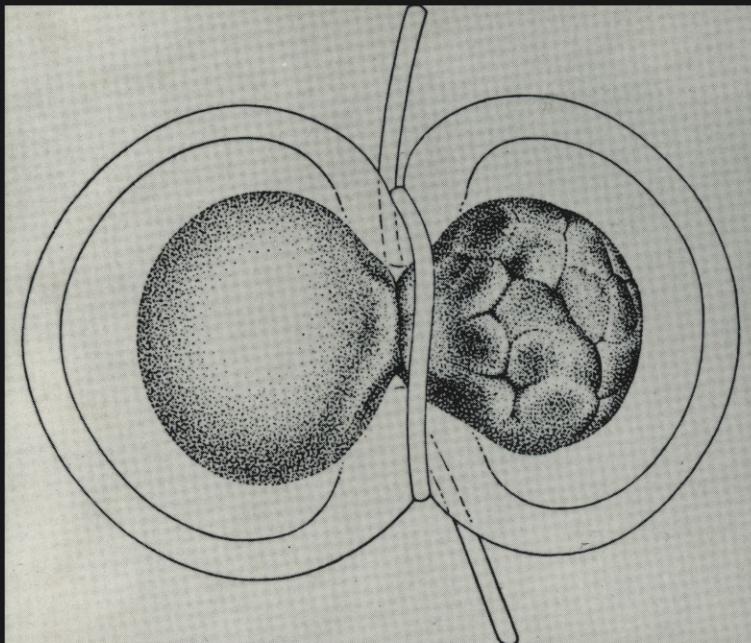


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LEON W. BROWDER

# Developmental Biology

A  
COMPREHENSIVE  
SYNTHESIS

Volume 7



## A Conceptual History of Modern Embryology

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Edited by SCOTT F. GILBERT

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## A COMPREHENSIVE SYNTHESIS

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**SCOTT F. GILBERT**

*Swarthmore College  
Swarthmore, Pennsylvania*

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# Preface

“Glory to the science of embryology!” So Johannes Holtfreter closed his letter to this editor when he granted permission to publish his article in this volume. And glory there is: glory in the phenomenon of animals developing their complex morphologies from fertilized eggs, and glory in the efforts of a relatively small group of scientists to understand these wonderful events.

Embryology is unique among the biological disciplines, for it denies the hegemony of the adult and sees value (indeed, more value) in the stages that lead up to the fully developed organism. It seeks the origin, and not merely the maintenance, of the body. And if embryology is the study of the embryo as seen over time, the history of embryology is a second-order derivative, seeing how the study of embryos changes over time. As Jane Oppenheimer pointed out, “Science, like life itself, indeed like history, itself, is a historical phenomenon. It can build itself only out of its past.” Thus, there are several ways in which embryology and the history of embryology are similar. Each takes a current stage of a developing entity and seeks to explain the paths that brought it to its present condition. Indeed, embryology used to be called *Entwicklungs geschichte*, the developmental history of the organism. Both embryology and its history interpret the interplay between internal factors and external agents in the causation of new processes and events. The embryologist, of course, has the advantage of seeing this “history” repeating itself every time a new organism is generated.

So it is not surprising that historians, embryologists, embryologists-turned-historians, and historians-turned-embryologists can collaborate on a history of embryology. This is, of course, a history of embryology, and there is no pretense that it is the history of the field (any more than the excellent volumes on oogenesis and morphogenesis in this series can be considered the complete texts in these areas). The predominant theme in this volume is the concept of induction. This was not the way the book was originally planned, but rather, a fortuitous accident of those who were able to write their chapters during this time. Other themes could have predominated, and I hope that there will be more historical volumes in this series that will address these areas. However, induction has certainly been a pivotal principle in the history of embryology, and it remains an extremely active field of contemporary research. Given the current interest in the molecular mechanisms of neural and “secondary” induction, this history becomes all the more timely.

One of the values of this book for contemporary developmental biologists

should be to clarify some of the concepts that embryologists have bequeathed to developmental biology. The volume begins with a chapter by Frederick Churchill that details the bases for the tradition of comparative embryology. The work of Baer, Pander, and Rathke is central to this endeavor, and it is here that the first observations of mutually interactive tissues were made. When Christian Pander discovered the primary germ layers of the chick embryo, he concluded that

a unique metamorphosis begins in each of these three [germ layers] and hurries toward its goal; although each is not yet independent enough to indicate what it truly is; it still needs the help of its sister travellers, and therefore although already designated for different ends, all three influence each other collectively until each has reached an appropriate level.

This quotation shows that both internal and inductive features of embryogenesis were recognized very early. Jean-Louis Fischer then provides the context of French comparative anatomy and teratology in which Laurent Chabry performed his experiments showing the mosaic development in tunicate embryos. Fischer also shows how personal and national ideologies can influence the interpretation of experimental results. Next, Jane Maienschein provides a history of the early work in *Entwicklungsmechanik*, belying the textbook notion of a few “founding fathers” and demonstrating the rich tapestry provided by the work of numerous investigators. One of these investigators, Curt Herbst, is given special attention in the chapter by Jane Oppenheimer. Her study delineates how calcium and lithium ions were first used to study morphogenesis, how the first concepts of induction entered into experimental embryology, and how physiological theories of tropisms influenced research into embryonic cell movements.

The work of Herbst was extremely important in the intellectual development of Hans Spemann. Margaret Saha documents the evolution of Spemann’s conceptualization of induction by discussing his experiments on lens formation. These studies were critical for the design of Spemann’s later experiments and provide insight into how he came to interpret them as he did. The next chapter is an autobiographical essay by Johannes Holtfreter in which he discusses his science and his art. He describes how he came to Spemann’s and Mangold’s laboratories, how his concept of induction changed over the years, and how he attempted to find the molecules that were responsible for the induction of the neural tube.

Several research programs came out of the studies on induction and morphogenesis that were performed by Spemann and Holtfreter. One of these was the study of the cell surface in development. The search for the processes and molecules responsible for intercellular adhesion is shown by Gerald Grunwald to have been full of surprising results and serendipitous observations. Another research program starting from the Freiburg group was Joseph Needham’s attempt to produce a biochemistry of the embryo. P. G. Abir-Am documents Needham’s attempts to place the biochemistry of the embryo on a solid theoretical and philosophical foundation. Here we see the complexities of forming a new interdisciplinary science and the need to free biological thinking from the paradigms of nineteenth-century physics.

Needham’s colleague in these endeavors, Conrad Waddington, attempted to weld *Entwicklungsmechanik* with genetics and evolutionary theory. Together with Salome Gluecksohn-Waelsch and Boris Ephrussi, he helped create the basic tenets of developmental genetics. Richard Burian, Jean Gayon, and Doris

Zallen discuss the ways in which Ephrussi attempted to synthesize genetics and development, while I relate the attempts of Gluecksohn-Schoenheimer and Waddington to use the concepts of induction and competence to reunite these disciplines. One of the most hotly debated topics in those early days of developmental genetics was whether all the genotype resided in the nucleus. Both Ephrussi and Waddington were initially partial to the presence of plasmagenes in the cytoplasm. Jan Sapp details the debates on this issue, especially as they pertained to protozoans, organisms that some scientists saw as models for metazoan development, but which other scientists saw as interesting exceptions to the general rule of nuclear inheritance.

Developmental biology is the anagenetic descendant of embryology. N. J. Berrill relates that Paul Weiss wrote to him asking Berrill to suggest a name for the science that included embryology and also gene activity, regeneration, cell movement studies, and other areas of developmental biology. Berrill sent the letter back to him with the last two words capitalized. We who study developmental biology are the inheritors of embryology's concepts, organisms, and sense of wonder. From other sources, we have received a new set of tools with a resolving power far greater than what was available a generation ago. Frogs, chicks, and sea urchins (along with nematodes, flies, and leeches) are now being dissected with monoclonal antibodies, antisense mRNAs, and confocal microscopes. We are presently seeing a return to those old embryological enigmas that were abandoned for lack of such specific tools. The morphogenesis of the discipline continues.

I thank all the authors for their contributions, and Marie DiBerardino, Leon Browder, and Mary Born for their roles in the conception and birth of this volume.

“Glory to the science of embryology!”

Scott F. Gilbert

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## Chapter 1

# The Rise of Classical Descriptive Embryology

FREDERICK B. CHURCHILL

### 1. Introduction

The chick egg is an enticing object for study. Nineteenth-century microscopists accepted the challenge with enthusiasm and exquisite results. They were, however, not the first. Aristotle, Fabricius of Aquapendente, and William Harvey had all immersed themselves in similar activities. Their investigations led them back to the very beginnings of an individual life. Harvey, for example, in the mid-seventeenth century spoke of the primordial heart as the *punctum saliens*, that is, the first leaping point of life, but years later he recognized blood islands in the *area opaca* at the periphery of the early embryo as living antecedents to the heart itself. With the advent of the early compound microscope, Marcello Malpighi charted a more exacting course through the early stages of development, which can still be traced easily through his incomparable illustrations. Eighteenth-century successors, Albrecht Von Haller, Lazzaro Spallanzani, and Caspar Friedrich Wolff, added further refinements in observations.

In all these undertakings, from Aristotle's to Wolff's, the mastery of details represented only a limited objective. Each investigator embellished his observations with comments about the coming-into-being of organic form and of the very nature of life. Their comments on development reflected certain commitments to a metaphysics of the organic and inorganic worlds, to a system of causation in mundane affairs, and to the structure of the cosmos itself. The reader comes away from Aristotle's *Generation of Animals* with a conviction that the Stagirite philosopher was delighted in the empirical support he found for his four causes, four primary substances, and a male-oriented hierarchical world view. Harvey found comfort in a time of civil discord in the chain of efficient causes stretching from the primordial heart, to the blood and spirit, ultimately to God. In the age of Newton, Malpighi envisioned mechanical and corpuscular events associated with the first signs of the primitive streak. Spallanzani, both cleric and experimentalist, argued for preformation, despite the fact his observations produced ambiguous results. Von Haller, an exemplar of the scientific enlightenment, envisioned the omnipotence and beneficence of God in the

amnion fold and vitelline membrane. Wolff became equally convinced of the active and generative capacity of matter with the formation and accretion of vessels, somites, and other early structures (1).

## 2. Descriptive Embryology in the Baltic Periphery

Despite all the compelling metaphysical and religious reasons for following the early steps of development, the details were hard to come by and equally difficult to communicate. Not until the second, third, and fourth decades of the nineteenth century did a combination of preparatory techniques and improved microscopes allow for reliable observations on the histological level, and only then did research and pedagogical demands require the construction of a suitable vocabulary. During these decades, the systematic use of engraved illustrations encouraged comparisons of microscopic and gross observations, which were a vast improvement over that which had come before (2).

Institutional factors during these decades also created a climate in which scientific investigations and publications thrived. Reforms at Prussian and other German universities in the wake of the Napoleonic wars encouraged a spirit of scholarship for its own sake, and the enduring fragmentation of the German states nurtured seminars and small laboratories at multiple centers of learning, fostering an independence and a new professionalization in science (3). How these institutional and cultural changes advanced the science of embryology in particular is less clear. Königsberg (now Kaliningrad), Würzburg, Breslau (now Wrocław), Bonn, and Berlin nevertheless became centers for some of the most innovative studies of animal development. It can be no accident that three of the most talented observers of the early embryo came from the Baltic region and were educated in northern German universities. Their crossing paths reinforced their common scientific concerns.

Christian Pander (1794–1865) and Karl Ernst von Baer (1792–1876) were born in Riga and Dorpat (now Tartu), respectively, and knew each other as young students at the newly reformed German university in Dorpat. Heinrich Rathke (1793–1860) grew up in Danzig (now Gdańsk) and was educated at Göttingen, where he knew Pander, and in Berlin. All three were medical students, and questions about generation and development were commonly broached in the anatomical and physiological texts they must have used. Pander and von Baer studied anatomy and physiology under Karl Burdach in Dorpat and met again in 1816 in Würzburg, where von Baer had been privately studying comparative anatomy under the guidance of Ignaz Döllinger. With Döllinger's encouragement and advice, Pander studied chick development and published his single, but influential, work in embryology, *Beiträge zur Entwicklungsgeschichte des Hühnchens im Ei*. Then, after 4 years of leisurely travel through western Europe accompanied by his illustrator, Eduard J. d'Alton, Pander settled in St. Petersburg in 1821, where he pursued an independent career as paleontologist and geologist. He eventually promoted a pre-Darwinian theory of evolution. Von Baer, who had enticed Pander to Würzburg, almost immediately moved to Königsberg, where he became Prosector at Burdach's newly founded Institute for Anatomy. Here he advanced through the academic ranks to become Professor of Zoology and

Director of the Zoological Museum. It was here, too, that he made many contributions to embryology, in particular his discovery of the mammalian egg, *De ovi mammalium et hominis genesi* (1827), and that he wrote his major embryological text, *Über Entwickelungsgeschichte der Thiere. Beobachtung und Reflexion*, 2 vols. (1828–1837). Rathke, who had assumed the position of chief physician at the city hospital in Danzig, found time to undertake and publish detailed embryological studies of vertebrates and the crayfish. In 1829 he was appointed Professor of Physiology and Pathology at the University of Dorpat. His *Abhandlungen zur Bildungs- und Entwicklungs-geschichte der Menschen und der Thiere*, 2 vols. (1832–1833), firmly established his reputation as an acute observer, and in 1835 Rathke succeeded von Baer in Königsberg after the latter joined Pander on a permanent basis in St. Petersburg. Neither Pander nor von Baer pursued embryological studies after leaving Germany. Rathke continued throughout his career to produce important monographs on development in fish, reptiles, and vertebrates in general. These set an empirical standard that influenced the course of descriptive embryology for the next half-century.

It is hard to measure the influence of Burdach and Döllinger on these three students. Both were human anatomists and physiologists with abiding interests in the function and development of the human brain (4). Both were also peripheral supporters of German romantic Naturphilosophie, a scientific movement that had fundamental concerns for the generation of form and for comparative morphology. Von Baer's *Autobiography* includes a humorous account of his youthful attraction to and prompt rejection of naturphilosophic teachings in general. He also recounts his later estrangement from Burdach because of a clash of opinion over the incorporation of von Baer's observations in Burdach's multi-volume *Physiologie als Erfahrungswissenschaft* (5). Burdach certainly promoted von Baer's career and at a crucial juncture sacrificed the female dog on which von Baer made his discovery of the mammalian egg. Burdach also provided Rathke with a forum in his *Physiologie*. Von Baer wrote warmly of his tutelage under Döllinger in Würzburg and attributed to Döllinger the development of the simple technique of releasing air from the end of the chick egg in order to cause the yolk to fall away from direct contact with the shell membrane. This important technique facilitated observations on the blastoderm (6). The forces that united and compelled these three contemporaries from the northeastern periphery of the German intellectual world to investigate development so avidly and effectively may be beyond our immediate grasp. An examination of their accomplishments, however, goes a long way in elucidating the goals of classical descriptive embryology.

### 3. Christian Pander

Pander completed his studies on the chick in less than 15 months. The published *Beiträge* consisted of a short monograph of 42 pages (7). In his narrative Pander concentrated on the structures of the undeveloped egg, the early development of the blastoderm, and the appearance of the primitive streak with its two primitive lateral folds. He described the beginning somites, which he identified as rudimentary vertebrae. He observed the blastoderm separating into

two germ layers, or, as Pander expressed it, into a “serous layer” (*Serosenblatt*) and “mucous layer” (*Schleimblatt*), and he explained how soaking the germ in water for 24 hr allowed him to tease the two layers apart. He later related the appearance of a third layer, the “vascular layer” (*Gefäßhaut* or *Gefässblatt*), from which, he asserted, the vascular system arose. He told of the rudiments of the heart and the early blood flow, said little about cephalic development other than describing a head fold, or that which we would consider the head ectoderm, and finally turned his attention to the amnion and other extraembryonic structures. In his description of early development, Pander had failed to mention details that would be traditionally examined in a beginning descriptive embryology course today, and he indicated only a vague understanding of the fundamental relationship between the primitive streak and the neural folds.

It was not, however, in the factual details but in his conception of the germ layers that Pander made his most valuable contribution. These were antecedents to later embryonic structures. It was in their growth and interplay that embryonic form came into being. “Actually a unique metamorphosis begins in each of these three layers,” Pander asserted,

and each hurries toward its goal; although each is not yet independent enough to indicate what it truly is; it still needs the help of its sister travellers, and therefore, although already designated for different ends, all three influence each other collectively until each has reached an appropriate level (8).

For Pander the germ layers were thus an essential precursor to later structures. In their movements and interactions was to be found the explanation for later form. Pander does not elaborate upon these germ layer interactions. Whether he intended mechanical processes, or whether in some prescient way he thought of the interplay of forces or exchanges of chemicals, we are unable to determine in his statements. Both Wolff and Haller, however, had misunderstood and passed by this critical juncture of the developmental process, and it was here that Pander thought the controversy between preformation and epigenesis would be resolved.

There exists in the *Beiträge* a mismatch between that which Pander covered in his account and that which d'Alton illustrated. Pander stopped with a roughly hewn description of the formation of the heart. D'Alton portrayed with great accuracy the development of the head region, limb buds, the atrium, ventricle, three aortic arches, the sinus venosus, and the fusion of the dorsal aortae. He drew an unprecedented plate of transverse and longitudinal sections of germ-layer formation. The legends for the illustrations, presumably dictated by Pander, explain in part what is portrayed, but the juxtaposition of the main text, the figure explanations, and illustrations raises anew the issue of the relationship between the artist and the anatomist or embryologist (9).

In this case, there is no reason to suppose that Pander's was not the guiding hand in the determination of what was being illustrated and what was being discussed. The vocabulary of the anatomist and writer could not match the subtlety of the artist, but that is only to say that neither embryologist nor artist understood what many of the rudiments were and what they would soon become. For example, D'Alton pictured the head region in a way that we can today confidently point to the telencephalon, diencephalon, mesencephalon, and

metencephalon (10), yet these regions were not separately identified and there is no reason to suppose they possessed, in the mind of either author or artist, an important future as separate entities. Until that moment, the artist's realism outstripped, but did not surpass, the scientist's understanding. D'Alton was to become an important anatomical illustrator, in his own right (11), but there is no reason to suppose that he understood chick development better than his companion and co-worker. With Pander's *Beiträge*, descriptive embryology was to become heavily dependent on its pictorial presentations, but the comprehension of events was still to be found in the word and its connotations.

#### 4. Karl Ernst von Baer

After leaving Würzburg and his comrade in August of 1816, von Baer lingered in Berlin, where he spent the fall studying clinical subjects. He assumed the position of prosector at Burdach's new anatomical institute in Königsberg the following July. For the next 17 years von Baer demonstrated a versatile mastery of biology. He taught a variety of subjects ranging from invertebrate anatomy and embryology to botany and anthropology. He established a zoological museum, acted as occasional director of the botanical gardens, and assumed the Professorship of Anatomy in 1828 when Burdach redefined his position as Professor of Physiology. Von Baer was clearly a successful teacher and one of the stars at this most northeastern university of the far-flung Prussian educational system. In part, to be closer to the family estate in the northernmost province of Estonia, and perhaps because of both ministerial uncertainties and political unrest in Königsberg, von Baer decided in 1834 to move permanently with his family to St. Petersburg, where he was already a member of the Academy of Science (12). The move brought von Baer new opportunities to explore the Arctic, to participate in the scientific charting of the interior of the Asian land mass, and to become a scientific leader in imperial Russia, but it also marked the end of von Baer's productive career as an embryologist. The scientific work for which he is best known issued solely from his Königsberg years.

In 1819 von Baer repeated Pander's investigations on the chick and clarified some important details. He identified the notochord as a distinct and primary structure that lay subjacent and prior to the neural tube. He recognized that Pander's primitive ridges were really neural folds that closed upon each other and eventually marked the spinal column. In contrast to Pander, who had envisioned the spinal column as a coagulation from the surrounding medium, von Baer saw the notochord as a product of detachment and the neural tube as the result of evagination (13). Von Baer's embryological examinations concentrated on, but were not confined to, the chick. Even at the outset of his studies he found the value of clarifying developmental processes by recourse to other vertebrates.

In the spring of 1827 von Baer discovered the mammalian egg, first in the dog, then in other mammals, and thus ended a search that had begun with William Harvey and Regnier de Graff in the seventeenth century and had been avidly pursued by Albrecht von Haller and others in the eighteenth and early nineteenth centuries. Harvey, who had insisted that all animals came from eggs and whose aphorism "ex ova omnia" became a shibboleth in biology, had killed

numerous does in the royal game preserve in an effort to comprehend the fertilization and generation of mammals. Harvey's efforts had been doomed to failure at the outset in no small measure because the initial development and implantation of the blastocyst in the genus *Cervus* is delayed for 7 or so weeks (14). De Graaf described a vesicle that is now designated the "Graafian follicle" but felt that this entire structure, when liberated into the Fallopian tube, corresponded to the ovum (15). He recognized, however, a problem in his account, for at a later stage the developing egg appeared smaller than the follicle. Von Haller, who considered the follicle a glandular body and hence gave it its current name, sacrificed 40 sheep in his quest for the unfertilized mammalian egg. His search was in vain, so he concluded that the ovum must form by a coagulation of seminal fluid in the oviduct. The Scottish anatomist William Cumberland Cruikshank, the Swiss physiologist Jean Louis Prevost, and the French chemist Jean Baptiste Dumas traced the developing mammalian germ backward and ever closer to its physical association with the follicle (16), but it was an analogy that provided the intellectual bridge for identification of the egg within the follicle. As Vladislav Kruta has pointed out, the Bohemian physiologist Jan Evangelista Purkinje discovered the germinal vesicle in the chick egg, or that which a decade later would be designated a nucleus. Purkinje felt this vesicle, which he recognized disappeared during ovulation, represented the initial locus of generation. It was this structure that von Baer set about to find in mammals (17).

Von Baer turned to canines, in which fortuitously the blastocyst quickly forms a distended circular structure and becomes quickly implanted in the uterus. He succeeded in identifying ever smaller germs in the Fallopian tube, but failed to establish their origins until Burdach offered a bitch that had just come into heat. Using a magnifying glass, von Baer identified what he thought was Purkinje's vesicle lying within the unruptured follicle. His retrospective account, encrusted with melodrama by nearly 40 intervening years, perhaps overly dramatic, reflects the initial uncertainty and surprise at the similarity between the mammalian and avian eggs:

I recoiled as if struck by lightning. . . . I had to try to relax a while before I could work up enough courage to look again, as I was afraid I had been deluded by a phantom. Is it not strange that a sight which is expected, and indeed hoped for, should be frightening when it eventually materializes? . . . I had never believed that the contents of the mammalian ovum would resemble the yolk of bird eggs to such a degree. . . . I had seen a sharply delineated regular globule, enclosed in a strong membrane and differing from the bird's egg yolk only in the coarse, somewhat loosely fitting membrane. The small, opaque ova I had found in the oviduct had had a yellow-white coloring, no doubt because the yolk was already dissolving; the larger ones were transparent (18).

Von Baer's discovery put to rest the contention that the mammalian germ formed from a coagulation in the oviduct of seminal fluid rather than from a genuine egg, as in all other animals. At that time, however, von Baer assumed that the egg he discovered was indeed the mammalian analog of the germinal vesicle Purkinje found in the chick egg. Thus it was natural for von Baer to report that "in mammals the innate vesicle contains a more developed vitellus and by reference to the future foetus proves itself to be the veritable ovum. It may be called the foetal ovum in the maternal ovum. Therefore mammals have thus an ovum within an ovum, or, if one may be permitted to speak in this way, an ovum raised to the second power" (19). It took another 5 years before three microscopists, one

of them working under Purkinje's guidance, independently sorted out von Baer's false analogy by identifying the germinal vesicle within the mammalian "ovum raised to the second power" (20). Equally obscure was the fertilization process. Endorsing a tradition that considered Leeuwenhoek's animalculae of the male semen to be parasites, von Baer coined the term "spermatozoa." He dismissed their possible effects as he spoke of "the female power of generation" residing in the germinal vesicle and of "the influence of the masculine generative fluid" (21).

His investigation of the mammalian egg was exemplary of von Baer's attention to details and ranging interest in generation and development of all kinds. During his years in Königsberg, von Baer studied the generation of internal parasites. He commented on the sudden appearance of cholera in the Baltic region and sided with the importationist interpretation of this feared disease. He took a keen interest in the finds of mammoths and other prehistoric animal remains. On the basis of comparative anatomy and embryology, he maintained that there existed four ideal basic animal organizations, which he later realized corresponded with Georges Cuvier's four embranchements (22), but unlike his French counterpart, he believed in a transformation over time of organisms within the same basic type. This "limited degree" of transformation could be charted along branching, temporally arranged taxonomies that also marked a teleological course from simple to complex developments. Humans were no exception to this natural process; they arose in some manner not from apes, but from an original human type associated with the ocean (23). Many of these subjects may seem a far cry from von Baer's descriptive embryology, but in his mind they helped forge an outlook that made the study of embryos compelling for interpreting a larger world picture. Ten months before he left Königsberg for St. Petersburg, von Baer delivered one of his many popular lectures; this one was entitled "The General Law of Nature in all Development" (24). Here von Baer both delimited and emphasized his belief in the universal and teleological change of all life forms.

Within this general developmental framework von Baer's most substantial work represented a model study of the coming-into-being of animal form (25). Unfortunately, the *Entwickelungsgeschichte* had a fitful publication history, which resulted in a disjointed presentation. Portions of the First Part appeared in the second volume of Burdach's *Physiologie*, but the collaborative publication arrangements with his former teacher proved trying, and von Baer subsequently decided to bring out his own separate work (26). The completed First Part of the *Entwickelungsgeschichte* appeared in 1828. This carried the description of the sequential development of the chick through its entire development from the appearance of the blastoderm to hatching. In addition, the First Part closed with an important 120-page section devoted to "Scholia and Corollaries," in which von Baer presented general observations about the nature of development. In 1837, after von Baer had left Königsberg and after he failed to send additions and corrections, his publishers, the Brothers Bornträger, brought out the Second Part of the work, which consisted of a series of "Lectures on Generation and Development" presented to physicians and naturalists and began with observations on the structure and formation of the avian egg in the oviduct. The lectures provided additional sequential details on chick development, on the development of specific chick organs, on reptilian and mammalian development, on the mammalian egg (including the human egg), and on the development of frogs and fish.

There was no concluding or summary statement. Only in 1898, 22 years after von Baer's death, did Ludwig Stieda, embryologist and von Baer's early biographer, edit and publish the concluding section of Part Two. With this, von Baer had intended to present his studies on the development of humans. Given this publication history, the *Entwickelungsgeschichte* can hardly be viewed as a systematic work. It was cobbled together as von Baer studied the material at hand. It was a work, however, that became the exemplar for and totem of nineteenth-century embryology, not only because of its carefully executed descriptions of developmental details, but because of its comparative concerns, its extended theoretical sections, and its comprehensive interaction with some major works of von Baer's contemporaries.

In order to come to grips with the totality of chick development, von Baer divided the embryological sequence into three basic periods. The first covered the first 48 hr, during which von Baer recognized that the blastoderm segregates into two germ layers, that the primitive streak and the notochord designate the longitudinal axis of the embryo, that the neural folds close, and that the head region becomes elevated and separated from the surrounding blastoderm. In addition, von Baer described the appearance of four head regions, the optic vesicles, the rudiments of the pulsating heart lying under the head, and the 10–12 visible somites, which mark the location of later vertebrae. At the end of this first period, von Baer pictured the embryo as "an inverted shoe" and concluded:

The history of the first period teaches us that the embryo is a highly independent part of the germ, that as its independence becomes evident, the vertebrate type emerges, that its development proceeds out of the rudiment—both upward and downward, and that a segmentation appears in the animal part that is built into the type of all segmented animals (27).

Even in sections of ostensibly factual presentation, von Baer could not resist drawing the reader's attention to larger comparative and taxonomic issues.

The second period of von Baer's tripartite division encompassed the third through fifth days of incubation. During this time the embryo becomes ever more separated from the blastodisc; circulation of blood is particularly evident as the pulsating chambers of the heart are discernible and vessels extend over the surface of the yolk. The gut becomes clearly demarcated from the animal half and increasingly separated from the yolk by a folding under and tucking in of the lateral portions of the germ layers (28). Lungs, liver, and pancreas appear as outgrowths of the gut. Von Baer distinguished the five primary parts of the brain and many details of the central nervous system. He deviated from Pander's threefold germ-layer system by envisioning each of the two primary layers dividing into two and producing a fourfold layering: the skin layer (*Hautschicht*), the muscle layer (*Fleischschicht*), the vessel layer (*Gefäßblatt*), and the mucous layer (*Schleimblatt*). Pander had envisioned the germ layers as the raw building material that, through tubulation and twisting, formed many of the primary systems of both the animal and plastic (vegetative) parts. Von Baer extended the operation of the germ layers to the formation and living processes of later structures and the extraembryonic membranes: i.e., the chorion, amnion, and allantois.

Overall, von Baer found three unique events in this second period of development: (1) the bifurcation of the embryo into animal and plastic (or vegetative)

parts, (2) the rotation of the embryo onto its left side, and (3) the concomitant displacement of the principal subjacent veins and yolk stalk to the left side. This asymmetrical shifting of the main organs of "ingestion" reminded von Baer of the molluscan pattern of development. "We therefore might conclude," he reasoned, "that in the second period the type of the mollusc replaces the previous symmetrical fundamentals of the vertebrate. We must not say, however, that the embryo of the chick stands at the developmental stage of the mollusc. The vertebral column, the spinal chord and the brain speak too much against it" (29). The vertebrate type was obvious to him from the first appearance of the notochord, and by the beginning of the second period all the characteristic features of the vertebrates were evident. Furthermore, with the appearance of the allantois, the chick took on the appearance of a land vertebrate. Von Baer had no intention of forcing the pattern of development into an ascending of an organic hierarchy.

The third period in von Baer's chronological survey covered development from the sixth to the twenty-first day of incubation, when the chick hatched. This is the period when the embryo grows at the expense of the yolk until the latter is encircled and consumed. The embryo begins as a dependent extension of the blastoderm; it completes its development by subsuming all but the extra-embryonic structures. All external and internal features become increasingly refined and individualized as particular structures of a particular embryo. Through the unique development of the respiratory organs and the development of wings, a beak, and feathers, the embryo becomes increasingly specialized as a bird, then as a land bird, and finally as a bird of a particular genus and species. "Finally, the individual characters take shape and will only become complete at the peak of life outside of the egg; for it is evident that freshly hatched chicks are more similar to one another than are matured chickens" (30). Embedded in this view of the increasing specialization and individualization of the embryo existed a general perspective to development. Von Baer made this explicit in the Scholia and Corollaries.

Specified as the second part of the first volume of *Entwickelungsgeschichte*, the Scholia and Corollaries occupy nearly half of the written text (31). On the basis of length alone, these generalizations were as significant as the descriptive portion. What is less clear, until one studies the Scholia and Corollaries in detail, is how extensively von Baer drew on his observations of other vertebrates (as well as of the chick) and of many invertebrates to establish general principles of development. By presenting his generalizations in this particular form, von Baer was associating his work with a tradition in scientific writing that stretched back through Isaac Newton's *Principia Mathematica* to Euclid's *Elements of Geometry*. Where the Scholia and Corollaries, and indeed Propositions and Theorems, formed however, an integral part within the structure of Newton's and Euclid's deductive argument, they were appended to von Baer's empirical descriptions and serve as generalizations that had emerged from the preceding empirical examinations. Biology could not be treated with the rigorous propositional style of a deductive science. Nevertheless, the association promoted the presumption that the principles of development were secure scientific generalizations. Jane Oppenheimer long ago pointed out that von Baer's genius and appeal was this juxtaposition and integration of both *Beobachtung* and *Reflexion* (32). The Scholia and Corollaries served, too, as a forum for commenting on many of the

preconceptions of von Baer's contemporaries. It is thus doubly important to review their general thrust.

Scholium I, entitled "On the Certainty of Observation of the Embryo," deals with von Baer's recognition that young embryos are coarser in outline than older ones and simply do not exist in miniature below the resolving power of the common microscope of the day. The assertion was fatal to the doctrine of preformationism, which had dominated eighteenth-century embryology and which Jean Prévost and Jean-Baptiste Dumas had recently reasserted.

Scholium II concerned "The Formation of the Individual in Relation to Its Surroundings." Von Baer observed that during development the embryo increasingly separates itself from the rest of the egg and then sequentially incorporates the remains of the blastoderm, other egg structures, and, upon hatching, some of the extraembryonic membranes. The same self-promotion also applied to the production of the ovum and offspring, so that "generation is here a direct relocation of growth past the boundaries of the individual[,] and propagation is nothing more than an extension of growth beyond oneself" (33). For von Baer these principles of individual aggrandizement and overgrowth were explicitly both mechanistic and teleological, and with reference to the goal-oriented development of chick embryos, which began with different crude shapes and material conditions, von Baer emphasized the latter: ". . . the essence (the idea according to the new school) of the generating animal form rules the development of the fruit" (34).

Scholium III deals with the "Internal Development of the Individual." Here von Baer pointed out that development is a process of differentiation from the homogeneous to the heterogeneous. According to von Baer, this process consists of the formation of two plates: the animal and vegetative (35). He felt each plate then divides, producing four germ layers: the skin, the muscle, the vessel, and the mucous layers. Next, histological differentiation occurs within each germ layer, and finally, the individualized tissues interact in a morphological differentiation that results in organs and other structures. Von Baer finds in these processes a transformation rather than new formations. "Each organ is thus a modified part of a more general organ, and in this respect one can say that each organ is already contained in the fundamental organs. . ." (36). Von Baer's perspective placed a premium on tracing the genetic derivation of advanced structures from particular germ layers. Like Pander before him, von Baer encouraged further considerations of the mechanical interplay between germ layers.

Scholium IV, a much longer and more complex section, presents the generalized "Scheme that Vertebrate Development Follows." Von Baer described a pattern of development, which proceeds (1) along the vertical axis of the embryo from the skin to the mucous layers, that is, from the dorsal to the ventral; (2) along the horizontal axis from the germinal area through the vascular area to the yolk, that is, from the center to the periphery; (3) and along the longitudinal axis from the brain to the heart, and then to the gut region, that is, from fore to aft. As the germ layers become distinct, von Baer also noted that they produce a double tubulation, each with an internal and external cylinder. The tubulations are positioned above and below the longitudinal axis indicated by the notochord. Limb development consists of irregular growth beyond these organic cylinders, or "fundamental organs." Their repeating patterns, von Baer argued, have significant implications that become apparent in the following scholium.

Scholium V, the longest of the scholia, deals with the taxonomic "Relationships of the Forms which the Individual Assumes in the Different Stages of Development" (37). Von Baer first set out to refute the widely held law of parallelism, which derived its name from the postulated parallel in form between the hierarchical scale of taxonomy and the teleological progression of development. Promoted by the German anatomist Johann Friedrich Meckel and the French anatomist Étienne Serres, this controversial law maintained in specific terms that the embryos of higher organisms advance through the adult stages of lower organisms. The law had received a significant boost from Rathke's recent discovery of gill slits in avian and mammalian embryos. Von Baer, however, countered with a wealth of details showing that structures of higher organisms may appear earlier or in different developmental patterns than in lower organisms. He argued, not against a limited parallel between the development of some structures and a taxonomic arrangement, but against the existence of a universal, uniserial hierarchy of all organic forms, which was traced in embryogenesis.

Instead of a uniserial taxonomic hierarchy, von Baer insisted on four basic types of animal organization, each of which had a radically different developmental pattern. Through his embryological studies he had independently arrived at the same fundamental organizational types worked out by Cuvier on the basis of adult structures. Von Baer's four archetypes corresponded to Cuvier's four embranchements, that is, to vertebrates, mollusks, articulates, and radiates. Unlike Cuvier, who based his delimited groups on mutually exclusive functional grounds, von Baer accepted the existence of intermediate forms that might combine aspects of two of the basic types (38). The study of individual development appeared to promote a unity among types more than did the study of physiology and adult anatomy. In addition to these contrasting types of development von Baer also distinguished that which he called the grade of development. He meant by this the degree of histological and morphological differentiation that the organism or organ achieved in the general process of developing from a homogeneous egg to a heterogeneous, complex adult. Always, it must be borne in mind, von Baer emphasized the teleological concept of the functioning and unitary whole of the embryos he studied (39).

Von Baer generalized the relationship between the type and grade of development in terms of four important laws of development:

1. The more general characters of a large group of animals appear earlier in their embryos than the more special characters.
2. The less general forms develop from the most general forms, and so on, until finally the most specialized form arises.
3. Every embryo of a given animal form, instead of passing through the other forms, rather becomes separated from them.
4. Fundamentally, therefore, the embryo of a higher form never resembles any other form, but only its embryo (40).

The first two of these laws spoke to the general pattern of the appearance of traits; the second two established the relationship between the development of a single embryo and its taxonomic position. The first two reasserted in a law-like fashion von Baer's rejection of preformationism; the second two reconfirmed von Baer's dismissal of the law of parallelism. In combination with the distinction between the type and grade of development, von Baer's laws of development provided the

conceptual tools to justify a branching taxonomic organization to the animal kingdom. In the second half of the century this pattern would play a leading role in the phylogenetic ordering of life (41). The creators of evolutionary phylogenies would agree with von Baer's programmatic exhortation that "the history of development is the true torchbearer for investigations of organic bodies" (42).

Scholium VI concluded the first volume of the *Entwickelungsgeschichte* by generalizing the results of the previous scholia: "The history of development of the individual," von Baer asserted in italic type, "is the history of its increasing individuality in all respects" (43). Only within the context of the preceding Scholia and the body of empirical studies presented at the outset could this aphoristic generalization have its full impact. Finally, von Baer closed his text with a reflective passage that reminded the reader that cosmic design, that is, a teleological perspective, was still very much a part of the study of life:

. . . there is one fundamental thought that runs through all forms and grades of animal development and regulates all their peculiar relations. It is the same thought that collected the masses scattered through space into spheres, and united them into solar systems; it is that that called forth into living forms the dust weathered from the surface of metallic planets. This thought, however, is nothing less than life itself, and the words and syllables, in which it is expressed, are the multitudinous forms of the living (44).

Von Baer's fame did not rest exclusively on the First Part of the *Entwickelungsgeschichte*, but there is no need to survey the Second Part beyond what we mentioned earlier. Von Baer's interest in tracing the development of the unfertilized egg and the separate organ systems of the chick became organizational themes in later texts of descriptive embryology. From a more general perspective, von Baer's extraordinary efforts to juxtapose the development of birds, reptiles, mammals—including humans—and some invertebrates established the importance of comparative embryology for anatomy, taxonomy, and eventually for the study of phylogenies. Above all, von Baer's understanding of early development in terms of the dynamics of germ layer development provided a later generation the empirical grounds for establishing homologies and a powerful tool for focusing on exclusive mechanical processes of development. Heinrich Rathke was to accelerate these trends.

## 5. Heinrich Rathke

Unlike Pander and von Baer, Rathke left the university with the intent of pursuing a career in medicine. During the 1820s he set up private practice, took on duties as the chief physician at the city hospital and as an official regional doctor, and gave school-level instruction in physics and physical geography. Despite these multiple responsibilities, Rathke continued the embryological work he had begun as a medical student in Berlin (45). At first his interests focused on the development of the sexual organs of salamanders, fish, frogs, toads, birds, and mammals (46). By the mid-1820s he discovered the gill clefts in mammals and birds, which emphasized the affinity between the embryos of higher and lower vertebrates (47). He also studied extensively the development of other vertebrate structures, such as the liver, the portal system, and the respira-

tory system in vertebrates. At the end of the decade Rathke investigated the development of invertebrates, in particular the embryology of crayfish (48).

By the time he assumed his first academic position in Dorpat in 1829, Rathke had established a characteristic two-tiered pattern in his embryological research. First, focusing on single organ systems, he examined their appearance in many types of organisms and through their comparisons drew general conclusions about the taxonomic hierarchy of developmental processes. Rathke, like many of his contemporaries, remained a mild adherent of the law of parallelism (49). Second, investigating the development of a single organism from the first discernible stages of development to hatching, Rathke strove to fathom the unity of the embryo and of its passage to the adult form. Thus Rathke began his career by concentrating on vertebrate development but soon expanded his purview to include echinoderms, mollusks, and many arthropods. Through his lifelong commitment to the study of development he became the “compleat embryologist” in a way that eluded von Baer after he moved to St. Petersburg. Although von Baer was not in the habit of citing in any systematic fashion the studies of his contemporaries, he acknowledged with praise many of Rathke’s observations in his *Entwickelungsgeschichte* and his *Autobiography* (50).

During 40 years of scientific research, Rathke published over 125 major and minor monographs. Besides his embryological and anatomical observations, he wrote numerous natural history pieces on subjects, including fossil materials, which he came across during his travels north to Scandinavia and south to the Crimea and Black Sea. In contrast with von Baer’s general concern for methods and generalizations in embryology and taxonomy, Rathke’s embryological accomplishments appear to focus more on the details of individual transformations. Nevertheless, it was out of these details that classical descriptive embryology had to be built. Rathke’s lasting contributions include his discovery of the transitory gill clefts and associated vascular arches in amniotes (51), the working out of early elements in the formation of the vertebrate skull (52), and a clarification of certain fundamental relationships in the vertebrate urogenital system. At the distance of a century and a half, his concentration on the basic details of development seems an exercise in endurance and single-mindedness, but these details contained important lessons for general biology. In order to provide a sense of the exacting demands of getting the details right, we turn by way of example to the third of Rathke’s significant areas of contributions.

The vertebrate urogenital system presents the student of embryology with a major challenge (53). Even today, the complex structural relationship between two systems with related but different functions is nearly impossible to grasp in the development of a single species, and in some of the finer details there exist confusing specialized deviations from the general pattern. Moreover, the nomenclature we now use makes transvertebrate comparisons possible by implying homologies and functions that are not readily obvious with gross microscopic inspection. The names of structures with their implied functions, however, became standardized only after a generalized pattern of development was recognized in an evolutionary context. Even as late as the 1870s intensive work on the urogenital systems of elasmobranchs, the chick, and mammals was necessary before some general relationships could be definitively established (54). Before then, a welter of names, a mixture of conjectured functions, and a confusion of

embryological derivations reigned. A generalized picture required the tireless and meticulous work of scores of comparative embryologists working over a span of half a century. No embryologist contributed more to the early attack on these problems than Rathke.

Rathke concentrated on the urogenital system during two separate periods of his life. The first set of studies consisted of a series of publications, that started with his dissertation on the sexual structures in salamanders in 1818, soon broadened to include fish and certain higher vertebrates, and came to a climax in 1832–1833 in a sequence on reptiles and mammals (55). Despite the intensity and brilliance of these investigations, Rathke at this stage made only modest advances beyond his contemporaries. He recognized that the structure known as Oken's body in mammals was the embryological equivalent to that which Pander and Wolff before him referred to as the kidney in the chick. Rathke redesignated both structures “the Wolffian body” and equated this with the persistent or true kidney of amphibians. Having straightened out one confusion, he created another by arguing that in birds and mammals the Wolffian body gave rise to the mature kidneys, to the adrenals, to either the oviduct or the ductus deferens and epididymis, depending on the sex of the organism, and to the gonads. Although at different times he seemed to recognize the rudiments of both the female and male gonaducts, he failed to see that they were contrasting structures derived from a longitudinal splitting of the segmental duct and existed simultaneously in the early embryos of both sexes. Instead he at first equated both ducts; later, when his contemporary Johannes Müller distinguished them, Rathke argued that the “false ureter” (i.e., Wolffian duct) disappears and the “filament” (i.e., the Müllerian duct) becomes the vas deferens and oviduct; still later he reversed his position and claimed that in both males and females the “filament,” i.e., Müllerian duct, degenerated and the “false ureter,” i.e., the Wolffian duct, metamorphosed to the vas deferens and oviduct. A concern guiding both Müller and Rathke was the teleological belief that all structures must have a function (56).

His second period of investigations of the urogenital system came after Rathke had moved to Königsberg and when he turned to producing a comprehensive embryology of the adder (57). Representing an intermediate group between amphibians, on the one hand, and birds and mammals, on the other, reptiles had already become one of Rathke's special objects of study. They also marked the crux of a disagreement between Rathke and Müller concerning the partial metamorphosis of the false kidney into the vas deferens and epididymis (58). Rathke was not to be disappointed in his more thorough explorations. The modern embryologist and historian Howard Adelmann comments on Rathke's accumulated experience and “greater assurance” (59). By 1839 Rathke had dispensed with the terms “false kidneys” and “Wolffian bodies” and replaced them with the functionally and ontologically more accurate German expression *Urnieren*, which carries the connotation of “primordial kidney.” Rathke also invoked Pander's tripartite germ layer conception while discussing the primitive kidneys' origins from the vessel layer (*Gefäßblatt*). But his major accomplishment was to complete that which Müller had initiated in 1830 by distinguishing between the male and female gonaducts. Rathke recognized that in males of the adder and other amniotes, the efferent ducts of the mesonephros become transformed into

the vas deferens of the testes and that as the mesonephros degenerates, some of the Wolffian tubules develop into the epididymis. He recognized, too, that in males the Müllerian duct, in contradiction to Müller's and his earlier functionalist beliefs, serves no purpose, but degenerates and disappears. His work clarified the contrasting origins of the vas deferens and oviduct and helped place in perspective the temporary nature of the mesonephros for all amniotes. Rathke, like Müller before him, however, failed to understand that the uterus arises out of a fusion of the two oviducts and remained uncertain about the independent origins of the metanephros or true kidney.

There is no better testimony, to Rathke's talents and perseverance as a descriptive embryologist than his account of teasing apart the Müllerian duct from the efferent tubule of the mesonephron before the latter became coopted in development by the testes and the reproductive system:

To find the efferent [mesonephric] duct, which each of these organs [the Urnieren] possesses, requires a very careful search and rather strong magnifications. One can detect it best when the Urniere has lain in alcohol for a time. Then with a fine forceps pull off the oviduct or ductus deferens [that is, the Müllerian duct] which covers it and examine this organ with the microscope under concentrated light falling from above. This duct is then seen as a very delicate vessel. . . . It projects hardly at all above the surface of the Urniere and has the same yellowish-white color, about the same caliber, and also the same fragility as the vessels peculiar to this organ (60).

