonic, especially the nuclear material that is responsible for the direct development of the individual by arrangement of the various separated materials which takes place at that time, and it determines simultaneously the position of the later differentiated organs of the embryo.

That is, the early embryo acts as a mosaic of independent parts, brought about by qualitative nuclear division.

In 1888, Roux did not conclude more generally from his evidence that he had shown all development to occur because of this qualitative nuclear division or independent self-differentiation. Of course, he did not reject such an idea. But he knew that he had not established anything larger with this experimental work

than what happens for the very earliest stages of development which he had examined directly. He acknowledged (39) that "how far this mosaic formation of at least four pieces is now reworked in the course of further development by unilaterally directed rearrangements of material and by differentiating correlations, and how far the independence of its parts is restricted, must still be determined."

As Roux continued with his experiments, he discovered some cases that did not fit his interpretation. Yet by that time he was sufficiently committed to it that he did not revise his conclusions. Instead he generated auxiliary hypotheses to fill the gap. For example, in the few cases in frogs in which a whole embryo did result from the one blastomere, he suggested that there exists a reserve idioplasin (or set of nuclear materials). This reserve comes into action in the special cases when regeneration or postgeneration (following injury) occurs. For Roux, his experimental approach, his embryological questions, and his interpretation fit together into a program he called "*Entwicklungsmechanik*."

His had called for a "physiology of development." And Pflüger, Born, and others had borrowed from physiology to pursue an experimental approach to embryological questions. Roux was therefore not doing anything completely new and different. Indeed, Roux's program might not have had the impact it did had not others already been pursuing their own experimental programs and had they not already been sympathetic to parts of something like Roux's *Entwicklungsmechanik*.

2.5. Hans Driesch (1867–1941)

Hans Driesch took up the call for just the sort of causal, analytical accounts of individual development that Roux was pursuing. In his first major series of experiments, Driesch (40) tested for the potency (the ability of the cells to differentiate into cell types other than those they would normally form during development) after the first cleavage stage. He clearly expected to reinforce Roux's results by looking at another organism which had more durable, available, and more easily observed egg cells. Since he was working at the Stazione Zoologica in Naples, he selected the widely available sea urchin, just as Roux had worked with the familiar frog in Breslau.

With sea urchin eggs, Driesch could actually separate the two blastomeres completely as Roux could not with frogs. As Oscar and Richard Hertwig had shown (41), vigorous shaking of the water containing sea urchin eggs resulted in separation of the blastomeres from each other. Their results suggested that each cell might remain functional and continue to develop on its own. While the other "shakers" had worked with the bits of unfertilized egg produced by vigorous shaking, Driesch studied the fertilized egg just after the first cell division. He shook the cells apart, placed them in glass dishes, and then waited expectantly. He reported that he had waited in excitement for the experimental results for "I must confess that the idea of a free-swimming hemisphere or a half gastrula with its archenteron open lengthwise seemed rather extraordinary. I thought the formations would probably die. Instead, the next morning I found in their respective dishes typical, actively swimming blastulae of half size" (42).

Instead of producing partial embryos, then, the sea urchin blastomeres developed into half-sized, normally formed embryos, which developed to the blastula stage, with a few also going on to the gastrula and eventually even larval stage. It seemed, after all, that the cells each retained what Driesch called a "totipotency," or the ability to respond to the needs of the whole and to become any part of the whole that the conditions demanded. Each cell was able, in effect, to regenerate the missing material. That seemed to be the case to the four-cell stage at least, though just as Roux had remained restrained in his conclusions to the experimental report, Driesch did not draw any wild interpretations that went far beyond his data at hand.

Driesch suggested that some predetermination along the lines Roux expected occurs normally, but that some regulative ability to respond to abnormal conditions remains as well. And he pointed out that his results did differ from Roux's, of course, but that "perhaps this difference is not so fundamental after all. If the frog blastomeres were really isolated and the other half (which was probably not dead in Roux's case) really removed, would they not perhaps behave like my *Echinus* cells?" (43). The results did certainly show that His's doctrine of preformed organ-forming germ regions already lying in the egg could not be right, or at least could not be the only factor directing development. But he was less clear about the implications of his results for Roux's hypothesis of the efficacy of qualitative nuclear division.

With time, however, he did go farther and concluded that the cells retain their totipotency and regulative capacities. Eventually, he moved to an anti-mosaic and antipredeterminist point of view which appealed to teleology to explain how organisms develop into the right sort of form (44,45). After 1900, Driesch turned increasingly from embryology toward philosophy and toward vitalistic views of life. Yet in the early 1890s he remained an enthusiastic supporter of experimental study of embryology. And he endorsed the call for a causal, analytical account of developmental processes, even when his own research results called Roux's interpretations into question.

2.6. Theodor Boveri (1862–1915)

Investigators into other areas widened the scope of developmental mechanics. Theodor Boveri, for example, sought to determine the relative contributions of nuclear and cytoplasmic material to development, as well as the relative contributions of the male and female parents. One experiment involved shaking unfertilized sea urchin eggs quite vigorously. This broke them into small bits, some with nuclear material in them and others with none. He then fertilized the bits with sperm from another species. Boveri predicted that if the pieces developed according to the normal pattern of the host (egg) species, then the cytoplasm must play at least a major role in determining development. If, however, they developed according to the donor (sperm) species, then the nucleus must have been primary since there was no cytoplasm from that species. Boveri concluded that the sperm determine heredity.

Yet, in fact, his results from this ingenious experiment remained inconclusive, partly because of some of the same sorts of difficulties that Roux had

experienced simply in getting the experiment to work and to produce sufficient numbers of surviving specimens. American embryologist Thomas Hunt Morgan pointed out the difficulties, for example, and called into question the interpretations, initiating a debate that continued for years. Morgan admired Boveri's work nonetheless, including the further addition of the magnificent set of "Zellenstudien," which revealed Boveri's commitment to experimentation for study of heredity and development (46,47).

2.7. Edmund B. Wilson (1856–1939)

A young American, Edmund Beecher Wilson, visited Europe after receiving his Ph.D. degree at The Johns Hopkins University and decided to work with Boveri. There, in 1882–1883, he learned about Boveri's cell studies and about the latest in cytological techniques. He then continued on to the Naples Stazione Zoologica, which he found quite exciting and the best place to learn about the current leading techniques and theories. As a result, in 1891–1892 when he had the opportunity to return to Naples, he eagerly took it. Driesch was then working on his isolated blastomere experiments, and Wilson joined in.

Roux had studied frogs and Driesch sea urchins. Wilson resolved to look at the same phenomenon in his own favorite organisms, including several different annelids and *Amphioxus*. By the time Wilson completed the work, Driesch and his friend Herbst had left Naples for a while, and Wilson had to return to the United States to take up his new position at Columbia University. Thus, Wilson wrote to Driesch when the latter returned in June 1892 to report, "It is very easy to shake the blastomeres apart and I have got numerous half- and quarter-embryos exactly like the usual ones but $1/2$ or $1/4$ as large" (48). He then succeeded in getting the eight-cell stages to give rise to what looked like they might be one-eighth-sized embryos. Thus, he concluded, "It looks as though any cell of the early cleavage-stages may, if slightly disturbed, give rise to an embryo." But the results for the later stages were not sufficiently clear as yet.

The eight-cell stage was particularly important, Wilson recognized, for here was the first time that the division produced cells that did not normally give rise to some part of all three germ layers. The first two divisions might result in four cells that retained just enough material that normally goes into each germ layer to make up for the losses in the abnormal experimental conditions in which it found itself. But he knew from his cell lineage studies that the eight-cell stage should not be able to do that. If each blastomere at this stage could produce a whole organism, then this case would be important. For if "a pure ectoderm cell can regenerate the whole, we shall have a demonstration of your views and a fatal blow to the theory of 'Keimplasm' and qualitative nuclear division," Wilson wrote to Driesch (49). This was clearly an exciting possibility.