Like Pander and von Baer, Rathke used the simplest techniques to achieve his goals. We have already mentioned Döllinger's method for opening chick eggs. Other eggs must have required specialized treatments, which have gone unrecorded, but needles, delicate scissors, incubators, and water baths were the extent of the embryologist's equipment. Occasionally, mention is made of injections of pigmented fluids to ascertain passages through the maze of vessels and tubules. The first serial sections made with paraffin and an automatic microtome were introduced in the early 1880s; prior to that time, embryologists practiced the art of freehand sectioning and used various sorts of sectioning machines in order to obtain cross-sections (61). In light of the techniques available, Rathke's achievements, those that seem to us both advances and errors, provide a sense of the extraordinary challenges that confronted the early comparative embryologists. These were mediated at every turn by uncertain microscopy, competing nomenclature, and theory-laden associations. For all their uncertainty, Rathke's descriptions and interpretations were admired and respected by his contemporaries. Von Baer relied on them in both volumes of his *Entwickelungsgeschichte*; Burdach appropriated many of them for his *Physiologie*; Müller praised them as he was modifying some of their details (62); and Adelmann comments that "it is difficult to see how Rathke could have done better, lacking as he did the fixatives, microtome, and stains of the modern worker. . . . Rathke's illustrations of gross relationships are so accurate that they can be used with profit today" (63). Rathke was not the only investigator at the time who pursued the details of development with extraordinary patience, skill, and insight. The number of his comrades at the microscope bench, including Pander, von Baer, Müller, and Purkinje, rapidly swelled as younger researchers followed the example of this first generation of comparative embryologists.

## 6. A New Theoretical Framework at Mid-Century

Comparative embryology played a central role in the shaping of nineteenth-century zoology. By providing a guide to structural affinities that adult anatomy alone could not furnish, it became an arbitrator between anatomy and taxonomy. It proffered an independent criterion for determining the relationship of forms. Although von Baer had debunked the law of parallelism, embryologists assumed, as we have seen in Rathke's discussion of the mesonephros or in his discovery of the transitory gill arches, that single developing systems told what was the primitive and what was a more advanced state. Von Baer's laws of development assured both a hierarchical status of individual embryological patterns and their teleological nature.

Other achievements in biology quickly followed the early efforts to catalogue and interpret embryological forms. The year Rathke published his study on the adder, Matthias Schleiden and Theodor Schwann, working in Müller's institute, presented two monographs that refocused attention on the cell as the basic unit of structure of plants and animals. Schwann's cell theory was above all else an embryological theory of the fine structure of the body. According to Schwann, cells crystallized out of a fluid cytoplasm, pursued specific patterns of development, and died after adulthood and senescence. Normal differentiation of tissues and organs followed a normal *Entwickelungsgeschichte* of the constituent cells. Abnormal growths and neoplasias followed an abnormal cellular *Entwickelungsgeschichte*. It was no accident that both Schwann and Müller quickly saw the implications of the cell theory for human pathology (64).

Shortly thereafter, Albert Koelliker earned his doctorate with work that traced the development of spermatozoa from stem cells to their release from the testes (65), and Robert Remak established the cellular nature of the egg (66). Both achievements provided an important new perspective for the embryology of sexual reproduction. How these gametic cells interacted with one another and what relationship existed between the fertilized ovum, i.e., zygote, the blastomeres of cleavage, and the early organs of development remained complex issues of debate for another 35–40 years. Until the mid-1870s, dynamic rather than material views of fertilization prevailed. These implied that spermatozoa (and pollen grains) provided a physicochemical stimulus or transferred a physical motion to the egg, as hypothesized by the chemist Justus Liebig and his anatomist/physiologist colleague Theodor Bischoff. In the 1860s and 1870s the evolutionist Ernst Haeckel, the physiological psychologist Oswald Hering, and the embryologist Wilhelm His promoted variations on the same theme (67).

Contributing to the success of the dynamic theories of fertilization was uncertainty about the nature of the cell. At midcentury Remak and Rudolf Virchow, two of the most noted microscopists and pathologists of the day, claimed that cells were derived from previous cells by direct division, a process codified in Virchow's aphorism "Omne cellule e cellule." But equally powerful voices, including those of the anatomist and embryologist Thomas Huxley and Max Schultze, Professor of Anatomy at Bonn, maintained that protoplasm, rather than the cell, was the basic stuff of life. Only between 1875 and 1880 did zoologists and botanists, such as Oscar and Richard Hertwig, Otto Bütschli, Hermann Fol, Eduard van Beneden, Walther Flemming, and Eduard Strasburger, all of whom had been trained in microscopy and were steeped in developmental

questions, ultimately resolve the processes of fertilization and cell division. They concluded that a portion of a single spermatozoon or pollen cell entered the ovum or ovule and that two pronuclei, one from each gamete, fused and began a chain of mitotic divisions that formed the embryo of the next generation (68).

Traditionally, this work in nuclear cytology has been viewed by historians and biologists as a prelude to “genetics,” but such an historical account reads history backward. The microscopic studies of the nucleus and the discoveries of given chromosomal stages in mitotic and meiotic divisions, which took place between 1875 and 1885, were refinements within the tradition of descriptive embryology and the microscopic studies of sexual cells begun in the 1840s by Koelliker and Remak. Despite these achievements, objections continued to be raised at the end of the century against construing the cell as the primary unit of life (69).

Contemporary investigations in natural history also focused on development as a key to understanding the nature of life. Throughout the 1830s marine biologists, again many from the Baltic region, began describing complex life cycles of coelenterates, echinoderms, and ascidians. Structurally different organisms were united through a chain of metamorphic stages. In 1842 Japetus Steenstrup generalized this embryological phenomenon in an influential, albeit somewhat romantic, work entitled *On the Alteration of Generation* (70). Some of the finest naturalists and microscopists of the period adapted the notion of the alternation of generations and the related phenomena of hermaphroditism, parthenogenesis, fission, gemmation, and other forms of imperfect sexual and asexual reproduction to many other invertebrates and protozoa, and it became an essential criterion for sorting out the various stages of plant development. Its wide application to taxonomy and its elucidation of sexual and asexual reproduction reinforced the conviction that the study of development was central to all other aspects of biology (71).

Besides fashioning the cell theory, promoting the study of gametogenesis, and unveiling multiple modes of reproduction, descriptive embryology shaped zoology of the nineteenth century in a fourth way. Pander, von Baer, and Rathke all visualized early development in terms of the growth, movement, and wrapping around of germ layers. This, after all, was the essence of von Baer’s new epigenesis. Although Pander and Rathke conceived of three primitive layers whereas von Baer thought of two and four, all three pioneers recognized the potential of determining the derivation of advanced structures from the primary germ layers. In the early 1850s Remak brought the cell theory and germ-layer doctrine together. He had been a young microscopist in Müller’s laboratory in the late 1830s when Schleiden and Schwann had announced the cell theory. He had readily applied the doctrine to his own anatomical and embryological work. From the outset, however, he had been skeptical of Schwann’s belief in exogenous cell generation from a uniformed extracellular matrix, and the more he examined the production of cells, the more convinced he became that cells were derived from cells through direct cell division. The suspicion of a continuous chain of cells reinforced earlier suggestions that the germ layers provided the means for organizing histological destinies, but now Remak could think in terms of cell lineages. He accepted and extended Pander’s and Rathke’s tripartite division of the primitive germ layers but made important revisions concerning the products of what he called the middle or motor-generative layer and the

lower or gut-glandular layer. His demonstrations that the sensory organs were ultimately derived from the serous layer and that the liver and intestinal glands originated from the middle layer reinforced the value of a sustained analysis of cell origins (72).

While Remak studied cells and their germ layer origins in Müller's laboratory and then at makeshift facilities in his Berlin home, a young surgeon on board the H.M.S. *Rattlesnake* halfway around the world employed the germ layer theory in a daring and successful effort to unify the polyps, jellyfish, and siphonophores. Thomas Henry Huxley had wanted to be a naturalist and anatoomist, but financial circumstances led him to a commission as a naval surgeon after completing 3 years of prescribed study and practice at Charing Cross Hospital in London. The position was an expediency that presented him with the opportunity to follow the Union Jack to the South Pacific and Australia and guaranteed him free time aplenty to pursue microscopic studies. He found that the "foundation membranes" provided the tools to make affinities between organisms and to compare adult with embryonic structures. "It is curious," he commented with respect to the adult medusa, "that throughout, the outer and inner membranes appear to bear the same physiological relation to one another as do the serous and mucous layers of the germ" (73). In 1853 the Anglo-Irish naturalist George J. Allman coined two of the designations we use today for the primitive germ layers: ectoderm and endoderm (74).

## 7. Evolution and Development

In the post-Darwinian period, descriptive embryology became an imperative. Darwin, in *The Origin of Species*, had discussed with triumph the similarity between embryos of diverse types in what he called "the law of embryologic resemblance." He was not an expert microscopist, nor had he studied classical descriptive embryology, but as early as his student days in Edinburgh and during the Beagle voyage he showed a keen interest in the life cycles of invertebrates, (75) and his 8-year examination of barnacle taxonomy relied heavily on his being able to work out the developmental stages of these crustaceans. Darwin exploited in a gross morphological fashion the metamorphic transformations among barnacles to determine their phylogenetic relationships (76). Indirectly, he had been a student of von Baer and knew the latter had maintained that the embryos of diverse vertebrates were strikingly similar (77). There was no question in Darwin's mind that embryological relationships helped document the evolutionary notion of common descent. "Embryology rises greatly in interest," he pointed out in *The Origin*, "when we thus look at the embryo as a picture, more or less obscured, of the common parent-form of each great class of animals" (78).

It is one of the ironies of nineteenth-century biology that, despite von Baer's extended refutation of Meckel and Serres, Darwin's "law of embryonic resemblance" was confounded with and then transformed into an evolutionary form of the law of parallelism. One of Johannes Müller's students, Fritz Müller, at the time a naturalist and resident of Brazil, encouraged the confusion. In a provocative monograph entitled *Für Darwin*, in which he detailed a comparative study of the embryology of crustaceans, Müller described two possible patterns of development. As he documented the common descent of the crustaceans, he found

that “Descendants . . . reach a new goal, either by deviating sooner or later whilst still on the way towards the form of their parents, or by passing along this course without deviation, but then, instead of standing still, advancing still farther” (79). According to Müller’s view, embryos either diverge in form from one another after traveling a pace on a common developmental path or the evolutionarily more advanced embryos trace the entire developmental path of their ancestors to the adult stage and progress beyond. Neither alternative reflected von Baer’s original assertions that different forms deviate from one another in development from the outset because they arise from eggs that are in essence, if not visibly, different. But in the heady days following *The Origin of Species*, when genetic continuity seemed the order of the day, no one noticed (80). Following Fritz Müller’s lead, naturalists, paleontologists, and embryologists all assumed a degree of physically manifested recapitulation of ancestral development. It was another student of Johannes Müller, Ernst Haeckel, who dramatized the obfuscation with the phrase that “ontogeny recapitulates ontogeny” and elevated the parallel to the status of “the fundamental biogenetic law” (81).

Since the biogenetic law became such an influential consequence of descriptive embryology, it is valuable to observe how Haeckel at first understood it. Although he recited some of the standard embryological features that suggested a recapitulation, Haeckel presented the law as a “causal nexus” (*Causalnexus*) that drew its validity from accepted biological processes. The biogenetic law was spawned by Haeckel’s acceptance of evolution theory and the recognition that there existed a phylogenetic history to trace. Its mechanism was explained in terms of cell division and a belief in the antithesis between the conservative nature of heredity and the progressive nature of adaptations, and it was ontologically validated by Haeckel’s belief that a bond of material and physical forces linked the stages of ontogenetic and phylogenetic development. This bond, in his mind, guaranteed that the forms generated by the one must parallel the forms already produced by the other. Furthermore, Haeckel believed that evolutionary innovations were added only at the adult stage and that as phylogenies progressed these terminal additions were continually condensed into earlier stages of development. Phylogenetic trees, so often illustrated by Haeckel, suited the process of terminal addition; they failed, however, to capture the condensation process in subsequent ontogenies.

In five “theses” Haeckel hammered out the implication of this causal nexus (82).

1. Ontogeny or the development of the organic individual, being the series of changes in form through which every individual organism passes during the entire span of its individual existence, is directly determined by the phylogeny or development of the organic lineage (*Phylon*) to which it belongs.
2. Ontogeny is the short and rapid recapitulation of phylogenesis, determined by the physiological functions of heredity (propagation) and adaptation (nourishment).
3. The organic individual (as a morphological individual of the first through sixth order) repeats during the quick and short course of its individual development the most important of those changes in form, which its ancestors traversed during the slow and long course of its paleontological development according to the laws of heredity and adaptation.
4. As ontogeny takes an ever shorter path, the complete and accurate repetition of phyletic by ontogenetic development is falsified and abbreviated by secondary contractions; therefore the more complete the repetition is, the longer is the series of successively transversed juvenile stages.

5. Since the organism during its individual development becomes adapted to new circumstances, the complete and accurate repetition of phyletic by ontogenetic development is falsified and altered by secondary adaptations; therefore the more accurate the repetition is, the more similar are the conditions of existence under which the organism and its ancestors have developed.

It is instructive to compare Haeckel's five theses with von Baer's four laws of development, presented earlier. Both, after all, aspired to generalize on the same process of development. The 40 years intervening between each statement witnessed, of course, substantive developments in biology that necessarily changed the focus of those generalizations, but the contrast is greater than this contextual change suggests. As we have pointed out, von Baer's laws were in large measure a summary corrective to previous theories of development: to preformationism and the law of parallelism. They were drawn directly from von Baer's own microscopic experiences and suggested a commitment to both a teleological and mechanistic explanation of form. Later stages of development were intimately connected with, and even guided by, preceding stages. Tim Lenoir has fortuitously associated von Baer's laws and the developmental morphology of many of von Baer's contemporaries with a "teleomechanistic" biology (83). Haeckel's theses are restatements of his biogenetic law. They are generalizations more by fiat than observation. They offer a formalized relationship between two processes that can only be tenuously connected. They profess a material foundation for biology but in reality provide an idealistic or intuited one, as though the very structure of the cosmos guaranteed a connection between ontogeny and phylogeny. It is worth noting that the fourth and fifth theses, however, blunt the certainty of recapitulation and provide rejoinders should observations threaten to falsify the first three (84).

## 8. Specificity of the Germ Layers

As he began touting the biogenetic law, Haeckel at first mentioned gross anatomical similarities, such as the embryonic gill slits in vertebrates and the progression of the heart from a tubular structure to a four-chambered organ. Oppenheimer has pointed out that Haeckel did not even refer to germ layers in the *Generelle Morphologie*, in which he first emphasized recapitulation (85). What was to him an acceptable argument for recapitulation in 1866, however, paled in comparison to the arguments proffered in the early 1870s. By that time three Russian naturalists, who had come to Germany to complete their scientific education, had refashioned the germ-layer doctrine into a powerful tool for establishing phylogenies. Alexandre Kovalevsky had attended the zoological lectures of Heinrich Georg Bronn in Heidelberg just when the latter completed the first German translation of *The Origin of Species*. Inspired by the theory of evolution, Kovalevsky had then refined his skills at microscopy under one of the masters of the art, Franz von Leydig, at Tübingen. Elie Metchnikoff, later to become a Nobel laureate for the demonstration of phagocytosis by white blood cells, had studied embryology under another German master teacher, Rudolf Leuckart, in Giessen. Nicolaus Kleinenberg had started out in botany but had been captivated by Haeckel's circle in Jena. All three had concentrated on

studying the fine structure of development of invertebrates for the purposes of determining phylogenies.

Both Kovalevsky and Metchnikoff converged on the Bay of Naples between 1864 and 1865, where together they developed special methods for hardening and sectioning the fragile embryonic material for their examinations. For the next 2 years Kovalevsky, on a sustained basis, and Metchnikoff, in a more fragmented way, examined the development of germ layers in a range of invertebrates and lower chordates. Kovalevsky received his masters degree for a classic study of the development of amphioxus and his doctoral degree for the study of the brachiopod *Phoronis*. During the same period he examined, as well, the development of tunicates or sea squirts, which were thought to be mollusks but which he showed, by examining the development of their larvae, were really chordates. The determination of the early germ-layer derivatives of amphioxus and ascidians in particular promised to shed light on the phylogenetic origins of vertebrates. Metchnikoff examined the development of insects, scorpions, and cephalopods. He was particularly effective in demonstrating that the tornaria larva of the acorn worm, *Balanoglossus*, which today we classify as a hemichordate, had features transitional between those of echinoderms and vertebrates. A recent reevaluation of his work has emphasized that Metchnikoff was intent on establishing structural and functional homologies of the germ layers throughout the animal kingdom (86). Both Russians won enthusiastic acclaim from other embryologists. Their embryological accomplishments were so substantial that when the two returned to St. Petersburg in 1867 to receive their respective doctoral degrees, they were jointly awarded the Karl Ernst von Baer prize by the 75-year-old von Baer himself. Inspired by the studies of Kovalevsky and Metchnikoff, Kleinenberg received his doctorate for a detailed study of the *Hydra* (87). Among other things he demonstrated through an examination of cell movements that the body cavity was not formed simply through a process of invagination, but that the adult ectoderm and oral opening were secondary to the formation of an external theca. Despite these complexities, Kleinenberg homologized the two germ layers of *Hydra* with the two initial layers found in the development of more complex invertebrates and vertebrates.

Meticulously illustrated and detailed to the point of presenting as many minutiae as possible of germ layer, tissue, and cell movements, the monographs of these three Russians were worthy successors of the descriptive tradition laid down 40 years earlier by Pander, von Baer, and Rathke. The advanced cell theory, the germ-layer doctrine, and evolution theory, however, had altered the goals of the enterprise. Whereas the earlier generation was concerned with establishing the rules of development first and passing judgment on the conclusions of anatomy and taxonomy second, Kovalevsky, Metchnikoff, and Kleinenberg became obsessed with documenting phylogenetic pathways. Their exacting studies of germ-layer homologies were quickly emulated by other embryologists, such as Anton Dohrn, Edwin Ray Lankester, the Hertwig brothers, Karl Semper, and Francis Maitland Balfour, many of whom worked in Haeckel's institute and all of whom recognized the evolutionary significance of this level of analysis. Haeckel became an enthusiast of germ-layer analysis and steered his own research onto the same track. In a morphological study of calcareous sponges published in 1872, he described the contrasting certainty with which this refined

approach endowed his biogenetic law: “In my General Morphology I sought to demonstrate synthetically that all the phenomena of the organic world of forms can be explained and understood only by the monistic philosophy; and now this demonstration is furnished analytically by the morphology of the Calcispongiae” (88). Two years later Haeckel presented his *gastreæ* theory to the public. This spectacular hypothesis attempted to identify in abstraction the ancestor of the metazoa. By then, Haeckel was convinced that invagination was the primitive mode of gut formation. By a logical abstraction Haeckel claimed that wherever the process of invagination occurred today, it did so because of the precedent set by ancestors and the conservative laws of heredity (89).

Haeckel exemplified an extreme advocate in the joining of biogenetic law and the germ-layer doctrine. Others used the combination more effectively and with *sotto voce*. Using both principles, Francis Maitland Balfour produced a detailed analysis of the vertebrate urogenital system, which answered many of the questions left by Rathke’s exemplary examination of the adder (90). Haeckel’s exact contemporary August Weismann, who had done important embryological work on *Diptera* in the early 1860s, combined the two beliefs in the early 1880s in a less doctrinaire way to arrive at the notion of the continuity of the germ cells. This concept, in turn, evolved into one of the fundamental pillars of classical genetics (91).

Other embryologists employed the techniques of microscopic analysis to modify and attack both the specificity of the germ-layer doctrine and the biogenetic law. Wilhelm His, another near-contemporary of Haeckel, helped develop a microtome and, with the sections this instrument permitted, restudied the development of the chick in a classic monograph that went far beyond the studies of von Baer and Rathke. When the germ-layer doctrine became the rage, His attacked the nomological implications of the biogenetic law. Instead, he argued and demonstrated through mechanical analogies that the causes of germ-layer movements must be sought in the mechanics of cell growth and the interacting pressures of expanding germ layers. He was in fact reinforcing, with all the authority of modern reductionistic biology, a perspective foreshadowed in Pander’s and von Baer’s description of the primitive germ layers (92). August Rauber, anatomist at Dorpat, developed his own brand of “cellular mechanics,” which also spoke to the proximal causes of the developmental processes (93). In the United States the first generation of embryologists, Charles Otis Whitman, Edmund Beecher Wilson, Edwin G. Conklin, and Frank R. Lillie, traced cell lineages of numerous organisms from early cleavage to their projected ultimate fates. This tedious exercise provided another refutation that ontogeny slavishly recapitulated phylogeny (94).

## 9. Conclusion

Between 1816, when Pander went to work with Döllinger in Würzburg, and 1880–1881, when Balfour completed the first comprehensive presentation of comparative embryology, classical descriptive embryology took shape and grew to maturity. Three Baltic region embryologists, Pander, von Baer, and Rathke, laid down the methodological lines that descriptive embryology was to take during the three score years under review. The three made it clear that exacting and

multiple microscopic examinations of many series of embryos provided the only secure route to a clear understanding of the sequential stages in development. They also made it clear that comparisons of development between major taxa provided the only way to attain an understanding of any individual developmental event. It was not that they demoted the chick egg in importance—Pander and von Baer could hardly be accused of that indiscretion—but as they elevated the importance of studying the development of other types, they made it possible to identify homologous structures and processes. In these they found an unrealized unity in diversity. This unity might have several explanations. For von Baer and Rathke it reflected a limited transformation and a unified cosmic arrangement, but for others, even before Darwin, the unity of development suggested a common genetic origin.

During this period the cell theory in all its variations, the multiple modes of reproduction in all their complexity, the formation of gametes and their value in fertilization, and the further articulation of the germ-layer doctrine added new dimensions to embryology. All four of these areas of study emerged out of the pursuit of a more complete descriptive embryology. They were actually consequences of the same developmental imperative and microscopic and comparative investigations that perfused anatomy, taxonomy, and natural history. To live was to develop; to understand was to map the course of that development.

Between the second and ninth decades of the century few voices were raised in opposition to the general thrust of descriptive embryology. During this period there existed a tradition of experimental teratology, particularly in France, and a tradition of regeneration studies that traced its heritage back to the early eighteenth century. In both traditions the objectives were to understand taxonomic relationships by creating malformations in embryos and adults (95). Neither tradition broke radically from the objectives of descriptive embryology. When the excesses of Haeckel's biogenetic law, however, became clear by the mid-1870s, grumblings within the ranks of descriptive embryology itself were evident. They urged a search for proximal rather than formal causes and challenged the specificity of the germ layers. The breakthrough came in the 1880s, when a younger generation of embryologists, including Wilhelm Roux, Gustav Born, Oscar Hertwig, Hans Driesch, and Thomas Hunt Morgan, inspired by the analytical successes of physiology, began reorienting embryology away from comparative descriptions toward an experimental enterprise. It is doubtful that the new approach, called variously "Entwickelungsmechanik," "developmental physiology," "Biomécanique," "regeneration studies," or simply "experimental embryology," would have disturbed the founders of classical descriptive embryology. After all, theirs had been a recognition of proximal causes even as they set out to establish the descriptive details of formal teleological patterns.

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20. The three microscopists were the French biologist J. V. Coste (1833), Purkinje's student A. Bernhard (1834), and Thomas Wharton Jones (1835). For further details see Kruta, 1971, p. 102.
21. von Baer, 1828, *Commentar*, pp. 116–117. In places von Baer is ambiguous about the role of the spermatozoa. In one rhapsodical passage he hypothetically suggests a correspondence between the “spermatic animalcules” and the germinal vesicle. It is likely that von Baer intended sarcasm at this point.
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25. von Baer, K. E., 1828–1837, *Über Entwickelungsgeschichte der Thiere. Beobachtung und Reflexion*, 2 vols., Gebrüder Bornträger, Königsberg. A convenient, though somewhat stilted, English synopsis of von Baer's classic may be found in Blaykher, L. Y., 1955, *History of Embryology in Russia from the Middle of the Eighteenth to the Middle of the Nineteenth Century*, trans. and ed., H. I. Youssef and B. A. Malek, with an Introduction by J. Maienschein, Smithsonian Institution Libraries, 1982, Chapters 14–24.
26. von Baer, 1986, pp. 232–241, describes at length the misunderstandings with Burdach and unsatisfactory arrangements von Baer had over his contribution to Burdach's *Physiologie*. The experience also led to an uneasy relationship between von Baer and Rathke.
27. von Baer, 1828–1837, Vol. I, p. 38.
28. In modern terminology we would talk about the successive tucking under of the splanchnopleure and somatopleure. This process would include elements of the endoderm, mesoderm, and ectoderm.
29. von Baer, 1828–1837, Vol. I, p. 88.
30. *Ibid.*, p. 140.
31. Whereas the description of the sequential development of the chick took 140 pages, the Scholia and Corollaries took 124 pages. The remaining 10 pages were devoted to explanations of the two copper plates and corrections.
32. Oppenheimer, J. M., 1955, Problems, concepts and their history, in: *Analysis of Development* (Willier, B. H., Weiss, P., and Hamburger, V., eds.), Saunders, Philadelphia, pp. 1–24.
33. von Baer, 1828–1837, Vol. I, p. 149.
34. *Ibid.*, p. 148. Emphasis is von Baer's.
35. von Baer often called the vegetative plate the “plastic” plate.
36. von Baer, 1828–1837, Vol. I, p. 157.
37. This was clearly the climax of the Scholia. The Fifth and Sixth Scholia are the only ones that have been translated into English. See Huxley, T. (trans.), *Fragments relating to philosophical zoology. Selected from the Works of K. E. von Baer*, in: *Scientific Memoirs, Selected from the Transactions of Foreign Academies of Science, and from Foreign Journals of Natural History* (Henfrey, A. and Huxley, T. H., eds.), Taylor and Francis, London, 1853, Vol. 1, pt. II, pp. 186–238.
38. von Baer, *Entwickelungsgeschichte*, Vol. I, pp. 208–209.

39. Lenoir, 1982, pp. 72–95.
40. These laws have been taken verbatim from Huxley's translation of the Fifth Scholium, p. 214.
41. von Baer, 1828–1837, Vol. I, p. 225, presents such a branching taxonomy shortly after enunciating his laws.
42. *Ibid.*, p. 231.
43. *Ibid.*, p. 265.
44. *Ibid.*, pp. 263–264. I have followed, with minor revisions, Huxley's translation, pp. 237–238.
45. Rathke, 1818, was a 24-page medical dissertation entitled "De Salamandrarum corporibus adiposis, ovarii, et oviductibus eorumque evolutione," with two tables (Berlin). Stieda, L., 1888, Rathke: Martin Heinrich R., *Allgemeine Deutsche Biogr.* 27:352–355, presents a brief but useful sketch of Rathke's life and career.
46. Key sections of works on the embryology of the urogenital system can be followed in Adelmann, H., 1966, *Marcello Malpighi and the Evolution of Embryology*, 5 vols., Cornell University Press, Ithaca, NY, Vol. 4. For Rathke see pp. 1801–1831, 1837–1851, 1931–1943, 1963–1976.
47. See particularly Rathke, H., 1825a, Kiemen bei Säugethieren, Oken's *Isis*, col. 747–749; 1825b, Kiemen bei Vögeln, *Ibid.*, pp. 1100–1101; 1825c, Beobachtungen und Betrachtungen über die Entwicklung der Geschlechtswerzeuge bei den Wirbelthieren, *Neueste Schriften. naturforsch. Gesellsch.*, Danzig, 1 (Heft 4):1–146; 1828a, Bemerkungen zu dem Aufsatze des Herrn Prof. Huschke: Ueber die Kiemenbögen und Kiemengefäße beim bebrüteten Hühnchen, *Ibid.*, 21:80–85; 1828b, Ueber das Dasein von Kiemenandeutungen bei menschlichen Embryonen, *Ibid.*, 21:col. 108–109; and 1832, *Anatomisch-Philosophische Untersuchungen über den Kiemenapparat und das Zungenbein der Wirbelthiere*, Eduard Frantzen, Riga und Dorpat. All these works are partly reproduced and translated in Adelmann, 1966.
48. Rathke, H., 1829, *Untersuchungen über die Bildung und Entwicklung des Flusskrebses*, L. Voss, Leipzig.
49. For example: "Since the true kidneys sprout and develop in addition to the Wolffian or Oken bodies, we may see a nice confirmation of the statement that the higher animals are only developments of the lower ones." Rathke, 1825c, quoted in Adelmann, 1966, Vol. IV, p. 1826.
50. von Baer, 1986, p. 237, makes a revealing comparison between his own and Rathke's style of research at the time he was writing critically of Burdach's treatment of his own contribution to the latter's *Physiology*: ". . . Rathke had always been in the habit of having his investigations published as soon as possible, while I, striving for general results which always require much comparative work, was in no hurry. . . ."
51. See Lenoir, 1982, pp. 96–102, for a detailed and philosophically oriented discussion of Rathke's discovery of the gill clefts.
52. See Russell, E. S., 1916, *Form and Function*, John Murray, London, pp. 151–156, for a discussion of Rathke's cautious criticisms of the vertebral theory of the skull.
53. In the development of the idealized vertebrate there exist sequentially three kidneys. (1) The *pronephros*, or "head kidney," opens into the body cavity via a segmental or primitive duct. With the exception of fish, the *pronephros* soon disappears in most vertebrates, but the primitive duct forms the foundation for later genital and urinary passages. (2) The *mesonephros*, or Wolffian body, consists of segmentally arranged glandular tubules that open at one end into the body cavity and at the other into an efferent common tubule, which eventually joins the primitive duct. The *mesonephros* functions as an embryonic kidney and remains a prominent structure in adult amphibians. (3) The *metanephros*, or proper kidney, of amniotes is a caudal outgrowth of the primitive duct and assumes much of the same tubular appearance as the *mesonephros*. What makes the relationship of these three sets of kidneys complicated is that they rarely appear together in an easily identifiable sequence. Furthermore, as development progresses in certain fish, amphibians, and higher vertebrates, the segmental or primitive duct soon divides longitudinally into an easily recognized duct, known as the *mesonephric* or *Wolffian* duct and the thread-like Müllerian duct. In male birds and mammals the anterior portion of the *Wolffian* duct becomes the *vas deferens*, which transports semen from the testes to the ureter, and the Müllerian duct degenerates. In female birds the Müllerian duct becomes the oviduct and the anterior portion of the *Wolffian* duct degenerates. In female birds furthermore, the system becomes asymmetrical as the right Müllerian duct and ovary disappear. In mammals the caudal ends of the two Müllerian ducts become the *vagina* and *uterus*. The *Wolffian* body in amphibians remains the functioning kidney and eventually develops Malpighian bodies and convoluted tubules,

- which together form the complex filtering system for the blood. True mammalian kidneys develop later as an offshoot from the posterior portion of the segmental, or primitive, duct. As the true kidneys assume their renal functions, the two Wolffian bodies degenerate, and their anterior ends along with the collecting and transverse tubules become appropriated by the testes to form the epididymides and the vas deferens.
54. See Balfour, F. M., 1881–1882, *A Treatise on Comparative Embryology*, 2 vols., Macmillan, London, Chapter 24 for a general account of the end-of-the-century understanding of the development of the urogenital system.
  55. Rathke, H., 1818, published his dissertation in Latin, *De salamandarum corporibus adiposis, ovariosis, et oviductibus eorumque evolutione. Dissertatio inauguralis*, Berolini, typis Heusleri viduae. Much of its contents appeared in an expanded form in Ueber die Entstehung und Entwicklung der Geschlechtstheile bei den Urodelen, *Neueste Schriften d. naturforsch. Gesellsch.*, Danzig, 1920:1–108. Later important works in this first stage of research include: 1825d, Beobachtungen und Betrachtungen über die Entwicklung der Geschlechtswerkzeuge bei den Wirbeltieren, *Ibid.*:1–146 and 1832–1833, *Abhandlungen zur Bildungs- und Entwickelungs-Geschichte des Menschen und der Thiere*, F. C. W. Vogel, Leipzig. The latter work consists of a series of monographs, including one of the urogenital system of snakes, lizards, and turtles and one on mammals. Since all of these works are difficult to find, I have followed the translations and discussion of them in Adelmann, 1966, Vol. 4, pp. 1758–2028.
  56. Lenoir, 1982, pp. 95–111.
  57. Rathke, H., 1839, *Entwickelungsgeschichte des Natter (Coluber Natrix)*, Gebrüder Bornträger, Koenigsberg. See also Adelmann, 1966, Vol. 4, pp. 1963–1976.
  58. See letter from Rathke to Müller, Feb. 18, 1829, portions of which are reprinted in Adelmann, 1966, Vol. 4, pp. 1860–1861.
  59. *Ibid.*, p. 1962; Brian Bracegirdle, 1978, *A History of Microtechnique*, Cornell University Press, Ithaca, New York, pp. 76–81.
  60. Rathke, 1839, in Adelmann, 1966, *Ibid.*, Vol. 4, p. 1966.
  61. Adelmann, 1966, Vol. 4, p. 2240.
  62. Müller, J. P., 1829, Ueber die Wolfschen Körper bei den Embryonen der Frösche und Kröten, *Arch. Anat. Phys.*, Jhg. 1829, pp. 65–70, and Müller, 1830, *Bildungsgeschichte der Genitalien aus anatomischen Untersuchungen an Embryonen des Menschen und der Thiere*, Düsseldorf. In 1829 and 1830 Müller made substantial modifications to the sequence envisioned by Rathke. He discovered the pronephros in frogs and toads but equated this erroneously with the Wolffian body of higher vertebrates. More significantly, he recognized that the segmental duct gives rise to two distinct ducts. His conclusions about their fates in birds corresponds to our modern understanding, but for mammals Müller failed to make a clear distinction between their ultimate fates. For an incisive discussion of Müller's achievements and confusions, see Adelman, 1966, Vol. 4, pp. 1851–1901. For a valuable discussion of the philosophical context of Müller's 1830 work, see Lenoir, 1982, pp. 103–111.
  63. Adelmann, 1966, Vol. 3, p. 1802.
  64. Schwann, T., 1839, *Mikroskopische Untersuchungen über die Uebereinstimmung in der Struktur und dem Wachsthum der Thiere und Pflanzen*, Sander, Berlin, the English translation appeared in 1847 as *Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants* (Smith, H., trans.), Sydenham Society, London; Müller, J., 1838, *Ueber den feineren Bau und Formen der Krankhaften Geschwülste*, G. Reimer, Berlin; for a discussion of these two works with relation to embryology, see Churchill, F. B., 1976, Rudolf Virchow and the pathologist's criteria for the inheritance of acquired characteristics, *J. Hist. Med.*, 31:117–148; Duschésneau, F., 1987, *Genèse de la théorie cellulaire*, Bellarmin, Montréal, provides a recent assessment documenting the importance of embryology for the cell theory.
  65. Koelliker, A., 1841, *Beiträge zur Kenntnis der Geschlechtsverhältnisse und der Samenflüssigkeit virbelloser Tiere, nebst einem Versuche über das Wesen und die Bedeutung der sogenannten Samentiere*, W. Logier, Berlin.
  66. Remak, R., 1850–1855, *Untersuchungen über die Entwicklung der Wirbeltiere*, 3 pts., G. Reimer, Berlin.
  67. Farley, J., 1982, *Gametes and Spores. Ideas about Sexual Reproduction 1750–1914*, Johns Hopkins University Press, Baltimore, pp. 34–71, and Robinson, G., 1979, *A Prelude to Genetics* Coronado Press, Lawrence, KS, pp. 47–70.

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71. See Winsor, M. P., 1976, pp. 44–72; Farley, J., 1982, pp. 72–109; Churchill, F. B., 1979, Sex and the single organism, biological theories of sexuality in mid-nineteenth century, *Stud. Hist. Biol.* **3**:139–177; and Churchill, 1989, The guts of the matter. Infusoria from Ehrenberg to Bütschli: 1838–1876, *J. Hist. Biol.* **22**:189–213.
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73. Huxley, T. H., 1849, On the anatomy and the affinities of the family of Medusae, reprinted in *The Scientific Memoirs of Thomas Henry Huxley*, (Foster, Michael and Lankester, E. R., eds.), Macmillan, London. Quotation appears in Vol. 1, p. 24,
74. Allman, G. J., 1853, On the anatomy and physiology of Cordylophora, a contribution to our knowledge of tubularian zoophytes, *Roy. Soc. Trans.* **143**:368.
75. Sloan, P. R., 1985, Darwin’s invertebrate program, 1826–1836: preconditions for transformism, *The Darwinian Heritage* (Kohn, D., ed.), Princeton University Press, Princeton, NJ, pp. 71–120.
76. Richmond, M. L., 1988, Darwin’s study of Cirripedia, in *The Correspondence of Charles Darwin* (Burkhardt, F. and Smith, S., eds.), Cambridge University Press, Cambridge, U.K., Vol. 4:1847–1850, pp. 388–409.
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78. Darwin, C., 1859, *On the Origin of Species by Means of Natural Selection*, John Murray, London, p. 450 (facsimile reprint by Harvard University Press, 1964).
79. Müller, F., 1964, *Für Darwin*. Quotation is taken from the English version: *Facts and Arguments for Darwin*, John Murray, London, 1869, p. 111.
80. von Baer was one of the most noteworthy holdouts against a material theory of evolution; thus he opposed any pattern suggesting genetic continuity.
81. For a lucid description of the transformation of von Baer’s laws to Haeckel’s biogenetic law, see Gould, 1977, Chapter 4. Haeckel did not use the expression “das biogenetisches Grundgesetz” in either his *Generelle Morphologie* or the first edition of *Natürliche Schöpfungsgeschichte* (1868), but it appears in the second edition (1870) of the latter work.
82. Haeckel, E., 1866, *Generelle Morphologie der Organismen. Allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von Charles Darwin reformierte Descendenz-Theorie*, 2 vols., George Reimer, Berlin, Vol. 2, p. 300. I have followed with minor changes the translation of Russell, E. S., 1916, pp. 253–254. See also Gould, Steven Jay, 1977, p. 81.
83. Lenoir, 1982, pp. 12–16.

84. Fritz Müller (1864) also recognized the exceptions to a strict parallel between ontogeny and phylogeny (p. 114).
85. Oppenheimer, J. M., 1940, The non-specificity of the germ-layers, reprinted in *Essays in the History of Embryology and Biology*, M.I.T. Press, Cambridge, MA, p. 263.
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90. Balfour, F. M., 1876, On the origin and history of the urinogenital organs of vertebrates, *J. Anat. Phys.* **10**:17–48. See particularly Balfour's comments on the relationship between the Wolffian duct in birds and selachians (pp. 46–48).
91. Weismann, A., 1883, Die Entstehung der Sexualzellen bei den Hydromedusen. Zugleich ein Beitrag zur Kenntniss des Baues und der Lebenserscheinungen dieser Gruppe, Gustav Fischer, Jena: for a recent analysis of this trend, see Churchill, 1987; Churchill, F. B., 1985, Weismann's continuity of the germ-plasm in historical perspective, *Freiburger Universitätsblätter*, Heft 87/88:107–124; and, 1986, Weismann, Hydromedusae, and the biogenetic imperative: A reconsideration, in: *A History of Embryology*, (Horder, T. J., Witkowski, J. A., and Wylie, C. C., eds.), Cambridge University Press, Cambridge, MA, pp. 7–33. For more negative evaluations of Weismann's use of the biogenetic law see Gould, 1977, pp. 102–109, and Berrill, N. J., and Liu, C. K., 1948, Germplasm, Weismann, and Hydrozoa, *Q. Rev. Biol.* **23**:124–132.
92. His, W., 1874, Unsere Körperform und das Physiologische Problem ihrer Entstehung. Briefe an einen befreundeten Naturforscher, F. C. W. Vogel, Leipzig.
93. Rauber, A., 1880, *Formbildung und Formstorung in der Entwicklung von Wirbeltieren*, Wilhelm Engelmann, Leipzig.
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## Chapter 2

# Laurent Chabry and the Beginnings of Experimental Embryology in France

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### 1. Introduction

The birth of experimental embryology is certainly one of the most important events in the history of biology (1). If we can date the origin of scientific embryology to the works of H. C. Pander (1817) and K. E. von Baer (1828), the birth of experimental embryology took place with the works of W. Roux (1881–1888); it should be noted, however, that the conception of developmental mechanics goes back to W. His (1874).

The origins of experimental embryology are essentially German, and the greatest names who worked for the development of this discipline—W. Roux, E. Pflüger, O. and R. Hertwig, T. Boveri, C. Herbst, H. Driesch—are all from the land of Goethe. However, during the years 1880–1900, there was no lack of practicing embryologists in other countries, and among those who distinguished themselves in France are L. Chabry, Y. Delage, and E. Bataillon (2).

### 2. E. Geoffroy Saint-Hilaire

Amongst these three, Chabry left the strongest impression on the history of the developmental mechanics. But before analyzing L. Chabry's research and the reasons that led him in the direction of scientific investigation, we should mention some French-speaking authors who preceded him in applying the experimental method to the embryo. In 1820, Etienne Geoffroy Saint-Hilaire unquestionably did this in order to resolve certain anatomical questions (3). Indeed, E. Geoffroy Saint-Hilaire belonged with E. R. A. Serres to those anatomists who, at the beginning of the nineteenth century, forged “transcendental anatomy” and essentialist morphology, which had many affinities with the German “philosophy of nature.”

E. Geoffroy Saint-Hilaire thought that all organisms were structured after an

ideal anatomical model. He could not describe this abstract model because it was of divine origin and thus inaccessible to the “learned.” God had created this model from which sprang the diverse forms of animals that populate the earth. There is therefore in his vision of the living world a unity of composition according to a unique plan recognizable in the entire animal kingdom. Furthermore, numerous animal varieties, including humans, were formed following embryonic transformations in the course of geological time. The embryos, thought E. Geoffroy Saint-Hilaire, were transformed because they breathed and were thereby sensitive to environmental variations. Thus when Lavoisier came to show the importance of oxygen for the maintenance of life, certain geologists, among them De Luc (1795), believed that the atmosphere was modified during these geological times and that these modifications in the composition of the atmosphere must have had an effect on the organisms.

E. Geoffroy Saint-Hilaire based his science on this theological scheme, and he conducted experiments in order to justify his theory of organic unity and to prove that organic modifications must have resulted from the environmental variations acting directly on the embryos. To this end, he covered chick eggs with strips of cloth to slow the embryos’ respiratory exchanges, or he cut the eggshells with a saw to accelerate them. To show the function of the attachments of the embryonic membranes on the embryo relative to teratological formations, he incubated eggs vertically. In other experiments he interrupted the incubation of the eggs between the seventh and tenth day in order to test the effect of hypothermia on the embryo.

E. Geoffroy Saint-Hilaire expected much of his experiments and would not have been surprised if he had found a reptile in one of his experimental eggs. According to the logic of transcendental anatomy and the unitarian model, a bird could give birth to a reptile, just as a reptile could produce a bird. Similarly, he did not see a contradiction in the transformation of a vertebrate into an invertebrate (4).

To call E. Geoffroy Saint-Hilaire’s work “scientific” invites a paradox. For although he was studying problems of a scientific nature, his theory was grounded in a theological model wherein the origins of developmental variations were inaccessible to science. Therefore, while the context played a role in the progress of comparative anatomy, it is not true to say that E. Geoffroy Saint-Hilaire did experimental embryology: he ignored scientific embryology because it could not enter his intellectual considerations. Likewise his transcendental anatomy does not permit the claim that it was evolutionary in the sense acceptable after the publication of Darwin’s *The Origin of Species* (1859). Like Serres, E. Geoffroy Saint-Hilaire practiced an anatomy of the embryo, but not an embryology. As far as evolution is concerned, his system led him to defend within the framework of an epigenesis a “transformation” of organisms in a circular fashion. We are in a closed system because a organism can transform itself in a “superior” or “inferior” form, and these “transformations” are possible only in the rigid framework of a unified plan.

E. Geoffroy Saint-Hilaire’s experiments did not produce any results, although he believed that he had obtained embryonic malformations. However, these malformations (exencephaly) were not provoked by his experimental procedures since he used crested chicks as experimental material. These crested chicks normally produce “exencephalic” embryos (5).

### 3. C. Dreste

Nevertheless, to understand the progress of the experimental method in French embryology, we must always mention the work of E. Geoffroy Saint-Hilaire because of its influence on Camille Dreste, who perfected experimentation in embryology. C. Dreste conducted his experiments with a well-defined goal of obtaining experimental transformation of embryos.

C. Dreste, like E. Geoffroy Saint-Hilaire, subjected the whole egg to a teratogenic action. These operations included the partial varnishing of the shell, refrigeration of the eggs, or else subjecting the eggs to concussion before incubation. As opposed to E. Geoffroy Saint-Hilaire, he obtained numerous malformations. However, although Dreste could obtain different types of monstrosities, he could not produce any one malformation specifically. Because his method did not allow him to predict the result, many of Dreste's contemporaries judged it nonscientific. Then, as now, the scientific community has more influence than the individual when it comes to judging the value of a work that appears too marginal in comparison with the prevailing rules of the contemporary sages.

However, C. Dreste's major investigations sought to discover whether racial and even specific characteristics were comparable to teratological characteristics. If so, research in experimental teratology should, he thought, enlighten the biologist about the past and present transformations of organisms (6): "The greatest question of the natural sciences is without doubt the origin of the innumerable forms in which life manifests itself on the surface of our planet." Thus, when, in 1888, the question arose whether to build a laboratory of transformistic experiments in Montsouris Park in Paris, as requested by Clémence Royer, Darwin's translator, C. Dreste joined in and proposed a plan of experimentation. In this project the indirect experimental method would have an important place, being the only one that could satisfy the mind of a transformist experimenter. Indeed, if the environment was preponderant in bringing about organic transformations, it could only act globally on individuals or embryos—hence the desire, of E. Geoffroy Saint-Hilaire as well as of Dreste, to make the embryo react to the effects of hypoxia or hypothermia which arise spontaneously in nature. Here the experimenter imitates nature, hoping that she will reveal her secrets. Thus Dreste and E. Geoffroy Saint-Hilaire shared this belief in the environmental transformation of embryos, although they placed it in very different contexts.