A few months later, Wilson wrote to Driesch again. He had continued his work, he reported, and could not get the eight-blastomere stage to develop further. Success came only with the two and four cells, which divided and produced small-sized, but perfectly normal-looking, embryos. There did not seem to be any accommodation for the fact that half the material was simply not there. Rather, each blastomere seemed to contain within itself sufficient material

and direction to develop properly. Thus, no "regeneration" takes place, and it seemed that only with the eight-cell stage had division produced a qualitative deficiency for which each cell could not itself compensate. But this did not lead Wilson to Roux's interpretation of qualitative nuclear division. As he reported in his letter and in an article that appeared soon after (50), he could only conclude that each blastomere did not require everything it would need for normal development. He saw no evidence that the lack lay in the nucleus or that any sort of nuclear division normally directs development.

Over the next few years Wilson, Driesch, and others continued to gather additional information about isolated cell divisions and differentiations. They compared notes and argued about the best conclusions. And they persisted in denying that the available evidence from the various different species they had studied pushed them in any way toward Roux's emphasis on nuclear division to explain development. They continued to look at the internal structure and the patterns of cell division for clues to the causes of embryonic differentiation.

Yet though they disagreed with Roux's particular interpretations, they agreed with the new experimental orientation toward embryology. As Wilson put it in a general lecture to the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, Pflüger's experiments on gravity had inaugurated a new approach in biology. For (51):

These pioneer studies formed the starting-point for a series of remarkable researches by Roux, Driesch, Born, and others, that have absorbed a large share of interest on the part of morphologists and physiologists alike; and it is perhaps not too much to say that at the present day the questions raised by these experimental researchers on cleavage stand foremost in the arena of biological discussion, and have for the time being thrown into the background many problems which were but yesterday generally regarded as the burning questions of the time.

2.8. Thomas Hunt Morgan (1866–1945)

Another young American, also a graduate of Johns Hopkins and a friend of Wilson's, Thomas Hunt Morgan joined the experimental group shortly after his graduation in 1891. In 1892, Morgan translated a major paper of Boveri's into English. The next summer, the translation appeared and Morgan began his own line of research on teleost fish, following, as he said, the experimental approach of Pflüger, Roux, Driesch, and the Frenchman Laurent Chabry. As he said, one reason for the translation was "to point out the new avenues of research that such work opens. Results of this kind are of the utmost importance, inasmuch as they touch the very heart of the question of Heredity. Each advance in our knowledge gained by experimental work of this sort, carries forward rapidly our understanding of the most vital phenomena of life" (52). Various experiments of Morgan's to assess whether the first cleavage plane corresponds to the median plane of the embryo and the resulting adult, as Roux had said occurs, showed Roux to be wrong. Other studies with isolated blastomeres in various species did not produce any reliable results, though they suggested that external conditions alone do not direct development. As Morgan realized, "Perhaps I have stated my conclusion too positively. Any one working at such problems will realize and appreciate the difficulty of correct interpretation of such evasive and compli-

cated phenomena. I wish therefore to offer the explanation attempted above as an alternative view that may help as a working hypothesis and give a stimulus to further inquiry along these lines" (53).

Morgan then undertook a series of studies of echinoderm eggs, following Boveri and Driesch. He could not find any clear cases in which the nonnucleated pieces of sea urchin eggs segment any further, as Boveri had tried to show. He did not agree with Boveri's interpretation and his emphasis on nuclear inheritance, but he admired the approach and began to pursue the questions raised. He found it likely (54) that instead of the nucleus and the chromosomes carrying the important material for development, "a simple mechanical explanation is probably at the root of the matter, but I do not feel warranted in suggesting one." Other experiments showed further that the sea urchin is already cytoplasmically differentiated by the two-cell stage and probably even before. Driesch's experiments to establish the early isotropy of the egg and its cleavage products were therefore not convincing. Several alternative hypotheses could explain the data. Morgan concluded, and there was not sufficient evidence to favor one over the others.

In still other experiments, Morgan followed up on other suggestions by Boveri, Driesch, Wilson, and his own colleague and friend at the MBL Jacques Loeb. By the mid-1890s he had run through the leading experimental results of the day, repeating, extending, and questioning the procedures and results (55). He took care to record the number of cases that failed as well as the number of successes, and he often offered several different possible interpretations for the data at hand. He then turned to frog development directly and then to regeneration (56,57). While endorsing the use of experimentation to tackle embryological problems, he clearly rejected Roux's particular interpretations and most of the other alternatives as well. For Morgan, more data were needed before any theory of the causes of embryonic development could be sufficiently well founded.

3. Experimental Embryology

3.1. Accepting Experimentation for Embryology

Oscar and Richard Hertwig, Oscar Schultze, Moritz Nussbaum, Curt Herbst, Jacques Loeb, and a number of others carried out a variety of experimental studies as well, each examining various aspects of heredity and development and each employing experimental approaches to their work. The move to experimental embryology was clearly "in the air," with each successful research project stimulating others to respond. Experimental manipulation promised control of the complex of conditions that surround development and otherwise made it appear possible to obtain results and answers to questions that seemed inaccessible otherwise. There was, that is, a general endorsement of experimental approaches by those interested in embryology. And this moved these researchers to a middle ground between what had been the study of morphology (including form and the development of form) and physiology (including the functional processes that produce the form). The work was variously labeled the "physiology of development," "experimental embryology," and "*Entwicklungsmechanik*."

Given the general move by a number of researchers with various goals and even different names for their work, then, why is it that textbooks today refer to *Entwicklungsmechanik* in particular and to Roux as the leader of the pack? Primarily because of his polemics in favor of a new program and his institutional successes. He convinced people, at least in retrospect, that his program offered a new epistemology for the study of development—and the rest of biology for that matter.

3.2. Roux's Program for *Entwicklungsmechanik*

In his papers, Roux had suggested what he saw as the advantages of experimentation, but it was really in the introduction to his new journal (58) that he had the opportunity to achieve the sort of full polemical attack he liked. Entitled *Wilhelm Roux's Archiv für Entwicklungsmechanik der Organismen*, the new journal experienced the heavy editorial hand of its founder from the beginning. In his essay explaining the purpose of the publication, Roux offered a manifesto for experimental work, and work in embryology in particular. Experimentation, he insisted, is the proper causal method of investigation. And given that causal investigation is the only legitimate study for science, experimentation must be the only method for science. Embryonic development is particularly difficult to study with direct observation. Roux insisted, because the processes and patterns lie largely hidden from sight within the embryo and change very quickly. The investigator has to devise alternative methods for obtaining information, and manipulative controlled experimentation was, for Roux, the obvious answer.

In addition, experimentation offers the major advantage over traditional forms of study in cytology, for example, that it is possible to work with living material. Cytologists must kill, prepare, harden, fix, slice, and eventually observe bits of the original material which has been far removed from its normal condition. Experimentation makes it possible to watch what is happening as it is happening. The major problem is to see "inside" the organism, and a properly designed experiment will allow just that.

Another advantage of experimentation is that if the researcher is sufficiently careful to keep the conditions of the material under control and to alter only one factor at a time, then it is possible to compare the experimental case with normal cases. The information thus derived will be reliable, as only experimental results can be, Roux insisted. He assumed that biological processes and patterns remain essentially constant from one organism to another, so that study of one artificially altered organism can yield general results that hold for all organisms under similar conditions. He also assumed that the processes of development and other living functions are mechanistic and can be understood in mechanical terms, which conform to general rules of mechanical causation. Otherwise there could be no science at all, he felt. Yet with the goal of searching for such causes, and with proper experimentation, developmental mechanics could answer tough questions and could begin to achieve a certainty as physics did, as an "exact science."

Despite his enthusiasm for experimentation, Roux was not naïve enough to think that every experiment would yield perfect results. Recognizing that life is

complex, he knew that it is difficult to identify what actually causes what. Two things may occur together, and one may appear to cause the other because it is slightly prior temporally. Yet both may result from some common cause, he realized, and may not have anything to do with each other except accidentally. Therefore, interpreting results of experiments would require the utmost care and vigilance. It was not the perfect method, but Roux certainly implied in a number of places that he considered experimentation the only legitimate method for biological science.