### 4. Stanislas Warynski and Hermann Fol

In 1884 two Swiss physiologists, Stanislas Warynski and Hermann Fol, published a study which—without diminishing the interesting work of the founder of teratogeny—contained a new experimental methodology that was to open new horizons in the interpretation and explanation of monster organisms (7). This technique, the direct method, allowed manipulations of embryos by means of an instrument placed directly on a precise point of the embryonic organism. This was in contrast to the indirect method, as employed by Dreste, who exposed an entire egg to a teratogenic factor.

The experimental technique employed by Warynski and Fol is simple. They

began by making an opening in the shell of the chick egg just over the 24–48-hr embryo. This opening of 2–3 cm in diameter would permit the investigator to place the point of a thermocautery on a precise spot in the embryo. The lesions thus produced were localized in the cephalic part, either by direct application, by heating from a distance, or, in later experiments, by simply pressing the unheated instrument on the cephalic anlage.

The results of these experiments are interesting, because these experimenters obtained specific malformations of the head (acephaly and microcephaly) in 48-hr embryos. From the 24-hr embryos they obtained a great number of omphalocephalic embryos as well as some cases of cardiac duplication and heterotaxies. Furthermore, in another study, operating on 30- to 36-hr embryos, Warynski obtained single-hearted omphalocephalic embryos when the lesion was produced over the “cephalic eminence” and double-hearted omphalocephalics when the lesion extended wider and deeper (8).

Henceforth, the experimenters could produce, almost at will, a specific type of malformation by precisely controlling the point of injury which, after a succession of morphogenetic events, would drive the embryo toward the malformation. Thus Warynski and Fol could explain the embryological processes that led to defects and could also better understand normal chick development. Their methods and modes of inquiry became part of the concepts of developmental mechanics.

Dareste and Warynski and Fol did not hesitate to criticize each other about the worth and interpretation of the results they had obtained (9). Without going into details of this polemic between the two groups, two quotations will suffice to underline the factor that distinguished Dareste from Warynski and Fol. In conclusion of his critique of the Swiss physiologists’ methods, Dareste wrote, “Ever since I began my research on teratogenicity long ago, I aimed at finding procedures to modify the evolution of beings, and to apply the experimental method to the evolution of transformism” (10), and according to him, in its ability to duplicate nature, “indirect experimentation” was the only means possible. Warynski and Fol, conscious of the methodological problems that their work could raise, wrote: “The disturbances of these laws of normal evolution interest us less for themselves than for facilitating the earlier analysis of normal phenomena.” Moreover: “We have completely liberated ourselves from imitating natural disturbances in order to produce perturbations whose causes would be simple and easy to analyse” (11). In these quotations we find two fashions of considering the experimental method, its aims as well as its means.

Furthermore, Dareste’s belief that the direct experimental method held no future prevented him from considering in 1880 the works of the new school of embryology begun in Germany by W. Roux and in France by L. Chabry. At that time, Dareste deliberately ignored all the new work based on direct destruction of embryonic cells.

## 5. Laurent Chabry

In turn, when Laurent Chabry (1885–1895) submitted his thesis “Contribution to the Normal and Teratological Embryology of Simple Ascidia,” he did not refer to the work of Dareste, Warynski, or Fol. Nevertheless, he could not ignore

the experiments (12) of Auguste Lereboullet with pikefish embryos (1864) as well as his studies in comparative embryology (1863–1864). The thinking of Lereboullet is close to that of Dareste: he was convinced that no experimental method would allow the “production at will of determined monster forms,” that the “cause” responsible for defects observed in the embryos was “inherent to the primordial constitution of the egg” and that the type of monstrosity could not in any way “depend on external conditions” (13).

Dareste, Lereboullet, Warynski, and Fol were pioneers of an experimental methodology for the embryo. There were practical differences in the application of their experimental method—“direct” method for one, “indirect” method for others—and in the interpretation of results. But, for Chabry, these differences were not a source of conflict. It was important to understand the mechanisms that permitted an egg cell to develop into a functional organism, and the experimental research of these authors offered the possibility to interpret their results in this way.

Chabry did not start his investigations through embryology, but through the studies of physiology and animal mechanics. He was influenced by the lectures given by Etienne Jules Marey at the Collège de France. The latter had already published *The Movement in the Vital Functions* (1868), *The Animal Machine* (1872), and *Graphic Method in the Experimental Sciences*, when Chabry submitted his thesis in medicine in 1881. This thesis, “Contribution to the Study of the Movement of Ribs and Sternum” was steeped in the methodology developed by Marey. Chabry continued his study of animal mechanics and authored papers (14) on leaping mechanisms (1883), the swimming mechanics of fish (1883), and the length of the legs of jumping animals (1885).

It was the impulse given by the “convinced positivist,” Georges Pouchet, director of the laboratory of marine zoology at Concarneau (15), that propelled Chabry, an associate director of that laboratory, toward cellular mechanics. The originality of Chabry’s 1887 thesis “Normal and Teratological Embryology of Ascidia” (16), lies not only in his scientific method, but also in his ability to manufacture the instruments indispensable for the progress of his experimentation. Again, we may recall how Chabry was influenced by Marey, who wrote: “The experimenter must know at every instant how to modify the instruments which he uses and often how to manufacture them himself” (17). Chabry’s thesis is concerned primarily with the teratology of tunicates produced by experimental techniques, and only secondarily with normal ascidian embryology.

Chabry collected *Ascidia aspersa* from the nearby Bay of Concarneau and artificially induced them to lay eggs. To observe the eggs he invented a new apparatus: the capillary object bearer. This allowed observation of the egg in a glass tube. This tube is linked to a handle which puts the tube in rotation and offers the observer a view of the egg on all sides parallel to the rotation axis of the glass tube.

The second apparatus invented by Chabry was the perforator, a modification of the “capillary object bearer” and “object rotator.” The capillary object bearer was given a fine glass needle, which Chabry manufactured by stretching a glass rod over a flame. Then one of the ends of a fine glass stylet thus obtained was positioned on an incandescent surface (platinum knife of a thermocautery) and briskly withdrawn. By means of this operation, invented by Chabry, one obtained microneedles. He went on to invent the microforge, as well as the micro-

manipulator, two instruments still invaluable to embryologists. The perforator allowed Chabry to destroy one or several blastomeres of a cleaving embryo.

While Chabry pursued his studies of the normal embryology of ascidia, the summer of 1884 found him harvesting egg deposits in which eggs, without any operations, presented abnormal cleavages. Although such eggs had been observed before, nobody had thought of studying them, as they were not considered "of great interest." Chabry's did not agree. As he had only this teratological material before him, he therefore started to study these "not very interesting eggs." In Chabry's words (18), this study was "more fertile in results than I had expected and more so than my investigations of normal cleavage." Here we have an example to show that negative judgments in biology must be taken with caution. With this new research Chabry inaugurated a study of abnormal invertebrate cleavage similar to the study that Rauber (1883) had just completed in his work on "monster segmentation" in a vertebrate, the frog (19).

To define the "monsters" to be studied, Chabry uses the term "hemiterity" in the sense that Isadore G. Saint-Hilaire (20) had used it, i.e., to mean a slight anomaly, not serious, generally compatible with life. Chabry noted seven hemiteric processes in *A. aspersa* (21): (1) deviation from a cleavage plan; (2) delay of cleavage; (3) cleavage limited to the nucleus; (4) absence of cleavage; (5) fusion of cells; (6) abnormal migration of cells; (7) death of part of the cells. Some of these anomalies could coexist in the same individual. Chabry also pointed out that these teratological processes might correspond to normal and regular developmental processes in other species. Therein lay the interest of teratological studies—using the abnormal to understand the normal. That was the path taken by Dreste, in particular, when he discovered the primitive quality of the heart in the chick embryo, as well as that of Fol and Warynski in their study of cephalic development. In any case, the experimental or mechanical embryology of development is a discipline that essentially will produce monsters, not for the purpose of studying the monstrosity itself, but to draw lessons about normal development.

The different teratological processes then became the object of a specific description. Chabry established a difference between the hemiterata, distinguishing between those slight abnormalities that appear spontaneously in nature and those that are produced by the experimenter. This standpoint is important for understanding Chabry's thinking, for if the experimenter can reproduce natural anomalies artificially, then the natural cause that creates the anomaly escapes him: "The natural and artificial monstrosities constitute thus two distinct series with only a certain number of similar or maybe identical terms" (22).

However, certain natural and artificial anomalies have an initial common "anatomical cause," e.g., "the death of part of the cells" of the egg. A blastomere that died naturally or was killed by the experimenter creates a causal chain of events that determines the same anomaly. "We have here identical effects which in no way prove identical cause" (23). The experimenter who turns to normal eggs may study and describe the anatomical concatenations produced by the puncture that killed the blastomere and lead to the final anomaly. But this experimental teratology remains mute about the "prime cause." Chabry wanted it understood that he was not talking about the "great prime cause," (as speculated on by Erasmus Darwin), which would be of theological interest.

In nature, it is not a puncture that kills one (or several) cells of the cleaving egg in the egg deposits of the “monstriparous” females. Rather, this “prime cause” is specific to the germ (embryo): The monster egg in nature contains within itself the monstrosity. Since the egg is a product of parents, the latter are biologically responsible from the start for the “anomaly” of these eggs. For Chabry, the natural monstrosity is original, i.e., depends on the parents. All other anomalies are artificial, from the moment that one can determine the effect of the origin that produced the monstrosity.

Chabry established a distinction between the hereditary monstrosity, originating with the parents, and the monstrosity whose origin does not depend on a physiological condition of the parents. Monsters were produced by two causes: “1) the abnormal condition of the germ; 2) the intervention of external causes during the evolution of a well formed embryo.” For Chabry, the monstrosities, the organic defects, were in the majority of cases the result of the fecundation of an abnormal egg by an abnormal sperm. This concept of monstrosity was important for Chabry because he warned that the investigator must be prudent when he thinks that he has imitated nature in all points of his experimental proceedings. Often, if not always, he imitates nature only in “its most simple behavior.” That is what Chabry had done and he was to stick to this opinion.

Chabry’s modesty about the abnormalities he unleashed with his glass needles remained intact. When he told the scientific community about the results of his experiments, he denounced the exaggeration of certain experimenters who “produced monsters through an intervention on normal eggs after fecundation, and believed that they imitated nature on all points while (like myself) they only imitated its most simple behavior” (24).

Pursuing the teratological tradition established by Etienne and Isidore Geoffroy Saint-Hilaire and by Daresté, Chabry evoked the problems of teratological classification of the different “monster species” which he observed in ascidian larvae, and he concluded that classification of monsters by arbitrary characterizations retained by the teratologists was impossible.

Chabry’s thesis would have remained merely an embryological and teratological monograph of *A. aspersa* if the abnormalities of some of the embryos he studied had not been caused by the natural death of one or several blastomeres. Study of the consequences of natural cellular death (“sphacelus”) on the progress of embryonic development, together with the relative ease with which the experimenter could reproduce these consequences through destruction of one or several cells, gave rise to a rebirth of a theory of preformation (neo-preformation).

Chabry’s thesis became the point of departure for a new theoretical conflict which was to grow with the progress of experimental embryology and with the birth of genetics at the beginning of the twentieth century. The study of monsters derived by “sphaceli” (sloughs, i.e., cellular death) showed that the death of one cell leads irremediably to an organic loss in the future larva. Chabry specified, “From that one draws easily the conclusion (which I believe to be valid only for the ascidia and those animals whose blastomeres differentiate early) that each blastomere contains the potential of certain parts which are irremediably lost by its death, and that the different parts of the animal are preformed in the different parts of the egg” (25).

Natural death at the two-cell stage of either blastomere leads to formation of a “demi-individual.” Death at the four-cell stage of the right anterior cell leads embryogenesis to the formation of a “three-quarter individual,” and so forth. When Chabry destroyed the right posterior cell of a four-cell embryo, the resulting larvae were always deprived of the otolith. Thus, the material necessary for formation of the otolith is contained in this cell. Destruction of the right anterior blastomere at the same stage would cause the failure of pigment cell (“eye”) development. The material necessary for formation of the “eye” is thus contained in this blastomere. Likewise, Chabry specified the origin of the notochord, the atrium, and the organs of fixation. Thus, Chabry concluded that each blastomere is normally characterized not only by size, a proper form, and position, “but it assumes also a succession of fixed forms which constitute a new point in its history” (26). Thus, as in the experiments of Warynski and Fol, the kind of trauma that one inflicts allows one to predict which monstrosity will be produced. Furthermore, the experiments confirmed the observation of an organic predestination specific for one or several blastomeres. However, Chabry insistently cautioned his readers that his experiments, in spite of appearances, do not prove “that the animal is preformed in the egg and that each part of the animal is preformed in a part of the egg” (27).

A fundamental problem at the beginning of the debates on causal embryology was preformation versus epigenesis, or perhaps, one ought to say neo-preformation and neo-epigenesis, as there are several concepts and possible interpretations in these theories. The thesis of Chabry provided arguments in favor of a theory of neo-preformation. Chabry’s experiments showed in effect that the ascidian egg is anisotropic. The egg can thus be compared to a mosaic; i.e., the different organic structures are contained in the egg since fecundation and successive divisions only separate the different elements of the future structure of the individual. The death of one of the early cells of the egg therefore leads to loss of the structure or structures that were contained therein. The egg does not regulate, it does not reconstitute what has been taken from it. Opposed to this idea is the concept of isotropy of the egg. The experiments of H. Driesch (1892) or of O. Hertwig in particular were interpreted to indicate isotropy. The absence of one or several blastomeres of the egg of the sea urchin did not prevent the occurrence of normal embryogenesis: the egg regulates, there is embryogenesis. Given this conflict of evidence concerning egg isotropy, biologists could choose the side they preferred.

Chabry’s experiments appeared to provide evidence for preformation, but Chabry was against generalization of this system by reason of the ideology he defended. Neo-preformation is a conservative system of constraints, since the whole being is predetermined in the egg. Again, we find in the last few years of the nineteenth century the ideological quarrel of the eighteenth century between the partisans of the preexistence of germs and those in favor of epigenesis. The preexistence of germs lent “scientific” support to the heredity of the privileged: the royal embryo could only be king, just as the embryo of a nobleman could only be a nobleman, and it went without saying, since everyone had to remain in his or her social condition, the embryo of a commoner could only be a commoner. To fight the preexistence of germs was to fight for freedom and the equality of men. Thus, it was not unusual that a Saint-Simonian like E. Geoffroy Saint-Hilaire in 1820 defended epigenesis against the preexistence of germs.

At the end of the nineteenth century we find again a debate between partisans of neo-preformation and partisans of neo-epigenesis. This debate was fueled by scientific facts drawn from experimental embryology supporting the discoveries of nascent genetics at the beginning of the twentieth century, and also by an ideological background. It stands to reason that the Neo-Lamarckians, French and Belgian, were more inclined to defend neo-epigenesis, while the preference of the Anglo-Saxon Neo-Darwinians leaned more toward neo-preformation.

Chabry refused to give his experiments all the theoretical importance they deserved, because his political ideology did not agree with the system of constraint and inequality that organic predetermination implied. He would not, therefore, agree with a social and intellectual predetermination containing the theory of neo-preformation. Chabry collaborated in the journal *L'Egalite*, which was the organ of the collectivist movement of Jules Guesde. He took an active part in forming socialism in France and was a supporter of the labor movement. Ideologically he fought against social inequality. Indeed, in biology, Chabry rejected an absolute theory, as we have already indicated, because he knew the limits of experiment in general and that his experiments in particular were only relevant to the egg of *A. aspersa*. Chabry was joined in this concept by Eugene Bataillon when the latter wrote: "It is true that our experimental determinisms are only imperfect notches on a general determinism whose realizations, scattered over a life-time, will always have an invaluable advantage over ours, that of duration" (28).

Six years after publishing his thesis, Chabry died in 1893 at the age of 38, following a kidney infection. His last published works (29) include the invention of a microsyringe, which permits injection of a liquid into a cell (1888), and investigations (which he published with G. Pouchet) on the teratogenic effect of artificial sea water on the development of sea urchin larvae (1889). The latter was not uninteresting, because it showed the important role of the environment on the developing organism: Pouchet and Chabry showed that the sea urchin larvae raised in sea water deprived of significant amounts of lime could not normally constitute their skeleton.

## 6. Edwin Grant Conklin, Albert Dalcq, and Oscar Hertwig

When in 1905 Edwin Grant Conklin repeated and confirmed Chabry's experiments (30), he, too, was not convinced of the reality of a biological determinism. On the contrary, he considered predeterminism a fatalism that did not have a place in science or in society. Against this absolutist theory, Conklin wrote:

Many of those who reflected on these questions apparently felt that there is no just middle between absolute free will and absolute determinism, man is sometimes "free" and sometimes a simple "automaton;" he is absolutely free or absolutely enslaved, completely undetermined or completely determined. But these extreme opinions are not exact, not scientific and untenable, because they are contradicted by the facts of experiments. Experience gives us the assurance that we are neither absolutely free nor absolutely enslaved, but part free and part enslaved; the alternatives are not liberty or determinism, but rather liberty and determinism (31).

In 1932–1938, the Belgian embryologist Albert Dalcq, using Delage's technique of merogony, showed that the ascidian egg could regulate. It is the consti-

tution of the egg cell, which at a given time is found to be regulative and at another time mosaic. Thus, it is a temporal and spatial relationship which determines the isotropy or anisotropy of that egg (32).

Maybe it was his refusal to accept an absolute determinism that led Dalcq to conduct his experiments with the ascidian egg. In spite of his marked inclination for neo-epigenesis, he had to give in to the evidence of a theoretical reconciliation; evoking the relationship between development and heredity and giving his advice on a general concept of biological theory. In summary, this concept boils down to admitting at the start only the preformed elements, as limited as possible, and then partaking in a widespread epigenesis, not only for the cytoplasmic events, but also for the awakening of the activities of the nucleus (33).

It is also interesting to recall that Oscar Hertwig, who was a defender of neo-epigenesis, did not neglect the deterministic aspects of heredity. We owe to him the following metaphor, to be used again, which he gave *a propos* the theory of Naegeli, "I would say that idio blasts are comparable to the letters of the alphabet which, although there are only a few of them, nevertheless, by combining differently, make up different words. In turn, different combinations of words make up sentences with different meanings" (34).

## 7. Conclusions

By inclination, embryologists favored a theory of neo-epigenesis, and geneticists one of neo-preformation. Without wishing to make a rigid and absolute affirmation, we can see that these theoretical tendencies were conditioned by the material studied by the embryologists and the geneticists. From the embryos subjected to experiments a certain plasticity emerges, which speaks in favor of epigenesis, and the experiments of hybridization give evidence of preformation. But beyond experimental data are the people who interpret them and give them the meaning that best corresponds to their ideology.

By his ability to take balanced theoretical stands in spite of the absolute nature of his experimental results, Chabry is indeed at the origin of this new biology which is destined to reconcile the apparently antagonistic theories of epigenesis and preformation.

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## Chapter 3

# The Origins of *Entwicklungsmechanik*

JANE MAIENSCHEN

### 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-

maining 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 idioplasm (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 antimosaic 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 researchers 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 Entwickelungsmechanik 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.

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## Chapter 4

# Curt Herbst's Contributions to the Concept of Embryonic Induction

JANE M. OPPENHEIMER

### 1. Introduction

A Nobel Prize was awarded to Hans Spemann over 50 years ago (1935) for his discovery of the organizer effect in embryonic development. The citation did not mention induction. During the following year, when he published his book summarizing his work and thought, its title in German was *Experimentelle Beiträge zu einer Theorie der Entwicklung* (*Experimental Contributions to a Theory of Development*) (1). It was only in the English translation (1938) that the word induction was introduced into the title: *Embryonic Development and Induction* (2). But it was the concept of induction that was celebrated by the prize.

The most celebrated of the induction experiments were described by Spemann and H. Mangold in 1924 (3). This was the study that defined the so-called organizer effect. When the dorsal lip of one amphibian gastrula, or part of it, was grafted to a region of another amphibian gastrula that would have been expected to form only body skin, a new embryo, or part of one, formed. That embryo presumably would not have formed had the graft not been made. It was important to have shown that the fate of one part of an embryo could be altered by the influence of another; that is where induction enters into the story. What was really dramatic about the experimental result was that the new embryo might be whole; the wholeness has never been adequately investigated, but it was dramatic and attracted attention.

What was investigated—or attempted to be—was the mechanism whereby the formation of the new embryo came about, a mechanism soon to be explained in terms of relations between embryonic parts. Spemann's explanation was made, ultimately, in terms of a process whereby a layer of cells that moved inward at a lip exerted an inductive effect on the cell layer that it came to underlie. More specifically, contact between what was to become chorda-mesoderm and any ectoderm lying above (of the same age) could result in the formation by the latter of central nervous system (spinal cord or brain) and sometimes also sense organs. The interaction between overlying and underlying layers was vigorously ana-

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lyzed in nonchemical experiments, by a whole generation of embryologists during the first third of this century, primarily in Spemann's laboratory in Germany and in Ross Harrison's in the United States. Although the most famous and critical early experiments were published in 1924, Spemann had postulated the explanation as possible as early as 1903 (4), when he described in detail some of his first experiments on amphibian gastrulae.

To oversimplify, the theory was that an underlying layer induced an overlying layer to form something that it would not have formed had there been no contact between the layers. I wish to discuss here the introduction of the concept of induction into embryological explanation. This was not a unique contribution by Spemann.

Let us begin by defining what embryologists mean by induction. We may perhaps do this most profitably by quoting from a chapter written by Holtfreter and Hamburger published in 1955:

Unlike hormones, inductive stimuli operate only at certain stages, as a rule, during early development, and they are normally ineffective unless there is an intimate contact between inducing and reacting tissues. The effects of the inductive tissues are undeniable, since in their absence [in amphibians] none of the ectodermal and probably few of the mesodermal differentiations would ever arise. Once stimulated, the cells proceed along their new course of differentiation independently of a continued application of the inducing stimulus. The newly acquired characteristics are self-maintaining and handed on to subsequent cell generations. In this respect, too, the inductive stimuli differ from hormones which must be applied continuously in order to sustain the differentiations initiated by them.

Thus, normally, "inductors" are living and as a rule embryonic tissues which determine the cytological fate of the reacting, adjacent cells (5).

Spemann's principal and original interest was in the establishment of the vertebrate body axis, and near the turn of the century he began to look into this problem experimentally by constricting amphibian eggs. Egg constriction had been first attempted for amphibians in 1893 by O. Hertwig (6), then without success, but later successfully by Endres (1896) (7) and Herlitzka (1897) (8), who had thereby produced twins. Spemann, too, produced twins, partial or complete, by constricting eggs. These results began to be published in extenso in 1901 (9).

But shortly after beginning the constriction experiments, Spemann also interested himself in the development of the eye, and in 1901 he reported that he had removed the optic rudiment and found that in the absence of the optic cup no lens formed, and he saw a possible causal relationship (10). Already at that time, he postulated that if the formation of the lens was in fact brought about by contact with the optic cup, that would have to be proved by artificially bringing the optic cup into contact with ectoderm that would not normally form lens. Such experiments would later be performed (see Chapter 5 in this volume). But in 1901, Spemann merely postulated the graft experiment, and 1901 is the year we are interested in.

Why 1901? In 1901 Curt Herbst, then a Privatdozent in Heidelberg, published a book entitled *Formative Reize in der tierischen Ontogenese* (*Formative Stimuli in Animal Ontogenesis*) (11). It was intended, as its long subtitle specified, to be *Ein Beitrag zum Verständnis der tierischen Embryonalentwicklung* (*A Contribution to the Understanding of the Embryonic Development of Animals*). As we shall see, it was not the first work to postulate the operation of formative stimuli,

but one reason for our interest in it at this moment is that here Herbst, too, postulated that the formation of the vertebrate lens is brought about as the result of contact between the ectoderm that forms it and the subjacent optic vesicle. He did not do his own experiment; he interpreted the results of one that Mother Nature had performed. There is a developmental anomaly in vertebrates that results in the presence of a single median eye instead of two lateral ones. In a cyclopic individual, as it is called, a single median eye is accompanied by a single median lens, and lateral eyes and lenses are absent. As Spemann was doing in 1901, Herbst, too, postulated a critical experiment: "If we could artificially divide each of the optic vesicles into two or in some other way cause (veranlassen) the formation (Entstehung) of four optic vesicles instead of two, according to my hypothesis, if all four of the vesicles were to touch (anlegen) the ectoderm, the formation of four lenses would result ("ausgelöst werden," in his words) (12). Spemann had not yet seen Herbst's book when he began his own experiments on the eye, as he wrote in a postscript added to the final proofs of the 1901 report (13). When Herbst's book was ready to be sent to the publisher in July 1901, he was able to add to it that he had just learned that Spemann had reported his defect experiments at a meeting in May.

## 2. Curriculum Vitae

Let us now desert Spemann and remain with Herbst, who has not recently received the attention he deserves; in fact, that was already true at the end of his long life. He was born in Saxony in 1866 and died 80 years later in Heidelberg (14) (Fig. 1). He first studied in Geneva with Carl Vogt (1886–1888) and then in Jena, where Ernst Heinrich Haeckel was among his teachers. Herbst was awarded his Ph.D. degree in Jena in 1889 under the sponsorship of Arnold Lang. His dissertation was a morphological study of a Myriapod, *Scutigera coleoptrata*, graded *magna cum laude* (15). He spent the summer of 1890 as an assistant to Lang in Jena and then briefly studied chemistry at the Polytechnical Institute in Zürich. While a student in Jena, in 1889, Herbst had become acquainted with a fellow-student, Hans Driesch. They developed, and maintained, such a close friendship that Richard Goldschmidt later described Herbst as Driesch's *alter ego* (16). They both had independent means; Herbst's father had been a manufacturer, Driesch's a merchant who dealt in silver and gold. Thus they were able to travel together widely before formally entering the academic hierarchy.

In 1889 they spent the Easter holidays near the Mediterranean; during the period from November that year through April the following year they went to Ceylon, Java, and India. Their travels continued through a number of years until ultimately they had included all the countries of Europe, Algeria and Tunis, Palestine, and Syria; they made two visits to Egypt and India. Their journeys together continued until Driesch married in 1899, and even shortly after. After attending an International Zoological Congress in Berlin in August 1901, the two Driesches and Herbst took the long way around to Naples, traveling in Russia and Turkestan for 2 months en route to Italy. This was far from their first visit to Naples. Driesch and Herbst often performed experiments on marine eggs at the Stazione Zoologica there during their *Wanderjahre*. Less frequently, they worked in Trieste or Rovigno. They were fortunate to have been spared military service.



**Figure 1.** Curt Herbst (1866–1946). (Photograph reproduced courtesy of the Universitätsarchiv, Ruprecht-Karls-Universität, Heidelberg, Germany.)

Initially, both were placed in a replacement reserve that did not involve actual training; when this assignment was later reviewed, they were completely freed of all military obligation (17).

Herbst became habilitated in zoology at Heidelberg in 1901, under the tutelage of Otto Bütschli. First a lecturer there (*Privatdozent*), he rose to the rank of Assistant (*ausserordentlich*) Professor in 1906, and in 1919 he succeeded Bütschli as Professor of Zoology. He became emeritus professor in 1935, at the age of 70, and remained in Heidelberg until he died in 1946.

Herbst was elected to membership in the Heidelberg Academy of Sciences in 1919. In 1914 he became a scientific member of the Kaiser Wilhelm Institute for Biology in Berlin-Dahlem, but remained in Heidelberg. He received a stipend from the Kaiser Wilhelm Foundation (4500 marks in 1914–1915, the equivalent of

around \$3712.50 in April 1915) (18) and had a small expense account. He had earlier been Roux's choice for the Foundation's first Director of the Berlin-Dahlem Institute for Biology (19). Boveri opposed the nomination, according to Horder and Weindling (20), because he did not want a third Jew as a Director of a Kaiser Wilhelm Foundation Institute. (Otto Warburg had already been chosen for Biochemistry, and Fritz Haber for Physical Chemistry.) I have been told on good authority, by a knowledgeable German Jew who emigrated here from Silesia, that Herbst is a Jewish name. But even that knowing person is certain that Curt Herbst cannot have been Jewish, since had he been, he would not have been allowed to remain alive in Heidelberg until his natural death. Viktor Hamburger, Jewish himself, who studied with Herbst, has expressed himself as "sure that Herbst was not Jewish, and nothing whatsoever about him, in his face, physical appearance, would indicate it. . . . I am pretty sure that he was an agnostic, like his bosom friend Driesch, and everybody else" (21). Herbst's mother had a rather French name, Henriette Martin; his father's first name was Heinrich, hardly a diagnostically Jewish forename. Perhaps his parents liked Faust.

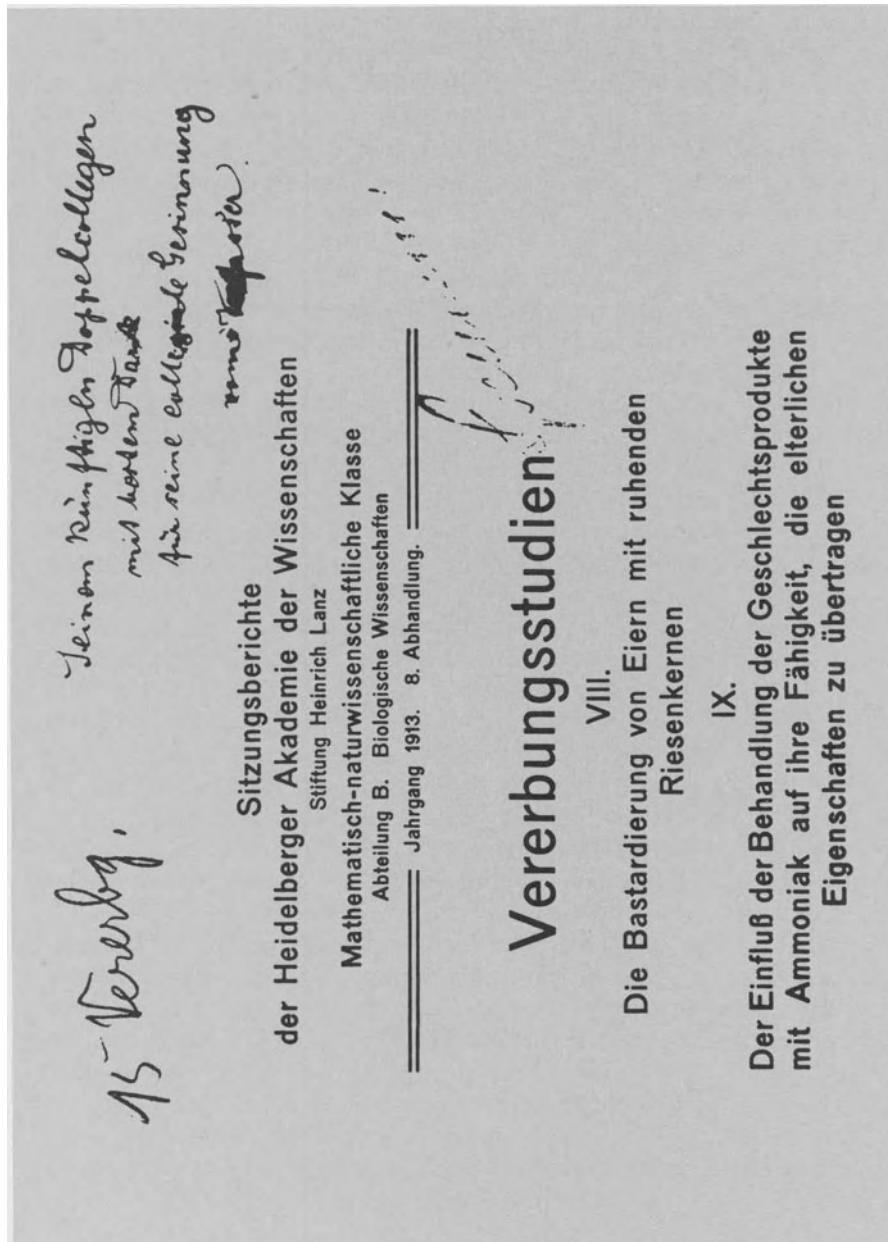
With respect to a possible move to Dahlem in the early days, there is a bare suggestion that it was considered; a friendly inscription on a reprint that Herbst presented to Spemann in 1913 suggested that Herbst might have had hopes that were not fulfilled (Fig. 2).

These hopes may have been raised again later. In 1918, when Spemann was Director of the Kaiser Wilhelm Institute for Biology in Berlin-Dahlem, he wrote to the President of the Foundation, referring to a recent meeting when it had been decided to invite Herbst, described as highly esteemed, to come to Dahlem to work as an associate of Spemann (22). That this did not come about may well have related to the conditions prevailing in Berlin and in the Dahlem laboratories in autumn 1918, when military collapse was imminent, and revolutionary riots frightening. According to Mangold, the Institute became converted to military headquarters and was used as a depository for armaments (23). Even in summer 1919, when Goldschmidt returned there, he found that "soldiers occupied some rooms on the first floor and filled the whole building with noise and evil smells" (24). What embryologist would have chosen to move there during those times?

In any event, in late 1918 Spemann received a call to leave Berlin for Freiburg. When he left the Kaiser Wilhelm Institute in 1919 for Freiburg, Herbst was mentioned as his possible successor, but by then he was considered too old to advance to the short list (25). Herbst resigned his scientific membership in the Kaiser Wilhelm Institute in 1919, when he took over Bütschli's chair. Several years later he was named a corresponding [*auswärtige*] scientific member, an honorary title he held until war's end (the capitulation papers were signed on May 7 and May 8, 1945, in Rheims and Berlin, respectively), almost exactly a year before the end of Herbst's own life on May 9, 1946.

### 3. Personalia

Unlike his friend Driesch, who had a particularly friendly personality (he even enjoyed an amicable personal relationship with Roux, in spite of their frequent strongly polemical disputes resulting from their opposed interpretations of embryological theory), Herbst seemed to a number of his contemporaries



**Figure 2.** Herbst's inscription to Spemann on a reprint published when there was contemplation of Herbst's moving to Berlin-Dahlem. (Reproduced courtesy of Professor Klaus Sander of the Institut für Biologie I (Zoologie), Albert-Ludwigs-Universität, Freiburg im Breisgau, Germany, where Spemann's reprint collection is housed.)

to be distant and conceited. Driesch was his only friend; he never married. According to Hans Querner, who, as an actor himself on the Heidelberg scene, knew colleagues who had clear memories of Herbst, "his personality was undistinguished. In contrast to such authorities as Alfred Kühn, Hans Spemann, Hartmann, etc., he seemed a comic figure" (26). I showed Querner's letter to Viktor Hamburger, who wrote, "I think the characterization of Herbst is correct. The 'comic figure' was due in part to his Saxon accent which was considered as 'funny' by all other Germans and imitated by everyone at parties, etc." (27).

Hamburger, who as one of his students was fairly well acquainted with Herbst, was perhaps slightly less critical of him in his recent book about Spemann than Querner had been, but saw him in much the same light. "As a person he was amiable and easy going but difficult to approach. . . . I never got to know him well. He was aloof and shared his life with only a few trusted friends" (28). An example: Hamburger wrote that when he, Hamburger, was in Heidelberg, Herbst took his dinner every day at the restaurant in the railroad station, "hardly a congenial-gemütliche place" (29).

Richard Goldschmidt, who also knew Herbst personally, said in his *Portraits from Memory* that when Driesch left Heidelberg for Leipzig in 1919, Herbst "changed considerably and grew beyond his former conceit and contempt for everybody but Driesch and his followers. He developed into a very fine person" (30). An image of Herbst as observed from Driesch's point of view might have portrayed him as exceptionally pleasant even at an earlier time. Dr. Gottfried Zirnstein, historian of biology in Leipzig, knows of him through his letters to Driesch on deposit in that University, and in his comments on some of Herbst's tastes has written that he was "interested in art history, . . . visited old churches, cloisters, enthused about beautiful landscapes. He obviously liked small quiet hotels more than large noisy ones. . . . He was moved by music; he wrote on 2.3.1901: 'The lamentations of Orpheus and lost Eurydice touch my heart. . . . I still hear them continually in my ear'" (31). Zirnstein had shortly before, in writing to me of Herbst's interest in operatic, concert, and theatrical productions, specified that he had particularly liked the operas of Wagner, including the Ring (32). The Ring would not have been to the taste of most conventional German university professors in Herbst's day.

The letter from Querner already referred to pointed out that Herbst was anti-Nazi. Goldschmidt too had something to say about that. "When the Nazis came to power," Goldschmidt wrote, "he showed the stuff he was made of. He never ceased denouncing them and made a point of visiting his Jewish friends when everybody could see it and of helping them to the last. Up to the last week of his life he wrote me letters which, had they been opened by the Gestapo, would have sealed his fate" (33).

The fact that Herbst was twice considered as a possible candidate for a Directorship of a Kaiser Wilhelm Institute, first by Roux in 1912 and later by Spemann in 1919, is an objective measure of the esteem in which he was held by his professional colleagues. His students, too, greatly admired him for his knowledge and wisdom. Hamburger said in his recent book about Spemann that it was as a result of attending one of Herbst's graduate seminars in experimental embryology that he, Viktor Hamburger, had decided to specialize in this field (34). Another of Herbst's students, Walter Landauer, who emigrated to America in

1924, was to carry out a program that was to exert considerable influence on the new biology. His Heidelberg dissertation had dealt with the effect of lithium ions on echinoderm hybridization; in America he became an innovator in studying teratology, mainly that of birds, in terms of biochemistry and genetics (35). He was one of the first investigators to apply such new interpretations systematically to vertebrate embryology.

## 4. Opera Magna

### 4.1. The Earliest Experiments

We first mentioned Herbst's ideas about induction in connection with their overlap, in theory, with Spemann's thoughts on inductive relations between optic cup and lens. The 1901 book, in which Herbst's ideas on the lens were expressed, did not represent his first publication. His earliest substantial contribution to the biological literature appeared in 1892 in the *Zeitschrift für wissenschaftliche Zoologie*; a sequel to it, a companion article, followed in 1893 in the *Mitteilungen der zoologische Station zu Neapel* (36). These two articles dealt with the effect of changes in the ionic composition of the surrounding medium on the development of echinoderm larvae, the 1893 article presenting further details regarding experimental protocol and otherwise amplifying the report published in 1892. In 1891, in Trieste, where von Baer before him had tried to study the development of echinoderms (unsuccessfully, since the maid threw away some of the eggs that von Baer kept in the hotel room where he lived and worked) (37), Herbst began his own experiments on sea urchin eggs by raising them in calcium-free sea water; the arms of the plutei were absent if their calcareous skeletal rods were defective or absent. Not only the place where these experiments were done, but also their time, is of interest as we look back; this was the same year that Driesch separated blastomeres to find that each could form a whole embryo.

Herbst's experiments repeated one that had already been performed by Pouchet and Chabry and reported in 1889 (see Fischer, Chapter 2 in this volume). Herbst's interest in such experiments had been aroused by Sachs's view that the morphogenesis of an organism is affected by its chemical constitution (38). But later in his analysis of Driesch's life and work, Herbst stated explicitly that he, Herbst, had begun his "experimental investigations on the influence of altered chemical constitution of the surrounding medium on the development of animals stimulated by three short communications by G. Pouchet and Chabry that dealt with the influence on sea urchin development of a greater or lesser degree of precipitation of the calcium from the sea water by potassium or sodium oxalate" (39), and he implies this also in this 1901 book.

Pouchet (40) and Chabry also began their experiments in order to test the idea that "morphological characters of living beings are a function . . . of their chemical constitution," choosing to study the effect of calcium deficiency on the calcareous skeletons of sea urchin larvae; they were the first to report that when skeletal formation was defective, retarded, or absent, the arms of the plutei were absent. They concluded that "the formation of the arms is subordinate to that of the spicules, because an arm is not found that is not supported by a calcareous

skeleton; and things come about as if the skeletal point pushed before it the ectoderm that covers it like the finger of a glove. . . ." Herbst confirmed these results and found similar conditions in larvae raised in sea water to which lithium had been added, but he felt that his own interpretation went beyond that of Pouchet and Chabry, in that he thought of the effect of the skeletal spicules not in a mechanical analogy, but in terms of morphogenetic stimuli and *Auslösung*.

I emphasized, [he wrote in 1901] what escaped Pouchet and Chabry, the character of *Auslösung*, . . . and I came to the conclusion that the pluteus larvae with skeletons rudimentary or completely lacking had formed no arms because the intensive growth of the part of the ciliary ring concerned did not take place on account of the cessation of the stimulus which the growing skeletal rods would otherwise have exerted upon them.

The reason that Pouchet and Chabry studied the effect of sea water with reduced calcium content on sea urchin development is obvious: the larval skeleton is calcareous. Herbst himself, in his 1892 communication, reported the addition of calcium salts ( $\text{CaCl}_2$ ) to the sea water in which he raised sea urchin embryos, rather than the removal of calcium from it; he also added a number of other salts, among them  $\text{LiCl}$ ,  $\text{LiBr}$ ,  $\text{LiNO}_3$ ,  $\text{LiI}$ ,  $\text{NaBr}$ ,  $\text{NaI}$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{NaNO}_3$ ,  $\text{KCl}$ ,  $\text{KBr}$ ,  $\text{KI}$ ,  $\text{KNO}_3$ ,  $\text{K}_2\text{SO}_4$ ,  $\text{RbCl}$ ,  $\text{CsCl}$ , and  $\text{MgSO}_4$ . Ions of sodium, potassium, calcium, and magnesium are normally present in sea water, as Herbst knew; lithium, cesium, and rubidium are all elements found in the same column of the periodic table as sodium and potassium. It was lithium that provided Herbst with the most interesting results, and thus that was the ion on which he concentrated his further efforts.

We shall in due time return to discuss other formative influences on the thought of Herbst, but meantime it is interesting to explore why Pouchet and Chabry should have altered sea water. In their paper in the *Comptes rendus* of the Academy of Sciences, describing their results, they stated specifically that: "The following experiments . . . were initiated in departure from the principle expressed by Chevreul (1824), and energetically defended by Ch. Robin, that morphological characters of living beings are a function . . . of their chemical constitution"; and in the next sentence they referred also to Gautier as having supported the same principle. Herbst complained in the introduction to his 1892 paper that Pouchet and Chabry failed to give specific references to specific works of these authors.

Chevreul, Robin, and Gautier were all scientists of great prominence; Chevreul, in particular, was one of the dominant and seminal thinkers of his day. He wrote many works of broad philosophical significance on general and special interrelationships between what we now call organic and inorganic chemistry. As early as 1813 he began to study the composition of natural fats, showing saponification to be decomposition of a fatty acid to an inorganic salt by a base which takes the place of anhydrous glycerin. These results were published in 1823; thus, five years before Wöhler's synthesis of urea, Chevreul demonstrated the strong possibility that organic and inorganic compounds obey the same laws. Gautier was also interested in organic chemistry as applied to vital phenomena; he worked on alkaloids. Robin was a highly distinguished professor of histology in the Paris Faculty of Medicine and a member of the Institute. It is puzzling that Herbst, who knew French as a cosmopolite (and besides, he had studied for

a year in Geneva), should not have known of the attitudes of these French thinkers, or if he did, that he should have wished specific references to them; this accentuates the width of the gap between French and German investigators of related subjects in the late 1800s.

However, had Herbst wished references to the work of Chevreul, Robin, or Gautier, he need only have browsed briefly in the *Journal de l'anatomie et de la physiologie*, in which the definitive paper by Pouchet and Chabry had been published, a journal called popularly in Herbst's time the *Journal de Robin et Pouchet*. The Journal was far from obscure; it published papers by Haeckel, His, Broca, Claude Bernard, Bert, Helmholtz, Balbiani, Ranzier, Marey, and Richard Owen, among others. The volume for 1874 contained a review of a book by Gautier entitled *Chimie appliquée à l'hygiène, à la physiologie, à la pathologie*. Robin died in 1885; the volume for 1886 contained his obituary, over 180 pages long, by none other than Pouchet himself. The obituary referred not only to relevant publications by Robin himself, several published in the same Journal, but also to some by Chevreul and Gautier, including the Chevreul 1824 publication presumably referred to by Pouchet and Chabry in their introduction to the sea urchin paper. One of the works of Chevreul that Pouchet referred to in the obituary was highly explicit as to the relationships between chemical constitution and vital phenomena:

There are two very different ways of studying and explaining the phenomena of life; in one, they are believed to depend, directly and indirectly, on a particular force, called the *vital principle*, which is often represented as being antagonistic to the forces ruling raw matter; . . . in the other, without prejudging the nature of the causes that produce the phenomena, one attempts, after having defined the phenomena as well as possible, to relate them to their immediate or proximate causes, and far from admitting *a priori* that they are the direct effects of a vital principle, one tends, instead, to refer them to the forces which rule raw matter.

Chevreul then himself refers directly to his 1824 book. Another article published by Chevreul the same year as that containing the quotation just cited spoke for the value of comparing the chemical constitution of egg and bird and should have been of particular interest to embryologists.

And if many of the works of Chevreul, Robin, and Gautier referred to in the *Journal* expressed general attitudes of great significance concerning chemical constitution of organisms, one paper by Chevreul, the very first paper published in the very first volume of the *Journal*, even suggested changing artificially the composition of waters—for medicinal rather than teratological purposes, to be sure, but a plan for studying the effects of chemically altered water was there.

It is an interesting aside that Chevreul's remarks on medicinal waters were appended to a series of articles on the chemistry of dyes; he had been for a time, before taking up an influential position in the *Muséum d'Histoire Naturelle*, director of the dye works at the Gobelins establishment; he worked on color contrast, and he published a book on it that is said to have influenced the development of impressionism. As a matter of fact, the passage we have quoted above as somewhat critical of the notion of vital forces was also appended to a memoir on dyes, which concerned itself with Prussian blue, among other substances. Gautier worked on artificial coloring of wines; and even Chabry himself wrote a paper on the chemistry of Prussian blue, without this time referring to

Chevreul. Thus rather technological, and even aesthetic, studies of color in France may have had their influences, at least indirectly, on the development of German experimental embryology.