Roux's manifesto received wide attention. William Morton Wheeler translated it into English the next year and discussed it at the MBL. Embryologists from Germany and elsewhere began to send their best articles to Roux's journal, thereby suggesting that they endorsed at least his basic approach, if not also the details of his interpretation. Roux's heavy editorial hand and his insistence that articles in his journal represent proper experimental work helped to ensure that his vision would gain more attention than it might otherwise have based on his research reports alone.

Richard Goldschmidt, himself a strong-willed man, reported one experience with Roux's editorial control that evidently parallels in kind, if not in detail, a number of similar episodes by others as well. Goldschmidt had found a book by A. Labbé on experimental cytology particularly important and decided to translate it from French into German. A series of monographs edited by Roux and published by Engelmann seemed the best place to publish the translation. Goldschmidt reported, probably with some exaggeration (59):

After some months the manuscript was returned with a letter from Roux, in which he said that this was indeed a very interesting book but that its value would be considerably enhanced if I would add a few notes which he, Roux, had written out for me. In the manuscript I found hundreds of notes in Roux's handwriting, some attached to practically every page, which uniformly ran like this: "At this point it should be emphasized that Wilhelm Roux stated already in 1894 that . . ." and then followed some quotation which fitted or did not fit the occasion but glorified the father of *Entwicklungsmechanik*.

After some continued correspondence, Goldschmidt claims to have tossed the whole thing, Roux's comments included, into the trash. Others found it more palatable to accept Roux's suggestions, but it clearly was easier if they accepted his standards and his goals from the beginning.

3.3. Experimental Embryology

It is not always exactly clear when a field becomes established as a new discipline. By 1909, experimental embryology had achieved full status with its own textbook. British embryologist J. W. Jenkinson, in his *Experimental Embryology* (60), explained that the field differed from experimental morphology in its emphasis on the "physiological point of view." Understanding the "causes which determine the production of that form, whether in the race or in the individual" was one of the two main problems of biology. The other was to explain how the organism functions in a way so that it maintains its form within its environment. The latter question must be approached physiologically; the

former, concerning the origin of form, is morphological, but approachable from that physiological viewpoint. Addressing that fundamental question is experimental embryology.

Experimental embryology, Jenkinson explained, was also known as the mechanics of development or the physiology of development. Although the text itself discussed the work of many different experimental embryologists, the first page states that the field "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." Roux's theory, Jenkinson explained, had attracted much criticism and controversy as well as support. And the attention had proved fruitful for the field generally. Roux's experiment of 1888 and his subsequent manifesto suggesting why that experiment had been so important gave the new field a focal point, or a rallying cry. It provided a provocative statement of purpose for others to attack, criticize, pursue, and in the process to explore further. Not many agreed with Roux's interpretations, and few accepted the exclusive emphasis he sometimes placed on an experimental epistemology. But many listened, questioned, and discussed within a shared framework which they could attack, revise, or extend. Statements like Jenkinson's produced the false impression that Roux alone—or at least primarily—had provided the framework.

4. Responses

Clearly, then, experimentation had begun to reach center stage in embryology in a number of people's work in the 1880s and 1890s. In fact, by 1900 experimentation was nearly universally accepted as a proper approach for embryological work. By the 1920s, the major textbooks on embryology each began with a chapter on "the experimental method" (61–63). Experimentation meant different things to different people, but generally included the artificial manipulation of conditions so as to control the complex of factors that shape development in order to test the altered effects of just one. Working hypotheses also provided a framework from which to use the experimentally derived data to test which interpretation best fit, and the whole manner of obtaining information was felt to be reliable since anyone should be able to repeat the process and obtain just the same results. Almost everyone agreed that experimentation in that sense could provide information in some cases where no other approach could do so.

For most embryologists, however, experimentation was not the only, or even always the best, approach. Careful observation and description of normal developmental patterns and processes could combine with comparison of those developmental details among individuals within the same species and across species to yield important results as well.

Faced with a plethora of competing theoretical interpretations, beginning with the Roux-Weismann mosaic theory, embryologists sought to decide "which, if any, of the tales were correct," as American biologist Herbert Spencer Jennings put it later while reflecting on the situation in embryology around the turn of the century. He continued (64):

Henceforth, they said we must so work that our results and conclusions can be tested; can be verified or refuted. We must be able to say: Such and such things happen under such and such conditions, and if you don't believe it you may supply the conditions, you may try it for yourself, and you will find it to be true. But that is precisely experimentation; and so they flocked with enthusiasm to experimentation.

The enthusiasm was there. And Roux served as the cheerleader for the experimental program in embryology. Yet others found that experimentation was not a perfect cureall. Not all the results were definitive or repeatable in the way Roux had imagined. Nor, as the differences in Roux's and Driesch's results with isolated blastomeres showed, did all data lead clearly and definitively to one, and only one, proper interpretation. Experimental manipulation did not point the way to truth about the causes of development and differentiation as easily as Roux had insisted it would.

Yet the rhetoric was inspiring, and experimentation did carry the researcher further in many cases than observation or comparison alone. So the new field of experimental embryology, couched in terms of *Entwicklungsmechanik* and building on the dynamic enthusiasm provided by Roux as polemicist, was established. It has made its way, perhaps not quite accurately, into textbooks as the starting point for modern embryology.

ACKNOWLEDGMENTS. The letter from Wilson to Driesch, June 15, 1892, is quoted with permission of the Stazione Zoologica in Naples. I thank the Archivist Christiane Groeben for bringing this and related material to my attention. The research for this project was supported by NSF Grant #SES-87-22231 and a summer grant from Arizona State University's College of Liberal Arts and Sciences.

Notes and References

1. Gilbert, S. F., 1988, *Developmental Biology*, Sinauer Associates, Sunderland, MA, pp. 285.
2. Haeckel, E., 1866, *Generelle Morphologie der Organismen*, George Reiner, Berlin.
3. Gegenbaur, C., 1876, The condition and significance of morphology, translated and reprinted in Coleman, W. (ed.), 1967, *The Interpretation of Animal Form*, Johnson Reprint Corporation, New York, pp. 39-54.
4. Nyhart, L. K., 1987, The disciplinary breakdown of German morphology, 1870-1900, *Isis*, 78: 365-389.
5. Nyhart, L. K., 1986, *Morphology and the German University, 1860-1900*, Ph.D. dissertation, University of Pennsylvania, Philadelphia.
6. His, W., 1874, *Unsere Körperform und das physiologische Problem ihrer Entstehung*, F. C. W. Vogel, Leipzig.
7. Oppenheimer, J. M., 1940, The non-specificity of the germ-layers, *Q. Rev. Biol.* 15:1-27, in: Oppenheimer, J. M., 1967, *Essays in the History of Embryology and Biology*, MIT Press, Cambridge, MA, pp. 256-294.
8. His, W., 1874, p. 144.
9. Bracegirdle, B., 1978, *A History of Microtechnique*, Cornell University Press, Ithaca, NY, pp. 129-130.
10. Churchill, F. B., this volume.
11. Fischer, J-L., this volume.
12. Ridley, M., 1985, Embryology and classical zoology in Great Britain, in: *A History of Embryology* (T. J. Horder, J. A. Witkowski, and C. C. Wylie, eds.), Cambridge University Press, Cambridge, MA, pp. 35-67.