Herbst's repetition of the Pouchet and Chabry experiments, and his reinterpretation of their results, had both theoretical and practical consequences, some of lingering and some of more immediate value. His 1892 article reported, as we said, that he had diminished the calcium ion concentration; later, in 1900, when attempting to ascertain at what stage its effect became operative, he found that when cleaving echinoderm eggs were raised in calcium-free sea water, the cleavage cells separated completely and cleanly from one another (41). Artificial separation of blastomeres was an important embryological practice at the time. It was then customarily achieved by shaking or other drastic mechanical means, and sources of experimental error were introduced, some of which might prevent valid interpretation of results. Thus laboratory procedures were considerably facilitated and improved by the introduction of Herbst's new method of making it possible for blastomeres to separate themselves more gently than embryologists had previously been able to do it for them.

When Herbst began his work, he first focused on the calcium ion, as did Pouchet and Chabry before him, partly because the skeleton of the echinoderm is calcareous in constitution, as already pointed out, but also partly because the chemists had found a way to precipitate it out of solution as an oxalate. His reasons for increasing or decreasing the concentration of other ions were also explained earlier, and it was there pointed out that it was the addition of lithium that seemed to produce the most interesting results. Among these some had considerable theoretical interest and influence unrelated to the formation of arms by plutei.

Some specimens that had been raised in sea water to which lithium salts had been added failed to form skeletal rods, as had those raised in calcium-free sea water, and these too lacked arms in the pluteus stage. But the addition of lithium ions also sometimes produced an even more striking effect. In some gastrulae that had been raised in sea water to which lithium ions had been added, no internal gut formed. The portion of the blastula wall that would have been expected to fold in (to line the ectoderm and form the digestive tube) turned outward instead of inward, forming an empty endodermal bag. These specimens had "exogastrulated," and they were called "exogastrulae" (42). What was most important here was that in many of the exogastrulae, the amount of endoderm was considerably increased at the expense of the ectoderm. This was one of the first and strongest blows at the doctrine of the fixed specificity of the germ layers, at that time maintained so dogmatically that some of its strongest proponents, such as Haeckel, ridiculed all attempts to analyze development experimentally, or to describe it in terms of any causality other than evolutionary. Herbst's experiments and observations were among those that led to others that reinforced the value of experimentation.

Herbst's studies, published in 1892 and 1893, on the effects of altered constitution of the ambient media on embryonic development were thus among the most important early contributions toward the initiation of chemical embryology; according to Viktor Hamburger (43), he "deserves credit as the first practitioner of chemical experimental embryology."

## 4.2. Related Theoretical Writings

Herbst soon also began to publish writings that were more theoretical than their predecessors. Two of them, entitled “On the significance of stimulus physiology for causal interpretation of processes in animal development” [Ueber die Bedeutung der Reizphysiologie für die kausale Auffassung von Vorgängen in der tierischen Ontogenese] appeared in the *Biologisches Centralblatt* in 1894 and 1895 (44). In the 1894 publication, Herbst considered directional stimuli [Richtungsreize]; in that of 1895, he concentrated on what he called formative or morphogenetic stimuli. These articles consisted principally of surveys of the literature, but Herbst also derived some theoretical generalizations from the data he collected and presented, especially in the latter portion of the 1895 article. In spite of the emphasis on animals in the title, most of the literature reviewed concerned plant material; botany, at the time, was the dominant biological discipline. The facts available were abundant; irritability and the not unrelated stimulus-physiology were also among the most popular subjects of the investigations of the day.

Herbst stated explicitly that he had been led into these theoretical considerations by the results of his studies using altered sea water. “An observation,” he wrote in 1894, “that I made during my investigations of the influence of altered chemical constitution of the ambient medium on the development of animals brought to me to the conjecture that directional stimuli most probably play a significant role in the achievement of ontogenetic processes” (45).

In 1894 and 1895, articles in the *Biologisches Centralblatt*, each subdivided into several separate sections in the volumes of the journal, covered in the aggregate nearly 140 pages. No attempt can be made here to summarize their specific content; that would be a task comparable to trying to recapitulate briefly all the factual (or then seemingly factual) data presented in a few chapters of *The Origin of Species*. One generalization about them can be made: the directional stimuli whose effects were enumerated in the 1894 article originated largely outside the organism (light, gravitational, mechanical, magnetic, or chemical factors, etc.).

If, as we have already pointed out, Sachs's views on the chemical constitution of organisms as related to their form contributed to turning Herbst's interests toward the experiments we have described, so also did Sachs's and Loeb's studies on tropisms influence his interpretations. Thus it is hardly surprising that Herbst should have chosen to begin his pair of theoretical articles by concentrating on directional stimuli comparable to those previously demonstrated by experiments on tropisms in plants. And tropism theory had also been applied to analysis of plant growth as well as movement. The extension of theory from plants to animals was given impetus by studies that Loeb began in 1887 (46). In 1894, without reference to Loeb, Herbst spoke of the “oxygenotropism” of migratory mesenchyme cells in echinoderm larvae (47). His devotion to the philosophically minded Driesch did not deter him from concentrating on physical rather than metaphysical attempts to explain developmental phenomena.

Let us here indulge in a brief digression that relates to both induction and directional stimuli. “A specific path of differentiation can be initiated in an embryonic part by a stimulus from an adjacent region,” said Viktor Hamburger in

his recent book: "The term 'induction' had been used by Driesch and Herbst," he said, "to denote such an interaction, but the concept was rather vague and rigorous experimental evidence was lacking" (48). How rigorous? I remember Harrison once saying that Driesch had provided the first experimental evidence of induction in an article published in 1896, without specifying how rigorous it was. In 1941, when Herbst discussed the same 1896 experiment in his obituary of Driesch, he did not relate it specifically to induction theory, but he called it "a simple but striking experiment" [einfachen wie schlagenden Versuch] (49). Herbst and Harrison were referring to an experiment described in Driesch's article entitled "Die taktische Reizbarkeit der Mesenchymzellen von Echinus microtuberculatus" [Tactile stimulability of mesenchyme cells in the sea urchin *E. microtuberculatus*].

The experiment was as follows. Driesch shook for a half-minute sea urchin blastulae containing primary mesenchyme cells that were not yet in their ultimate locations [geordnet]; he then isolated specimens in which they had been appreciably displaced by the shaking and made drawings of them after successive intervals of time. He found that in a number of cases the cells, after having been displaced, ultimately moved to the positions to which they normally would have migrated to form the spicules of the larval skeleton. Driesch explained the results in terms of response to tactile stimuli emanating from the ectoderm, but of course the supposed contact implied by his use of the word "tactile" was the result rather than the initiator of the response (50).

Actually, when Driesch described these experiments, he began his article by quoting a passage in which Herbst himself had discussed the apparently directed movements of the primary mesenchyme cells, referring to their formation of the skeleton under conditions in which "a specific cell group grows toward a very specific location, as though driven by an unseen force" (here Herbst's words were *richtende Kraft* rather than *richtende Reiz*) (51).

To return to Herbst's articles in the *Biologisches Centralblatt*, the one published in 1895 concentrated, as we have said, on formative or morphogenetic stimuli, including those originating within the organism itself. Herbst had been thinking about such internal stimuli at least since the beginning of his work on sea urchin development.

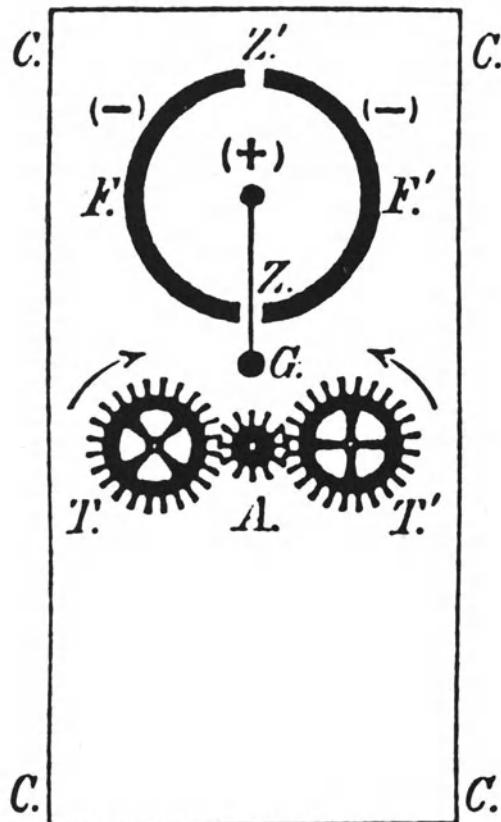
As already pointed out, in 1892 Herbst emphasized that he interpreted the results of the experiments using calcium-free sea water differently from the manner in which Pouchet and Chabry had done so. Pouchet and Chabry had made a mechanical analogy. In contrast, Herbst interpreted the result as indicative of a growth phenomenon: no stimulus, no growth. That was, in fact, his first example of the effect of one internal part on another. An external condition, the absence of calcium ions, prevented skeletal formation, but it was the absence of skeleton, an internal condition, that affected growth; the chemical factor acted only indirectly on growth through the mediation of an internal condition, a fact that Herbst did not choose to emphasize at the time. In 1893 he pointed to another example of the action of one internal part on another, in the passage that we have already quoted, on the directing effect of the ectoderm on the migration of mesenchyme cells, which moved "as though driven by an unseen force." On the same page he also added as an example of a related phenomenon the growth of a nerve to the "right" [richtige] muscle. Again he did not here discuss in detail the

fact that the interactions he named were between internal parts. That remained for 1895. It is in a way somewhat puzzling that he should have waited until the fourth time that he wrote on *Auslösung* to enter into detail on the manner in which inner factors evoked growth responses, since this is what he had been thinking about all along. But it was only toward the end of the 1895 paper that he came to the point. In the fourth of its six sections he at last addressed his interest in “[i]nner formative (morphogenic) stimuli (qualitative correlation or induction of specific configuration through inner factors)” (52). Note Herbst’s use of the word “correlation;” that was a usage then fashionable that has not survived. Herbst thought it included all cases where “an organ directly influences another in its morphological character . . . by contact or by means of a specific substance, or in some other material manner. . . . Correlation and induction of specific configurations are identical concepts” (53).

By using the word “material” he may have implied it to include the mechanical in a narrow sense. More than once in the 1895 article he spoke of a stimulus acting as though it were “opening a valve” (54). “Let us think of a steam engine connected to two different mechanisms in such a way that by the turning of one or another valve either one or the other mechanism is set into action, thus we have in principle the best representation of the essence of [a particular] group of morphogenic stimuli” (55). On the next page, and elsewhere, he wrote of the “switching character” of still unknown factors, of “switching stimuli,” and of the interpolation [*Einschaltung*] of something. “For instance, should it prove to be the case that sex often, or even usually, is determined by internal formative stimuli, in this event it would be a matter of internal switching stimuli” (56). Here he was using a hard analogy; in the next paragraph on the same page he used an example of chemical reactions in a retort.

The machines had been in his mind for at least a year. In 1894 (57) he had referred to the work of Noll, a botanist, who in 1892, in a book entitled *Ueber heterogene Induktion* (58), had gone so far as to design a machine that could react in various ways (positively, negatively, crosswise) to gravitational stimuli (Fig. 3). Herbst liked but did not completely adopt Noll’s mechanical analogy: “Of course it would not occur to any one to think that positively or negatively geotropic organs in particular must be constructed exactly as in Noll’s machine, for then he would be in the position . . . of the peasant who when he first sees a locomotive would wager that there is a horse inside of it” (59).

To return from material to mental machinery, Herbst took great pains to emphasize that what formative stimuli evoked were “qualitatively new formative processes” (60). If his stress on the induction of modalities that differ qualitatively shows how deep were his insights into inductive phenomena, so also does his appreciation of the fact that specificity may be accounted for by the reacting entity. “I understand . . . by the specificity of reaction to a stimulus that it is determined by the inner disposition which the reacting body exhibits at the time of the stimulation” (61). But he understood, too, the subtleties that might characterize the processes: “One and the same indifferent organ rudiment can be incited by the same organ-forming factor—whether it be internal or external—to form different types of organs under differing conditions” (62). And he envisioned the possibility that at least in some situations stimuli might set into action developmental mechanisms that are at rest but that have been in readiness to



**Figure 3.** Noll's induction machine, designed to illustrate geotropism. The diagram is found on page 20 of his book (reference in my note 58). In the book, the illustration is not accompanied by a caption, but is explained and discussed in a five-page section of the text. C, cylindrical housing; A, horizontal axis, in center of a cogwheel that meshes with cogwheels T and T'; F and F', somewhat less than semicircular metal strips that represent stimulus fields, each free at one end (positive pole) in interspace Z; at their negative poles they can each connect with an electric motor, F in connection with T, F' with T'; G, a metal rod that is freely mobile. When the long axis of the machine is perpendicular, the positive pole of rod G is suspended in interspace Z, the circuit is not closed, and the machine is at rest. If the position of the machine is changed, for instance if its left side is turned downward, rod G turns, and its contact with wheel T results in activation of the electric motor. The activity of the machine continues until the apparatus has returned to its resting position. It is my suggestion (not Noll's) that the reader may more readily understand the action by rotating the page 90° to the left.

function: this is where the valve opens. Thus there was room in his scheme for pre-formation as well as epigenesis. He was still writing about the valve in 1901 (63).

As we provide evidence of the delicacy of Herbst's understanding of the intricacies of the induction process, we must also admit how far he was from viewing them as his successors were to do. When Hamburger and Holtfreter defined induction in 1955, they emphasized their belief that "inductors" are living and as a rule embryonic tissues which determine the cytological fate of

the reacting, adjacent cells" (64). Herbst did not interpret induction in terms of cells. When speaking, in his 1901 book, of the effect of the skeletal rods of the plutei, he explicitly repudiated cellular differentiation as significant in producing it. "What justifies us," he inquired, "in considering this stimulus as formative? It certainly does not release a new tissue differentiation that consists of a particular form and shape of particular cells. What it does is simply foment growth in a particular place in the ring of cilia" (65). "The reference of developmental processes to the cell," wrote Harrison in 1937, "was the most important step ever taken in embryology" (66). Herbst stopped short of taking that step. By the way, I have commented elsewhere (67) that even Spemann only sparingly expressed his concepts in terms of cells, as opposed to cell territories.

During the summer of 1895, the year that saw the appearance of the second theoretical paper, Herbst began the preparation of his final publication dealing expressly with formative stimuli; in fact, he wrote a considerable portion of it during that summer. This was to become the book about which we started our discussion, the work in which Herbst's thoughts about lens induction converged with those of Spemann. In this little book, his theoretical *Programmschrift* as he called it [Hamburger translates this as manifesto (68)], he was still drawing support for his postulates from the literature, this time concentrating mainly on evidence from the animal world. The 1892 article in the *Zeitschrift für wissenschaftliche Zoologie* had covered about 140 pages, the Naples publication about 84, and the two communications in the *Biologisches Centralblatt* together about 140, a total of approximately 364 pages. The 1896 publication referred to in note 36 as describing further variations on the experiments included 61 pages; thus these early experimental papers contained in the aggregate 425 pages. The 1901 book contained only 125 pages, but they were alone between their own hard covers. Herbst said in its introduction that it was being published separately because it would have been too long and cumbersome for the *Biologisches Centralblatt*.

In content the book differs from the earlier periodical articles primarily in that in it the author cast his nets far more widely to obtain his evidence. We mentioned earlier that as early as 1893 he had exemplified relationships between parts by the growth of a nerve to the "right" muscle. In the first section of the small book, which concentrates on the effects of external stimuli, he selected a wide variety of such examples: determination of sex by external factors [already mentioned tentatively in 1895 (69), remember]; causes of the development of different castes in colonial insects; the effect of different temperatures on color and pattern in butterflies; the influence of nutrition on hypotrich infusorians; the influence of gravity on cleavage and organ formation in frogs. Of these, only sex determination and the early experiments on frogs had been mentioned in his previous writings. He concluded the book's section on external stimuli by emphasizing that these can result in quite complicated effects, pointing out, for instance, that some formative processes may be inhibited or suppressed, while others can be led to increased activity or deviated to other paths.

The second portion of the book, concentrating on internal formative stimuli, begins with a brief summary of the old experiments on the effect of altered sea water on the development of plutei and then introduces new subjects into the discussion. Among these are the formative influence of the nervous system on

the regeneration of crustacean appendages, a subject that, as we shall see shortly, he had begun to explore experimentally in 1896, and the dependence of vertebrate muscle formation on innervation by spinal nerves. Next comes the section on the vertebrate lens to which we have already alluded. Herbst then took up the influence of the gonads on primary and secondary sexual characteristics, on the external genitalia, and on the reproductive ducts. There are no question marks in the various subheadings relating to the gonads, but when he framed his next topic, it was in the form of a question: is the effect of the thyroid gland to be considered as formative stimulation? He answered it in the negative. He next described briefly the formation of sensory epithelial cells and of tactile corpuscles under the influence of sensory nerve endings, and he closed the section by postulating that the mammalian decidua forms as a response to the presence of the egg membranes. In discussing this particular example of the participation of formative stimuli in morphogenesis, he made an analogy to gall formation. Gall formation had been a popular subject of investigation in the 1880s, following particularly upon papers published by Adler (1881) and Beijerinck (1883) (70) and had already been discussed by Herbst, as an example of the effect of chemical constitution on morphogenesis, as early as 1892 (71). He was still returning to it in 1895 (72), so that he was not being in any way innovative in commenting on it again in 1901.

A brief conclusion, just 10 pages long, added little in the way of new generalization. Herbst emphasized that he could not provide specific information regarding the character of the formative influence. He recognized that a question arises with respect to the role of heredity in the causation of developmental phenomena, but he neither expressed it in exact terms nor chose to attempt an answer to it even in vague terms. At the very end, he tries to accommodate his ideas to those of Roux and Driesch; considerable polemic interplay between their ideas had characterized the literature. And at last, he abandoned his views favoring mechanical (*maschinellen*) over vitalistic interpretation of induction. We have seen how on occasion he seemed to be choosing the former alternative. Yet when he wrote the last six sentences of the book, he lost his nerve. He began the passage by repeating his example of the role of the vertebrate eye cup in inducing the lens. He then pointed out that Gustav Wolff had shown that regenerating salamander lens forms in an entirely different way and confessed that he envisioned the possibility that various different particular releasing factors may be subsidiary to some other hidden factors, still unknown, to which they are all subject. What kind of factor might such a ruling one be? He declined to attempt to reply to this question, and amazingly, after all that has gone before, referred the readers for an answer to what he calls Driesch's "vitalistic" writings (73).

## 5. Obiter Dicta

We have so far chosen for comment selected publications by Herbst that we have taken up more or less chronologically. But his series of writings (in fact much longer than we have indicated even in note 36) was from time to time interrupted by other writings that seemed to deal with very diverse subjects, but

that proved ultimately to be derivative from, and supportive of, his interest in the concept of inducing factors. Had he performed only the experiments and attempted only the interpretations that we have already enumerated, his work would no doubt have earned a chapter in this book. But most of the work about to be reported now would probably not have resulted in our remembering him. It was all related fairly intimately to his earlier work, but because of other developments within the field of biology, it proved to be less influential at the time.

Between 1895 and 1916, he produced a long series of articles on heteromorphosis in crustacean regeneration (74). "Heteromorphosis" was the word that Jacques Loeb had introduced to describe the condition in regeneration wherein when one type of appendage is amputated, another kind regenerates in its place (75). Bateson reported in 1894 that the condition arose spontaneously in *Palinurus penicillatus*; he called it homoeosis (76). Herbst mentioned rather routinely, without special emphasis, in the 1895 article in the *Biologisches Centralblatt*, that sometimes in plants one organ forms in place of another under atypical conditions (77). Herbst's experiment was to remove a crayfish's eye. He did this not to ascertain what would regenerate, but rather to test the influence of external conditions on the ability of the eye to regenerate at all. He was looking for additional evidence for the action of external morphogenic releasing factors. Would a crayfish regenerate its eye? And if so, would it do so in darkness where the eye would not be usable and where therefore the regeneration would be functionally superfluous? Heteromorphic structures, later recognized as antennulae, formed whether the eye was removed in the light or in the dark. Herbst concluded at the time that light had no influence on regeneration, but that some internal factors, possibly chemical in nature, were at play (78).

However, he soon found new and different formative factors to be involved. When he had first removed the eye, in the experiments described in 1895, he had been careful to remove along with it the optic stalk and the optic ganglion. In 1889 he showed that if he did not remove the optic ganglion, but left it intact, a new eye formed (79). By 1901 he was saying the origin of a new eye in the place of an amputated one is to be considered the effect of a formative stimulus exerted on the wound surface by the central organ of light perception (80). He had started this series of experiments in order to test a possible action of external formative stimuli; his results had by a circuitous route led him back to internal ones. These questions of heteromorphosis—which we call homeosis—have become central to contemporary developmental genetics.

Heteromorphosis was not the only new subject begun to be later explored by Herbst as a result of his early interest in formative stimulation. Sex determination was another. We remember that in 1895 he commented that sex might be switched in direction by some mechanism involving chemical releasing factors. Beginning in 1928 and continuing to 1940, Herbst produced a long series of articles on sex determination in *Bonellia*, which will not be discussed in detail here. *Bonellia viridis* is a Gephyrean worm whose larvae become dwarf males if, when still sexually indifferent, they settle down on the proboscis of an adult female. This had been known since 1879 (81). In 1928 Baltzer expressed the belief that the male-determining factor was chemical in constitution (82). Herbst showed that such simple chemical substances as carbonic acid or dilute nitric acid might act as such effective male-determining agents (83). To readers during this last decade

of the twentieth century, accustomed to thinking of intercellular communication in terms of protein receptors and chains of messengers, analyzed in molecular detail, the action of such simple agents may not sound exactly like induction, but to Spemann, in his day, when he was beginning to think of the means of induction, it did: "The analysis of a chemical inductive stimulus," he wrote in his Silliman Lecture on the means of induction, "had been attacked systematically and successfully . . . on the larvae of *Bonellia viridis*" (84), referring by name to both Baltzer and Herbst.

Herbst's final publication appeared in 1943 (85). It was entitled "The significance of experiments using salts for the question of the mode of action of genes," but the article was confused, even semimystical. Its principal outcome was demonstration of the sad fact that Herbst's arteries had hardened and that his fine mind had softened.

## 6. Ab Origine

Herbst's contribution to induction theory was fleshing it out into an elaborately conceived hypothesis. He was, of course, not its only begetter. Wilhelm Pfeffer is usually given credit for having introduced the word and concept of induction into biology in his 1881 *Pflanzenphysiologie, Ein Handbuch des Stoffwechsels und Kraftwechsels in der Pflanze* (86). Spemann's book lists Pfeffer's *Pflanzenphysiologie* among the references, but does not refer to it in the text. When Roux's dictionary of embryological terms appeared in 1912 (87), it attributed the source of the term to Pfeffer and also gave the date as 1881; it defined the term as the effect of one structure on another. When Pfeffer wrote in his 1881 book of the induction of specific configuration in discussing the effects of light or gravity on plant growth, he was speaking of external, not internal, inducing agents: he also used the word *Auslösung* in the 1881 book. In fact, Roux was not wholly accurate in giving the date as 1881. Pfeffer had used both words 10 years before, in 1871, in connection with the effects of the external environment, in an article on specific causes of plant growth. In describing the effects of moisture, temperature, light, and gravity on the production of root hairs in *Hepatica* or liverwort (*Marchantia*), the bilaterality of the brood bulbs is not induced [*induziert*], he said, but inherent, like that of the lateral shoots. A few pages later he changes his terminology. "A certain amount of illumination is necessary to evoke [*hervorzurufen*] a considerable growth of root hairs" (88).

Thus the contemporary preoccupation of botanists with stimulus physiology, and with tropisms, and the directive effects of environmental agents on plant movement and growth were among numerous possible points of departure for Herbst's thought. But they were not the only ones. He wrote in 1894 that, so far as he knew, the first investigator who suspected that there might be a directive influence of external factors on morphogenetic processes in the embryo was Wilhelm His (89). In 1878, His explained the spreading of the teleost blastoderm over the yolk by postulating that it could be understood if one attributed to its cells an endeavor to increase the surface of the cellular blastoderm as much as possible. Where the layer is thick, he surmised, the deeper cells work their way to the surface, resulting in the spreading away of the others. The greater the surface

area, the greater the access to oxygen (90). Was this one source for Herbst's emphasis on what he called oxygenotropism?

Herbst first used the word *Auslösung* in 1893. Weismann used it in the Romanes lecture in 1894, which discussed "External influences as developmental stimuli" (91), but he was then thinking of development in the evolutionary sense. He spoke of the environment as the releasing stimulus for change.

Herbst was also aware of the other examples that preceded his own that demonstrated that salt solutions could exert an effect on developing form in animals. In the introduction to his 1892 paper, he mentioned specifically "the well-known investigations" (92) by Schmankevitch, who, in Russia in the 1870s, had shown that altered salinity changes body shape in the brine shrimp *Artemia* (93). This was, of course, before polymorphism was recognized as such. Schmankevitch's observations seemed then to confirm that animal form might be a function of chemical constitution. This was not directly related to embryology, but was one more indication of the growing conviction of an interrelationship between morphology and chemical makeup.

The extension of the concept of formative stimuli from external to internal did not have to wait long for formulation. Herbst in 1895 raised the question why, if external stimuli could affect growth, might not also internal stimuli from other parts of an embryo do so also? This had seemed to be what had happened in the case of the pluteus's arms. Actually Roux himself in 1881 had in *Der Kampf der Theile* (94) expressed the idea of mutual interaction between embryonic parts, as analogous to selected evolutionary processes. Ideas develop in minds, not in the air, but sometimes they do seem to leak out into it.

In 1895 Herbst made very clear that he then felt that the strongest influence on him emanated from the work of Rudolf Virchow. It was Virchow, claimed Herbst, who introduced the idea of formative stimuli into science: "The concepts," he wrote, "of a formative stimulus and of the ability to react to it [Reizbarkeit] were introduced into science by Virchow . . . in the year 1858" (95). Again, in the first paragraph of the Introduction to his 1901 book, he wrote more specifically of the direct relationship of Virchow's ideas to his own theories of development. The first sentence of the 1901 Introduction begins by commenting that in 1894 and 1895 Herbst had written on formative (morphogenetic) stimuli. The text next says: "In amplification of an old article of Virchow's . . . and a newer one by Billroth, . . . in the beginning of the second article I advanced the concept of a formative (morphogenetic) stimulus, by which I understand every cause for *Auslösung* which initiates morphogenetic process definitely characterized in a qualitative regard" (96).

What Virchow emphasized in the article referred to by Herbst was that the concept of stimulation leads to the concept that something can be stimulated. There is, according to Virchow, something that changes as a result of stimulation. "In my opinion," he wrote in 1858, "there are namely two phenomena that demonstrate most clearly the peculiarity of vital metabolism in the relationships in question here, namely that the alteration is always limited to cell territories, simple ones or groups of them; and that the most varied kinds of stimuli have the same effects. . . . Every formative process is to be considered as an active accomplishment of tissue elements and as evoked by stimulation" (97).

Virchow did not use the word *Auslösung*; the participle I have here trans-

lated as evoked was *hervorgerufen* in Virchow's article. But in his emphasis on stimulability as logically related to stimulation, he implied something that was meaningful to Herbst. In thinking of formative stimuli, Virchow the pathologist was thinking of the formation of tumors; Herbst applied the idea to embryonic development. Virchow, the cell pathologist *par excellence*, emphasized cell territories. Here unfortunately Herbst did not follow him.

When Virchow used the word evoke, he was using a common German word, not a technical expression. Even von Baer before him had used it, in a biological description of developmental events, just 30 years before. "The eye," he wrote in 1828, "seems to be an outgrowth of the nerve tube that protrudes through the muscle layer as far as the skin layer, and the outer parts of the eye are changes in the skin evoked as a result" [dadurch *hervorgerufen*] (98). It is not clear what von Baer meant by outer parts of the eye. He thought that the lens is formed by an albuminous mass that also forms the vitreous body, and he believed that the choroid and sclera split off from the retina. (He did know that the cornea was part of the sclera.) By outer parts he possibly meant the nictitating membrane and eyelids, which he called outer parts of the eye in a different passage in which he discussed its development. We know only that he wrote in no uncertain terms that the developing eye evokes the formation of other structures by the skin.

When Herbst in 1901 set Virchow upon his stage, he placed him in the company of Billroth. Virchow is well remembered today; not so Billroth. Billroth, born in Germany, became a surgeon and pathologist in Zürich and Vienna. His interest to Herbst, and to us, is that in 1890 he published a small monograph, *Ueber die Einwirkungen lebender Pflanzen-und Thierzellen auf Einander* (99). In it, he described some of the effects of bacterial action in producing irritation and inflammation. He thought of the formation of tubercles and nodules as a result of "the fact that plant cells can exert a purely formative stimulus on the cells of animal tissue," which he thought a remarkable phenomenon. "We are so accustomed," he wrote, "to thinking of bacteria as destructive enemies of animal cells that it is new to think of them as exerting formative influences" (100). And then he launched into extravagant panegyrics praising Virchow and his concept of the formative stimulus:

Formative stimuli and formative stimulability! This concept was first developed by Virchow in his thoughtful article "Stimulation and Stimulability." . . . Our generation can have no idea of the impression that this made on the young people who worked with Virchow then and on those who succeeded them. . . . "Stimulation and Stimulability," once a slogan, the Alpha and Omega of a whole school of medicine! We cannot dispense with this concept if we are concerned with organized Nature. Therefore Virchow's clarification of it can never go out-of-date; it is even today the basis of what we mean by this expression. . . . Indeed we here recognize the fundamental influence the Virchow exerted on pathological anatomy and then on our whole comprehension of the processes that take place in living organisms. Virchow was the first to clarify that not only nerve and muscle react to stimuli, but that the substance of every individual cell reacts to stimulation, and that this can be expressed in alterations of function, nutrition, and formation. The stimuli can be thought of as only instigating transitory specific physical or chemical changes in the living substance, or as exerting them permanently (101).

To try to pin down the ultimate origins of the concept of induction would be a silly exercise; in logic, the idea reaches back into antiquity. As for hard science,

in our sense of science, we may defer to Faraday. In the first sentence of his first article on electromagnetic induction, published in 1812, he wrote that at that time “the term had been received into scientific language” (102). Where and how and whether that may have related to biology and embryology is another story that must await another telling.

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11. Herbst, C., 1901a, Formative Reize in der tierischen Ontogenese, Ein Beitrag zum Verständnis der tierischen Embryonalentwicklung, Arthur Georgi, Leipzig.
12. *Ibid.*, p. 66.
13. Spemann, 1901b, p. 79. “Shortly after I had demonstrated . . . that the formation of the lens is brought about [ausgelöst] by the eye cup, Herbst also [in 1901] expressed the same opinion independently of me.”
14. Printed sources of information about Herbst's life are rare. His death was close to the time that World War II ended; therefore, the obituary notice that would normally have appeared in *Naturwissenschaften* was never published. A short, unsigned notice appeared in *Sitzungsberichte der Heidelberger Akademie der Wissenschaften, Jahresshefte 1943/55*, Heidelberg, 1959, pp. 41–42. Hans Querner prepared a short biographical summary for *Neue deutsche Biographie*, 1969, Duncker & Humblot, Berlin, **8**:593. Information about his personality has been provided not only by the printed sources specified in the following notes, but also by private correspondence in letters from Viktor Hamburger, once a student of Herbst; from Hans Querner, who, like Herbst, was a professor in Heidelberg; and from Dr. Gottfried Zirnstein of Leipzig, who has access to Herbst's letters to Driesch written between 1902 and 1924. One of Querner's personal sources of information was Erich von Holst, who came to Heidelberg in 1946 and who was interested in Herbst; Querner had been von Holst's assistant. Dr. Marion Kazemi provided photocopies of documents relating to Herbst as a member of the Kaiser Wilhelm Foundation and as a possible candidate for office in the Kaiser Wilhelm Institute in Berlin-Dahlem. I am grateful to them all.
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18. Akten der Generalverwaltung der Kaiser-Wilhelm-Gesellschaft, No. 1557: “Professor C. Herbst 1.10.1914 bis 31.3.1915 . . . 4500 M.” The exchange rates published in the New York Times on five Fridays in April 1915 (4/2; 4/9; 4/16; 4/23; 4/30) were used to convert German marks to American dollars.
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21. Hamburger, V., letter to J.M.O. 2/17/87.
22. Spemann, H., letter to the President of the Kaiser Wilhelm Foundation, Berlin, April 13, 1918; photocopy provided by Dr. M. Kazemi.

23. Mangold, O., 1953, Hans Spemann, *Ein Meister der Entwicklungsphysiologie, sein Leben und sein Werk*, Wissenschaftliche Verlagsgesellschaft, Stuttgart, p. 40.
24. Goldschmidt, R., 1960, *In and out of the Ivory Tower. The Autobiography of Richard B. Goldschmidt*, University of Washington Press, Seattle, p. 188.
25. Aufzeichnung über die Sitzung des Kuratoriums des Kaiser-Wilhelm-Instituts für Biologie . . . den 13. März 1919 . . . in der Akademie der Wissenschaften. Aus Akt II 11/3 p. 120c. “Von Herbst und Brauss sei zu sagen, dass sie den Zenith ihres Lebens überschritten hätten.”
26. Querner, H., letter to J. M. O., 2/25/85.
27. Hamburger, V., letter to J. M. O., 3/11/85.
28. Hamburger, V., 1988. *The Heritage of Experimental Embryology. Hans Spemann and the Organizer*. Oxford University Press, Oxford and New York, p. 15.
29. Hamburger, V., letter to J. M. O., 9/15/88. In 1988 Hamburger may have been thinking of restaurants in railroad stations as they had become by that year. I remember well that even in the 1940s the Savarin Restaurant in Pennsylvania Station was, if not the most elegant dining place in Manhattan, at least sufficiently pleasant to choose unapologetically as an agreeable meeting place for dinner with friends.
30. Goldschmidt, 1956, p. 70.
31. Zirnstein, G., letter to J. M. O., 3/21/89.
32. Zirnstein, G., postcard to J. M. O., 5/15/89.
33. Goldschmidt, 1956, p. 70. In 1971 The Mendel Newsletter described the Goldschmidt papers housed in the Bancroft Library at the University of California (Berkeley), stating that nine Herbst items (1907–1939) were included in the collection (p. 2). Photocopies of these, which I have seen, indicate that their content was exclusively scientific, not at all political. Perhaps Goldschmidt destroyed the later letters he received from Herbst. Goldschmidt had emigrated from Germany to the United States in 1936.
34. Hamburger, 1988, pp. 15–16.
35. Oppenheimer, J. M. 1981, Walter Landauer and developmental genetics, in: *Levels of Genetic Control in Development* (S. Subtelny and U. K. Abbott, eds.), Alan R. Liss, New York, pp. 1–13.
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38. Herbst, 1892, p. 446.
39. Herbst, C., 1941, Hans Driesch als experimentellen und theoretischen Biologe, *Wilhelm Roux' Arch. Entwicklungsmech.* **141**:111–153. Quotation from p. 145. The three short publications by Pouchet and Chabry referred to by Herbst all appeared in 1889, as follows: Pouchet, G. and Chabry L., *Sur le développement des larves d'Oursin dans l'eau de mer privée de chaux*, *Compt. rend. Soc. biol.* **41**:17–20; *De la production des larves monstrueuses d'Oursin, par privation de chaux*, *Compt. rend. Acad. sci.* **108**:196–198; *L'eau de mer artificielle comme agent tératogénique*, *J. anat. physiol.* **25**:298–307.
40. This passage is reprinted, with the permission of The Johns Hopkins University Press, from Oppenheimer, J. M., 1970. Some diverse backgrounds for Curt Herbst's ideas about embryonic induction, *Bull. Hist. Med.* **44**:241–250. The passage reprinted here is found on pp. 243, 244–248. It concludes here with the sentence at the top of p. 73.
41. Herbst, C., 1900, *Ueber das Auseinandergehen von Furchungs- und Gewebezellen in kalkfreien*

Medium, *Wilhelm Roux' Arch. Entwicklungsmech.* **9**:424–436. A recent article on sea urchin morphogenesis refers to Herbst (1900) when stating that hyaline layer (HL) can be dissolved in  $\text{Ca}^{2+}$ -free medium, and points out that “Herbst's (1900) experiments and later observations indicate[d] that microvilli link the apical surfaces of the blastomeres with HL” (Adelson, D. L. and Humphreys, T., 1988, Sea urchin morphogenesis and cell-hyalin adhesion are perturbed by a monoclonal antibody specific for hyalin, *Development* **104**:391–402; quotation from p. 392). It is correct that Herbst described the layer surrounding the blastomeres as hyalin (the same word in German) and as containing radiating fibers, and his drawings indicate clearly and indisputably that he saw delicate fibers that protruded into the layer; in his text (1900, p. 430) he said that here and there they could be seen to come and go. These observations antedated by decades, however, the use of the word “microvilli” to define cytoplasmic cell protrusions.

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48. Hamburger, 1988, p. 18.
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## Chapter 5

# Spemann Seen through a Lens

MARGARET SAHA

### 1. Introduction

In 1924 the results of perhaps the most publicized and seminal experiment in embryology were published, those of the “organizer” grafts performed by Hans Spemann and his graduate student Hilde Mangold (1). Not only did these investigations result in a Nobel Prize for Spemann, but more important, they fostered a heightened interest in the phenomenon of embryonic induction and led to establishment of a prolific school of research into the nature of the organizer (2). Despite its notoriety, the organizer experiment was not the first instance of induction to be experimentally demonstrated, nor did it come to serve as the classic textbook paradigm for general embryonic induction. In both respects this position is reserved for induction of the lens.

In a series of experiments published in 1901, Spemann, in an effort to demonstrate the dependence of lens formation on contact by the optic vesicle, ablated the presumptive eye rudiment on one side of the embryo with a hot needle and observed that lens formation consistently failed to occur on the operated side (3). Historically, this serves as the earliest example of an inductive event to be demonstrated experimentally, one which fostered considerable interest among Spemann’s colleagues. More important, the phenomenon of lens induction quickly came to serve as a prototype for other tissue interactions. In the 1938 work *Embryonic Development and Induction*, which encapsulated his life’s work, Spemann chose to use lens induction as a model system with which to introduce the general concepts of embryonic induction (4). Moreover, lens induction has continued to serve as the classic textbook paradigm for embryonic induction (5).

Despite its historical and practical significance, the history of lens induction has been relatively neglected by historians. This chapter will focus on the early history of lens induction experiments, tracing Spemann’s intellectual odyssey from the turn of the century, when he performed his first ablation experiments, to the early 1920s when the organizer experiments were conducted. Not only do these experiments possess an intrinsic interest for the history of induction (for they comprise the bulk of all induction studies in the first two decades of this

century), but more important, they provided the necessary intellectual background for interpreting data arising from other transplantation experiments, namely, the organizer grafts. Here it will be argued that between 1900 and 1912, the 12 years Spemann devoted primarily to the lens problem, he attained an increasingly refined interpretation of embryonic lens induction; it was this conceptual framework which enabled him not only to analyze the organizer experiments in terms of an inductive interaction, but also to interpret them in the particularly subtle fashion in which he did.

## 2. Early Years: The Search for a Problem of General Interest

Spemann's achievements in the area of induction did not occur in an intellectual vacuum. Rather, they derive from the fortuitous combination of a receptive mind and his fortune to work in a scientific environment in which the ideas of correlative development and progressive determination were topics of intense interest (6). His introduction to contemporary scientific thought occurred in the autumn of 1891, when he matriculated at Heidelberg to commence studies in the medical faculty (7). Here he attended the lectures of some of the most stellar scientific figures of the day, the botanist Ernst Pfitzer, the zoologist Otto Buetschli, the physiologist Kuehne; but it was the renowned comparative anatomist Gegenbaur who exercised the most profound influence over Spemann. Spemann speaks of the "great moments" when, from an evolutionary viewpoint and with expert assistance from his préparateurs, Gegenbaur would dramatically compare various aspects of human anatomy to that of animal ancestors. Although Spemann never intensively pursued comparative anatomy as an area of research, and later became quite critical of much of the contemporary conceptual framework employed in the field, he retained an abiding awareness in the discipline which would add to the breadth of his interpretations in his later studies. In his lens induction studies, for example, incompatible results from different, but closely related species were often viewed from a comparative anatomical vantage point.

Yet another potent influence on Spemann's thinking was his close friendship with Gustav Wolff, whom he met through an informal Heidelberg scientific society of which both were members (8). Wolff's self-imposed goal was to determine the essence of organic purposiveness in nature, and toward this end he chose to investigate regeneration phenomena. In 1895 he published his rather startling results concerning regeneration of the lens from the upper margin of the iris following its extirpation. It is highly likely that given their close friendship (30 years later Wolff was a frequent guest of Spemann's at the Freiburg zoology colloquia), Wolff discussed his ongoing research with Spemann, introducing him to eye/lens formation as a model system for asking fundamental questions in development. Spemann maintained that it was Wolff's results which initially led him to undertake his first lens induction experiments. That Wolff's work exercised a lasting impact is revealed by the fact that in Spemann's subsequent work all discussions of lens-forming potential invariably include an extensive treatment of the role of the upper lip of the iris. Moreover, as discussed later in more detail, his intimate knowledge of lens regeneration from the iris contributed

significantly to his formulation of the “double assurance” concept and his “embryonic field” ideas.

In the winter of 1892/1893 Spemann moved to Munich in order to complete his studies (9). There, at the recommendation of Wolff, Spemann met August Pauly and developed a deep admiration for the charismatic Larmackian zoologist, who was a major proponent of psychic factors operating in the process of adaptation. Throughout his career Spemann would also liberally employ psychic analogies for biological processes. But unlike many of his contemporaries, he never abandoned his analytical, data-driven approach to scientific problems in favor of a psychical one; for Spemann, psychical analogies simply reflected his lifelong conviction that at least certain aspects of development could never be reduced to cellular analysis.

Perhaps Pauly’s most lasting impact was his recommendation that Spemann, now firmly committed to pursue biology rather than medicine, continue his studies at Würzburg with Theodor Boveri (10). Following this advice, in 1894 Spemann commenced his doctoral work under Boveri’s direction with a descriptive embryological study of the nematode *Strongylus paradoxus*, a project designed to instill acute powers of observation and reconstruction—traits of paramount importance to Boveri and later to Spemann. But Spemann learned more than microscopy and experimental technique under Boveri’s tutelage; through daily theoretical discussions with Boveri (whose interests were at the very heart of current embryological research), Spemann was introduced to the fundamental problems of the field as formulated by Weismann, Roux, and Driesch.

Of particular relevance were Roux’s ideas, formulated in the 1880s. In an attempt to simplify the conceptual framework of embryology and render it more amenable to experimental manipulation, Roux drew the distinction between dependent differentiation, in which interactions with other parts of the embryo were necessary, and independent differentiation, in which development proceeded in the absence of such interactions. These represented clear and testable alternative mechanisms of development and evoked considerable theoretical discussion. Both Driesch and Herbst speculated on the respective roles of nucleus, cytoplasm, and various stimuli (both external and internal) on embryonic development. Herbst, who was particularly intrigued with the role of various chemical and mechanical stimuli, cited a number of examples of what were loosely termed “correlative development”: blood vessel supply influencing bone structure; the development of taste buds being determined by innervation; dependence of the lens on the eye; the effects of lithium salts and gravity on embryonic development. In a now famous treatise, Herbst went so far as to argue that it eventually might be possible to resolve the entire process of embryogenesis into a series of inductions exerted by one region of the embryo on another (11).

It is clear that the frequent discussions of such issues exercised a potent impact on Spemann. For his Habilitation lecture, he chose to discuss the Roux/Driesch experiments in a seminar entitled “Critical consideration of experiments on the effects on development of removal or killing of single blastomeres.” For his disputation, he proposed theses that encompassed the basic issues of contemporary biology: the inheritance of acquired characteristics, the role of mimicry, Roux’s principle of self-determination, and the relationship between the humanities and the sciences (12).

Following his Habilitation, which completed his period of formal training, Spemann was expected to embark on an independent research program. Once again, he wished to pursue a problem of general biological interest requiring technical expertise. He chose to investigate in more detail the problems of why Roux failed to obtain regulation in his blastomere experiments by retarding the development of one of the blastomeres with respect to the other by the application of lower temperatures. In the course of these experiments, Spemann, presumably using a hair loop for orienting embryos, fortuitously obtained embryos with anterior duplications, an intriguing result which promised to shed light on some of the fundamental problems in embryology, such as symmetry, individuality, and cytoplasmic determinants. Spemann therefore undertook an exhaustive series of ligation experiments in which he constricted salamander embryos in various orientations and at various stages.

The results of these studies were published in three lengthy studies between 1901 and 1903 (13). He observed that with all frontal constrictions only that part containing the dorsal region gastrulated and developed normally, whereas the ventral half formed a “belly piece” lacking all axial structures. He declined to speculate on the material differences between dorsal and ventral blastomeres, suggesting only that in the latter either the formative impulse or some material capable of self-differentiation was lacking. With his medial ligations, Spemann obtained a series of anterior duplications, with the degrees of duplication dependent on the extent of the original constriction; a slight ligature resulted in the anterior head being duplicated, whereas a deep constriction produced two embryos joined only at their tail regions. Confirming Dreisch’s results, complete separation produces two entirely normal embryos. Spemann explained these data by correctly postulating that during gastrulation the deeper the constriction, the more the invaginating mesoderm was divided into two, leading to the various duplications. When these constrictions were performed subsequent to gastrulation, only slight indentations rather than duplications occurred, leading Spemann to assert that the axial structures such as brain, eyes, and spinal cord were irreversibly determined by the end of gastrulation.

Yet another feature of these studies that intrigued Spemann was the Cyclopean eyes produced by extremely slight constrictions, a topic to which he devoted an entire monograph. He was particularly fascinated with the fact that despite the eye’s ectopic position, a single lens was perfectly situated with respect to this single medially located eye. In this respect Spemann was able to draw on a large, established body of research on the development of the vertebrate eye, which by the turn of the century was becoming an increasingly attractive model system for comparative anatomists and morphologists.

At the point at which Spemann was searching for an independent research program, a number of factors combined to make lens induction a logical and perceptive choice. From the stimulating environment of the Boveri laboratory, Spemann became familiarized with the ideas of Weismann, Roux, Dreisch, and Herbst and insisted on a project that would involve technical prowess and respond to the most fundamental issues of contemporary embryology. The vertebrate eye appeared—as his own work had already revealed—to show promise in addressing the issues of progressive determination, individuality, and correlative development. Moreover, the phenomenon of Wolffian lens regeneration, with which Spemann was familiar on a firsthand basis, provided an added attraction

of this system. Finally, by the turn of the century, the concepts of “dependent differentiation,” “correlative interactions,” and “induction” had entered the realm of general scientific consciousness. Employed interchangeably and quite vaguely, these concepts would be molded by Spemann into a more refined and precise analytical tool—one which could be applied to yet other aspects of determination in order to create a more unified picture of embryonic development.

### 3. Lens Induction: A Paradigm for Vertebrate Cell Determination

As indicated earlier, Spemann’s decision to study lens induction was based on an intuitive conviction that this problem would shed light on a number of significant contemporary embryological issues. His intuition proved accurate. Although not the first scientist to introduce the concept of induction into embryological thought, Spemann, in the course of his lens induction studies, popularized and refined the concept, rendering it one that might serve as a unifying principle for vertebrate development.

#### 3.1. Initial Experiments (1901–1906)

In the course of his constriction experiments, Spemann became convinced that he was able to identify the presumptive eye rudiment at early neural plate stages (14). The combination of his intellectual background and a prepared mind immediately suggested the feasibility of an experiment that would conclusively test the loosely formulated hypothesis that lens formation was dependent on some form of contact with the optic cup, namely, removing the eye rudiment at early stages. Not only was this phenomenon mentioned by Herbst as an example of “correlative development,” but it was also frequently cited in the vast body of literature dealing with descriptive morphology of eye formation. Already in 1898 the anatomist Theodor Rabl was sufficiently aware of this relationship between the eye and the lens to report a possible exception to it: in one of his specimens he mentioned a lens-like structure that was shifted away from the developing eye. He declined to speculate further and his observation of this developmental anomaly remained only a minor point in his lengthy monograph (15).

Against this intellectual background, Spemann embarked on his first lens induction experiments in the spring of 1899 and published the results 2 years later (16). He was quite clear concerning the intent of these experiments: to determine whether processes in eye development occur dependently or independently of one another, that is, whether “there exists some causal relationship or a harmony dating back to the egg.” He aimed to determine whether the lens was required for eye cup formation as well as whether the optic vesicle was a prerequisite for lens formation. Toward this end he conducted two series of experiments. In the first set, he partially injured the eye rudiment at the closed neural fold stage, preventing it from reaching the epidermis; the emergence of optic cups lacking lenses led him to conclude with certainty that a lens was not essential for the development of the optic cup from the optic vesicle.

For the second, scientifically more consequential, question, Spemann ab-

lated the presumptive eye rudiment in an open neural plate stage embryo employing Roux's method of a hot needle. In each instance where the optic rudiment was successfully destroyed, a lens failed to develop. He therefore concluded that this experiment demonstrated indirectly, but with sufficient certainty, that contact by the optic vesicle was necessary for lens formation. Spemann tempered his conclusion by cautioning that his experiments revealed nothing concerning the lens-forming potential of various regions of the ectoderm or whether the optic vesicle was sufficient to induce lens formation. Answers to these important questions would await transplantation experiments in which the optic vesicle was moved to ectopic locations or non-lens epidermis was transplanted over the optic vesicle. He admitted that he had already attempted such technically challenging experiments, but as yet with little success. Spemann was equally intrigued by the question of whether, during embryogenesis, a lens could be "regenerated" from the upper margin of the iris in the absence of a lens of strictly ectodermal origin. Unfortunately, a series of negative results made it impossible for him to draw any conclusions on this point.

Following a discussion of the necessity of pursuing transplantation experiments, he concluded this elegant paper with a caveat characteristic of one who routinely considered all possibilities allowed by the data; he cautioned that although the optic vesicle was required for lens formation, it was not yet determined whether the stimulus it provided came directly from the eyecup or originated in another region of the embryo and simply detoured through the eyecup.

Although Spemann did not employ the term "induction" or even precisely define his usage of the terms "dependent" and "independent" differentiation and correlative development, this 1901 paper remains one of the most significant and seminal in the history of embryology. Prior to this publication, it was still possible that all the examples of correlative development cited by Herbst, Roux, and others were nothing more than vague reciprocal relationships, incapable of being separated and analyzed, necessary to the development of any complex organism. But following Spemann's lens induction experiments, it appeared that in this instance and perhaps others it might prove feasible to dissect these interactions with the hope of actually constructing the type of hierarchy envisioned by Herbst. Spemann had provided the first experimental proof of this possibility; but he was too prudent a scientist to articulate this possibility in detail. He first wished to pursue lens induction from the perspective of the lens-forming potential of the responding ectoderm.

Spemann's work received immediate and widespread recognition, not only because of its topical nature, but also because it was first presented before the annual meeting of the German Anatomical Society. His paper fostered a heightened interest in the lens problem, and during the course of the next several years, many investigators attempted to repeat his experiments with the goal of testing and extending his conclusions.

### 3.2. Mencl's Challenge

In 1903 an article written by the Czech biologist Ernst Mencl appeared in *Archiv für Entwicklungsmechanik*, in which he rather boldly attempted to refute the conclusions Spemann had reached in his 1901 paper (17). Mencl began with a

statement concerning the general significance of his results for Roux's question of dependent as opposed to independent differentiation, specifically on the correlation between nervous and epidermal derivatives. But he rapidly proceeded to deny the existence of any such correlation, at least in the case of lens formation. For supporting evidence he cited a number of mutant salmon embryos that possessed lenses in the total absence of any eye tissue. In addition to his own observations, he mustered all the available evidence supplied by other investigators who had encountered a similar phenomenon to bolster his case; he vehemently argued that there existed no causal relationship between the eye vesicle and the lens.

Spemann immediately responded to this challenge with a note to *Anatomischer Anzeiger* in which he specifically addressed Mencl's claims (18). Lacking the vitriol typical of other investigators who would soon join this controversy, Spemann first considered potential flaws in his own work, namely, that in the course of ablating the eye rudiment he damaged the presumptive lens cells. He also entertained the possibility that his ablation process interfered with yet another process that was instrumental in lens determination, one involving the mesoderm or endoderm (although this had never been suggested in the literature). Considering these alternatives unlikely, but not fully dismissing them, he proceeded to a detailed criticism of Mencl's work, criticism that hinged on interpretation of Mencl's histology. After examining the sections in question, Spemann maintained that pieces of eye tissue were indeed present. Mencl quickly responded with an emotional rebuttal in which he presented no new information, but merely restated his earlier position more emphatically (19).

Shortly thereafter, the American embryologist Warren Lewis entered the field of lens induction with a paper that argued (vehemently against Mencl) for an even greater role of the optic vesicle in lens formation (20). Successfully performing the transplantation experiments that Spemann had suggested in his 1901 paper, Lewis removed the optic vesicle from a late neurula embryo of *Rana palustris* (a frog species native to America) and transplanted it caudally to a location beneath head ectoderm that was not fated to form a lens. Lewis reported that lenses failed to develop in their normal location but that lens-like structures did develop directly over the repositioned optic vesicle in several of these experiments. When the presumptive lens epidermis was removed and a piece of belly epidermis was positioned in its place over the optic vesicle, lenses likewise developed. Based on these findings, he asserted that not only was the optic vesicle necessary for lens formation, but, given its ability to elicit a positive response from non-lens ectoderm, it was also a sufficient inductor of the lens.

The publication of Lewis' data provided Spemann with the incentive to report the results of additional ablation experiments that confirmed those of Lewis as well as extended them (21). In essence, this 1904 paper served more as a theoretical treatise than one designed to convey results, one intended both to raise questions and to clarify issues by synthesizing all the available data from the various investigators. For Spemann the most important of these issues was the following: although previous experiments made it possible to claim with a reasonable degree of assurance that lens formation was dependent on the stimulus provided by the optic vesicle, there was no evidence to answer the question of whether the epidermis is thoroughly indifferent or whether it already possesses a lens-forming potential but must await a "cue" from the optic vesicle.

In an attempt to address this problem, he designed a series of experiments to compare the lens-forming potential of head ectoderm as opposed to trunk or body ectoderm. If the head ectoderm possessed a more pronounced ability to generate lenses, one could conclude that it does indeed harbor some sort of predisposition. In order to test the lens-forming potential of the head region, Spemann removed the presumptive lens cells and let the surrounding non-lens head ectoderm heal over in its place. The fact that lenses formed in a high percentage of these cases indicated that non-lens head ectoderm clearly possessed lens-forming potential. But since Spemann had not yet completed his own set of experiments testing the ability of trunk ectoderm to form lenses, he relied on the data of Lewis, who had claimed that lenses formed from trunk ectoderm when provided with the stimulus of the optic vesicle. Given these results, he was obliged to conclude that head ectoderm did not appear to be any more endowed with a lens-forming predisposition than trunk ectoderm; all regions of the ectoderm were thoroughly indifferent and equally capable of responding to the instructive role of the optic vesicle with a lens.

Lewis' results also addressed a related question that Spemann had already raised in his 1901 paper, namely, whether the causative influence proceeds directly from the optic cup to the epidermis or whether it originates in another region of the embryo and simply travels through the optic vesicle. The fact that a detached optic vesicle moved to an ectopic location was able to stimulate lens formation from the overlying epidermis strongly suggested that the stimulus emerged directly from the vesicle.

Yet another critical question for Spemann was whether lens formation required merely a single stimulation from the optic cup or whether a lasting influence was required. To address this problem, Spemann proposed a series of experiments in which the optic vesicle would be removed at different stages of development. Having not yet completed his own studies, Spemann drew on the results of an experiment (conducted for an entirely different purpose) performed by Schaper (22) in which he removed the spinal cord and hind- and mid-brain. Based on Schaper's results—in which the optic vesicle initially contacted the presumptive lens ectoderm but later pulled away due to the operation—Spemann conjectured that the process of lens determination began as dependent differentiation but was able to continue, in part at least, as self-differentiation. For Spemann, Roux's alternatives were by no means mutually exclusive. Moreover, since the lens eventually did begin to degenerate in the absence of an eye, he hypothesized that lens induction involved a continuing reciprocal interaction between the eye and lens primordia.

Finally, after some brief speculation concerning the possible chemical nature of the signal from the eye vesicle, Spemann raised the question of how regeneration phenomena can be explained in the context of current knowledge. In the course of his experiments Spemann had obtained several instances of lenses arising in the absence of optic vesicle contact, cases he cautiously attributed to regeneration. Here, as throughout his career, accurate interpretation of the data took precedence over any philosophical predilection; he persuasively argued against Wolff's psychic teleological interpretations of lens regeneration, maintaining that it was scarcely surprising that lenses would arise from the dorsal iris margin.

I am therefore of a different view than Wolff's; I differ from his view that the actual organic events are something which only could be understood according to a psychic analogy. It is surprising to me that this way of viewing things, which is older than its modern representative, still appears to cause so many difficulties. Simple reflection on the explanation of the capability of the upper ridge of the iris to build a lens reveals that these cells are of the same ectodermal origin as the cells of the epidermis, and they are even more closely related to the cells of the brain. Thus lens regeneration from the upper iris ridge arises from the same forces of the optic cup which already have been confirmed for normal development (23).

Although Spemann did not yet employ the term, this represented an incipient version of an embryonic field idea, the concept he would later use to interpret both lens determination and the organizer experiments.

While continuing his transplantation experiments throughout the breeding seasons of 1905 and 1905, Spemann published a methodological paper, detailing the techniques available for addressing some of the fundamental questions raised in his previous publication (24). He praised Harrison's work and emphatically counseled others to apply such techniques not only to the lens system, but also to other aspects of development.

Thus in the 5 years since the publication of his first induction experiments Spemann had succeeded in clearly formulating and articulating the fundamental issues in this field and to a large extent molding it as an area of study. He endowed the concept of induction with more precision and drew critical distinctions that created the context for subsequent studies of induction. For example, Spemann framed the distinction between the inducing and reacting system (the notion of competence) and the distinction between thoroughly indifferent tissue or "primed" tissue simply awaiting a "cue" (the notion of bias or predisposition). Yet even though he drew such distinctions on paper, he was acutely aware that in reality they might well be blurred; already he had hypothesized that lens induction represented a combination of dependent and self-differentiation. Although the concept of the necessity of the optic vesicle for lens formation appeared to be sufficiently grounded in a bedrock of experimental evidence to be considered a tenet, Spemann, unlike Lewis, refused to dismiss categorically other viewpoints. Revealing his background in comparative anatomy, he issued the insightful and prophetic caveat that as yet too few species had been investigated for him to conclude that the necessity of the optic vesicle for lens formation was a universally applicable phenomenon. It was still possible that the requirement for optic vesicle contact might be species-specific.

### 3.3. “Double Assurance” and a New Perspective on Lens Induction

In 1905 the American embryologist Helen King repeated Spemann's optic vesicle ablation experiments on the American frog *R. palustris* (25). Contrary to Spemann's earlier results on *Rana fusca*, King obtained "free lenses," that is, lenses that developed in the absence of optic vesicle contact. These data appeared to provide the first experimental verification of Mencl's claims, and King's publication provoked an immediate response from both Lewis and Spemann.

Lewis responded with harsh criticism of King's work and emphatically restated his position concerning the necessity and sufficiency of the optic vesicle for lens formation (26). Not only did he claim to confirm his original ablation experiments, but he also maintained that lenses were induced from trunk ectoderm regardless of whether the optic vesicle was transplanted to the belly region or whether belly was ectoderm placed over the optic vesicle of neurula stage embryos. For Lewis, lens induction was a simple one-step inductive event in which the optic vesicle was able to evoke a lens from any piece of epidermis.

Spemann, on the other hand, even though for technical reasons he deemed King's results unconvincing, responded quite differently. Although the cumulative evidence still remained uncompelling, there was an ever-growing number of reports of "free lenses" in the literature, including observations from Rabl, Schaper, and Mencl (27). This list, in conjunction with Spemann's more open-minded temperament, persuaded him that it would be scientifically prudent to repeat his original ablation studies in a variety of species. He undertook these experiments throughout the breeding seasons of 1905 and 1906, and in 1907 he published the surprising results of these studies, results that necessitated a radical shift in his thinking concerning the nature of induction.

Opening his paper "New facts on the lens problem" (28) with a brief historical treatment of the problem and an admission of the impact of King's work, Spemann proceeded to describe the fateful experiment in which he ablated the presumptive eye rudiment of a neural-plate-stage *Rana esculenta* embryo and obtained free lenses in a significant number of cases. Since these results initially cast some doubt on the technique itself, he immediately attempted to repeat the results of his 1901 *R. fusca* experiments. Not only did Spemann ablate the eye rudiment with a hot needle, but he also surgically removed the entire rudiment; in neither case did he obtain lenses. The ability to generate free lenses appeared to be species-dependent. Additional evidence that lens formation in *R. esculenta* was not dependent on contact with the optic vesicle derived from the fact that unlike in other species, the size of the lens did not seem to correlate with the size of the optic cup; there were several instances in *R. esculenta* in which the lens was considerably larger than the optic cup. Further experimentation revealed that the majority of other species lay somewhere between *R. fusca* and *R. esculenta* in terms of their ability to produce free lenses, with most possessing some degree of self-differentiation tendencies but still requiring contact with the optic vesicle for optimal lens formation.

Spemann limited his conclusions to hypothesizing that lens cells were determined (he did not speculate how) during the open-neural-plate stage, but that different species required differing degrees of optic vesicle contact for complete lens differentiation. Eschewing further theorizing, he merely indicated ongoing lines of experimentation that would shed additional light on his surprising results: interspecific transplants of lens epidermis and optic vesicles; rotation of the presumptive lens ectoderm and optic rudiments; determination of the extent of lens-forming potential in a species such as *R. esculenta* in which the presumptive lens ectoderm possesses significant self-differentiation tendencies.

It was in a talk presented the same year before the German Zoological Society, entitled "Concerning the problem of correlation in animal development" (29), that Spemann attempted to place his new findings in a larger concep-

tual and biological framework. Following an elaborate historical overview in which he traced the concept of correlation or correlative development back to Cuvier and Geoffroy St. Hilaire, Spemann presented an extremely detailed survey of all known examples of correlative development: innervation and muscle, nerves and sense organs, muscle and skeletal development. Within this context he discussed in considerable detail the history of lens induction experiments leading up to the discovery of the dual nature of lens formation from both the inherent self-differentiation tendencies acquired by the presumptive lens ectoderm and contact by the optic vesicle. Claiming that this might turn out to be a general phenomenon in development, he compared lens determination with the closely analogous case of the development of the operculum opening reported by Braus in the previous year. In amphibian embryos it was thought that the opening of the operculum was caused by the pressure of the forelimbs, which must break through this sheet of tissue, but Braus discovered that even when the forelimb rudiments were removed in the early stages of development, the opening emerged in its proper location. In order to explain this phenomenon, Braus introduced the term "double assurance," a term borrowed from the engineering discipline to denote that in the eventuality of one structure or process failing, another would be able to take its place. Spemann maintained that this concept of double assurance was equally true for the process of lens determination.

Simultaneously with Spemann's confirmation of Mencl's and King's free lens results, he made another discovery, which, although largely neglected by subsequent scientists and historians alike, was of equal significance for the field of lens induction and experimental embryology (30). In the course of his transplantation experiments, Spemann was acutely aware of the difficulty of removing the presumptive lens epidermis cleanly away from the optic vesicle. In order to ensure this, he performed the clever control of removing an optic vesicle from a late-neurula-stage embryo and transplanting it into the belly region; in half the cases he implanted the optic vesicle facing outward toward the ectoderm, and the remaining were placed facing inward toward the gut. Surprisingly, the percentage of lenses arising was nearly equal in both groups. On closer histological examination, Spemann observed presumptive lens cells firmly attached to the detached "clean" optic vesicles. To complicate the matter even further, he discovered that in many species of frogs the ectoderm consisted of two layers, a thick pigmented outer layer, which gave rise to epidermis and cornea, and a thin transparent inner layer, which gave rise to the lens. The latter was not only extremely adherent to the optic vesicle, but exceedingly difficult to detect. Given these technical problems, Spemann cautioned that the results of such transplantation experiments must be viewed with skepticism.

Incorporating his results concerning the dual nature of lens formation and the difficulty of ensuring clean, uncontaminated transplants, Spemann concluded the bulk of his own lens induction experiments by 1909. He devoted the next few years to a thorough analysis of his (and others') data and published a comprehensive synthesis of the field in a lengthy monograph in 1912 (31). Here he reported the results of several thousand transplant experiments he conducted on a wide variety of species. He conclusively confirmed his earlier results demonstrating that in certain species the optic vesicle was essential for lens formation, in other species its role was less than negligible, while the majority of species

investigated lay somewhere in between. Having investigated the extent of lens-forming potential in these same species, Spemann observed an interesting correlation; the greater the ability of a particular species to form free lenses, the more restricted was its lens-forming potential to the actual lens region. The more dependent the organism was on the optic vesicle for lens formation, the more extended was the region with lens-forming potential.

But for the majority of species lying somewhere between these extremes of complete self-differentiation and total reliance on the optic vesicle, Spemann maintained that a fairly large area of head ectoderm possessed lens-forming potential. Here Spemann detected "circles of diffusion" of lens-forming potential radiating from the presumptive lens region itself, with the most potent lens-forming tendencies at the center and growing progressively weaker toward the periphery. Thus two mechanisms of lens determination were operative, with their relative contributions varying among species. The first of these was induction by the optic vesicle and by this he meant the contact of one tissue with another, the former changing the developmental fate of the latter. The second was the presence of self-differentiation tendencies in the presumptive lens ectoderm, tendencies that were partially derived from the egg itself or (more likely) were gradually acquired throughout development.

Thus by 1912 Spemann developed an analytical and sophisticated view concept of lens determination. He abandoned the simplistic view of Lewis that stipulated that lens determination was the result of a one-step process in which the optic vesicle acted on indifferent or uncommitted ectoderm to form a lens. Retracting his earlier conclusions (which were based on Lewis' data), Spemann now argued that a large region head ectoderm did indeed possess a lens-forming predisposition while trunk ectoderm did not possess such a tendency. By 1912, Spemann (unlike many others in the field) ceased to use the vague terms of correlative development and dependent differentiation. He employed the term "induction" in the more modern sense of a given tissue contacting a neighboring tissue, resulting in a change of its fate. But unlike the majority of his contemporaries, he did not regard induction as the sole component of the determination process. In the case of lens determination, it was complemented by the presence of self-determination properties of the presumptive lens ectoderm, tendencies acquired earlier in development which possessed a field-like quality. By 1912 Spemann labored with the firmly rooted assumption that most, if not all, developmental processes would employ both the above mechanisms.

#### **4. Determination at Gastrula Stages: Application of the Lens-Induction Paradigm to the Organizer Experiments**

Although Spemann was primarily occupied with issues related to lens induction during the period between 1901 and 1912, he never totally abandoned his earlier interests concerning determination during gastrula stages. One should, in fact, regard these mutually symbiotic interests as outgrowths of the same fundamental concern for the mechanisms underlying the problem of restriction of developmental potential in a given region of the embryo. The constriction studies he completed at the turn of the century had provided him with

his first fortuitous data concerning the development of the eye and lens, while his extensive research on lens induction supplied both the technical expertise and the conceptual framework for interpreting his gastrula determination experiments.

Spemann decided against pursuing the series of constriction experiments further because of the technical limitations inherent in the technique. A more precise localization of developmental potential or centers of differentiation was impossible employing these rather crude ligations. But the techniques he perfected and, in some instances, pioneered in his lens induction studies enabled him to focus more precisely on regional developmental potential. Moreover, his intriguing findings regarding the circles of diffusion of lens potential diminishing not only over time, but also space, impelled him to investigate similar phenomena in other systems.

Already in 1905 and 1906, Spemann had attempted a series of experiments in which he rotated various regions of the neural plate (32). During the next few years Spemann's interest in determination at the gastrula stages intensified. In 1908 he received a professorship at the Zoological Institute of Rostock and wished to establish a new research program. Moreover, by this time he had completed most of his experimental work on lens induction and was now immersed in the task of analyzing his data and attempting to determine its relevance to larger concerns within the field of embryology. With regard to this latter pursuit, the problem of determination at gastrula stages was certainly in the forefront of his thinking.

He therefore embarked on an extensive series of more refined experiments in which he made reciprocal exchanges between various regions of gastrula ectoderm within the same embryo. Regional pigment differences within the embryo which lasted for several days following the operation allowed Spemann to distinguish host and donor—an issue to which he was particularly sensitive from his lens induction studies. The results of these experiments whose aim was, first, to define precisely the time when self-differentiation replaces the original ability of the embryo to regulate and, second, to locate the "center of differentiation" for neural plate structures were reported in two papers published in 1918 and 1919 (33). His initial results elegantly confirmed his earlier constriction experiments: exchanges between presumptive epidermis and presumptive neural plate during early gastrula stages resulted in both pieces acquiring the fate of the host region, whereas transplants performed at later stages resulted in the tissues retaining their original identity.

As part of this series of experiments Spemann also transplanted a portion of the upper blastopore lip to the trunk region of another embryo and obtained a number of neural tubes and other neural-like structures. In order to explain this phenomenon, he invoked the same two mechanisms that he did for lens determination, namely, self-differentiation and induction. He suggested that the dorsal lip might serve as a (self)-differentiation center, speculating that determination spread in wave-like fashion, posterior to anterior, entirely within the sheet of ectoderm. He also hypothesized that a signal from the underlying mesoderm

induce neural structures. Given the preliminary nature of the data, I do not speculate further concerning the relative contributions of these two mechanisms of determination.

In addition to reciprocal exchanges, Spemann also conducted experiments in which he halved gastrula stage embryos, with the cutting being performed in a variety of different planes, and fused them back together. The most significant conclusion emerging from these experiments was the idea that neural ectoderm already possessed a bias or predisposition—an idea also garnered from his lens induction studies.

Spemann's interpretation of his results was still limited by the lack of a definitive host and donor marking system, a deficiency he remedied in 1916. After that he employed heteroplastic transplantation between the darkly pigmented newt *Triton taeniatus* and the lightly colored *Triton cristatus* species. Reporting the results of these experiments in 1921 (34), not only did he elegantly confirm the results of his earlier experiments, but he presented interesting observations of relevance for evolution and gene activation. But more important, the use of heteroplastic transplants in his dorsal lip grafts allowed him to determine with certainty the source of the tissue comprising the secondary embryo. It was as a footnote to this 1921 paper that Spemann first reported preliminary data on the organizer experiments.

It was another 3 years before Spemann and Hilde Mangold accumulated sufficient data to publish their results in a formal paper (35). For Spemann these results once again confirmed that determination was not a simple one-step process; as with lens determination, two mechanisms appeared to be operative. First, it became evident that the chorda-mesoderm which was brought into contact with the ectoderm during the course of gastrulation induced neural structures. But, in addition, the action of the organizer could exert its effect by serving as a self-differentiation center propagating its effect through the ectoderm. Spemann concluded his analysis by remarking on a phenomenon that would later be recognized to characterize many inductive interactions, namely, reciprocal interactions between the induced and inducing tissues. Just as the eye and the lens exercised mutual effects on each other, he postulated that the same held true for the underlying mesoderm and the presumptive neural plate. "Apparently the inducing part, while in action, was subjected to a counter-action by the induced part. Such reciprocal interactions may play a large role, in general, in the development of harmonious equipotential systems" (36).

For the remainder of his career Spemann continued to concentrate on discerning the nature of the organizer. Although he continued an active research program through the early 1930s, papers were increasingly of a theoretical and synthetic nature. In this respect his work on lens induction and neural induction exercised a reciprocal effect on each other, with the conceptual framework derived from his lens induction experiments applied to neural induction and the terminology of the organizer experiment applied to lens induction.

Already in 1924 Spemann published a more integrative paper entitled "Organizers in Animal Development" (37) designed to accompany the technical paper he coauthored with Hilde Mangold. Here he referred to the optic cup as the "organizer of the lens," but since the optic cup and its organizing capabilities do not arise exclusively through self-differentiation, but rather by induction, he termed it an "organizer of the second degree." Somewhat analogously, Spemann demonstrated that the same applies to neural induction; if a piece of presumptive epidermis is implanted near the dorsal lip of another embryo, it invaginates to

form archenteron roof, which, when excised, is able to induce neural plate. From this Spemann concluded that the principle of progressive determination through organizers of ever-rising degrees possessed a general validity, at least among amphibian species.

By the late 1920s Spemann began to apply the concept of double assurance to the organizer experiments explicitly rather than implicitly. Citing the experiments of Goerttler, he argued that just as the optic vesicle does not exercise its effect on totally indifferent epidermis, neither does the underlying mesoderm. Following the publication of Holtfreter's first paper documenting the ability of a dead organizer to induce neural tissue, Spemann became disillusioned and effectively ceased active research. He devoted full time to distilling and refining his ideas on induction and determination—an effort that culminated in the publication of *Embryonic Development and Induction* (38).

Here, more than in any previous publication, Spemann reveals the potent effect that the conceptual framework provided by his lens induction studies exercised over his thought on induction and determination in general. Not only does he begin his treatise with a thorough discussion of lens induction as a means of introducing the basic concepts and terminology of embryonic induction, but he proceeds to apply nearly all the fundamental ideas arising from his lens induction studies to the phenomenon of neural induction: double assurance, the existence of a predisposition in the responding tissue, reciprocal interactions between the induced and inducing tissues, the presence of a determination field.

## 5. Postscript: A Modern Perspective

In the years following the publication of Spemann's book the majority of embryologists interested in problems of induction and determination focused their attention on the questions of the nature of the organizer and regionalization in neural induction. Data quickly accumulated demonstrating that a "dead" organizer, as well as a host of other tissues from the animal and plant kingdoms, and even inorganic substances, were all equally capable of inducing neural tissue. Spemann, who regarded the concept of a "dead organizer" as a contradiction in terms, remained quietly skeptical of the feverish attempts to identify the chemical nature of the inducer, efforts he viewed as both overly reductionist and vastly premature. As is well chronicled elsewhere, by the end of the 1940s, research on the nature of the organizer resulted in a long-lasting stigma being attached to the phenomenon of neural induction, and to a more general disillusionment with the entire field of embryonic induction (39).

Despite the initial surge of interest in neural induction following publication of the Spemann and Mangold paper, a number of investigators continued to pursue questions relating to lens induction throughout the 1930s and 1940s (40); the majority of these, unfortunately, had both technical and theoretical flaws. They failed to heed Spemann's caveats regarding the dangers of contamination and the necessity of host and donor marking in their tissue transplants, and they also lacked much of the subtlety of interpretation that Spemann endowed on the field. As a result, the majority of these studies uncritically accepted the simpler

view of Lewis that the optic vesicle was both necessary and sufficient for lens formation. There were, nevertheless, several notable exceptions; scientists such as Liedke, Reyer, and Jacobson (41) not only attempted to employ more stringent criteria for judging the authenticity of a lens response, but also argued that lens induction was a progressive, multistep process. Yet with none of these investigators directly refuting Lewis' data and neural induction still in disrepute, the textbook paradigm for general embryonic induction has remained Lewis' model of the optic vesicle inducing the lens in a single step.

Within the past decade the study of embryonic induction has experienced a resurgence of interest among embryologists. This renaissance is attributable to a number of factors: the use of more precise molecular markers rather than morphology; the employment of cell autonomous host and donor marking strategies; more detailed biological studies; above all, the elaboration of a molecular framework for embryonic induction, namely, growth factor receptor systems. Beginning with mesoderm induction, this resurgence has spread to lens and (even) neural induction. Recent work on lens induction has demonstrated that the optic vesicle is neither necessary nor sufficient for lens formation; rather, it is a progressive, multistep process beginning early during gastrulation when critical interactions between the very early presumptive eye rudiment and the presumptive head ectoderm take place and confer a placodal (including lens, but not exclusively) bias on a large region of head ectoderm. The optic vesicle acts on this predisposed ectoderm to complete determination and promote differentiation (42). The data pertaining to neural induction remain more preliminary, but many of these same ideas are emerging, for example, the hypothesis of dorsal ectoderm possessing a bias or predisposition toward neuralization on which the chordamesoderm acts to refine the inductive stimulus (43).

When analyzing Spemann's work from this more contemporary perspective, it is clear that many of his ideas appear—given the limitations imposed by the era in which he worked—uniquely insightful and perceptive. For neural as well as lens induction Spemann postulated a predisposition of the responding ectoderm prior to the action of the inducer and, as a corollary to this, the idea of induction as a multistep process. He viewed induction not only as a subjacent tissue inducing an overlying one, but also in terms of signals traveling laterally through a single tissue layer. One can, of course, rightfully note that many of Spemann's formulations of these ideas were not as sufficiently grounded in experimental evidence as their modern counterparts and that Spemann was often inconsistent when weighing the relative contributions of induction versus self-determination. But Spemann's most perceptive insight, one beyond reproach even from a modern perspective, was not his identifying specific mechanisms of determination, but rather his insistence that induction, or, more appropriately, determination, was a complex, multistep process involving a delicate interplay between the inducing and induced tissue—a conviction that first arose from his study of lens induction.

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## Chapter 6

# Reminiscences on the Life and Work of Johannes Holtfreter

JOHANNES HOLTGRETER

### 1. Editor's Note

One of the pleasures of being this book's editor has come from corresponding with Dr. Johannes Holtfreter and his wife, Hiroko. It is hard to think of a person who has contributed more to the conceptual foundations of modern developmental biology than Dr. Holtfreter. It was his work that "devitalized" the concept of the organizer by devitalizing the organizing tissue itself. His also was the "solution" that enabled experiments to be performed on amphibian embryos without enormous mortality. Dr. Holtfreter has contributed to this series before, discussing the work done in Spemann's laboratory and the evolution of the organizer concept. But the following essay is both a companion piece to that and an evocation of a life well lived in science and art. I have been privileged to receive some of this artwork, which Dr. Holtfreter has permitted me to publish here.

This essay flows from the informality of its context. It was part of a letter that Dr. Holtfreter sent to Dr. Katsutoshi Ishihara of Saitama University in Urawa, Japan, on July 22, 1981. Dr. Ishihara has graciously allowed us to print this letter, and the Holtfreters have been kind enough to review it, changing only a few details for publication. The opening paragraph of salutation, for instance, has been eliminated, and in its place Dr. Holtfreter has added a foreword and three paragraphs of biographical introduction. Hiroko Holtfreter's thesis research that is mentioned therein has been summarized by Viktor Hamburger, in *The Heritage of Experimental Embryology* (Oxford University Press, New York, 1988).

### 2. Foreword

I wish to say a few words about the way this narrative has come about. In 1981, Professor Ishihara of Saitama University, Urawa, Japan, invited me to give a few lectures at several universities in Japan, and to discuss with him and his students problems of developmental biology. I declined this offer due to my

advanced age. Instead, I wrote him a long letter in which I dealt with some of the highlights of my life and some of the scientific problems that have occupied me for many years. This letter Ishihara found sufficiently interesting to be translated into Japanese and published in the monthly journal *Shizen*. Meanwhile, friends of mine urged me to publish the original English version of the letter. So far I have declined to do so, but now, many years later, I accept the invitation of Dr. Scott Gilbert to publish the letter in this book.

### 3. Reminiscence

#### 3.1. Introduction

I do not intend to enlarge on the experiences I had as a boy and adolescent. I want to mention, however, that I was born at the very beginning of this century in the small town of Richtenberg, in the northern part of Germany, not far from the Baltic Sea. The rural environment of this place gave me ample opportunity to develop my inborn inclination as a naturalist. I was proud of my large collection of butterflies and moths, which contained representatives of almost all species found in this region. I took care of a terrarium of lizards and snakes and was happy when I discovered the pretty little salamanders, those creatures which later became the subject of my research. I dreamed of becoming the director of a zoological garden or, better still, the explorer of the remotest places of Africa and South America.

When World War I broke out in 1914, our family moved to the larger city of Stralsund, where my sisters and I could attend high school. I passed the grades without trouble, taking French and English for 6 years. But I did poorly in mathematics. Some of our teachers and several of my older school comrades were drafted to serve in the war, and I never saw them again. Eventually, the last class (Prima) I attended was closed altogether, and the remaining boys, including myself, were sent into the country to help the farmers harvest their crops.

Finally, in 1918 the war came to an end. But this was not at all the end of deprivations and shortages of all sorts. Nevertheless, the coming years were also the time when I attended the German universities. In the first 2 years, I became a student of natural sciences at the Universities of Rostock and Leipzig. Then I was attracted to the picturesque city of Freiburg im Breisgau and its mountains of the Schwarzwald, where one could enjoy hiking and skiing. I expected to find at the University of Freiburg the renowned naturalist Professor Doflein, as director of the Department of Zoology. To my dismay, I found that Doflein had died suddenly and had been replaced by Professor Hans Spemann, whose work I was not familiar with. Spemann was not a naturalist. Actually, he was one of the founders of experimental embryology. In his lectures, he dealt mainly with comparative anatomy, evolution, and Mendelian genetics. Experimental embryology was still too much in its infancy to be taught at the universities. There were, in addition, the assistant professors, Mangold, Geinitz, and Baltzer, who supervised the laboratory work of the graduate students. My doctoral thesis, sponsored by Spemann, concerned the early development of the liver in the frog embryo, a subject that was not of great interest to me or to Spemann.

Following my studies at Freiburg, as a young man of 25, I was given the chance to live for about a year in Italy, presumably with the aim of studying the marine fauna at the Stazione Zoologica in Napoli. There I met Hörstadius and von Ubisch, and they tried to get me interested in the development of sea urchins, but I did not catch on. Instead, I soon started making excursions into the beautiful and fascinating environment. How could I resist the temptation to visit Pompeii and Pozzuoli, to climb up the slope of Vesuvius, or to enjoy the magic beauty of Capri and Sorrento? And so, instead of diligently sitting behind the microscope, I absented myself more and more from the noble institute in the Villa Nazionale.

My excursions carried me farther afield. With little money in my pocket, I roamed all over Italy, took an all-too-superficial glimpse at the fabulous art treasures in Florence, Venice, Rome, and Naples, and, mostly on foot, I wandered through the remotest regions of Calabria and Sicily. I found this to be far more exciting than exploring the secrets of sea urchin development.

To me, this was a new stage of my life. You should realize that I had lived through the awful years of World War I and its aftermath, the period of utter bankruptcy of Germany. I had gone through the ordeal of producing a doctoral thesis of which I was (and still am) not very proud. Spemann was a remote person to me, and he did not think much of me as a prospective scientist; I myself was very insecure about my future role in society. Suddenly, here I was in Italy, the proverbial land of eternal sunshine, the land of *la dolce vita* and *far'niente*. No wonder, then, that I indulged in this newly found life of freedom and of unashamed, romantic sensuality. I took to writing poems and to painting pictures in oil and in watercolors.

I settled down for a long while in a small, picturesque village, called St. Angelo, which is situated on the rocky coast of Ischia. The place was quite inaccessible except by boat, and it had, at that time, not yet been discovered by the German tourists. The friendly people, who were mostly fishermen or had their vineyards above the houses, up on the flanks of volcanic Epomeo, accepted me as native son, known to everybody as Giovanni il pittore. (See Figs. 1–3.) This was the true paradise for a painter. But was I happy? *Die Heimat* was far, far away. The bitter truth was that I had exiled myself, that I lived in a dream, and that the dream had to come to an end. To soothe my bad conscience I painted for our little village church the holy angel, Saint Angelo himself—a blond youth in golden armor, slaying with his sword the fiercely squirming dragon, the incarnation of all evil.

Alas, my escapade into sunny Italy turned out to be a sad fiasco. I had lost contact with the scientific community, and I did not become a professional painter either. My good father, who had paid for my upkeep in Italy, had good reason to consider me a total failure. Staying then at my parents' home in Stralsund, I made some money by painting the portraits of some of the solid, respectable citizens of the town. I also participated in a real expedition to the remote tundras of Finnish Lapland, where I became acquainted with the last nomads of Europe, the Lapps, and their reindeer herds. This expedition had been organized by Professor Braun, a geographer at the University of Greifswald. I even published an entertaining article about Lapland, with drawings and colored pictures of mine in it.

Then I spent a few weeks on the desolate, windswept island of Helgoland. There I was the guest of an institute of fishery and marine biology. Since there was



**Figure 1.** “Two souls are dwelling in my breast.” (This self-portrait changes dramatically depending on which end the reader holds up.)

nothing else to do on this tiny island, I became a really devoted, hard-working student of marine life. I also became somewhat engaged in looking after the tanks of oyster larvae that were cultured in that institute. The person who had been in charge of the oysters was Mr. Erdmann, a former student of von Ubisch. He had been dismissed because he had succumbed to alcoholism, a disease that afflicted many inhabitants of that dreary, godforsaken rock in the midst of the stormy sea. Apparently, the director of the Institute was favorably impressed by my dedication to work. And that was it. There was no job for me in Helgoland.

And so, in desperation, I became again a university student, studying this time at Greifswald, an ancient little town close to Stralsund. There I worked for a diploma that would qualify me as a teacher at German high schools. I obtained the diploma with honors, but then I panicked. Oh my God, I thought, what a horrible prospect to spend the rest of my life as a schoolteacher!

So I ran away once more—now to Holland. There I stayed at the home of an



Figure 2. A street in Napoli.

old friend of mine, Nico van Harpen, a painter and art dealer, who lived a retired life in a pretty house in Laren. Meneer Nico, when I showed him some of my paintings from Italy, made only a few, rather noncommittal remarks about their artistic quality. However, more important to me was that he introduced me to somebody at the ministry of colonial affairs in Amsterdam. My secret hope was to find a position in tropical Java, at the famous Botanical-Zoological Garden in Buitenzorg (which, in English, means "Without worries"). But, of course, they had no place for me in Java. Ruefully, I returned to my parents' home and my old worries. Although schoolteachers were in great demand, I still had not the courage to apply for an appointment.



Figure 3. The happy painter.

Suddenly, things happened. To my surprise, I got a letter from Helgoland, in which I was asked whether I would accept the position of a caretaker of the oyster cultures. Yes or no? I was in a quandary. Immediately, I thought of Mr. Erdmann and his sad fate. I was afraid that that fate could easily befall me also. But I had no other choice, so I accepted the offer.

My suitcases were packed and I was almost on the way to Helgoland when another letter arrived. It came from Otto Mangold. He had become chairman of a department at the prestigious Kaiser Wilhelm-Institute (the present Max-Planck-Institute) in Berlin-Dahlem. He remembered me from our times in Freiburg, 4 years earlier. And he now offered me the position of an "assistant," a position that had been held before by Viktor Hamburger, who had returned to Freiburg.

Promptly, I took the train to Berlin and visited Mangold in his laboratory. I expressed to him my delight at being offered the opportunity to do research under his guidance.

Let the oysters take care of themselves; my future now lies in the amphibian egg! I promised Mangold to forget about my adventurous life style, and to walk in the footsteps of master Spemann. Our common task would be to delve into the mysterious machinations of the "organizer."

Here I must record a new dramatic twist of events. While I was still having a friendly chat with my prospective boss, the secretary came and delivered the mail. Among the letters there was one from Spemann. Mangold opened it and

read it, and made a long face. After some hesitation, he was frank enough to reveal to me the content of the letter. It concerned me, and the message was about as follows: Spemann had heard about my sitting unemployed in Stralsund and he had recently been approached by the director of the fishery institute in Helgoland, who asked him whether he could recommend me as caretaker of the oysters. Yes, he had recommended me. Now, he wanted to advise Mangold not to consider me as his research assistant, for, in his opinion, Holtfreter might do well as an outdoor biologist but not as a laboratory-bound experimental embryologist.

That sounded very bad. There was nothing for me to say. I realized that if this letter had arrived a few days earlier, Mangold would have faithfully followed this advice, and I would not now be sitting in his office. I knew that Mangold greatly admired—almost worshipped—Spemann and that he would never have done anything without the master's consent. It came therefore as a great surprise and relief to me, when, after having taken a long puff at his cigar, Mangold addressed me with these fateful words: "My dear Herr Holtfreter, I leave it to you. Do you want to go to Helgoland, or do you prefer to stay with me?" "Of course," I almost shouted, "I want to stay here in Dahlem and work with you." He nodded and we shook hands. This, then, was the end of my *Wanderjahre* and the beginning of my career as a scientist. This was 4 years after I had obtained the Doctor of Science degree.

I got along fine with Mangold. Altogether, our institute of biology was an ideal place of research, and I spent the most fruitful years of my life there. Like the other young "assistants"—Curt Stern, Joachim Hämmerling, Karl Belar, and others—who worked at the departments of professors Carl Correns, Richard Goldschmidt, and Max Hartmann, I was living in this same institute, in the attic, where each of us had a small room with just a bed in it. Consequently, we hardly ever left the institute and worked zealously in the laboratory, often until late into the night.

Dahlem was an attractive suburb of Berlin. There were still open fields around us, and not far away were the pine forests and lakes of Grunewald. We could reach the center of Berlin in half an hour by subway. Berlin of the 1920s had much to offer in the form of theaters, operas, cabarets, art exhibits, and other entertainments. But, as a country boy, I did not care much for the hectic and depersonalized life of Berlin and other big cities.

Mangold was a generous boss. He gave me full freedom to work on anything I wanted. Unfortunately, I was quite ignorant of what had been going on in developmental biology, and I had no definite plans. Then I remembered that our experimental work in Freiburg had been severely handicapped by the high mortality rate of our operated amphibian embryos. There was, for instance, Hilde Pröschold (who later married Mangold), with whom I shared the same work-bench. She had been given by Spemann the assignment to transplant the blastoporal lip of a *Triturus* gastrula into the belly side of another gastrula. This she did in several hundreds of cases, but only some five of them survived to the early tail bud stage, when they were fixed and prepared for microscopic study. It was then on the basis of these five barely differentiated specimens that the famous Spemann–Hilde Mangold paper was written (1).

Spemann himself had no remedy for this high mortality rate. Well, I found a

cure for it, and that was simple enough. Obviously, the denuded and defenseless embryos died of bacterial infection. Therefore, following the example of the medical profession, I proceeded to sterilize the instruments and glassware we used and to raise the embryos in a sterile medium. In addition, I devised an appropriate culture medium (2). Having observed that embryos with open wounds disintegrated quickly in plain water, even if the water had been sterilized, I reasoned that water was strongly hypotonic, hence toxic, for the embryonic cells. This led me to experiment with salt solutions of different concentrations until I found an apparently isotonic, balanced salt solution which turned out to be an ideal culture medium (my “life elixir”) for our experimental embryos, even for isolated fragments of the embryo. Under the label “Holtfreter solution,” it became a widely used medium for explantation experiments. It amused me later to observe that it was this solution, perhaps more than anything else, by which I became known among the embryologists.

These methodological improvements opened up new avenues of experimentation which could not have been pursued before. For instance, they enabled me to undertake extensive investigations of the differentiation capacities of small fragments that had been excised from frog or salamander gastrulae and then cultured for long periods in this medium (3,4). From these experiments, a number of important conclusions could be drawn concerning the state of determination of the various parts of the early gastrula.

During my sojourn in Dahlem (1928–1933), I was indeed a prolific worker, tackling simultaneously a variety of problems and publishing a long series of papers, most of which dealt with questions of embryonic induction, cellular differentiation, morphogenetic regulation, and an analysis of the complex organizer phenomenon. The horizon that had been opened up by Spemann’s experimental work was so vast and uncharted that whatever you did in this field was novel and exciting, and in those early days, there were hardly any workers outside the Spemann group to compete with.

Finally, I may entertain you with a historical note, which concerns my personal involvement in the frantic efforts of a handful of researchers to discover the nature of the factors that induce the formation of the neural plate.

It all started with the pioneering publication of Spemann and Hilde Mangold (1) in which Spemann speculated that there are possibly two different ways by which the organizer exerts its neuralizing effect on the reacting ectoderm: either it operates by producing a chemical agent that is transmitted to the ectoderm, or it acts by way of its very physical “structure.” Spemann never explained what he meant by “structure.” It seemed to me, however, that the hypothetical structure had something to do with the axial polarity and the living state of the so-called organizer (represented by the primordial chorda-mesoderm). Spemann argued that if this structure is destroyed, that is, if the organizer tissues are devitalized by crushing or by extreme heat or cold, they would lose their inducing (and organizing) capacities.