13. Oppenheimer, J. M., this volume.
14. Pflüger, E., 1882a, Hat die Concentration des Samens einen Einfluss auf des Geschlecht? *Pflüger's Arch.* 29:1-12.
15. Pflüger, E., 1882b, Über die das Geschlecht bestimmenden Ursachen und die Geschlechtsverhältnisse der Frösche. *Pflüger's Arch.* 29:13-40.
16. Pflüger, E., 1882c, Die Bastardirung bei den Batrachien. *Pflüger's Arch.* 29:48-75.
17. Pflüger, E., 1883, Über den Einfluss der Schwerkraft auf die Theilung der Zellen and auf die Entwicklung des Embryo. *Pflüger's Arch.* 32:1-79.
18. Pflüger, E., 1884, Über die Einwirkung der Schwerkraft and andere Bedingungen auf die Richtung der Zelltheilung. *Pflüger's Arch.* 34:607-616.
19. Born, G., 1884, Über den Einfluss der Schwere auf das Froschei. *Breslauer ärztlich. Zeitschr.* (reprint):1-14.
20. Born, G., 1885, Biologische Untersuchungen I. Über den Einfluss der Schwere auf das Froschei. *Arch. mikroskop. Anat.* 24:475-545.
21. Born, G., 1886, Biologische Untersuchungen II. Weitere Beiträge zur Bastardirung zwischen der einheimischen Anuren. *Arch. mikroskop. Anat.* 27:192-271.
22. Born, 1884, p. 2.
23. Oppenheimer, J. M., 1966, Ross Harrison's contributions to experimental embryology. *Bull. Hist. Med.* 60:525-543. Also in Oppenheimer, J. M., 1967, *Essays in the History of Embryology and Biology*, MIT Press, Cambridge, MA, pp. 92-116.
24. Horder, T. J., and Weindling, T. J., 1985, Hans Spemann and the organiser, in: *A History of Embryology* (T. J. Horder, J. A. Witkowski, and C. C. Wylie, eds.), Cambridge University Press, Cambridge, MA, pp. 183-242.
25. Roux, W., 1883, Über die Zeit der Bestimmung der Haupttrichtungen des Froschembryo, reprinted by Wilhelm Engelmann, Leipzig, pp. 1-29.
26. Roux, W., 1885a, Beiträge zur Entwicklungsmechanik des Embryo, *Zeitschr. Biol.* 21:411-526.
27. Roux, W., 1885b, Über die Bestimmung der Haupttrichtungen des Froschembryo im Ei und über die erste Theilung des Froscheies, *Breslauer ärztlich. Zeitung* (reprint):1-54.
28. Oppenheimer, J. M., 1964, Questions posed by classical descriptive and experimental embryology, in: Oppenheimer, 1967, pp. 62-91.
29. Churchill, F. B., 1966, Wilhelm Roux and a program for embryology, Ph.D. dissertation, Harvard University, Cambridge, MA.
30. Churchill, F. B., 1973, Chabry, Roux, and the experimental method in nineteenth century embryology, in: *Foundations of the Scientific Method: the Nineteenth Century* (R. Giere and R. S. Westfall, eds.), Indiana University Press, Bloomington.
31. Churchill, 1966, Chapter 4.
32. Weismann, A., 1885, *Die Kontinuität des Keimplasmas als Grundlage einer Theorie der Vererbung*, (translated by E. B. Poulton, S. Schönland, and A. Shipley, eds.), Clarendon Press, Oxford, 1889.
33. Churchill, F. B., 1968, August Weismann and a break from tradition, *J. Hist. Biol.* 1:91-112.
34. Churchill, F. B., 1970, Hertwig, Weismann, and the meaning of reduction division circa 1890. *Isis* 61:429-457.
35. Roux, W., 1888, Beiträge zur Entwicklungsmechanik des Embryo. Über die künstliche Hervorbringung halber Embryonen durch Zerstörung einer der beiden ersten Furchungskugeln, sowie über die Nachentwicklung (Postgeneration) der fehlenden Körperhälfte, *Virchows Arch. pathol. Anat. Physiol. klin. Med.* 114:113-153. Translated in: Willier, B., and Oppenheimer, J. M. (eds.), 1974, *Foundations of Experimental Embryology*, Hafner Press, New York, pp. 2-37. Page citations here refer to this more accessible English translation.
36. Roux, W., 1894, Einleitung zum Archiv für Entwicklungsmechanik. *Arch. Embryol.* 1:1-42.
37. Roux, W., 1895, The problems, methods, and scope of developmental mechanics. Translated by Wheeler, W. M., 1894, *Biological Lectures Delivered at the Marine Biological Laboratory*, pp. 149-190 and reprinted in Maienschein, J., 1986, *Defining Biology*, Harvard University Press, Cambridge, MA, pp. 107-148.
38. Roux, 1888, pp. 25-26.
39. *Ibid.*, p. 30.
40. Driesch, H., 1891, Entwicklungsmechanische Studien. I. Der Werth der beiden ersten Furchungszellen in der Echinodermentwicklung. Experimentelle Erzeugen von Theil- und Dop-