In the late 1920s, Spemann himself and, later, Krämer, a student of his (5), tested this hypothesis. They found that organizer tissue that had been thoroughly crushed and then implanted into a live gastrula had lost inductive capacity. This seemed to indicate that it is indeed some living “structure” and not a chemical agent which is at the basis of the induction phenomenon.

In 1931, at a meeting of the German Zoological Society held in the beautiful city of Utrecht, Spemann lengthily reported on his negative results with crushed blastoporal lips. I attended the meeting and, like so many others in the audience, was rather disappointed. I thought: Is this the end of all endeavors to analyze the mechanisms of neural induction? I could not believe it. Spemann's metaphysical concept (6) of the organizer as being a vitalistic agency, a sort of planning and disposing manager who instructs and organizes the adjacent, as yet undetermined, tissues, did not appeal to me. Would it not be more reasonable to assume that the chorda-mesoderm primordia operate somewhat like the endocrine glands which affect the differentiation and growth of other tissues by means of chemical messages?

Here was a problem of fundamental importance that cried out for a solution. I decided to do something about it myself. When I returned to Dahlem, I immediately began to work on this project.

An isolated piece of blastoporal lip (or other tissues) was devitalized by boiling, freezing, or desiccation and then placed in contact with fresh gastrula ectoderm. The contact was brought about in three different ways: (1) Following the customary procedure of testing the inductive capacity of an embryonic tissue, the killed tissue was implanted into the blastocoel of an intact gastrula. (2) The piece was wrapped into a mantle of isolated pure ectoderm, and this explant was cultured *in vitro*, a method that later became known as the sandwich method. (3) The isolated blastoporal lip (or other tissues) lying at the bottom of a glass dish were desiccated at 60°C. The dish was then filled with my new culture medium and an explant of living ectoderm was loosely placed on top of the rehydrated dead tissue. Thanks to the sterile methods employed, the mortality rate was extremely low.

### 3.2. Experimental Results

In all three experimental series, the killed tissues invariably induced in the adjacent ectoderm large masses of neural tissue. In some instances, especially in experiment No. 1, the neural tissue was "organized" so as to form a complex brain and typically differentiated eyes. Here, then, was clear evidence that heating, boiling, freezing, or desiccation did not abolish—or even diminish—the capacity of the chorda-mesoderm to elicit in the adjacent ectoderm massive neural formations which could be just as highly organized into brain and eye structures as those that had been induced by an implanted live organizer. Of further interest was the finding that the various devitalizing treatments converted the normally noninducing ectoderm and endoderm into neural inductors.

These results constituted a heavy blow to Spemann's vitalistic concept of "the organizer," for they indicated that the chorda-mesoderm tissues, alias the organizer, are merely the source of certain chemical substance(s) that initiate neural differentiation in the responding ectoderm. From these and other data (3,4) obtained later by myself and Chuang (7,8), a student of mine, it could be inferred that the organizer tissues do not actually organize the cell material whose new trend of differentiation they have induced. Rather, the induced cells organize themselves into complex organs. Therefore, in later discussions of this issue, I went as far as to declare the term "organizer" to be a misnomer.

I maintain, however, that there is sufficient evidence in support of the notion that the chorda-mesoderm area has the properties of a self-regulatory "morphogenetic field." Substantial evidence for this concept has come from my wife, Hiroko's, doctoral thesis, which has unfortunately not yet been published.

Now the question arose what to do with these revolutionary data? I had not behaved like a faithful disciple of Spemann; I had failed to tell the master what I had been doing lately, and doing it without his explicit consent. Now Mangold, who so far had been a benevolent observer of my work, urged me to communicate these findings to Spemann. This I did with some trepidation. Spemann applauded but did not actually congratulate me. He pointed out in his letter that some 3 years earlier, Hermann Bautzmann had been given the green light to work on heat-killed inductors. Although Bautzmann had as yet not published anything about this work, I should consider his right of priority and should not publish my findings until I had discussed them with him. So I traveled to Kiel to see old friend Bautzmann, carrying with me some of my prettiest microscopic slides. When he examined these slides, he wondered how I had been able to raise my experimental material up into highly advanced stages of differentiation. So I told him about my new culture method. He, however, had been plagued by the old trouble: His operated embryos had usually perished before they had reached the tailbud stage. Therefore, as to the effect of heat-killed organizer, his results were not very impressive. There were only a few cases which, at best, showed the induction of small lumps of barely differentiated neural tissue, but there were none of the massive brain formations I had obtained. It was a friendly meeting. We agreed to publish our findings jointly.

In the meantime, Mangold had been busy. My experiment No. 3 had indicated that the neuralizing agent is water-diffusible. Mangold now had the idea to let the hypothetical agent, derived from an isolated piece of inductor, diffuse into a piece of agar and then implant this agar into the early gastrula. He did not have time to make more than a few preliminary experiments of this kind.

Then Spemann, whom I had informed about my agreement with Bautzmann, expressed his wish to join us and to report on some pertinent finding of a young student of his, Else Wehmeier.

Thus it came to pass that the four-man communication on the inductive mechanism in embryonic development saw the light of publication (9). In this communication, Bautzmann cautiously reported on a few cases which he, after having seen my more convincing preparations, would interpret as showing neural inductions caused by heat-killed organizer implants. Spemann reported on a single case of Wehmeier's in which she had obtained the induction of neural tissue by application of an alcohol-killed implant of organizer tissue. Mangold briefly reported on his afore-mentioned experiments with agar. But his claim that he had obtained positive results remained questionable (this claim has never been confirmed by Mangold himself or by others).

In this short article, I could report on hundreds of well-documented cases of neural induction obtained with the methods I have outlined above. In subsequent, more elaborate accounts (10,11) of these findings, I also reported that all parts of the unfertilized *Triturus* egg, when boiled and solidified, can induce neural tissue, and that the neuralizing capacity of the various parts of the gastrula is not abolished by treatments with ethanol, HCl, xylol, or boiling ether. The

neutralizing agent proved to be extremely heat-stable, but dry heat above 140°C inactivated it.

This joint communication of four authors (9) heralded the beginning of a new era of research which may be titled: In search of the chemical constitution of the inducing agent(s). This quest expanded into a drawn-out, arduous, often tortuous campaign in which, at one time or another, investigators from all over the world became engaged. The campaign, which began in a mood of great expectations, lasted for some three decades, then petered out and ended, in the 1960s, on a note of despair and resignation. Today there is barely anybody around who is still active in this once so exciting field of research.\*

Spemann, Mangold, and Bautzmann discontinued working along these lines. Miss Wehmeier became associated with a biochemist, F. C. Fischer, and his co-workers; this "Freiburg group" was in the forefront of the campaign for a short time only and then disappeared from the scene. A harsh competition arose between this group and the "Cambridge group," whose leaders were Waddington and Needham. The latter worked for a spring season at our institute in Dahlem. As the news about inducing substances spread, investigators from other parts of the world entered the arena. To mention only the most prominent ones, these were: the Finnish workers Toivonen, Saxén, and Kuusi; the Americans Barth and Niu; the Belgian biochemist Jean Brachet; the Japanese Yo Okada, Yamada, Hayashi, and Kawakami; and, last but not least, Heinz Tiedemann and his wife, from Mangold's laboratory.

By now, many of the old-timers have died; others, like myself, have moved off in new directions, and the younger generation of biologists has been lured away into the more fertile grounds of modern molecular biology. Unfortunately, the old and fundamental problems of ontogenesis are still waiting to be resolved. What went wrong with the campaign?

It was perfectly legitimate and desirable to approach the problems of induction with the tools of biochemistry that were available in those times, but the problems turned out to be far more complex than had been anticipated, and the methods employed were not versatile and discriminative enough to bring them closer to a solution.

There was, first, the task of characterizing the constitution of "the inducing agent," that is, the agent thought to be emitted by the chorda-mesoderm district (the organizer) and to "induce" the adjacent ectoderm to develop into neural plate and its various neural derivatives. This task has unfortunately never been accomplished. The nature of the factor, or factors, which, in the normal embryo, brings about the observed transformation of the ectoderm into neural tissue or other tissues has as yet not been determined. Instead, experimental work has revealed that there is a great diversity of foreign tissues, chemical substances, and environmental conditions which can perfectly imitate the action of what we may refer to as the "genuine" inducing agent. The story, as I see it, runs as follows.

Earlier work of Spemann, O. Mangold, and Holtfreter had left no doubt that the ectoderm is incapable of differentiating into neural tissue and any of its other derivatives unless it is subjected to the inductive action of the subjacent

\* Certainly, much more work is going on in this area now than when Dr. Holtfreter wrote this letter!

chorda-mesoderm. Experiments of Mangold (12) had shown that whereas the gastrula ectoderm itself does not have the properties of a neural inductor, this same cell material acquires neuralizing capacities as a result of its differentiation into neural plate. A piece of neural plate that had been implanted beneath the ectoderm of a gastrula induced the latter to form another neural plate (homogenetic induction). Strangely enough, subjection to all sorts of devitalizing treatments (heating, freezing, alcohol) also gave neuralizing capacities to the ectoderm (Holtfreter). These treatments seemed to have liberated or activated the neuralizing agent which had existed in the ectoderm in a masked or inactive condition. The situation became still more perplexing when I discovered (13) that neuralizing agents (sometimes also mesodermizing agents) are present in practically every tissue of all animals—vertebrates and invertebrates—that were tested (including various tissues from a human corpse).

These findings raised many new questions which I cannot discuss here. From a practical point of view, this discovery of the ubiquitous occurrence of neuralizing (and other) agents in all kinds of foreign tissues (that are readily available in large quantities) was, of course, of great importance. Now, in order to isolate and chemically identify the agent, or agents, it seemed advisable to leave the precious little embryos alone and choose instead foreign tissues, such as calf liver or mouse kidney, as the source from which to extract the agent.

Thus, several groups of workers, using different kinds of foreign tissues and different methods of extraction, proceeded along these lines of approach. Among the various fractions that were extracted, there was one set of substances, generally described as proteins or nucleoproteins, which, when applied to the amphibian embryo, elicited conspicuous neural formations. This was an interesting result, but was it permissible to conclude that the neuralizing agent which is normally employed by the embryo is likewise a nucleoprotein or protein?

Another line of approach consisted in applying to the ectoderm substances of a chemically well-defined composition. Surprisingly, a great diversity of substances that were not related to nucleoproteins or proteins and which were unrelated to each other were found to be likewise capable of converting the ectoderm into neural tissue. For example, neuralizations have been obtained with the application of the following compounds: fatty acids (Fischer et al.); steroids, various polycyclic hydrocarbons (Waddington, Shen); alloxan, cysteine, cystine (14); cyclic AMP derivatives (15). Less well documented was the claim of Okada that Fuller's earth can act as neural inductor and the claim of Barth that cephalin and digitonin have neuralizing capacities. The assertions of Brachet and Niu that pure RNA can neuralize the ectoderm seem to have been definitely refuted.

Here again, the question arises: what is the significance of these data with reference to the genuine process of neural induction? Clearly, not all experimentally effective substances can be expected to be engaged in this process. Indeed, it is sheer guesswork which, if any, of them plays a role in normal embryonic induction.

The situation became still more confusing when I found (16) that explants of gastrula ectoderm of *Triturus torosus* which when cultured in Holtfreter's solution develop into epidermis, will, in part, differentiate into neural tissue when they are subjected to a brief treatment with calcium-free salt solution, with high

alkalinity, 10% alcohol, or distilled water. In the same breath, I should mention the subsequent experiments of Barth and Barth (17,18) and others which dealt with the effect of various ions on development and, especially, with the conversion of ectoderm into neuroblasts and other cell types under the influence of LiCl. Of particular interest was that in these experiments no particulate inducing substances were employed.

The above results of mine indicated that the treatments merely operated like an unspecific trigger, setting in motion a preexisting, pent-up mechanism which, through unknown chains of events, led to neural differentiation. I therefore referred to this phenomenon as "autoinduction." However, how could it be explained that such a diversity of environmental interferences elicited the same end-result—neural differentiation?

Perhaps, so I thought (19), the situation is analogous to the one in experimental parthenogenesis of the sea urchin egg, where a variety of exogenous chemical and physical stimuli can imitate the action of the penetrating spermatozoon, namely, to activate egg development. What these activating agencies seem to have in common is that they change the structure and permeability of the plasma membrane. This, in turn, would entail the same kinds of physiological and morphogenetic chain reactions that occur in the normally fertilized egg.

There were signs that, in a comparable way, the aforementioned neuralizations of ectoderm explants by means of a shock treatment with 10% alcohol, distilled water, high pH, or Ca-free salt solution were likewise based on a common mechanism. All these treatments primarily affected the integrity of the plasma membrane. The cells lost adherence to each other, they swelled because of uptake of some of the ambient salt solution, and they would have suffered irreversible cytolysis if the treatments had been continued for a longer period of time. It was—so I argued—this brief exposure to injurious environmental conditions which, among other dissociative processes, caused a liberation or activation of a cell-intrinsic physiological process that steered the cells, after their recovery from the shock, into neural differentiation.

All this is, of course, speculative, and these considerations are of no help if we wish to connect these experimental data with what goes on in the natural induction process of the embryo.

It seems unlikely that in the process of natural induction any of the exotic, though experimentally effective, agents, such as alcohol, distilled water, high alkalinity, or LiCl, are actively involved, or that, normally, neuralization is preceded by the kind of precytolytic shock reaction that was observed in the foregoing experiments. However, as already noted, we are not certain either whether any of the other artificially effective substances, such as fatty acids, steroids, alloxan, and so on—and, for that matter, nucleoproteins—are operating as normal inductive agents. To repeat: we do not know the nature of the normally active neuralizing agent. (See Figs. 4 and 5.) The trouble is that in all those years, when the hunt for the mysterious inductive substances went on, the real problems of induction as they are posed by the embryo were almost totally neglected. It is true, we have learned that neuralizing agents are water-diffusible, but this information is quite irrelevant since, normally, the inducing and the reacting tissues are so tightly applied to each other that the hypothetical inducing agent can pass directly from cell to cell. We have no idea of the molecular machinery



Figure 4. "Time is ill."



Figure 5. "The worries of an embryologist."

that is set in motion nor do we know what "competence" means in cell-physiological or molecular terms.

In our discussions of the mechanisms of induction, we should not lose sight of the fact that not only the entire neural system, but also all the other tissue derivatives of the ectoderm, are likewise products of induction. I should mention further that the derivatives of the neural crest, such as the cartilage of the visceral skeleton, the pigment cells, the dentin tooth papillae, and the mesenchyme of

the tailfin, owe their existence to inductive stimuli. I, therefore, maintain that embryonic induction is the most important device by which differentiation comes about in amphibian development.

Here would be the place where I, as a chronicler, should delve into the involved story of the “mesodermizing agent” and all the conceptual implications related to it. But enough is enough. Some day I may consider writing a book myself. At the moment I am busy preparing for print the vast body of observations I have collected on the slime mold *Physarum polycephalum*. I am in good health but greatly handicapped because the lenses of both my eyes have been removed and replaced by plastic lenses, with the result that my reading capability has become very poor.

Gradient theories have, I think, a legitimate place in sea urchin development and, with less justification, in attempts to describe polar differences in the regeneration of hydrozoa and flatworms. But I do not know of any solid material foundation for the assumption that gradients of any sort are present or operating in amphibian development. There are quite a number of such gradient theories as applied to the amphibian egg or embryo—those of Child, Dalcq-Pasteels, Yamada, Toivonen-Saxén, and, more recently, Wolpert. The authors differ greatly as to the nature and the localization of their gradients. In my opinion, none of these gradients exists in reality—at least, their presence has not been proven by biochemical, physiological, or morphogenetic data. To be sure, there is a gradient-wise distribution of the yolk platelets and the pigment granules in the frog egg, but this animal–vegetal distribution pattern has no distinct morphogenetic significance. In the gastrula there are also regional patterns as regards prospective significance, range of developmental potencies, and induction capacity, but these patterns do not exist in the form of linear or polar gradients. To speak, in connection with induction phenomena, of vegetalizing or animalizing factors or processes (or of “positional information”) is empty rhetoric; these terms explain nothing and do not even serve as useful working hypotheses. Perhaps the most fundamental problem of embryology is: how do cytoplasmic patterns of potentialities and of actual differentiation come about and what is their relation to the genetic information?

### 3.3. Biographical Notes

In 1933, our department of experimental embryology in Dahlem was dissolved. Otto Mangold became Ordinarius of the Zoological Institute in Erlangen, and I was called to München, where Karl von Frisch was the head of a large and beautiful Institute of Zoology. Von Frisch was a wonderful person whom I greatly admired.

With my avalanche of publications in the early 1930s, I suddenly became famous, even making headlines in the daily newspapers. I was invited to give talks at the various Dutch universities, at Brussels, Cambridge, and, later, at Santander (just before the Spanish Civil War started). These were great experiences. But the climax came in the years 1935–1936. Then, thanks to a Rockefeller Fellowship, I spent a year in the United States, staying at Harrison’s institute in New Haven, at the Carnegie Institute in Baltimore, and for a while at Woods Hole.

I must confess that, scientifically, I profited little from this visit to America. But I had the opportunity to visit the magnificent landscapes of Arizona and California, and it was a pleasure to meet so many friendly and interesting people.

Instead of returning to Germany right away, I traveled westward all around the globe on a leisurely voyage of about half a year. At that time, commercial airlines did not yet exist, the pretty places on earth were not yet overrun by tourists, and the big nations lived in an interim of peace.

My trip to the Orient was most generously financed by an unknown German private foundation, the Gwinner Stiftung. I could tell a long and amusing story of how I obtained this support. It amounted to a ticket, first class, all around the world, and generous daily allowances in addition—no strings attached to it, no obligation to do research or to write a progress report, no account to be given of how I spent or did not spend all that money. My benefactor, Dr. Gwinner, wished me to consider this trip as part of my general education. I felt as though I had become the prince in a fairy tale.

This trip brought me through the following places: the paradisian Hawaiian Islands; Japan, before its industrialization (Tadao Sato and Hidemiti Oka and their wives, whom I knew from Berlin, were my cherished hosts); from there rather brief visits to the strange cities of Macao, Shanghai, Canton (China was at that time not yet invaded by the Japanese); then to Saigon, Cambodia (the fantastic temples and palaces of the ancient Khmers in Angkor!); by bus to Bangkok, another wondrous place; and then by train down the peninsula to Kuala Lumpur, Penang, Singapore, with several stops in between to enjoy the mountainous landscapes, the tropical jungle, and the charming Malaysian people in their peaceful villages along the seacoast. Then I traveled through Java, climbed, by horse, up to the summit of one of the gigantic volcanoes, and also made a trip to the place I had once dreamed of and which I could now see with my own eyes, namely, the famous botanical garden of Buitenzorg. Now, indeed, I was a happy man, *ganz ohne Sorgen*.

But the most happy time of all my life came when I stayed for some 2 months on the island of Bali. To live with those gentle brown-skinned “natives,” who lived in peace with each other, in harmony with a gorgeous nature that was not spoiled by the contraptions of Western civilization, and who knew how to please their gods and to subdue their demons—all this was to me an exhilarating experience. Being accepted as friend, I stayed much of the time at their family campongs, in different villages. The Balinese have a unique sense of beauty and artistic expression. I had the opportunity to see much of their ancient cultural festivities: the temple festivals, the dramatic or comic theater plays, the divine dance performances, with the gamelan music in the background, the nightly long-lasting shadow plays and the other rituals and joyous celebrations connected with a wedding or a funeral.

I hurry to come to the end of this so-called letter, the longest letter I ever wrote in my life. Back in München, back to my activities at the university, I encountered an entirely different world, a very ugly world in which decency, mutual trust, and Christian love were replaced by brutality, intolerance, and fear. Hitlerism was triumphant; it celebrated orgies of maniacal furor. I was full of hatred and disgust against the regime, but I felt helpless. I knew that I was spied on and that sooner or later the Gestapo would get hold of me. I saw the war

coming. In 1939, shortly before the war started, I managed, "by the skin of my teeth," to escape from Germany. Thanks largely to Joseph Needham, I found a refuge in Cambridge, England. Then, when the Germans made preparations to invade England, I was interned and, together with thousands of other German refugees, I was shipped to Canada, where I lived behind barbed wire for almost 2 years. Released from internment in 1942, I found another asylum at McGill University in Montreal. There, supported again by a Rockefeller fellowship, I took up my research work once more until, in 1946, I was called to Rochester.

And here I am, still in Rochester, although for many years a retired professor. I am happily married to Hiroko, who has the position of a research associate with a young and promising scientist who works on questions of immunology. Maybe, sometime, we can pull ourselves together and visit once more Hiroko's homeland, Japan.

During the past few decades, interest in the problems of induction has faded away. The probable reason is the failure of the workers in this field to chemically characterize the nature of the naturally occurring inductive agents. Let us hope that the new generation of embryologists will find a way out of this dilemma.

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## Chapter 7

# The Conceptual and Experimental Foundations of Vertebrate Embryonic Cell Adhesion Research

GERALD B. GRUNWALD

Oh that this too too solid flesh would melt,  
Thaw and resolve itself into a dew!  
*Hamlet, Act I, Scene II*

### 1. Introduction

As embryology underwent its transformation from a descriptive to an experimental science, new research programs began to seek a causal understanding of the striking morphogenetic changes that had been observed to occur during embryogenesis. Whether these underlying morphogenetic forces were intrinsic properties of individual cells or whether they were some holistic property of the embryo *in toto* was a question fueled by several sources. On the one hand, His (1) hypothesized that morphogenetic movements occurred as a result of unequal growth among different populations of cells which led to bending and stretching of tissues, much as pushing and pulling on a sheet of some deformable material could lead to the production of complicated shapes. Roux (2), on the other hand, claimed to have observed specific positive and negative cytotropisms among populations of individual cells isolated from early amphibian embryos. The issue of developmental regulation as a cell-autonomous property, as opposed to a holistic expression of special attributes of embryos, was further exacerbated by the disparate interpretations of Roux (3) and Driesch (4) regarding their experiments on the developmental fate of isolated amphibian and sea urchin embryo blastomeres. While Roux's interpretation of independent cellular development in these specific cases were not supported by later investigation, his methods nevertheless served as a potent stimulus for further investigation on the individual cellular behaviors that contributed to the overall program of morphogenesis.

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A century later, His's morphogenetic models of rubber, paper, and clay have been supplanted by a number of theories which attribute the forces that shape the embryo to cell interactions mediated by discrete molecular entities, the cell adhesion molecules, whose structure and function are currently the target of intense investigation at the cellular, biochemical, and molecular genetic level. Indeed, the analysis of cell adhesive interactions and the identification of the underlying molecular mechanisms have grown from a zoological curiosity to an immense field of research which addresses problems in embryology, immunology, cell biology, pathology, oncology, neuroscience, and evolution. Several factors appear to have contributed to this explosive growth in cell adhesion studies. Progress in the general fields of membrane biology, protein biochemistry, immunology, and molecular genetics have enhanced both our conception of the cell surface and our ability to manipulate it. With the gift of hindsight not available to pioneers in the field of cell adhesion, one might today identify the two most fruitful and widely applied approaches to the identification of cell adhesion molecules as manipulation of the cell surface via immunological probes and determination of the role of calcium ions in mediating adhesive cell properties. The historical background to these two approaches is highlighted in this paper, focusing on the conceptual and experimental paths linking turn-of-the-century experiments on the regenerative properties of moribund poriferans with current studies on the molecular genetics of cell adhesion molecules. Other reviews of historical interest (5–7), reviews of recent advances in cell–cell adhesion (8–10), and reviews that extend to related areas such as cell–substrate adhesion and specialized cell junctions (11) are available.

## 2. The Invertebrate Zoological Roots of Intercellular Cell Adhesion Studies

In his paper “On some phenomena of coalescence and regeneration in sponges” (12), marine biologist H. V. Wilson describes the original observations that led to the studies often cited as the first experiments in the field of cell adhesion. Wilson observed that when marine sponges were kept for extended periods in an aquarium, they entered a degenerative state in which the loose tissues were easily teased apart into a cell suspension. The isolated viable cells could be recombined and reformed functional sponges. Wilson later discovered that it was not necessary to allow the sponges to enter into a degenerative state in order to obtain cells for his experiments. Rather, cells could be obtained in quantity from fresh normal sponges by straining them through bolting cloth, a method that Wilson himself described as rough, but effective. Wilson went on to describe how these clusters of fused cells combine into larger masses and ultimately form small, functional sponges. Thus by expression through a fine silk cloth was the field of cell adhesion born. While many embryologists would later cite this work as one of the foundations of the field of embryonic cell adhesion studies, Wilson himself did not see it this way. Wilson, in fact, distinguished his sponges from the embryos of higher organisms, remarking that his sponges would regenerate with

no definiteness of size or shape. . . . as a tiny lump may metamorphose into a sponge, or may first fuse with many such lumps, the aggregates also producing a sponge

although a larger one. In a word we are not dealing with embryonic bodies of complicated organization but with a reproductive or regenerative tissue which may start on its upward path of differentiation in almost any desired quantity (12).

However, Wilson does note that sponge larvae may be fused and will develop into sponges of large size but normal organization. The regulative capacities, whatever their biological basis, may extend between embryos and adults. Thus, "even where the embryonic bodies of sponges have a fixed structure and size, as in the case of the ciliated larvae, the potential nature as displayed in later development is not fixed in the matter of individuality" (12).

A major theme developed in the field of cell adhesion, to be discussed further later, is that cells not only possess adhesive qualities, but that the adhesion between cells is selective. Wilson's 1907 paper is also cited as an experimental foundation for the analysis of adhesive selectivity. However, it is clear from Wilson's writing that he did not see his observations as the cornerstone of a new science. Indeed, Wilson introduces his experiments almost apologetically:

I shall here briefly record some experiments which gave only negative results but which under circumstances admitting of a wider choice of species, ought to yield returns of value. These experiments were based on the assumption that if the dissociated cells of a species will recombine to form a regenerative mass and eventually a new sponge, the dissociated cells of two different species may be made to combine and thus form a composite mass bearing potentially the two sets of species characteristics. It is clear that such an organism would be analogous to one produced by an association of the blastomeres of the two species. Pending the successful carrying out of this experiment, it would be idle to discuss further the nature of the hypothetical dual organism (12).

Wilson briefly describes his experiments in which dissociated sponge cells were obtained from three different species with different coloration, allowing for recognition of the distinct cell types on a species basis. As in his earlier experiments, the cells went on to reconstitute sponges, but only within a species and not between species, thus forming a suspension of small sponges of each distinct type. Wilson clearly saw this as a failure, stating that "the more promising task is however to find allied species and subspecies, the regenerative tissue of which will combine under natural conditions." No mention is made of the possible physiological basis for this lack of interspecies coalescence, and its analysis is never mentioned as a worthy endeavor. The nature of the ability of similar cells to fuse is regarded not as a property of the cells as units, but rather as a holistic property of the protoplasm of which the species is composed. Indeed, Wilson believed that aggregates of sponge cells formed a syncytium, and that redifferentiation occurred, rather than any sorting out of already determined cell types, as would be later advocated by Galtsoff (13).

Little further light is shed on the subject of sponge cell adhesion or its biological significance by Wilson's later and more complete report (14), and it would be almost two decades after his initial study before the phenomenon would be reexamined in any detail by Galtsoff. Historians of science have noted that Wilson's observations of adhesive selectivity did not seem to excite much interest among embryologists at the time, perhaps because they were made "too soon" to be appreciated (15), or perhaps because phenomena of reconstitution and regeneration were thought to be more relevant to the biology of lower organisms than to the embryos of vertebrates (7). It may rather have been Wilson's negative presentation of his results, which clearly emphasized his interest in the

generation of heterospecific chimeras rather than in the utility and significance of the species-specific sorting out of cells, that may have failed to ignite greater interest in his observations. A further reason for a lack of appreciation of these cellular behaviors, and any appreciation of the role of the cell surface in mediating them, may have been due to the lack of focus on the cell surface as a distinct entity worthy of study in animal cells. In early writings, the term “cell membrane” was used to refer to the cell wall, originally identified in plants during Hook’s initial description of the cellular basis of living matter. The embryologists of the time, who for a generation learned their cell biology from E. B. Wilson’s “The Cell in Development and Inheritance,” could read:

From a general point of view the cell membrane or intercellular substance is of relatively minor importance, since it is not of constant occurrence, belongs to the lifeless products of the cell, and hence plays no direct part in the active cell life. In plant tissues the membrane is almost invariably present and of firm consistency. Animal tissues are in general characterized by the slight development or absence of cell walls. Many forms of cells, both among unicellular and multicellular forms, are quite naked, for example amoeba and the leukocytes; but in most, if not in all, such cases, the outer limit of the cell body is formed by a more resistant layer of protoplasm. . . . that may be so marked as to simulate a true membrane, for example, in the red blood corpuscles and in various naked animal eggs (16).

As Baker noted in his series on the history of the cell theory:

A clear picture of the nature of the cell could not be obtained so long as the wall was regarded as an essential part. It was necessary to realize that the wall was sometimes present and sometimes absent, while the cell itself was always bounded by a special membrane, not mechanically separable from the ground-cytoplasm within. This advance could not be made in one step. It was necessary to first discard the cell wall as unessential to the idea of a cell, which was then looked upon as consisting of naked protoplasm. The discovery of the cell membrane came much later (17).

Even in the third edition of Wilson’s book, by which time the plasma membrane had been so named and recognized as a distinct entity common to all cells (18), the cell surface received scant more attention than it did in 1896. The evolution of the concept of the cell surface will be returned to later.

In any case, it was almost two decades after the initial observations of Wilson that more pointed questions with respect to the cell biological and embryological relevance of sponge cell adhesion were asked by Galtsoff (13). Commenting on the phenomenon described by Wilson and others, Galtsoff raised questions that harkened back to Roux:

The phenomenon opens up several questions of general interest, both physiological and morphological. First, is the approach of separated cells to one another due to some kind of directive force (chemotropism, cytotropism), or is their aggregation and coalescence merely a result of accident? Second, what is the behavior of various cells after their dissociation and to what extent may it be affected by external factors (13)?

The two key advances evident in Galtsoff’s work were, first, analysis of the effects of various conditions on cell reaggregation and, second, development of an assay to quantitate these effects. These themes recur often during the advance of cell adhesion studies toward a mechanistic understanding of the phenomenon. Galtsoff investigated the effects of temperature, osmotic pressure, altered substrates, altered sea water composition, and effects of various monovalent and

divalent cations, pH, and the presence of foreign bodies and cells from other species on sponge cell aggregation. For quantitation, Galtsoff simply recorded the number of aggregates per unit area of his cultures. It is of interest that Galtsoff acknowledges the interest and criticism of both E. B. Wilson and T. H. Morgan, whose embryological inclinations may have helped turn Galtsoff toward the questions he addressed. Among Galtsoff's major conclusions were that cells coalesced through active amoeboid movements, that these movements were slowed by low temperatures, and that calcium was the most effective cation at promoting coalescence. Galtsoff observed that cells of different species, when mixed together, would come in contact as a result of amoeboid movement but would not stick together. These observations were one more nail in the coffin in which Roux's cytropisms would be buried.

Galtsoff, in considering the nature of specific molecular forces that could underlie cell adhesion of the sort he was analyzing, questioned the view of Leo Loeb (19), who suggested that tissues are formed by the agglutination of cells. Galtsoff argued that cell adhesion occurred due to properties of the outer protoplasm, but argued against L. Loeb's view due to the lack of evidence for any external factor such as is required to cause agglutination, as in agglutination of bacteria by immune sera:

I think the coalescence of cells which occurs as a result of their active ameboid movement is a phenomenon quite different from agglutination. The latter suggests the presence of a specific agglutinin, while no such substance exists in a suspension of separated cells. Besides ameboid movement, the formation of aggregates from separated cells is due to the adhesive properties of their protoplasm. Apparently the protoplasm was sticky before the separation of the cells and retains this property in a new environment. Accordingly, the cells stick and coalesce as soon as they have a chance to touch one another. There is no reason to suggest the existence of a specific substance like agglutinin that would cause the coalescence (13).

This view of Galtsoff's is surprising in light of the work of Lillie, who had been investigating the mechanism of fertilization in sea urchins. Galtsoff was aware of Lillie's work, but his only reference to it is in citing Lillie's admonition against confusing aggregation and agglutination. Galtsoff, like Lillie, used "agglutination" to refer to the adhesion of living cells by specific and nondestructive mechanisms, while Jacques Loeb used the term to include events such as the clumping of cells by alkali treatment. Galtsoff preferred the term "aggregation" for the activities of his sponge cells. This confusion over terminology reflects a more deeply seated controversy between Lillie and J. Loeb. As Gilbert and Greenberg pointed out (20), these two investigators fell on different sides of the "structuralist-physicalist" boundary, in that Lillie believed the specificity of sperm-egg binding occurred due to the interaction of complementary cell surface macromolecules, while J. Loeb (21) emphasized the role of physicochemical interactions of smaller molecules. Lillie's studies of the interactions of sea urchin egg and sperm led to the conclusion that the eggs possess and can secrete a substance that agglutinates sperm, and that the species specificity inherent in sperm-egg adhesion was mediated by these substances. Lillie interpreted his observations in light of emerging immunological concepts of antibody-antigen interactions:

The existence of sperm isoagglutinins in ova offers the possibility of an explanation for the specificity of fertilization on the basis of the laws governing antigens and antibodies, if these agglutinins are specific, as is so strongly suggested by the experiments. The union of ovum and spermatozoon is not a process in which the sperm penetrates by virtue of its mechanical properties, but one in which a peculiarly intimate and specific biochemical reaction plays the chief role (22).

Lillie elaborated his model in a later publication (23), drawing heavily on Ehrlich's side-chain theory of antibody formation, and hypothesized that sperm–egg interactions were dependent on lock-and-key binding of cell surface macromolecules. During this period, work in neurobiology also led to proposals that cell surface receptors played a critical role in cell recognition phenomena. Regarding his studies on the development of nerve fibers, Harrison concluded:

There is nothing in the present work which throws any light upon the processes by which the final connection between the nerve fiber and its end organ is established. That it must be a sort of specific reaction between each kind of nerve fiber and the particular structure to be innervated seems clear from the fact that sensory and motor fibers, though running close together in the same bundle, nevertheless form proper peripheral connections, the one with the epidermis, the other with the muscle. . . . The foregoing facts suggest that there may be a certain analogy here with the union of egg and sperm cell (24).

This view had been espoused earlier by Langley, whose studies of axon growth led him to conclude that “there is some special chemical reaction between each class of nerve fiber and each class of nerve cell, which induces each fiber to grow towards a cell of its own class and there to form its terminal branches” (25). Langley’s later studies of the specificity of drug–muscle interaction led him to conclude that such specificity resided in the cell surface and that it arose during ontogeny:

It follows then that there are considerable differences in the receptive substance on different muscles. And it seems to me probable that we must regard the embryonic muscle protoplasm as forming several receptive substances responsive to different chemical stimuli. . . . The varied effects produced by poisons show that the receptive substance varies in different cells (26).

The works of Ehrlich, Lillie, Harrison, and Langley, as pointed out by Gilbert and Greenberg (20), are remarkable in that they contained the germs of several concepts that form the basis of much modern research, including macromolecular stereocomplementarity, hormone–receptor interaction, allosteric control of protein function, cell-type–specific recognition, transmembrane signaling, and the dynamic nature of the cell surface. However, it would be several decades before these ideas would take hold among embryologists and several more after that before the tools became available to analyze these biochemical phenomena in the context of cell adhesion.

### 3. The Recognition of Differential Cell Adhesion as a Central Mechanism of Morphogenesis

The very different fates of two 1939 publications indicated, on the one hand, how much intellectual inertia had to be overcome to place the role of the cell

surface in proper perspective and on the other, how much promise the study of the cell surface held for understanding developmental phenomena. The *Biology of the Cell Surface*, the publication of which capped the career of expatriate black American biologist E. E. Just (27), promoted the view that the cell surface was a dynamic structure with a key role in developmental and evolutionary processes. Just discusses results of his studies of starfish development, first published in 1931 (28), which can be cited as the first experiment demonstrating developmental changes in the adhesiveness of cells.

Normally during early cleavage the blastomeres of this egg lie within the vitelline membrane apart from each other; later, regaining contact they develop into one embryo. When after the second cleavage the four blastomeres lie apart, they may with care by puncture of the vitelline membrane be removed as four independent cells. If brought together again and kept in close contact . . . they unite and develop into a single embryo. Also, two blastomeres from one egg when brought together with two from another in some cases united and developed into one embryo; often however union failed to take place. I found that this failure resulted whenever the transferred blastomeres were not in exactly the same moment of development. . . . To my knowledge, there exists no observation which so clearly and so beautifully shows the quickly occurring changes in ectoplasmic activity (27).

To Just, the role of the cell surface was paramount, superseding even that of the genes:

The ectoplasm stands not simply as a barrier of the cell against the outside world; it is also the medium of exchange between cytoplasm and environment. As such, it is the first cell-region to receive impressions from the outside world; through its delicacy of adjustment and fineness of reaction, it constitutes the first link in the chain of cytoplasmic reactions and sets the path for the orderly succession of events comprising the course in the differentiation of development (27).

However, for several reasons discussed by Manning (29) and by Gilbert (30), Just's work was largely and unfortunately ignored. The initial analysis of vertebrate embryonic cell adhesion in its modern form has been traditionally attributed to Johannes Holtfreter, beginning with his 1939 paper "Tissue affinity, a means of embryonic morphogenesis" (31). However, although certainly aware of the aforementioned studies by H. V. Wilson, Lillie, and Just, Holtfreter makes no reference to these studies in the introduction to his work. Rather, Holtfreter builds on earlier work of the German *Entwicklungsmechanik* school, beginning with Roux's cytrotropisms, the attractive and repulsive forces between cells which Roux believed to act at a distance as a mechanism guiding embryonic morphogenesis. Although Holtfreter believed Roux's original concept to have been largely disproven by detailed observations of vertebrate embryonic cells in culture, he hesitated to discard the baby with the bath water. Rather, Holtfreter believed that "the pioneering investigators of developmental physiology were often guided intuitively by considerations of analogy, searching in their own special field for phenomena already known in analogous form in other fields, thus attempting to explain biological phenomena by comparing them with similar ones observed in inorganic systems," and that this was "more fruitful for research than the resigned attitude of a Driesch who in view of the complexity of developmental events felt compelled to renounce causal analysis *a priori*" (31).

Holtfreter appeared comfortable with earlier attempts by Pfeffer, Butschli, Rhumbler, and especially His and their morphogenetic models based on physical properties of inanimate matter to explain the bendings, folding, and reorganization of embryonic tissues during development. Holtfreter credited Roux with the first attempt to experimentally explore these phenomena with isolated amphibian embryo cells, but believed that Roux overinterpreted his data on cells in culture in concluding that attractive and repulsive forces between cells were at work:

Roux advanced the dictum of an elective capacity of self-ordering of embryonic cells. In his opinion the embryo has “the tendency to arrange its cells appropriately according to their qualities, and to rearrange them correspondingly after their qualities have changed during development” (31).

In Holtfreter’s 1939 study, tissue segments derived from distinct amphibian germ layers were recombined *in vitro* with the result that they would adhere to form a composite mass within which distinct cell groups sorted out from one another to result in a topographically appropriate mixed aggregate within which considerable differentiation would take place. The reorganization of tissues within these aggregates was reminiscent not only of the topography, but also of the timing of morphogenetic movements which would have occurred *in situ*. Holtfreter interpreted these cell and tissue rearrangements to reflect attractions and repulsions between cells which were, unlike those proposed earlier by Roux, contact-dependent.

It seems advantageous to us to introduce a more fitting term for the forces that are instrumental in these processes of attraction and repulsion. Henceforth we shall apply the term *affinity*, which partly substitutes for the terms of Roux and which may serve as a reminder for the existence of analogous phenomena in chemistry. Affinity may be either positive or negative, it may be graded in its intensity, and may approach the point of neutrality. These graduations may change during development, increasing or decreasing between two cell generations, or they may repeat themselves in cycles. . . . On the basis of the few data presented here in rough outline, a general rule can already be established, namely, that when a morphological segregation occurs in a hitherto undivided cell layer, there is also a change in affinity between the new derivatives. . . . These changing boundaries do not respect germ layers. . . . (31).

Holtfreter considered these affinity changes to play a central role in morphogenesis:

As a means of self-ordering of embryonic regions these phenomena are of great significance. They lead to the anatomical segregation of physiologically different organ primordia and to their recombination with other parts of the embryo. They provide a unified explanation for local migration and constriction movements in whole cell complexes, starting with those in gastrulation and being continued during organogenesis. The processes of induction and subsequent formative influences would not be possible without a positive affinity between the reacting material and the inductor. In view of the transparency of the situation and the ready availability of material there should be no difficulty to explore the problem of affinity by means of further experiments and thus obtain new, well-documented support for a theory of development (31).

The relevance of such a theory extended beyond embryology, as Holtfreter realized, and he would later express the view that “the phenomenon of cellular adhesion is the prerequisite for the evolution and ontogenesis of multicellular organisms” (32). Holtfreter’s prescient view of the primacy of selective cell

affinities as setting up appropriate positioning for subsequent inductive interactions, coupled with his emphasis on the fundamental relationship of cell adhesion to both ontogeny and phylogeny, would be recapitulated many years later as the two central tenets of the Regulator Hypothesis of Edelman (33).

Holtfreter had thus established that selective cellular associations, while not apparently controlled through action at a distance, as favored by Roux, could be demonstrated to occur between cells at the tissue level. The question remained as to whether these tissue affinities were a holistic property of the embryo or were indeed the summation of individual cell behaviors. In his two-part study on the mechanics of gastrulation, Holtfreter (34,35) analyzed amphibian morphogenesis through an examination of cell morphology both of isolated cells and of cells remaining within whole embryos.

Here, then, we are confronted with one of those mysterious looking cases where an organismic principle, not explainable by the combined function of the parts, seems to pervade the system and to exert its dictatorial power. Vitalistic skeptics will strongly doubt that further dissecting analysis of the single processes will lead to a deeper insight into the apparently superimposed principle of the whole. However, the results of such a more intimate analysis of the problem here at hand refute an over hasty skepticism. The following sections give evidence that the directed movements of embryonic regions can actually be traced back to basic faculties of the single cells and to their specific response to changes of environment. The unitarian character of their combined effort is mainly the result of the predisposed arrangement of cells with a locally different kinetic behavior. The controlling supercellular forces can be localized and defined in physico-chemical terms (34).

Holtfreter discussed these physicochemical terms in a fashion which would be championed two decades later by Steinberg in the presentation of his differential adhesion hypothesis:

Roux (1894) and Rhumbler (1899) brought forward convincing evidence that the aggregation of cells into solid bodies, and their characteristic arrangement is mainly determined by the forces of surface energy. To these forces we must also attribute the calming effect of the cellular contact upon the amoeboid movements. The restless motions indicate that the isolated cell is unable to arrive at an equilibrium with the surface tensions of the surrounding medium and the substratum of glass. When, however, contact is established with another cell, the specific properties of the cell surfaces make a mutual adhesion and spreading possible. According to what can be predicted when two liquid bodies which have a higher molecular attraction to each other than to the surrounding medium, are in contact, the surface tensions at the interfacial cell surfaces will be lowered. This will reduce the surface energy of the whole system. The process of spreading will continue until the potential energy has reached a minimum, at which point equilibrium is attained (34).

However, the dynamic nature of the cell and its distinction from an oil droplet was not lost on Holtfreter:

It would divert us too much from our main problem if we were to attempt to analyze further the behavior of isolated cells from the viewpoint of surface tension. Rhumbler (1898-1910) and others have shown that such an interpretation goes a long way. One can hardly doubt that the motility of solitary cells, their tendency to aggregate or spread over some other surface are phenomena largely determined by differences of surface energy which tend to an equilibrium. Yet, such considerations can be regarded only as a first approximation. They miss the fundamental point that the cell is a heterogeneous organized system which, more often than not, evades the compulsion of these general rules. We should look more for the factors making for instability than for those tending

toward stability, for it is these continuous deviations from an equilibrium emerging from the specific asymmetrical structure of living matter which are the essential features of morphogenesis (34).

Regarding the separation of tissues during morphogenesis, as exemplified by the sorting and separation of germ layer recombinants *in vitro*, Holtfreter suggested that

the driving force behind the process of detachment is probably an alteration of the molecular characteristics of the cell surface which progressively reduces the reciprocal adhesion. As a method of sorting out physiologically related from unrelated cells the principle of tissue affinity becomes more dominating at the stage of organ formation (35).

The singular role of surface tension, however important for the determination cellular movements, even when enhanced by additional theories of the role of localized cell multiplication or water imbibition, was deemed insufficient by Holtfreter to explain morphogenesis. Holtfreter was convinced by his observations of cells that there was some intrinsic property of cells which added to the other surface and external forces as a driving mechanism (36). He cited work on neurulation from various laboratories suggesting that "alterations in cell shape are due to changes in protoplasmic structures, such as an oriented cytoskeleton" and asserted that this "new conception, superimposed over that of surface tension differentials, has strong claims to becoming a useful guide for interpreting morphogenetic movements" (35).

The ultimate analysis of these phenomena as cellular properties necessitated further experiments with dissociated cells. An important advance was made by Holtfreter when he developed a method for the dissociation of embryo cells with good yield and viability, although, as Holtfreter recalled some years later, his own viability was at one point at some apparent risk:

As to cell disaggregation, this was not something that I planned ahead and set out to do. It happened quite by chance. I am an intuitive scientist. I am also an artist and in my artistic drawings I often start out with doodling lines and when they begin to suggest something, I follow. I was trying to repeat someone's work on the effects of cyanide on amphibian embryos. To my surprise the cells separated. This was wonderful, but I almost got poisoned. Fortunately, I soon found that early amphibian embryos could be disaggregated into cells simply by raising the pH of the medium. This was a very useful finding because it enabled me to investigate self-differentiation of isolated cells. It also helped me to study cell movement which had always interested me greatly (37).

In a later study, using these dissociation methods, Holtfreter and his student Philip Townes (32) took advantage of Holtfreter's observation that early amphibian embryos could be dissociated into single cells by the elevation of pH, and upon return to physiological pH the cells would readhere to form tissue-like aggregates. This technique allowed for the analysis of cell affinities as well as tissue affinities, which had been studied earlier. An extensive analysis of a variety of recombinant tissues and cells from the embryos demonstrated that even dissociated cells, when randomized and recombined, could sort out among themselves and reform groups and layers reminiscent of the normal histological organization of the embryo. Holtfreter concluded that two distinct phenomena, directed migration and selective adhesion, worked in concert to produce morphogenetic movements:

The different cell types of the amphibian embryo, whether present as single cells, cell sheets, or globular cell masses, exhibit tissue-specific tendencies of moving either centrifugally or centripetally within a composite cell aggregate. Directed movements are followed by the phenomenon of cell-specificity of adhesion. The combined effects of these processes necessarily result in segregation and recombination of tissue primordia or individual cells. . . . These considerations do not tell us why, in embryos or in composite aggregates, certain cell types move inward and others outward. It seems necessary to assume the existence of a concentration gradient of some sort between inner and outer milieu of the aggregate towards which the different cell types react differently. . . . As has been suggested by Rhumbler, the forces of molecular interfacial tension cannot be ruled out in the kinetics of embryonic cells whose surface is of a semiliquid nature. Yet the well attested structural properties of the cell membrane would necessarily resist a free display of these forces since the latter presuppose freely mobile molecules. Further work is required to clarify the significance of interfacial tension in morphogenesis and to define the factors which direct the tissue-specific movements here recorded (32).

Holtfreter's discussion of "hypotheses concerning the molecular factors engaged in cellular adhesion" favored the interlocking of complex molecules, although the lability and nonstatic nature of cell adhesions suggested something other than rigid lock and key models. He expressed a reluctance to build such models, observing that "in view of the rather complicated nature of the phenomena observed and of our ignorance concerning the physicochemical factors involved, one could think of a diversity of symbolistic schemes which might tentatively cover the actual situation without, however, explaining it." Nevertheless, he succumbed to temptation and constructed a model wherein cell surface molecules mediating weak, nonspecific intercellular bonds are converted during development into stronger, tissue-specific bonds, which, superimposed on the inherent migration tendencies of cells, leads to the observed morphogenetic movements. Holtfreter, however, warns:

At present, it would be futile to speculate further upon the possible subcellular factors that are engaged in cellular adhesiveness. It should be pointed out however that this principle is of universal significance in morphogenesis, and that, in connection with directed cell movements, it is deserving of more attention than it has received (32).

Holtfreter clearly was concerned with the cell biology and biochemistry of the phenomena under consideration, but he lacked the tools and training to explore the subject further. In a recent reflection on his work, Holtfreter stated:

The idea of tissue affinities grew out of some earlier work that I had done with amphibian embryos, but it was not entirely original. In 1939 I wrote a short paper on this and I have been surprised by the attention it started to receive many years later. I knew that this problem called for a biochemical approach, but I did not have the training for this. In 1955 my student Townes continued this work using my methods, but then he went to medical school. . . . I had to stop. Everything required chemistry and electron microscopy. I had no training in chemistry and, probably, no strong inclination for it (37).

#### **4. Qualitative and Quantitative Quarrels over the Quintessence of Cell Adhesion**

The central conceptual question that stimulated Holtfreter had been to determine whether morphogenetic cell interactions reflected cell-autonomous

or higher-level phenomena. Holtfreter concluded that the victor was cell autonomy. This victory raised a new question: whether the differences between cells that resulted in their greater or lesser mutual affinity resided in a qualitatively or quantitatively distinguishable cellular property. Elements of both these possibilities could be detected in Holtfreter's musings on the matter, as when he suggested that both biophysical parameters (such as the solvation state of the membrane as affected by calcium) and molecular components (such as proteinaceous cementing substances) could both play a role in cell adhesion (38).

The matter of quality versus quantity in embryological investigations had earlier concerned Roux, who, according to Oppenheimer, felt that

[the] analysis of development must first be qualitative, a breaking down of complicated events into different modes of action and the factors related to them. Only when one of these factors is ascertained can the magnitude of its effect be investigated. Thus, Kant's dictum that science is only true science insofar as it is mathematical is not pertinent to developmental mechanics. A correct qualitative analysis of developmental events is also true science, and in fact in this science it is primary . . . (39).

Two influential successors to Holtfreter, Malcolm Steinberg and Aron Moscona, simultaneously pursued studies into the mechanism of cell adhesive interactions, each respectively championing either quantitative or qualitative considerations.

Malcolm Steinberg's first publication on the subject of cell adhesion, with the distinctly Paulingesque title "On the Chemical Bonds between Animal Cells. A Mechanism for Type-Specific Association" (40), established the tone of his work for many years to come. Steinberg considered various available techniques of tissue dissociation, reasoning that knowledge gained from the requirements of separating cells would shed light on what held them together. He dismissed mechanical dissociation methods that, while of experimental utility, did not in themselves shed light on the nature of cell attachments. However, three other methods of tissue dissociation in use at the time, the removal of calcium ions from tissues, exposure to alkaline solutions, and incubation with the protease trypsin, all had to be explained by any theory on the nature of intercellular bonds. Two factors regarding the structure of cells also weighed heavily in Steinberg's analysis: (1) evidence that at physiological pH, animal cell surfaces are generally found to be negatively charged, and (2) evidence largely obtained from studies of red cell membrane ghosts and myelin that cell membranes are highly ordered structures. Steinberg thus proposed:

Ca (or Mg, or both), in addition to desolvating the cell surfaces, associates with the surface anions. If certain of these anions are sufficiently separated from one another, the formation of slightly soluble salts involving two anions and one Ca would not be possible. When two cells in such a state collide, however, the formation of slightly soluble salts between the two cell surfaces becomes possible, the two valences of Ca being satisfied by an acidic group on the surface of each of the two cells. I wish to propose that this is the mechanism by which cells are held together. . . (40).

This early model constructed by Steinberg takes the cell surface to be a paracrystalline array with a particular distribution of surface charges which will differ between cells. The particular distribution of cell-surface-negative charges determines the number and pattern of possible links between two cells. Thus the strength of adhesion between two cells is maximized when the coordinate

positions of charges in the cell surface arrays correspond spatially. Differences in cell adhesive strengths result from greater or lesser degrees of correspondence between these arrays of charges. Thus distinct cell types with different arrays may adhere to one another, but they will gradually become displaced by cells with a better fit. This behavior could explain cell sorting phenomena in composite aggregates.

According to Steinberg, the removal of alkaline earth ions resulted in cell dissociation due to the requirement of these ions in forming charged bridges between cells, and exposure to alkaline solutions causes cells to dissociate due to distortions of the cell membrane and resulting distortion of the paracrystalline array of charges, decreasing the fit between cells. The dissociating effect of trypsin is attributed to its use at elevated pH, or to the presence of other activities than proteolytic ones (such as the degumming effect of crude trypsin, now recognized to be due to contaminating DNase). Steinberg stated:

I do not wish to deny the possibility that trypsin may also have a more direct action than the one attributed to it here. I only wish to draw attention to the conditions under which it has appeared to be so effective and to those under which it appears to be ineffective, since a lack of appreciation of those conditions may conceivably mislead us in our conclusions concerning the bonds which unite animal cells into tissues (40).

The calcium-bridging hypothesis elaborated by Steinberg required a relatively fixed array of surface charges to maintain different charge distributions in the plane of the membrane. This was in tune with then current notions of cell surfaces, which, although considerably more advanced than the one cited earlier from E. B. Wilson, were still quite different than the more dynamic concepts of today. It was not until the early 1970s that a revolution occurred in our concept of the cell surface membrane, stimulated greatly by the observations of Frye and Edidin (41) on the mobility of cell surface receptors and culminating in the fluid-mosaic model of Singer and Nicolson (42). It is only with the gift of hindsight that one can read statements in current textbooks such as *Molecular Biology of the Cell* (43) that "it is hard to imagine how a cell could live, grow and reproduce if its membrane were not fluid!" In fact, a dynamic concept of the cell surface has been in and out of favor more than once. As cited by Moscona (44), T. H. Huxley wrote in 1853 that "the periplast . . . which has hitherto passed under the names of cell wall, contents and intercellular substance, is the subject of all the most important metamorphic processes, whether morphological or chemical, in the animal or the plant. By its differentiation every variety of tissue is produced." That this view would change back and forth over the years was in part due to the choice of experimental material used in membrane studies. During the early years of the most recent swing in opinion, Moscona observed that:

In recent years there has been something of a revolution in the science of cell surfaces. . . . Like most revolutions, this one too has its roots in history. The classical embryologists and immunologists, whose concepts often converged, thought of the cell surface in terms of a dynamic structure involved in interactions with the environment, in cell guidance, and in intercellular communication. Later, as embryology, immunology, and cell physiology drifted apart, this foresighted viewpoint gave way to narrower interests. Models of the erythrocyte "ghost" became the dominating prototype of the animal cell surface which was thus pictured as a static, uniform "pellicle" essentially similar in all types of cells, and serving primarily as a permeability barrier. The advent of modern molecular and developmental biology and immunology revived a broader

interest in animal cell surfaces and prompted reexamination of the older concepts in light of biological realities. The resulting tide of information—physical, biochemical, cytological and immunological—has led to renewed awareness of the cell surface's significance and complexity. It is now abundantly clear that the cell surface is a dynamic system, which is heterogeneous in structure and composition and diverse in different kinds of cells. It undergoes changes in embryonic differentiation and metaplasia, and is subject to genomic regulation and environmental modulation. Its versatile functions play decisive roles in the control of cell growth, cell movement and cell recognition, and therefore in morphogenesis, differentiation, and immune response (44).

As the prevailing concept of the cell surface changed from a relatively static to a dynamic one, so too evolved Steinberg's models of cell adhesion. Several papers that followed were designed by Steinberg to address alternative hypotheses which had been put forward to explain cell sorting within mixed aggregates. While Holtfreter had invoked similar biophysical considerations to those cited by Steinberg, he had also interpreted the movements of cells in reconstituted tissues to indicate the presence of gradients that directed the migration of cells deeper or more superficially within an aggregate. Steinberg addressed this question by varying the relative proportions of cells within a mixed aggregate, finding that below a certain limit, cells that at higher proportions would sort to the interior of an aggregate now remained randomly dispersed through the aggregate (45). This result mitigated against a gradient and against directed migration but was consistent with the notion that different strengths of adhesion could by themselves account for the final configurations of cells within a mixed population. Curtis (46) had raised the possibility that the sorting of cells in mixed aggregates could occur due to different times necessary for the repair of damage that occurs to cells during their dissociation from tissues. Steinberg addressed this possibility by histological examination of aggregates at various stages during the course of sorting out and concluded that the kinetics of sorting of the two populations was inconsistent with Curtis's model but was consistent with sorting due to selective cell adhesion (47).

The ability of selective cell adhesive interactions as necessary and sufficient to explain cell sorting, and predictions resulting from this model, such as the independence of the final equilibrium condition of cells within an aggregate from the initial conditions (i.e., single cells or tissue fragments), were further tested and given an elaborative quantitative treatment by Steinberg (48,49) which extended and refined the conception of Holtfreter regarding the role of surface free energy (34,35). The central tenet which Steinberg explained in detail in 1963 was that the behavior of the cells in sorting is dependent neither on directed migration nor on qualitatively different adhesive affinities. Rather, purely quantitative differences in adhesive affinities could explain the observed sorting phenomena. Extending Holtfreter's idea, Steinberg proposed that purely thermodynamic considerations could explain the behavior of cells in mixed cell aggregates. The relative interfacial free energies of strengths of adhesion between cells A and B, which were a function of the relative strengths of adhesion of these cells to themselves and to each other, were the critical parameters. Based on this hypothesis, Steinberg made a number of predictions regarding the behavior of cells in mixed aggregates. Among these important predictions was that there would be a hierarchy of cell sorting between different tissues and a transitive

property in the sorting relationships. In this paper and in an extensive later study (50) a large number of cell combinations are shown to obey these rules. In the later paper, Steinberg makes the first reference to his theory of cell sorting as the "differential adhesion hypothesis."

While having a strong predictive component and the ability to explain the behavior of cells in mixed aggregates, the hypothesis was criticized by some at the time as doing little to explain, and seeming to reject, a role for specific molecules in cell adhesive interactions. However, this was not Steinberg's intention:

This analysis shows, then, that (i) the mutual sorting out of two kinds of cells to reconstitute tissues, one of which encloses the other, and (ii) the spreading of an intact fragment of the one tissue to envelope an intact fragment of the other are precisely the phenomena which are to be expected, in accordance with the principle of minimization of free energy, in the total absence of selectivity in the adhesion mechanism itself. Only quantitative differences in adhesion are necessary. The "information" required in the adhesion mechanism is, in such cases, restricted to "more" and "less." This does not mean, of course, that molecules of different sorts, on the surfaces either of cells of a given kind or of cells of differing kinds, may not in such cases participate directly in the mediation of adhesions. It merely means that whatever the chemical nature of, or diversity among, the adhesives themselves, the quantitative adhesive relationships among the cells which bear them would be expected to approximate . . . the relationships derived from the simple postulates which have been outlined (49).

Special significance is given to the transitive nature of the cell-sorting hierarchy because it suggests that "the property immediately responsible for the tendency of one cell population to envelop another is universal amongst all of the cell populations used in these experiments." This property is the cohesiveness of cells within a population, meaning "the work (or energy) of adhesion of one kind of cell surface to another of its own kind, averaged over the area of apposition" (50).

The differential adhesion hypothesis was not meant to supplant, but rather to complement, a role for specific and qualitatively different adhesion molecules. So much was made clear by Steinberg:

A not uncommon view seems to hold that some day either the differential adhesion hypothesis or the "specific adhesive factor" hypothesis . . . will win the field. In fact, these two hypotheses are pitched at two different levels of explanation and are in no way mutually exclusive. The chemistry of cell adhesion and the physics of morphogenetic assembly processes both enter into the explanation we are seeking, but in different roles. . . . What the chemistry will help to explain, as it comes to be understood, is why a particular set of adhesive relationships, and not some other set, exists in any particular case. The differential adhesion hypothesis will provide the link connecting the chemistry of intracellular adhesiveness to the morphogenetic behavior of the cell population. . . . In the last analysis, the role of differential cellular adhesiveness in governing any particular histogenetic process must, of course, be determined empirically. There can be little doubt that other kinds of morphogenetic mechanisms will claim a prominent share of the responsibility for determining the architecture of tissue and organs (50).

Thus the chemical nature and possible diversity of the cellular adhesives themselves remained, for the present, unknown. The idea that proteins were involved in binding cells together into tissues evolved over an extended period. Schiefferdecker (51) used a pancreatic extract to isolate epithelia from underlying connective tissue, and Rous and Jones (52) used pancreatic trypsin in the preparation of single cells for culture. However, they did not apply the trypsin

directly to tissues, but rather first placed tissue explants into plasma clot cultures and allowed cells to migrate into the clot from the tissue. Trypsin was then employed to dissolve the clot and free the cells for subculturing. Trypsin was first used to directly reduce chick embryonic tissue into its constituent cells by Moscona (53), who found that earlier methods of calcium deprivation and alkaline pH treatment, which worked for early amphibian embryos, were not alone sufficient for tissues from later stages of amniote embryos.

The availability of dissociated chick embryo cells was used by Moscona and Moscona in a further study to “find whether the cells of these disrupted tissues could become reorganized into integrated systems and resume their presumptive histogenetic development” (54). As in the case of Holtfreter’s amphibians, the embryonic chick cells did indeed aggregate and reestablish an organized tissue pattern. Moscona cites the “contiguity effects” and “tissue affinities” of Holtfreter as the basis for the cells’ behavior.

These early experiments of Moscona, like those of Holtfreter, were carried out in stationary cultures which relied on cell motility and random collisions to bring cells into contact. Moscona modified the aggregation assay by placing the cell suspensions on a rotating platform, eliminating the need for cell migration, as cells were brought into contact via centripetal forces and proceeded to form highly regular, spherical aggregates whose size was taken as a measure of mutual adhesiveness (55). This simple technical advance permitted the maintenance and aggregation of cells under considerably more controlled conditions.

Moscona went on to use his new assay conditions to examine the effect of various factors on cell aggregation. He found aggregation to be impaired at reduced temperatures, suggesting a role of metabolic activities and supporting a “role of specific surface products of cellular activity in the mechanisms of bonding and formative association of embryonic cells” (55). Further support for this idea came from studies showing that inhibitors of protein synthesis had an immediate effect, and inhibitors of RNA synthesis had a delayed effect, on aggregation. These data were interpreted by Moscona and Moscona as suggesting a requirement for the synthesis and replacement of materials removed from the cell surface by trypsin (56). Based on these results, Moscona questioned “whether the mechanisms of cell bonding and histogenetic association can be visualized solely in terms of cationic linkages or rheological schemes” (55), as had been proposed by Steinberg. Rather, Moscona emphasized the importance of qualitatively distinct cell surface proteins in cell adhesion and sorting. The resulting extracellular material (ECM) or specific ligand hypothesis would form the basis for his further studies.

Convinced of a role for specific cell surface proteins in cell adhesion, Moscona set about to identify them. His search was begun with the idea, perhaps inspired by Lillie’s extraction of sperm–egg agglutinins a half-century earlier, that cells in culture prevented from adhering due to high shear forces would shed adhesive components into the medium. Moscona had at one time considered the viscous material found in cultures of trypsinized cells to be involved in adhesive interactions; this material was later shown to be degraded by DNase (57). The identification of less visible cellular exudates that might indeed be involved in cell adhesion was assayed for by adding conditioned culture medium back to cells, and Moscona found that aggregation could be promoted in that larger

aggregates were produced (57). Importantly, this enhancement effect was reported to be tissue-specific. The approach of identifying vertebrate embryonic cell adhesion molecules by addition of exogenous ligands obtained from cell-conditioned medium was pursued further by both Lilien and later Hausman, first in Moscona's laboratory and then independently. Moscona and his student Jack Lilien (58,59) again demonstrated the enhancement of the size of aggregates of cells in a tissue-specific fashion by added conditioned medium, and many such experiments were subsequently done in Moscona's laboratory, each demonstrating exquisite tissue-type specificity of aggregation enhancement, not only between such disparate tissues as retina and liver, but between different central nervous system tissues such as retina and brain as well. This approach led Hausman and Moscona, using as an assay the direct promotion of cell aggregate size, to report the isolation of a protein from cell-conditioned medium (60) and later from cell membranes (61), which possessed the ability to enhance aggregate size in a tissue-specific manner.

Lilien independently pursued the nature of the adhesion-enhancing components obtained from cell and tissue-conditioned medium through a number of independent approaches, although direct enhancement of live cell aggregate size, as had been demonstrated in Moscona's laboratory, was not further tested. These various approaches included binding assays of radiolabeled extracellular material to cells, which demonstrated the binding materials to be specific for either retina or brain cells, and which were but one of three components necessary for cell adhesion to occur (62–64). The degree of specificity of binding was such that it correlated with the pattern of retinal–tectal innervation (65). The latter property, in conjunction with the reported inhibitory effect of factor binding on the mobility of cell surface membrane receptors (66), led to the proposal of a role for these factors in regulating neurite growth and the retinal–tectal innervation pattern. Biochemical analyses of the binding materials indicated a role in the binding for carbohydrate moieties, and the kinetics of factor release from cells suggested possible control by glycosyl transferase activity (67). These observations were synthesized by Lilien into a model that proposed that adhesion occurred via a three-component system consisting of a cell surface glycosyl transferase receptor, a heterobifunctional ligand which bound to the surface receptor, and a soluble homobifunctional agglutinin which linked the ligand–receptor complexes together (68). This model thus combined elements of Moscona's specific cell ligand hypothesis with the hypothesis put forth by Roseman (69), who had earlier proposed that changes in cell adhesion during development were caused by the regulation of cell surface glycosyl transferases and the carbohydrate moieties they recognized.

During this period, two in-depth reviews appeared that critically examined the existing experimental evidence for specific cell adhesion and the models used to explain it (5,7). It is apparent from these sources that cell adhesion came to mean many things to many people. Cell adhesion could be monitored in a variety of ways, and several factors could affect the way in which cells behaved in these assays. The variables included the method of tissue dissociation, either mechanical or using a variety of proteolytic enzymes, different culture conditions, including the presence or absence of components as simple as calcium or as complex as serum, temperature, metabolic inhibition, and the length and end-

point of the assays, which ranged from initial single cell kinetics to the formation of reconstituted tissues. One bright source of hope, if not light, which refocused and energized experimental efforts during this period were the experiments of Roth and Weston (70), who designed the first assay to demonstrate adhesive specificity in the absence of cell sorting. By using preformed aggregates of different cell types to pick up single labeled cells, these experiments demonstrated that differential cell adhesion indeed existed, was tissue specific, and that such adhesive specificity existed between cells before they were incorporated into aggregates. While the various approaches during this period all contributed to an understanding of the phenomenology of cell adhesion and sorting, they only hinted at the underlying biochemical mechanisms. Indeed, in retrospect, it seems that with each new assay, more highly specific cell recognition phenomena were ascribed to a growing list of putative adhesion factors. However, by the mid-1970s, no such adhesion factor had been characterized in any biochemical detail.

## 5. Immunological Approaches to the Analysis of Cell Adhesion

The complexity of cell adhesion and sorting phenomena necessitated the application of techniques with greater resolving powers to provide insights into the molecular mechanisms controlling cell adhesive interactions. In 1945, the Society for the Study of Development and Growth (the forerunner of the Society for Developmental Biology) held a symposium on "Specificity." Paul Weiss was a speaker at the meeting, and his talk was later expanded into a paper on "The Problem of Specificity in Growth and Development" (71). Weiss opened by stating:

The frequency with which such terms as specificity, selectivity, conformity, correspondence, etc., appear in biological literature is ample proof that they denote a universal and fundamental trait, running like a common theme through all manifestations of life. Yet, they are used with so many different shades of meaning and degrees of precision that it is impossible to tell whether the various phenomena to which they are applied bear a purely formal resemblance to each other or whether there is essentially a single principle in back of them all. . . . In particular, let us explore the pertinence of the serological specificities as a model of developmental processes, inasmuch as recent studies in immunochemistry have brought these specificities within our grasp. . . . It is to the exposed cell surfaces that we must look for the revelation of the factors which make or break specific cellular associations (71).

With respect to cell adhesive interactions, Weiss distinguished three types of tissue affinities: homonomic (selective combination with self), complementary (combination with other than self), and active detachment, all of which had been earlier observed by Holtfreter. Weiss recognized the fundamental questions as being (1) how do these various affinities arise ontogenetically, and (2) what is their nature, and can they be expressed in terms of known properties of a simpler order? Weiss went on to suggest:

Unless we want to invoke entirely unknown principles, no other explanation of such specificity seems at hand than one based on a concept of interlocking molecular configurations. This concept, traceable to Ehrlich, and culminating in the recent work of Pauling, maintains that the specificity of intermolecular relations is based on "steric

conformance," i.e., corresponding or complementary spatial configurations between molecules, or certain exposed atomic groups of them, enabling them to conjugate in key-lock fashion. The theory is that such structural fitting allows the fitting particles to come within ranges of strong binding forces (71).

As initially suggested by Ehrlich, Lillie, Langley, and Harrison, such interactions were believed to occur in antigen-antibody systems, enzyme-substrate systems, hormone-effector cell relations, drug action, and cell interactions. Weiss chose the immune system as his prototype:

. . . the cell can not be considered as an antigenic unit. It contains numerous and diverse molecular species, which if steric properties are a prerequisite of antigenic action, represent a wide variety of antigenic agents . . . it is reasonable to assume that any given cell harbors an infinitely greater variety of specifically configured proteins than we can reveal by present immunological techniques . . . our concept postulates that those [specifically shaped units] occupying surface positions can act as links between the cell and its surrounding structures. The clumping of scattered cells and blood corpuscles by agglutinins or precipitins is evidence that comparatively large bodies can become affixed to one another by intermediary molecules of fitting complementary configuration. Obviously, there is thus no fundamental difficulty in envisaging bonds between cells in general as effected by a similar principle (71).

However, in 1945, limited experimental evidence existed to support this idea, the only one Weiss discusses being sperm-egg interactions, as studied by Lillie and later Tyler. Indeed, Tyler's experiments on sperm-egg interactions led him to propose a model of cell interactions similar to Weiss's, but stronger in its implication of molecules related to antibodies, which he described as "An Auto-antibody Concept of Cell Structure, Growth and Differentiation" (72). Tyler's model paralleled that of the instructive model of antibody formation. In the absence of foreign antigens, self-antigens would serve as the model substrate for antibody formation. The interaction of such autoantibodies with their antigens was deemed to be involved in the maintenance of cellular integrity as well as in intercellular interactions.

The possible relationship of cell adhesion and recognition systems to the immune system was recognized by Sir McFarlane Burnet, who, in his December 1960 Nobel Prize acceptance speech, mused over the evolutionary origin of the immune system. While an obvious survival value could be attributed to the immune system, Burnet was aware that such a system was unlikely to arise *de novo*:

To provide an evolutionary interpretation of a physiological process, requires something more than the demonstration that it has survival value to the possessor. We must also offer some hint as to how it might have developed from pre-existent faculties. Here there is an obvious suggestion that immunological recognition is an inevitable derivative of the basic requirement for any integrally organized, multicellular organism—the existence of an elaborate system of information and control, of receptor, effector, and feedback mechanisms, that is needed to maintain morphological and functional relationships between cells. Some of this—perhaps a large proportion—must be mediated, as Paul Weiss has suggested, by complementary pattern relationships between macromolecular constituents. This may seem to be a very thin speculation which could not possibly stimulate a line of experimental inquiry (73).

Burnet's pessimism was not universally shared, and the influence of these immunological models, particularly in the weaker form put forward by Weiss, is certainly evident in the program pursued by Moscona. However, the immune model had another influence in that it stimulated experiments using immu-

nological reagents to explore the mechanism of specific cell adhesion. The first descriptions of the use of antibodies as tools came in the form of a two-part study by Spiegel (74,75). The introduction to the study quite clearly shows the linkage with earlier analyses:

In the autoantibody concept of Tyler and the molecular ecology of Weiss one important part is the hypothesis that contiguous cell surfaces are normally held together, at least partly, by forces like those between antigens and homologous antibodies. The forces are assumed to be associated with specific macromolecules, of at least two stereochemically reciprocal types per cell, so held in the cell surface that they can combine from cell to cell (74).

Utilizing as experimental material the sponges and amphibian embryos that Wilson, Galtsoff, and Holtfreter had earlier employed, Spiegel set out to do “one type of experiment . . . suggested by the proposal that macromolecules are involved. Such molecules might act as antigens when injected from one species into another. Antisera so produced should affect, in a predictable way, processes involved in cell adhesion” (74). Spiegel made antisera in rabbits by immunization with extracts of sponges or amphibian embryos and used the antisera in cultures of reaggregating sponge or amphibian cells. Normal serum had no effect, but the antisera were reported to inhibit aggregation in a species-specific fashion. The cells remained viable, as aggregation could occur if the antibodies were washed out.

Despite these promising results, it was some time before this approach was taken up by others. Moscona and Moscona reported in an abstract (76) that they obtained antibodies raised against chick embryonic liver cells which inhibited the aggregation of liver but not retina cells. Lilien, in his 1969 review article (6), refers to unpublished experiments of his where rabbit antisera were raised against various tissues of the embryonic chick. Following cross-absorption, these antisera were capable of specifically inhibiting aggregation of the cell type used for immunization, and the cells would resume aggregation when the antisera were washed out. Further experiments are mentioned in which antisera were raised against the aggregation-promoting material found in retina cell conditioned culture medium, and these antisera would also inhibit retina cell aggregation but not liver cells. However, neither Spiegel, Moscona, nor Lilien reported on the use of these immunological probes to explore the biochemical nature of the antigens recognized by these antisera.

As was the case in the original cell-sorting studies, an invertebrate, in this case the cellular slime mold *Dictyostelium*, looms large in the first successful application of the immunological approach to identification of a defined cell adhesion molecule. Making reference to the “Tyler–Weiss hypothesis,” Gregg (77) produced antibodies against slime molds from various developmental stages and concluded that their surface antigens changed during development in a temporal pattern which suggested they might be involved in cell adhesion. However, Gerisch and colleagues were the first to use such antibodies to analyze cell adhesion and develop a method for identification of the target antigens:

Our purpose was to block selectively cell contact formation of aggregating cells by antibodies attached to the cell surface. The principle question to be answered was: does binding of antibodies to any cell surface component prevent cell aggregation; or must the antibody be attached to specific sites in order to inhibit aggregation? A second

question was: is it possible to block cell contact formation independently from chemotactic reactivity? Antisera of different specificities were used to answer these questions. Since intact bivalent antibodies usually agglutinate the cells (except in extremely high concentrations) univalent fragments (Fab) of IgG antibodies have been used in order to make the observation of cell aggregation in the presence of antibodies possible. An additional advantage is their lower molecular weight—about 50,000 [daltons]—so that the target region which is covered by attached Fab molecules is smaller than occurs when intact bivalent antibodies are used (78).

Using Fab fragments produced from rabbit antisera raised against *Dictyostelium*, adhesion-inhibiting activity was obtained. Several important conclusions emerged from these studies, among them that Fab must be bound to specific sites to inhibit aggregation, that simply coating the cell surface with Fab does not inhibit aggregation, and that aggregation could be inhibited while other functions involving the cell surface, such as chemotaxis, were not. The results of the study further suggested that the target antigen was a glycoprotein and that its expression was under developmental control. Of key importance was the observation that specific Fab neutralizing activity could be demonstrated in membrane preparations from *Dictyostelium*, since this indicated that an assay could be developed for the purification of the target antigen. Such an assay was indeed developed and used to purify the adhesion protein which was the target of the inhibitory Fab fragments (79,80). The power of Gerisch's technique lay in two key features. First, the assay did not require monospecific antisera, since only the effect of a subset of the total Fab fragments in the antisera was measured. Second, since only the antigenicity of the target antigen, and not its biological activity, was crucial for Fab neutralization, the antigen could be identified even in an inactive form as might occur during its biochemical isolation. While cell adhesion molecules of slime molds will not be further considered here, Gerisch's work represented a major breakthrough in the analysis of cell adhesive interactions which would be fruitfully applied to the problem of analyzing vertebrate embryonic cell adhesion.

Before leaving *Dictyostelium*, however, there is another lesson that the slime mold studies held for the analysis of embryonic cells. In the course of Gerisch's studies, he observed that the cells of the slime mold were polarized and exhibited both head-to-head and side-to-side adhesions. Furthermore, these two types of adhesion were distinguishable by the morphology of the cell aggregates mediated by these two systems. They also differed in sensitivity to divalent cation chelators, indicating a differential dependence on calcium for function. The two adhesive systems differed in time of developmental expression, could coexist on cells simultaneously and function independently, and could each be selectively blocked by appropriate Fab fragments (81). The importance of these observations for embryonic cell adhesion was that they indicated that the disparate results obtained by the various conditions and assays for embryonic cell adhesion could be due in part to the existence of multiple, biochemically distinct and functionally independent adhesive systems residing on the surface of a single cell, a possibility which had not been explicitly stated before, and which will be discussed further later.

This immunological approach to the analysis of cell adhesion was first applied to vertebrate embryonic cells by Gerald Edelman's laboratory. Espousing

the view that "in no case has rigorous and convincing evidence been reported that a particular molecule is actually involved in adhesion" (82), these workers rejected Moscona's approach of the addition of exogenous ligands on the arguments that irrelevant substances may affect adhesion, that the addition of exogenous ligands to cells may not *a priori* be expected to enhance their adhesive ness, and that such hypothetical ligands may not retain activity if released from cells. Using the Fab neutralization technique, they reexamined adhesion among chick embryo neural retina cells, the favorite target of Moscona, Steinberg, Lilien, and many others, and discovered the molecule now known as N-CAM (82,83). N-CAM can justifiably be cited as the first discrete molecule with a demonstrated role in vertebrate embryonic cell adhesion to have been characterized sufficiently to allow for stringent comparison of experimental results between laboratories. Research on N-CAM emanating originally from Edelman's and now many other laboratories has given us more knowledge about the biochemistry and molecular biology of this molecule than any other ever proposed to be involved in cell adhesion (84,85). Among the interesting properties of N-CAM and a number of related adhesion and recognition molecules is a high degree of molecular homology with immunoglobulins, and all are now referred to as members of the immunoglobulin supergene family (86). Burnet's "thin speculation" of 1960 regarding the evolutionary origin of the immune system was insightful indeed.

## 6. The Role of Calcium in Cell Adhesion

Calcium has long been associated with vertebrate embryonic cell adhesion and has already been discussed in several contexts, without, however, providing a satisfactory explanation for its functional role. Many early observations pointed to its importance. Sydney Ringer, of solution fame, established his famous brew for the maintenance of beating frog hearts in a defined saline. He later extended his studies to test the effects of various solutions on the survival of fish and ultimately frog eggs and tadpoles, noting the enhanced survival in solutions containing calcium. The absence of calcium from the solution was noted to cause the separation of epithelial cells, leading Ringer to conclude that the major effect was on the "cement substance binding the animal cells together," with removal of calcium causing separation of the cells from one another rather than the general disintegration of the cells themselves (87). Roux (2) had noted that amphibian embryos were more easily dissociated into cells in calcium-free medium, and Herbst (88) made a similar observation on sea urchin embryos. The dissociation of tissues into cells, whether enzymatic or mechanical, was generally carried out in calcium-free medium, as by Moscona for chick embryos (53), and sometimes with the addition of chelators, such as EDTA for rat livers by Anderson (89) or for chick embryos by Zwilling (90).

The key to calcium's protective effect against tissue dissolution was deduced by L. Rinaldini, an Argentinian histologist (91). In an extensive review of methods for the isolation of living cells from animal tissues, Rinaldini discussed both the state of knowledge regarding the nature of "intercellular matter" between animal cells and the effects of various agents, including proteolytic enzymes and calcium ions, on tissue dissociation. Trypsin was extensively dis-

cussed, in terms of both its enzymatic properties and its use for tissue dissociation. It was noted that calcium enhanced the activity of trypsin, although it was made clear that this is due to a protective effect against autolysis by trypsin and not to a classical ion activation, as is the case for other enzymes. The enzymological evidence suggested that calcium combines with trypsin and protects a portion of the molecule that would otherwise be subject to proteolysis. Rinaldini noted that this effect might be similar to the protective effect of calcium on serum albumin against proteolysis. Importantly, he realized that

this effect is of practical and theoretical interest in connection with the digestion of the ground substance by trypsin. . . . The elimination of calcium and magnesium salts from the dispersant medium was found by Moscona (1952) to enhance the dissociation of early embryonic rudiments by tryptic digestion. These observations would appear *prima facie* to contradict the notion that calcium ions activate trypsin, but we have already seen that this effect is not a true activation but merely a protection exerted on the protein molecules against its own proteolytic activity; therefore it is conceivable that a similar protection may be exerted on other protein substrates and that the removal of calcium from the tissues may render them more susceptible to tryptic digestion (91).

A not unrelated suggestion had been made by Steinberg, as an alternative to his calcium bridge hypothesis, that "one might still contend that the bivalent cations induce in the cell surface a unique condition in which nonionic binding sites are properly displayed," although Steinberg (40) considered this to be a more complex explanation than a simple calcium bridge.

The role of calcium remained unresolved, such that Trinkaus could observe that "the calcium problem is symptomatic of our knowledge of cell adhesions in general" (7). Steinberg reexamined the role of calcium in a series of experiments concerning the kinetics of cell adhesion and the recovery of adhesiveness following trypsinization, making the observation that cells trypsinized in the absence of calcium exhibit a lag period, while cells trypsinized in the presence of calcium show no such lag and are able to aggregate immediately. Steinberg argued that

if one knew why cells are not adhesive during the first half hour following exposure to trypsin and the nature of the changes that take place during this period that allow cells to recover adhesiveness, the information would be directly applicable to problems of the mechanism of cell adhesion. . . . attempts to elucidate the biochemistry of inter-cellular adhesion through a study of the aggregation lag would hold the greatest promise if it could be demonstrated that the changes responsible for the initial inability to aggregate were caused by an effect of trypsin directly on the cell surface (92).

In an attempt to measure surface changes, Steinberg analyzed cell surface charges by cell electrophoresis but detected no differences between cells trypsinized with and without calcium. Steinberg concluded that perhaps there was a difference in the distribution of charges, rather than in their amount, which was responsible for the different adhesiveness of the two cell populations. No indication is given that Steinberg considered the possibility earlier suggested by Rinaldini that a qualitative difference due to effects on a specific surface component was responsible.

Biochemical evidence to support Rinaldini's idea finally arose from the work of Masatoshi Takeichi. Not unlike the contemporaneous view expressed by Edelman, Takeichi also had a sense of dissatisfaction with the prevailing state of understanding of cell adhesion, observing that "although there have been many

studies of the adhesive properties of cells, the reports from different laboratories are often conflicting" (93). Noting that the discrepancies included opposite conclusions regarding the effects of temperature and divalent cation dependence, Takeichi wrote that these conflicting results could be due either to a difference in the adhesive properties of different cells and/or to the existence of multiple cell adhesion mechanisms the detection of which would depend on the mode of cell preparation and assay. Takeichi's approach to the problem, not unlike that of Holtfreter's work on cell dissociation, had a serendipitous foundation:

I found the effect of calcium by chance. I was in the Department of Embryology, Carnegie Institution, and used a solution of trypsin available there for dissociation of Chinese hamster V79 cells. These treated cells never reaggregate in a balanced salt solution, although I had an experience in Kyoto that trypsinized cells generally reaggregate in a  $\text{Ca}^{2+}$ -dependent manner under the same conditions. Then, I checked the difference between the trypsin solutions in Carnegie and Kyoto. The difference was that the Carnegie's contained EDTA but Kyoto's did not. Then, I examined the chelation of which ion is essential. It was  $\text{Ca}^{2+}$ . After all, I found that the presence of  $\text{Ca}^{2+}$  prevented a  $\text{Ca}^{2+}$ -dependent cell adhesion molecule from trypsin digestion. This was the story of how I found cadherin activity. After these findings, I prepared antibodies which can block the activity of  $\text{Ca}^{2+}$ -dependent cell aggregation, and then identified the antigens, that were termed cadherins (94).

Takeichi's experiments (93) showed that calcium protected a specific cell surface protein against tryptic digestion as well as against enzyme-catalyzed iodination, suggesting that the conformation of the protein is calcium-dependent. Furthermore, the cells could be manipulated to express either of two distinct adhesive systems, one of which was resistant to low levels of trypsin treatment even in the absence of calcium, was functionally calcium- and temperature-independent, and permitted the loose association of cells; and a second, which was sensitive to low levels of trypsin when no calcium was present but resisted even high levels of trypsin if calcium was present, was functionally both calcium- and temperature-dependent, and permitted the tight association of cells to the point of membrane deformation (93). These results echoed those obtained earlier by Gerisch on slime mold adhesion and showed for the first time that a single type of vertebrate cell could indeed possess functionally independent adhesive systems.

While the initial studies were done on fibroblastic cell lines, Takeichi extended his method to embryonic cells. As these methods allowed for the preparation of cells that expressed either or both of the distinct adhesive systems, cell populations could be prepared, labeled, and mixed back together in aggregation culture. Using such an approach, Takeichi demonstrated that cells possessing one or the other of the two systems would sort out from one another and form separate aggregates, while cells expressing the same system would form a mixed aggregate, even if composed of very different types of cells (95). That antibodies could independently block one or the other of these two adhesive systems suggested that discrete antigenic targets, and hence distinct cell surface molecules, might subserve the two adhesive systems (96). Takeichi noted that "these results indicate that the conditions used for cell dispersion are most critical in the experiments for determining the adhesive selectivity of cells. . . . Some inconsistencies found among published results concerning the adhesive selec-

tivity of cells in initial cell adhesion is probably due to the difference in dispersion conditions employed in different laboratories. Very extensive previous data dealing with the specific cell adhesions must be carefully reexamined in light of the present results" (95).

Indeed, such a reexamination was stimulated by Takeichi's observations, leading to a rare confluence of consistent findings from the laboratories of Lilien, Steinberg, and Edelman. Subsequent reports from all three groups, each analyzing the adhesive interactions of chick embryo neural retina cells, concluded that embryonic cells within a single tissue indeed possessed multiple adhesive systems which were immunochemically and functionally distinguishable and under distinct developmental regulation (97–103). In addition to the calcium-independent cell adhesion molecule N-CAM described earlier, these retinal cells were shown to express the calcium-dependent cell adhesion molecule now known as N-cadherin (104–107).

## 7. Epilogue: From the Embryo to the Gene and Back

The past decade has seen an explosion of research in cell adhesion, in no small part due to the application of technological advances such as monoclonal antibodies and recombinant DNA. These and other techniques have permitted the identification, and subsequent structural and functional analyses, of an increasingly large array of molecules believed to regulate various aspects of cell interactions during development. Recent experiments from the laboratories of Takeichi and Edelman (108,109) demonstrate the power of modern molecular biological techniques and also bear directly on many of the central questions raised earlier by Holtfreter, Steinberg, and Moscona. By transfecting poorly adhesive fibroblastic cells with cDNAs coding for various cell adhesion molecules, the cells were rendered strongly adhesive and adopted a more epithelial behavior, thus directly demonstrating the ability of cell adhesion molecules to serve as intercellular ligands. Furthermore, when these cells were mixed with reconstituted embryonic organ rudiments containing epithelial and mesenchymal components, the untransfected fibroblastic cells sorted preferentially with the mesenchymal components, while the transfected epithelialized cells sorted with the epithelial component of the tissue. Thus, Holtfreter's hypothetical tissue affinities, which he earlier succeeded in attributing to the properties of individual cells, have in turn become attributable to the properties of individual molecules. These experiments have shed light as well on the issue of the primacy of qualitative or quantitative differences as a basis for cell sorting. Several different lines of transfected cells were constructed, expressing either similar levels of qualitatively different cell adhesion molecules or quantitatively different amounts of the same cell adhesion molecule. By producing various combinations of mixed cell aggregates, it was demonstrated that either quantitative or qualitative differences alone in the expression of cell adhesion molecules both resulted in the sorting out of cells. Thus, elements of both the differential adhesion hypothesis of Steinberg and the specific ligand hypothesis of Moscona stand vindicated.

Additional recent experiments continue to link together earlier general

concepts of cell adhesive interactions with current biochemical and molecular genetic studies of the above mentioned cell adhesion molecules. The role of cell surface charges in regulating cell interactions, emphasized by Curtis and others, has been brought back into focus by Rutishauser and colleagues with the demonstration that the polysialic acid moieties of N-CAM may have profound effects on the cellular behaviors influenced by this adhesion protein beyond the primary effects of cell adhesion per se (110). The role of cell surface glycosyltransferases in regulating cell adhesive interactions, discussed by Roseman and others, has also been revitalized by the observations of Lilien and colleagues of direct interactions between this class of enzyme and N-cadherin (111). Finally, direct manipulation of cell adhesion molecule expression during embryonic development by Kintner and Takeichi and their colleagues, through injection of N-cadherin mRNA transcripts into *xenopus* oocytes, leading to ectopic expression and abnormal development, has indeed begun to bring studies of the role of cell adhesion in vertebrate embryonic development full circle (112,113).

One hundred years after Roux could dissect embryos only with a needle and his intellect, the tools available to embryologists today cut much more finely. However, as Paul Weiss recognized, the path ahead must include a return to the embryo, "lest our necessary and highly successful preoccupation with cell fragments and fractions obscure the fact that the cell is not just an inert playground for a few almighty masterminding molecules, but is a system, a hierarchically ordered system, of mutually interdependent species of molecules, molecular groupings, and supramolecular entities" (114). The manner in which different cell adhesion molecules interact with each other and with other cellular elements such as the cytoskeleton, how their expression is regulated genetically and epigenetically, and how their function is integrated on the cellular, tissue, and organismal levels during development remain active areas of investigation.

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## Chapter 8

# The Philosophical Background of Joseph Needham's Work in Chemical Embryology

P. G. ABIR-AM

### 1. Introduction

Throughout the 1930s, Joseph Needham (1900–) was the foremost champion of chemical embryology. During the period 1931–1942 he authored three large books, *Chemical Embryology*, *A History of Embryology*, and *Biochemistry and Morphogenesis* (1); a series of collaborative papers on “Physico-chemical experiments on the amphibian organizer” (2); and many annual reviews of this field for the *Annual Review of Biochemistry*.

Needham worked in the Dunn Laboratory of Biochemistry at Cambridge University, where, since 1933, he was the Sir William Dunn Reader. By his own account, Needham’s colleagues in the Dunn Laboratory were chiefly interested in enzyme kinetics, and, with the exception of Sir Frederic Gowland Hopkins, Professor of Biochemistry, they did not consider his biochemical forays into the complex embryological material as likely to illuminate biochemistry’s disciplinary aspiration (3). At the same time, many descriptive embryologists questioned the value of Needham and his collaborators’ contributions to embryology as those contributions were both theoretically oriented and limited to physicochemical experiments (4).

Why did Needham, then the “second-in-command, in British biochemistry,” choose to focus his entire research effort on unifying two antagonistic biological subdisciplines: biochemistry, known as the most reductionist, and embryology, known as the most “irrational and irreducible”? Needham’s scientific agenda in biochemical embryology, deemed unusual by most of his colleagues in both biochemistry and embryology, was both enabled and constrained by his philosophical interests in reconciling mechanism, which Needham considered to be the best methodological approach to biology, with organicism, which Needham came to regard as a metaphysically superior approach, as a result of his contact with the philosopher of biology Joseph Henri Woodger (1894–1981) (5).

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The following examination of the philosophical rapprochement between Needham and Woodger, as expressed in their correspondence in the late 1920s and early 1930s, sheds light on the formation of some of the philosophical commitments that informed Needham's pioneering quest for a union between biochemistry and embryology in the 1930s. The correspondence reveals a gradual pattern of rapprochement between Woodger, a once classical or descriptive embryologist turned theoretical and philosophical biologist, and Needham, a modern biochemist whose historical and philosophical inclinations increasingly came to substitute for his scientific and experimental problems (6).