- pelbildung, *Zeitschr. wissenschaft. Zool.* 53:160-178. Translated and abridged in Willier and Oppenheimer, 1974, pp. 38-50. Page citations here refer to this more accessible English translation.
41. Hertwig, O., and Hertwig, R., 1887, Über den Befruchtungs und Teilungsvorgang des tierischen Eies unter den Einflüssen äußerer Agentien, *Jenaische Zeitschr. Naturwissen.* 20:477-510.
 42. Driesch, 1891, p. 46.
 43. *Ibid.*, p. 48.
 44. Driesch, H., 1893, Entwicklungsmechanische Studien. VI. Über einige allgemeine Fragen der theoretischen Morphologie, *Zeitschr. wissenschaft. Zool.* 55:34-62.
 45. Churchill, F. B., 1969, From machine-theory to entelechy: Two studies in developmental teleology, *J. Hist. Biol.* 2:165-185.
 46. Boveri, T., 1887, 1888, 1890, 1901, 1905, 1907, Zellenstudien I-VI, *Jenaische Zeitschr. Naturwissen.*
 47. Baltzer, F., 1967, *Theodor Boveri. Life and Work of a Great Biologist 1862-1915*, University of California Press, Berkeley, especially "Part Two: Scientific Work."
 48. Wilson, E. B., 1892, letter to Hans Driesch, Naples Zoological Station Archives.
 49. *Ibid.*
 50. Wilson, E. B., 1893, Amphioxus, and the mosaic theory of development, *J. Morphol.* 8:579-630.
 51. Wilson, E. B., 1894, The mosaic theory of development, *Biol. Lect. delivered at Marine Biol. Lab.* 1893:1-14.
 52. Boveri, T., 1893, Ein geschlechtlich erzeugten Organismus, translated by Morgan, T. H., as "An organism produced sexually without characteristics of the mother," *Amer. Naturalist* 27:222-232, Morgan quotation p. 222.
 53. Morgan, T. H., 1893, Experimental studies on the teleost eggs, *Anatomisch. Anzeiger* 8:803-814 p. 814.
 54. Morgan, T. H., 1894, Experimental studies on echinoderm eggs, *Anatomisch. Anzeiger* 9:141-152, p. 145.
 55. Allen, G. E., 1978, *Thomas Hunt Morgan. The Man and His Science*, Princeton University Press, Princeton, NJ.
 56. Morgan, T. H., 1897, *The Development of the Frog's Egg. An Introduction to Experimental Embryology*, Macmillan, New York.
 57. Morgan, T. H., 1901, *Regeneration*, Macmillan, New York.
 58. Roux, 1895.
 59. Goldschmidt, R., 1956, *Portraits from Memory. Recollections of a Zoologist*, University of Washington, Seattle.
 60. Jenkinson, J. W., 1909, *Experimental Embryology*, Clarendon Press, Oxford, p. 1.
 61. Morgan, T. H., 1927, *Experimental Embryology*, Columbia University Press, New York.
 62. De Beer, G. R., 1926, *An Introduction to Experimental Embryology*, Clarendon Press, Oxford.
 63. Huxley, J. S., and De Beer, G. R., 1934, *The Elements of Experimental Embryology*, Cambridge University Press, Cambridge.
 64. Jennings, H. S., 1926, Biology and experimentation, *Science* 64:97-105, p. 98.