The Needham–Woodger rapprochement is important not only as an input into their respective endeavors in biochemical embryology and philosophy of biology, but also as the would-be axis for the Biotheoretical Gathering, a group of ideologically sensitive scientists and philosophers whose avant-garde vision of nonhierarchical, interdisciplinary science at the interface of the physical and the biological sciences contributed decisively to the rise of molecular biology in the 1930s (7).

## 2. Joseph Henri Woodger and the Emancipation of Biology

Woodger, who graduated with honors in zoology from University College, London, in 1914, served as Reader in Biology at the Middlesex Hospital Medical School, London, since 1922 and Professor since 1949. He spent most of World War I with the regiment of his county of Norfolk in the Mesopotamian Desert, where his interest in philosophy was aroused by long conversations with Dr. Ian Suttie during the long battle at Shumran Bend and the subsequent march on Baghdad. After the war, Woodger returned to research at University College in 1919 as Assistant in Zoology. In 1922 he was appointed to a new Readership in Biology at the Middlesex Hospital Medical School (8).

In addition to his heavy teaching duties in the early 1920s, Woodger also managed to write a textbook for his students and to become fluent in German. The turning point in his professional life came in 1926 when he was given a term-leave to study with the zoologist Hans Przibram at the University of Vienna. There he was exposed to the philosophical interest of the staff in exploring the relationship between physics and biology along sophisticated neoreductionist lines (9). Shortly after his return to London, Woodger began to write a critique of biological theory very much in the style of the British analytical philosopher and editor of *Mind*, G. E. Moore, of Cambridge University.

Woodger's *Biological Principles*, for which he received a D.Sc. from University College in 1929 and which launched his career as a philosopher, started with a general survey of the historical background and present position of biology in the natural sciences and the relationships between natural sciences and logic and metaphysics. Part I of this 500-page treatise dealt with general problems on the relationships between data and systematization in natural sciences, while concentrating on a detailed presentation and critique of phenomenism and related doctrines and a discussion of the key problems of causation and substance in the natural sciences (10).

Part II, which occupied three-fifths of the book, dealt with "Problems of Biological Knowledge." In a chapter dealing with the mechanist–vitalist dilemma, which he explained as resulting from a semantic confusion caused by mechanism (which had four different meanings), Woodger discussed "The Theory of Biological Explanation." This theory, the main original contribution of Woodger, will be discussed later.

Other problems of biological knowledge discussed by Woodger included five famous antitheses: "Structure and Function"; "Organism and Environment"; "Preformation and Epigenesis"; "Teleology and Causation"; and "Mind and Body." Woodger believed that these antitheses had been imprinted on biological knowledge for centuries and attempted to explain them away with the help of new concepts from the theory of relativity, especially the concept of space–time (see below).

He concluded his book with a chapter on "The Future of Biology" in which he outlined three essential aspects of science, namely, investigatory (i.e., empirical), speculative (i.e., theoretical), and critical, and made a plea for recognition of the importance of the critical aspect, which he had pursued in his book. Woodger further argued that among the then prevailing characteristics of modern scientific thought, the following were of special significance for the progress of biology: (1) logic and epistemology; (2) empirical sciences; and (3) the "organic" view of nature (i.e., the new cosmology of Whitehead, which put the physics and biology on an equal metaphysical footing) (11).

Woodger saw the rationale for his "critical" approach to biological theory as lying in the clearing that had recently been opened for examination of the foundations of science by the conceptual and epistemological implications of the theory of relativity. At meetings of the Aristotelean Society in London, as well as by the semipopular expositions of relativity by Russell, Whitehead, and Eddington, Woodger was exposed to the special preoccupation of British philosophers with the epistemological status of the theory of (general) relativity (12).

This dual context of dramatic philosophical rhetoric and cultural resonance at large endowed the message surrounding the theory of relativity with new meanings for those like Woodger who were troubled by the traditional relationships between physics and biology. He inferred that if the very foundations of physics were in a process of profound change, then it was desirable to attempt the same for biology. Furthermore, new forms of abstraction seemed not only legitimate, but logically more attractive. Once the apodictic certainty of physics proved shakable, reasoned Woodger, biology was free to formulate its own laws without any longer fearing that in doing so it might defy the "perfect" epitome of certainty, lawfulness, and order.

Woodger also noticed that then-recent developments in symbolic logic, namely, the integration of pure mathematics with formal logic, demonstrated that physics did not possess exactness in a mathematically absolute sense. Therefore, physics could not look down at biology at being inexact and unscientific. Based on these considerations, the traditional disparity between physics and biology was thus reduced considerably.

Woodger further reasoned that mathematics was not the key to all under-

standing. He suggested that what both physics and biology needed most were new concepts, possibly similar new concepts. As suggested by Whitehead, the most relevant new concepts for both modern physics and modern biology were the concepts of “organisms” and “events.” Although only a short time earlier such concepts had been declared unscientific because they existed outside the atomistic picture of reality, the recent demise of the atomistic paradigm opened the way to new mutual relationships between physics and biology.

Biology was becoming an equal partner of physics, epistemologically, if not methodologically. The hope for new biological laws could now be entertained, because physics, too, required new laws if its macroscopic and microscopic events were to be encompassed under one system of laws. Continuing this line of thought, Woodger stressed that new biological laws would have to be laws of organization—the central new concept of both physics and biology—and, as such, the search for laws in biology would not involve the traditional threat of reducing biology to physics. This new possibility of asserting the logical independence of biology from the rigid lawfulness associated with classical physics opened the road for biology to progress as an independent, i.e., irreducible, science.

Another major premise that Woodger drew from the revolution in physics was that biology too would have to learn to operate with the concept of space-time. From the viewpoint of the newly perceived four-dimensionality of the world, space and time were not mutually exclusive phenomena, but rather complementary aspects of the same phenomenon. This novel synthetic perspective gave Woodger a rationale for advocating the integration of structure and function in biology. Thus, one could now advocate the unification of fields that pursued structure in separation from function, for example, anatomy and physiology, or cytology and biochemistry.

In what stands as his most original contribution to the critique of biology’s theoretical foundations, Woodger defined “organization” as “the mode of spatial and temporal differentiation of those events which are ‘known as’ individual living things,” while exclaiming that “biology would have to become biological in spite of itself.” Obviously, Woodger’s statement reflected how dependent biology had become on physics for its epistemological status. Even a traditional biological notion such as organization would have to first be ratified by physics before it could become a basis for a new scientific conceptualization in biology. Organization and relations, which physics had just shown to be internal and multiple (rather than external), would be, in Woodger’s view, the pillars of a new synthetic theoretical biology aimed at unifying fields formerly separated by a naïve realist conception of structure or by the mechanist-vitalist gulf.

Toward the end of *Biological Principles*, Woodger declared that he was not concerned with such mundane matters as heuristic success or prediction for utilitarian purposes. Rather, he remained primarily interested in a critique of biology’s foundations, the logical purification of its concepts, and the erection of axiomatic systems for biology in order to establish its philosophical and metaphysical respectability. To summarize, Woodger saw the confluence of modern thought progressing toward realizing the possibility of a new theoretical, scientific, yet irreducible, biology. This new biology would have two sources. First, symbolic logic would be used for working out the consequences of a theory or a

system of premises. Second, new developments in epistemology and metaphysics (namely the perceived inadequacy of the notion of causation and of particles), as well as the “organic” view of nature (based on the sharing of the key concept of organization by modern physics and modern biology) would restructure the meaning of experimental data.

### **3. Joseph Needham, 1925–1933: Mechanism, Organicism, and the Philosophical Status of Biochemistry**

Unlike Woodger, who upon his return from Vienna late in 1926 became fully absorbed with philosophy, while limiting his biological practice to teaching, Needham pursued a prestigious research career in biochemistry at Cambridge University. There, under the direct auspices of Sir Frederick Gowland Hopkins, the “father” of British biochemistry (13), Needham, who enrolled as a medical student like his father, eventually became a biochemist. After having received his Ph.D. in 1925, Needham developed a parallel interest in the philosophy of biology, being especially concerned with resolving the mechanist–vitalist debate.

Needham’s obsession with this debate was probably derived from his strong identification with Hopkins, a beloved teacher and parent substitute to whom Needham would refer as “*in loco parentis*” even 60 years after their initial encounter (14). Hopkins’ earlier career had been plagued by the lack of philosophical credibility and respectability, which biochemistry, as a new discipline, then faced in the philosophy-dominated academic circles. Indeed, it took Hopkins 27 years to establish a Chair of Biochemistry at Cambridge University (dedicated in 1925) (15). Thus, defending the philosophical integrity of biochemistry emerged as a dominant theme in Needham’s writing in the period 1925–1933, which culminated in his rise to the position of the “second-in-command” in British biochemistry when he was chosen for the position of the Dunn Reader in Biochemistry at Cambridge early in 1933.

Biochemistry’s ideal of explanation was extrapolation from the chemistry of the dead to that of the living, as espoused by F. G. Hopkins in a long address before the Physiological Society in 1913 and repeated often in his lectures. These lectures were attended by J. Needham in the early 1920s (16). Thus, he received a first-hand introduction into the fact that biochemistry was strategically situated between biology and the physicochemical sciences. However, the gap between these two domains, created by a conception of scientific order dominated by classical physics’ methodological and metaphysical mechanism, posed a critical dilemma for the biochemist’s endeavors. If they adopted mechanism as their philosophical guide to scientific practice, and they did so in order to be accepted as “scientific,” then their relevance to biology would be questioned, because most biologists held that mechanism was insufficient to account for biological phenomena. On the other hand, if they stressed their biological orientation and concerns, they would open themselves to the charge that biochemistry was not real chemistry, since chemical analysis of complex biological material could not match at that time the standards of accuracy prevailing in “pure” chemistry.

The efforts of biochemists to chart a course on the borderline between two powerful and tradition-bound systems of scientific authority, namely, the chemi-

cal sciences and biology, though prevailing for the most part of the nineteenth century, were finally recognized in the social system of science only in the twentieth century. Opposition to this biochemical enterprise prevented its institutionalization in academia for a long time. For example, as mentioned earlier, it took Sir F. G Hopkins 27 years to establish a Chair at Cambridge University (17). Indeed, it was not until after World War II that departments of biochemistry became a regular feature of universities and research institutes. In the period before World War II, a great deal of biochemical research was conducted in departments of chemistry, physiology, and others (18).

Needham's first written attempt to cope with the problematic philosophical status of biochemistry, "The Philosophical Basis of Biochemistry" (19), revolved around arguments designed to overcome the supposed contradiction in biochemistry's simultaneous claim that it could retain its biological relevance and be scientific (i.e., pursuing a mechanistic philosophy). While relying on the unique precedent set by D'Arcy Thompson's *On Growth and Form* (20), which tackled biological problems, especially the problem of form, using analytical techniques from mathematics, physics, and engineering, Needham sought a compromise so that the ontological uniqueness of biology could be compatible with the (dominant) scientific methodology of mechanism. His solution involved a dissociation between mechanism and materialism while advocating a "mechanical theory of the organism" according to which the uniquely biological was compatible with mechanism since mechanism was depicted as a "mere" methodology.

Three years later, Needham further pursued his quest to bridge the metaphysical gap between chemical and biological realities. This gap impacted directly on his scholarly standing as a career biochemist (because this gap, if real, deemed biochemistry's *raison d'être*—the extrapolation from the chemistry of the dead to that of the living—all but impossible). In his "Recent Developments in the Philosophy of Biology" (21). Needham tried to reconcile the supposedly contradictory requirements for being biological and scientific by utilizing Whitehead's new "organic theory of nature," which stated that all nature, encompassing both physics and biology, was composed of "organisms" of various degrees of complexity and was best understood in terms of "events." A direct outgrowth of this theory was a continuous world picture which held the promise of relieving biochemistry from its formerly peculiar status as a borderline instance between two, presumably discontinuous realms (22).

In 1925, Needham believed that mechanism alone was a sufficient mode for the scientific account of nature. However, by 1928 he espoused organicism as a complementary explanation. Needham's "solution" rested on removing the mechanist–vitalist dualism from within science to the no-man's-land between science and philosophy. He argued that mechanism should be retained only as a methodology and only for "experimental and exact biologists," whereas organicism should be recognized as the superior metaphysical system since it alone could properly deal with the metaphysical problems of both physics and biology (23). Needham, a founding member of the Society of Experimental Biology, which was founded in 1923 to foster scientifically respectable research in this field, felt that "experimental and exact biologists" would be relieved that the concept of organism became common to both biology and physics (24).

In probing Needham's philosophy, one finds that he distinguished between the scientific and the philosophical mind. The scientific mind saw the living and nonliving as forming one continuous series of systems with various degrees of complexity interpretable in terms of material macroscopic mechanism, with mechanism being reaffirmed as the "only working principle" for scientists. The philosophical mind saw the entire universe as being composed of organisms of all kinds. Far from solving the dualism mechanism–vitalism, Needham merely created another one by reinforcing the demarcating line between science and philosophy—a convenient solution for a practicing scientist (25). As Woodger phrased it in an unposted letter of May 28, 1928 (which was sent to Needham only after they became friends in the course of 1929), Needham tried to save the contested scientific status of biochemistry by labeling the rest of biology as philosophy (26).

#### **4. Process and Structure in the Woodger–Needham Rapprochement: From Philosophical Misunderstandings toward a Theoretical Integration of Biochemistry, Embryology, and “Logistics”**

Woodger and Needham started corresponding early in 1929 when Needham replied to an inquiry by Woodger concerning the addresses of the philosophers of biology A. Meyer and L. von Bertalanffy (27). In his letter Needham addressed Woodger's charge (in *Biological Principles*) that biochemists had been overlooking cell organization (and thus had no relevance to biology). Needham offered several counterexamples, especially Otto Warburg's work on cell respiration (28) and contemporary views in biochemistry which held that enzymes were a set of conditions associated with colloidal aggregates (i.e., not distinct chemical entities). Needham further stressed that no one had yet succeeded in isolating a pure enzyme (29). Another important example brought forward by Needham in support of the biochemists' concern with structural differences between living and nonliving cells was the work of Vles and Gex, which suggested striking differences between the spectrophotometric results obtained with intact versus cytolysed echinoderm eggs (30).

Needham and Woodger finally met on November 25, 1929, when Woodger came to talk on "Science and Religion" before the Zoological Society in Cambridge, and Needham offered him the hospitality of his College (31). J. Needham was elected as a fellow of Gonville and Caius College in 1923 (32).] Interestingly, Woodger declined to talk on Needham's "neomechanism" as reflected in the latter's publications of 1928 and 1929 (33), or on his own alternative organicist viewpoint outlined in 1929 in *Biological Principles* and in a paper to the *Proceedings of the Aristotelian Society* (34).

Their initial personal meeting was followed by an intense correspondence, revolving to a large extent around an exchange of comments on each other's writings. This ongoing exchange eventually resulted in a rapprochement that would become the basis for the Biotheoretical Gathering in 1932. However, one can gauge the gulf between their initial positions best by considering that un-

posted letter which Woodger wrote on May 28, 1928, as a comment on Needham's "Recent Developments in the Philosophy of Biology," and appended to his letter to Needham of October 8, 1929:

I am afraid I totally disagree with your view of the future of biological methodology. . . . I fully agree that the strong point of "mechanism" is on the heuristic side and that none of the other alternatives have yet offered anything in that direction which is fit to hold a candle to it. But I deny that the "organic" view has been wholly devoid of heuristic merit. . . . I think it is fair to say, too, that some biological discoveries have been made in the teeth, so to speak, of mechanism rather than by its aid. And although an investigation must always begin with analysis there is no reason why it should not be guided by the organic point of view as well as by any other. . . . I am afraid you have hopelessly misunderstood a review of mine in the *Quarterly Review of Biology*. I think it is plain from the context of the passage you have quoted that I am not at all denying. . . , the possibility, legitimacy and utility of applying the mechanistic methodology to man or any other organism.

Woodger was referring to Needham's earlier defense of mechanism in biology as the only solid methodology and his implied statement that biology was scientific only to the extent that it was biochemistry or biophysics. Needham's efforts in this regard were considered by Woodger to be misguided, since just at that time mechanism had been dismissed by recent advances in theoretical physics as the absolute and only criterion of scientific status. Thus, biologists no longer needed to cling to it.

Apparently, until the end of 1929, Needham, steeped in scientific but not philosophical practice, had missed the fact that a new world picture had been replacing the formerly exclusive mechanistic one. Having been primarily concerned with a defense of biochemistry as both scientific and biologically relevant, Needham had not yet grasped that a new option had just opened up for biology to become scientific without having to assume a mechanist posture. Woodger further remarked that Needham misunderstood his position on mechanism, since he did not profess to be antimechanist but only wished to point out its insufficiency, methodologically as well as metaphysically, for biological explanation. Woodger concluded that Needham's emphasis on mechanism as the only scientific methodology would make the greater part of biology nonscience.

Replying on October 12, 1929, Needham indicated his interest in the unposted letter (which contained the above-mentioned criticism) and remarked regretfully that his collection of essays (*The Sceptical Biologist*) could not be modified in light of Woodger's comments as it was scheduled to appear that month. In a revealing remark, Needham stated that unlike E. S. Russell and W. McDougall, two organicist philosophers who denied that mechanism had any value and with whom he thus could not find any basis for discussion, Woodger and the theoretical biologist von Bertalanffy offered an opportunity for insightful debate. These two did not deny the heuristic value of mechanism and merely pointed out its limitation in the biological realm (35).

Other differences in orientation toward the philosophy of biology between Woodger and Needham were further clarified when Woodger took the opportunity to comment on Needham's *The Sceptical Biologist* first in a letter to Needham on January 25, 1930, and later in print when Woodger was asked to review the book for *Mind*. Woodger's criticism revolved around several key issues which together revealed Needham's philosophical naïveté and disclosed that his

philosophical efforts were primarily designed as an ideological defense of the scientific discipline he practiced—biochemistry.

First, Woodger argued that Needham failed to distinguish between the metaphysical and methodological aspects of the mechanist–vitalist controversy. In a chapter of his book *The Sceptical Biologist*, entitled “Organicism in Biology,” Needham tried to reconcile the heuristic success of mechanism with its metaphysical inadequacy by restricting the meaning of mechanism to a “mere” scientific methodology which had no metaphysical claims, or rather was metaphysically untrue. Metaphysical truth belonged to organicism, which admittedly was heuristically weaker. Woodger criticized Needham’s verbal “compromise” by asking how mechanism could be heuristically successful if it had no counterpart in external nature.

In a similar vein, Woodger pointed out that in his strong desire to “hunt the vitalistic phoenix” (this was also a title of a chapter in Needham’s *The Sceptical Biologist*), Needham failed to distinguish between the diverse meanings of mechanism and organicism. Woodger was especially sensitive to such a negligence because of his then emerging view that language analysis was the basis for the reconstruction of biology’s theoretical foundations.

Continuing his critique, Woodger questioned Needham’s attitude toward scientific method. He claimed that Needham treated it as if it was a matter of divine revelation. This attitude of reverence was derived from Needham’s belief that the only biology worthy of the name of science was biochemistry and biophysics. Woodger regarded this conclusion as questionable because it would exclude the greater part of biology from natural science.

However, the most important criticism made by Woodger from the viewpoint of their future collaboration was his emphasis that both realism and scientific method were in a process of change and expansion. Woodger referred to the “modern movement towards realism” implying that the repertoire of real scientific concepts had come to include new items such as “organisms” and “events.” Moreover, scientific method had just been expanding by accommodating biological organisms as a scientific object. Woodger made these facts clear when he reminded Needham that the ideas of physics and chemistry, the product of a supposed divine method, were subject to profound change at that very time.

Finally, Woodger criticized Needham on moral grounds for suggesting that the pursuit of knowledge, i.e., being a biochemist, and not knowledge in itself, was of value. He ascribed Needham’s position to a reaction against the “simple minded metaphysics of the 19th Century,” rather than guessing that Needham’s main preoccupation at the time was with projecting himself as a defender of biochemistry in scientific circles that had been considering the staffing of the position of Reader in Biochemistry (which had been unofficially vacant since 1926) (36).

Therefore, Woodger urged Needham to abandon his defensive attitude on behalf of biochemistry and focus instead on the analytical examination of chemical methodology in biology and its limitations (i.e., not on its already well-known successes as antivitalistic evidence). He further asserted that the method of the day was critical analytical reflection concerned with logic and epistemology, not with “belief and dogmas.” This was why, he said, he deplored Whitehead’s move to synthetic concerns with metaphysics and cosmology in his

*Science and the Modern World* and regarded Whitehead's crucial importance to lie in his earlier analytical demonstration of the limitations of Aristotelian logic and its extension into a logic of relations, not merely of classes.

Despite his previous comments, Woodger's philosophical orientation to biology was specifically disclosed in his critique of Needham's usage of the term "living substance." Woodger asserted that "the pattern of the organization was alive," not the "living substance." Therefore, he asserted that the important question was not the traditional mechanistic one of "whether the organism can be analysed in terms of physico-chemical entities," but whether the physicochemical concepts were adequate to express relations between the elements into which an organism was analyzed. The difference between living and non-living objects was not only a matter of degree of organization, but a fundamental difference in hierarchical order. What mattered was the type of organization, not its degree.

Even crystals, which Needham presented as examples of a lower degree of organization than that of living organisms (but higher than that of noncrystalline states of matter), were seen by Woodger to reflect a different type of organization, not a difference in degree. Moreover, even crystals, because of their internal organization, could not be adequately described in chemical terms. Woodger's conclusion was that the then current physicochemical notions were not capable of dealing with problems of organized entities, as they were not invented to deal with those kinds of objects. They merely abstracted certain aspects from the particular types of organization manifested by living organisms.

Hoping to correct this inadequacy, Woodger began exploring the potential of a new symbolic logic, with a view toward discovering types of order that were adequate to express what had been observed in biological organization. Like Wittgenstein, to whom he referred specifically, Woodger advocated that the structure of knowledge should reflect the structure of fact. Thus, he searched for manifold knowledge which could reflect the manifoldness or diversity of biological organization.

His first project along this line would be the development of an "embryological logic," which would consist of developing the general scheme of embryological and genetical theory "more or less deductively from the very few initial empirical data and the causal postulate." He promised to send to Needham the result of this work, which Woodger believed would put an end to what he called the "fruitless discussion on genetical matters by neo-Lamarckians" (37).

In writing his concluding remarks, Woodger reiterated his conception of a critical theoretical biology which he had previously espoused in his letter of November 29, 1929. He reaffirmed that the problem of organization was the central and distinctive problem to be tackled by theoretical biology and emphasized the dependence of the properties of a part on their place in the hierarchy of biological levels, as a logical deduction awaiting experimental demonstration.

Of special importance in this "dialogue" with Needham was Woodger's distinction between intrinsic properties, which did not change in isolation, (presumably those investigated by biochemists) and relational properties (deriving from the position in the organized biological level), which did change upon isolation. This distinction rescued Needham, the biochemist, from the paradox posed by his biochemical practice and Woodger's theoretical biology. Woodger

further stressed that he did not intend his philosophical critique of the biologists' practice to be destructive, as Needham seemed to have intimated. His purpose, emphasized Woodger, was simply to do for biology what philosophers had been doing for physics, namely, analyzing the meaning of terms and their relation to phenomena.

Therefore, he suggested that Needham write a systematic work on the logical epistemology of chemical theory. However, he insightfully added that before beginning, Needham would be better off waiting until he had retired from active biochemical work! (original exclamation). Woodger reasoned that as long as one was working in the laboratory one was confined to "thinking for use," while "pathbreaking thinking" required a quality of life, which he thought was not offered by modern life but might have been available in ancient Greece and in the "spacious days of the Renaissance" (38).

Woodger was preoccupied with the exact meaning and uses of terms:

. . . but I find it necessary to be constantly asking myself in regard to every term I use: What exactly do I mean by this? Is it using me or am I using it? Also in regard to every concept I use: How did I come by this? In relation to what sphere of fact has it been developed and what will be the range of its application? Also "How is it related to what is observed?" (39).

This attitude reflected the influence of the Vienna Circle to which he had been directly exposed during his Viennese sojourn in late 1926. Yet, he was not fully aware that he was on his way to becoming a full-fledged philosopher primarily interested in logical possibilities rather than in experimentally constrained actualities. He saw his efforts as leading to a new theoretical biology, some sort of hybrid enterprise between philosophy and experimental biology, which would combine the logical rigor of philosophy with science's world of observational facts in a system of theoretical deductions. This uneasy alliance between logic and fact, or philosophy and experimental science, would characterize the endeavors of the Biotheoretical Gathering formed by Woodger, Needham, and others in 1932.

The first "rapprochement" between Woodger and Needham was evident when Needham replied to this long letter of Woodger on February 1, 1930. Needham asserted that he was convinced by Woodger that biochemistry needed criticism, not defense. Thus, he added, the book he was writing at that time (*Chemical Embryology*) reflected the fact that 99% of the embryologists were "depressingly morphological and static," while Needham was advocating "dynamic biochemistry" as a superior methodology. Needham's problem surfaced when he informed Woodger that his fellow biochemists were not interested in his excursions into embryology (though his professor encouraged his "digression," having had a broader vision of the discipline than the disciples). "It is too biological for them," complained Needham. He added that biochemists were captivated only by the kinetics of enzyme-substrate, the focus of biochemical research at that time.

On the other hand, Needham's plea to embryologists to accept the virtue of chemical embryology would soon be perceived as a reductionist threat. As a result of his dual interest, Needham stumbled on the problem of the compartmentalization of scientific knowledge into disciplines, and he was confronted with the dilemma of falling between two antagonistic groups of reference. By

virtue of technique and official career he belonged to biochemistry, yet he wanted to solve the problems of embryology, which offered a unique opportunity to apply new revolutionary ideas from theoretical physics such as the concept of space-time. At the same time, Woodger pressed him to abandon his concern with heuristic affairs within science, which “has become so narrowly specialized,” and pay more attention to broad philosophical matters of analysis.

Indeed, on the same day, February 1, 1930, Woodger wrote to Needham that there was difficulty with his treatment of the preformation–epigenesis dichotomy (in *Biological Principles*) and that this was “largely a question of language.” Woodger also noted a change of mind in Needham toward a more biological orientation. However, unlike Needham, Woodger felt the key to embryological puzzles resided not in chemical analysis, but in accounting for the “spatial repetition of cell patterns.”

Two months later, on April 12, 1930, Woodger wrote again asking Needham to comment on his new elaboration of the relationship between embryology and genetics for his essay for the *Quarterly Review of Biology* (40). While commenting on a paper by the Oxford biochemist R. Peters, which Needham sent to him as evidence for a biochemist’s occasional interest in problems of cell organization (41), Woodger remarked that they were far from understanding the connection between the “highest chemical level” and the “lowest biological level” or, for that matter, the connection between physical and chemical levels (e.g., in adsorption phenomena).

Also included in this letter were Woodger’s thoughts about his new idea that change in organized entities might best be captured through an analytical scheme encompassing at least two entities and one relationship (and that analyzability in such terms could be the criterion for the occurrence of intrinsic change). He further complained that such an accurate analysis was impeded by the muddled notion of the predicate, “bequeathed to us by the Aristotelian metaphysics and perpetuated by Aristotelian logic upon which our language is founded” (42).

Replying on the following day, April 13, 1930, Needham responded that he did not contest Woodger’s facts and that putting biological facts in accurate form was a great advance. However, he was not clear about the precise nature of the organizing relations espoused by Woodger, an oversight that Needham politely attributed to an obscurity in reality, not in Woodger’s representation of it. He also mentioned that Wittgenstein, “a most remarkable person, even more than his book,” would be in Cambridge for the next term and asked Woodger whether he had met Wittgenstein.

Further articulation of Woodger’s differences with Needham came in an exchange of letters concerning their mutual book reviews, in particular Needham’s review of Woodger’s *Biological Principles* (43). This review triggered a letter to the editor of *Mind* by an angry Woodger, who felt that Needham entirely misunderstood his scope and goals. While Woodger intended his book to be a philosophical contribution in the tradition of Wittgenstein’s “logical clarification of thoughts” and C. I. Lewis’s “study of the *a priori*,” Needham evaluated it as if it were a scientific contribution. Needham concentrated on Woodger’s sparse remarks on empirical matters (to which Woodger admitted to have assigned little importance) and ignored Woodger’s original contributions to a philosophy of

biology, namely, his proposals on how to transcend the mechanist–vitalist dichotomy (44).

Nevertheless, Needham was impressed with Woodger's severe criticism of the methodological deficiency of biology, especially with Woodger's assertion that biology was still following the dictates of nineteenth-century physics instead of reexamining its foundations in the way twentieth-century physics did after the advent of the theory of relativity and quantum theory. He agreed with Woodger that it made no sense for biology to continue imitating what physics had just dismissed, though he apparently overlooked the fact that he had only recently defended mechanism as the only sound methodology in science.

Indeed, Needham complained that Woodger did not provide a sufficient number of examples to illustrate his new principles. Obviously Woodger could not find such examples, since, as he states, the biologists were too busy imitating classical physics. Therefore, Needham clung to the few concrete examples mentioned by Woodger, such as the dichotomies structure–function, embryology–genetics, and morphology–biochemistry. These were all examples that Woodger believed could be shown, through the deployment of the concept space–time, to be the product of outdated and incompatible modes of abstraction, as well as nonexistent in nature.

Needham continued his critique by identifying the major innovation of Woodger's book, namely, that a new theoretical biology should revolve around the concept of hierarchy of levels of organization. This concept of organization could be reformulated so that it would encompass logical and analytical approaches, while at the same time being distinctly biological and scientific. Such a reformulation was possible because the concept of organization had recently been “cleared” from its vitalistic and nonscientific connotations by the then recent epistemological and conceptual upheaval in physics. It eventually acquired respectability and “reality” when Whitehead designed a new cosmology based on his “organic theory of nature,” in which physics and biology, rather than being antagonistic, came to resemble each other. After all, both were concerned with organisms and events as the ultimate units of reality, and both needed new concepts and new laws.

Needham considered Woodger's emphasis on the probability that biology's problems were absolutely unsolvable using the classical lines of thought as one of the best aspects of the book. This statement was no longer a source of despair since classical physics had been demolished as the supreme foundation of the scientific outlook. Biology could now expect to profit from the recent development in theoretical physics, logic, and epistemology.

Despite these praising views, Needham's review upset Woodger, who felt he was misunderstood. Woodger's goal had been to convince philosophers of the philosophical prospects of biology (as they had been focusing on physics and mathematics only). However, Needham had judged it primarily from the viewpoint of the practicing scientist and so, he remarked, Woodger was not offering enough guidance to the experimental biologist to follow. Once again, the different outlook of Woodger and Needham came publicly into focus and suggested that they approached their joint interest in theoretical biology from two opposite poles.

As Woodger explained, his task was to clear the way for biological progress

by using philosophical methods and by examining the methodological, ontological, and epistemological assumptions of practicing biologists. Biologists, like most scientists in other disciplines, were too busy imitating the successes of physics to understand the recent profound changes in physics' own presuppositions, asserted Woodger. In view of what he felt to be an entire misunderstanding of his effort by Needham, "missing the wood for the trees," Woodger stated that a rapprochement between the philosophical and natural sciences along the lines he had attempted in his book still encountered difficulties. He felt that practicing scientists had misconceptions about philosophy. Most of them thought of it either as an inferior rival or as an alternative endeavor to science, if not as a mere "constable who keeps peace between science and religion." Expounding on this point, Woodger wrote that he merely wished to deploy new methods of philosophical analysis in treating problematic concepts in biology, which scientists did not and could not treat. In a later letter, Woodger further amplified his position on what philosophy alone could do for natural sciences and what they could achieve on their own (45). He concluded his discourse by telling Needham that only von Bertalanffy understood his interest in theoretical biology, i.e., to pave the way before biological progress by using philosophical methods (46).

Woodger continued to think of his enterprise as a mixture of philosophy and natural sciences. Although his methods remained philosophical, he also wished to maintain relevance and connection with empirical progress in biology. He wanted to establish theoretical biology as an integral part of biological science, much as theoretical physics was an integral part of that science. Naturally, in light of the then recent ontological and epistemological priority accorded to theoretical physics, he might have entertained the hope that theoretical biology, too, would become the determinant of biological, and even scientific, reality. To be sure, this would occur only if theoretical biology could both anticipate experimental results and order them in a logically coherent and mathematically consistent scheme of laws.

Two months later, on October 2, 1930, Woodger turned more explicitly in favor of theoretical biology, i.e., thinking about biological data in biological terms without reference to other sciences. He asserted that there ought to be such a thing as "pure biology," as no one in the physical sciences knew enough biology while the biologists themselves were too busy imitating the physical scientists and, thus, had not developed, as yet, any purely biological system of thought (47).

In line with his stated priorities (of logic as primary and experiments as secondary), Woodger requested that Needham secure the addresses of several embryologists who would eventually supply the "facts" that Woodger was to fit into a "logical skeleton" or "framework." By 1931 the recent discovery of the "organizer" had captivated many embryologists. This discovery would also become the cornerstone of Woodger and Needham's theoretical, philosophical, and experimental aspirations (48). The problem of organization, on which they wished to erect their new theoretical biology, seemed to have found an exciting empirical system at the very frontier of embryology.

The year 1931 also marked the initiation of an important new influence on Woodger's view with regard to theoretical biology. Previously, he had thought about building a theoretical biology based on the example of what the theory of relativity had done for physics. However, in 1931, he proceeded to seize on the

relevance of quantum theory as well. In particular, Woodger was captivated by Harold Jeffreys' formulations of the lack of individuality of the electron in the atom, if the atom was conceived of as a system. Quoting Jeffreys' then recent discussion of quantum theory in his book *Scientific Inference* (49), Woodger noted that one only needed to substitute "cell" for "electron" and "whole organism" for "atom," and then the quantum physics picture, which "only when the electron emerges from an atom and travels freely . . . behaves as an individual," would be perfectly analogous to what "organicists" were trying to say about living organisms (50).

Despite his interests in keeping abreast of recent advances in both biology and physics, Woodger remained captivated primarily by philosophical concerns. Thus, in the last letter he wrote Needham before they decided to establish the Biotheoretical Gathering, on January 8, 1932, Woodger mentioned his then growing interest in problems of causation (a typical philosophical trapping, especially for a scientist). He further indicated that he had been attempting to devise a theory in which causation appeared as a result of an *a priori* postulate, rather than as a starting point (51). Woodger's absorption with philosophical problems in their own right, not merely as means for speeding actual biological progress, would have a significant impact on future members of the Biotheoretical Gathering. Woodger's influence would result from his personal position as host and informal leader, as well as from his being philosophically situated in a middle-of-the-road position between two major orientations prevailing in the Biotheoretical Gathering. On one hand, members who were philosophers and mathematicians were primarily inclined to explore possibilities rather than actualities. On the other hand, members who were experimental scientists remained primarily interested in the pragmatic heuristics of laboratory life and its daily production of plausible "results."

## 5. Conclusions: The Philosophical and Social Context of Biochemical Embryology in the 1930s

Needham and Woodger's private rapprochement of the diverse world views and disciplinary ideals of embryology and biochemistry in the period 1928–1931 was grounded in their joint philosophical interest in reconciling mechanistic methodology with organicist metaphysics in biology. By 1931, however, this philosophical problem and centuries-old controversy, which Needham and Woodger came to share in the late 1920s, was transformed into both scientific, historiographic and policy agendas. Thus, the Presidential Address to the Centennial Meeting of the British Association for the Advancement of Science held in London in September 1931, which has traditionally signified central issues of science policy, focused on restructuring the relationships between physics and biology, of which the relationship between biochemistry and embryology was a particular case (52).

Similarly, the Second International Congress for the History of Science, also held in London in summer 1931, featured a special symposium on the "Historical and Contemporary Relationships between the Physical and the Biological Sciences." Woodger and Needham were among its eight speakers which also in-

cluded British biologists J. S Haldane, E. S. Russell, and L. Hogben (53). While Woodger's contribution was primarily concerned with the philosophy of biological language, especially with semantic distinctions between propositions in the physical and the biological sciences (54), Needham addressed specifically the problem of the unity of the scientific method for biology and the physics of the future (i.e., nonclassical physics). In Needham's view, this methodological unity further enabled the resolution of the mechanist–vitalist controversy and a union between biochemistry and embryology.

Needham, whose two books *The Sceptical Biologist* (1929) and *Chemical Embryology* (1931) had included large historical sections, gave a presentation notable for its historical erudition. Besides referring to illustrious contemporaries (such as Whitehead, Eddington, and Driesch), Needham also made reference to an impressive gallery of scholars or cultural figures, Greek, Renaissance, and modern, including, among others, Galen, Democritus, Aristotle, Herophylus, Leonardo, von Haller, His, and Thomas Hardy. Analogies to Hellenistic concepts and Old Testament events further colored Needham's paper, entitled, like those of other speakers, “The Historical and Contemporary Interrelationship of the Physical and the Biological Sciences” (55).

Like his friend Woodger, Needham began his presentation by noting the confusion and absurdity surrounding the historical formulations of the mechanist and the vitalist positions on the relationship between physics and biology. While the mechanist position sought to base biology entirely on physics while ignoring the specificity of biology, the vitalist position denied any relevance of physics to biology while ignoring the heuristic importance of physical methods and concepts in biological research. In Needham's opinion, the relationships between physics and biology had to be recast in a way that would allow biology to continue to base itself on physics, especially the physics of the future, but at the same time allow biology's major problem to be recognized as the problem of organization. Essentially, he advocated a new empirical attack on the problem of biological organization.

In support of his position Needham quoted A. N. Whitehead's new scientific metaphysics, which regarded organisms as the ultimate components of scientific reality. According to Whitehead, physics should deal with simpler organisms and biology with the more complex ones (56). This formulation conveyed a status of metaphysical equality on physics and biology, while also justifying, in the name of their ultimate isomorphism, the mutual sharing of methods and concepts. Another crucial implication of Whitehead's metaphysics was its projection of a continuity between the physical and the biological realms. The biological realm was defined as an extrapolation of the organic and the more complex from the inorganic and the simpler. Of course, all these components were products of the same fundamental laws (57).

Needham's emphasis on Whitehead's metaphysics was understandable, especially from the viewpoint of his scientific practice as a biochemist. Biochemistry's legitimacy as a science rested precisely on the recognition of a continuity between the physicochemical sciences and biology, which alone could justify the extrapolation of findings from the chemistry of the dead to that of the living. During the 1920s, Needham had encountered the attempts of his beloved Professor of Biochemistry, F. G. Hopkins (58), to consolidate biochemistry as meta-

physically respectable. During these forays, Hopkins had to counteract the authority of philosophically dominant circles which denied, despite biochemistry's heuristic successes, its *raison d'être* (i.e., the extension of the chemistry of the dead to that of the living) as being philosophically impossible (59).

Indeed, as Needham commented, the relationship between physics and biology involved two items of faith: the first involved the possibility of explaining the more complicated in terms of the simpler, while the second involved the "essential unity of the scientific method." Thus, Needham's position could be termed a form of modified mechanism, as opposed to the crude mechanism (or crude vitalism and passive organicism) which he deplored in his talk. While accepting both "items of faith" as essential ingredients of the scientific method, associated historically with mechanism, he further insisted that the major problem of biology was organization, a problem that fell outside the scope of the traditional capacity of the mechanist method. Therefore, Needham placed his hopes in the physics and metaphysics of the future, which, unlike classical physics, accepted organization as a scientific problem.

Needham then proceeded to give a concrete example that further illuminated to what extent his philosophical orientation toward a synthesis between mechanism and vitalism had been influenced by his scientific practice on the borderline of biochemistry and embryology. He noted the possible promise of the concept of space-time in solving one of the then greatest puzzles in experimental embryology, namely, the observation that the geometrical position of a part often determined organizational features displayed by the whole (60). Unlike the embryologists of the prerelativity era, for whom geometrical position was absolute, Needham and other embryologists of the postrelativity era could hope to solve the "grave" problem of the relationship between the parts in the developing egg by exploring the new concept of spatial relations as an internal phenomenon, rather than an external one. Thus, the physics of the future gave embryology a new theoretical scope, said Needham, by freeing it from pseudoscientific entities, such as the Drieschian entelechy (61).

To be sure, it was the future of theoretical biology as the key to solving the problem of biological organization which most preoccupied Needham. Like Woodger, he was very impressed with theoretical physics' capacity to solve the major problems of physics, such as the structure of the atom. The new isomorphism between physics and biology, which had been explicated philosophically by Whitehead, gave both Woodger and Needham a reason to believe that a similar coup could be accomplished in biology. In this respect, Needham noted the existence of two schools of theoretical biologists at the time. One school, he said, regarded organization as a basic fact of life and of biology, a fact which required no further explanation. He named two former speakers, J. S. Haldane and E. S. Russell, as well as a Professor Thomson as being associated with this school, which Needham further characterized as "neo-vitalist" and as "passive organicist" (62).

The other school was represented by Needham's friend Woodger, the Viennese theoretical biologist Ludwig von Bertalanffy, and Professor L. G. M. B. Becking (a projected speaker at the Congress who failed to appear and one of the commentators on Needham's *Chemical Embryology*) (63). They believed, together with Needham, that organization and organizing relations should be the

object of further explanation. Needham castigated the first school for treating organization as “a golden calf” and stopping the scientific inquiry “precisely where the scientific worker ought to begin” (64). Quite evidently, Needham was seeking justification for his own then recent interest in experimental embryology, a field which had not yet been established in England and which Needham and two collaborators would import from Germany in the following 2 years (65).

Finally, Needham came to perhaps the most original part of his argument when he sought to counteract J. S. Haldane’s insistence on the right of the conception of life to a separate interpretation within science. At the same time, he argued for a similar right for crystallography, since crystallography, like biology, had its *raison d'être* in the study of organization. The crystal, said Needham, was more organized than inorganic matter, but less organized than biological organisms. As such, by possessing a level of organization of its own, crystallography was similarly entitled, as much as biology, to a separate interpretation, asserted Needham. His purpose for invoking crystallography’s concern with organization was to combat the dualism or pluralism in scientific method implied in the neo-vitalist positions of advocates such as Haldane, by diluting the unique association between biology and organization. Furthermore, crystallography both indicated that organization was open to empirical investigation and strengthened the argument on the continuity of the scientific realm. This resulted from its units of analysis, which were intermediary in their degree of organization, and because of its plausibility of obtaining exact, analytical results. Indeed, Needham’s colleague and friend at Cambridge University the crystallographer J. D. Bernal would present at this Symposium new experimental findings of direct relevance to the questions raised by Needham on the unity of the scientific method and of science.

In concluding his talk, Needham expressed the hope that the pendulum, which had swung between crude mechanism (or a “blind belief in physics”) and crude vitalism (or a “total scepticism for physics”) throughout the history of biology, would now shift in favor of “the existence of exact biology, and the unification of science” (66). Interestingly, a rather similar hope and rather forceful assertion was expressed at the Symposium by the Soviet speaker. Unlike Needham, who drew his confidence in the unity of science and the lawfulness, but distinctiveness, of biology from recent changes in theory and epistemology of physics, the Soviet speaker Boris Zavadovsky drew his confidence from the then recent and “official” position of Soviet dialectical materialism. That all-embracing philosophical system was supposed to “cover” not only the key issues in history, society, politics, and the economy, but also the specific problem of the relationship between physics and biology.

Following the turning point of 1931, when the restructuring of the traditional relationships between physics and biology was recognized as a scientific, historical, and policy priority, Woodger and Needham became the main organizers of the Biotheoretical Gathering. This group of scientists and philosophers set out to explore, both theoretically and experimentally, the then new agenda of recasting the traditional relationships between the physical and the biological sciences. By mid-1930s, their group was poised to conduct large-scale interdisciplinary research in “mathematico-physico-chemical morphology” with support from the Rockefeller Foundation (67).

By the late 1930s, a variety of policy-related problems—pertaining to the support of Needham and his collaborators' innovative project in physicochemical morphology at tradition-bound Cambridge University, the radical reputation of Needham and his collaborators in social and political matters, and the Rockefeller Foundation's inconsistency in the face of controversy stirred by the threat of interdisciplinary research to disciplinary authority in science—ultimately led to termination of research in physicochemical embryology. Only a generation later was this line of research revived (68). By then, molecular biology had already taken off at the simpler level of biological organization of unicellular organisms, especially bacteria. Needham and his collaborators' interwar physicochemical embryology was not easily accepted by either biochemists or embryologists, as evidenced by the dissent among the many advisors consulted by the Rockefeller Foundation in this matter. Yet, paradoxically, physicochemical morphology pioneered the colonization of the “no man’s land” conceptual space at the interface of physics, chemistry, and biology, which in due course became known as molecular biology (69).

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29. See Enzymes, viruses and other proteins (essays in honor of J. H. Northrop), 1962, *J. Gen. Physiol.* **45**(Suppl):1–265; Northrop, J. H., 1961, Biochemists, biologists and William of Occam, *Annu. Rev. Biochem.* **30**:1–10; Fruton, J. S., 1977, Fifty years ago: Willstatter's lectures on enzymes,

- Trends Biochem. Sci. 2:210–211; Nachmansohn, D., 1979, German-Jewish Pioneers in Science, Highlights in Atomic Physics, Chemistry and Biochemistry, 1900–1933, Verlag, New York.
30. Needham to Woodger, Feb. 26, 1929; the reference is to Vles and Gex's paper, 1928, in C. R. Soc. Biol. 98:853.
  31. Woodger to Needham, Oct. 8, 1929, Needham's Archive, Box 2.
  32. See biographical documents in Needham's Archive; also in Werskey, 1978, Chapter 2.4.
  33. See Needham, 1928, 1929.
  34. Woodger, J. H., 1929, Some problems of biological methodology, Proc. Aristotelian Soc. 29: 331–358.
  35. Needham to Woodger, Oct. 12, 1929, Needham's Archive, Box 2; see also L. von Bertalanffy to J. Needham, Oct. 7, 1929, in Needham's Archive, Box 2. For a retrospect on von Bertalanffy's (1901–1972) various contributions to systems theory and theoretical biology see Gray, W., and Rizzo, N. D. (eds.), 1973, Unity through Diversity, A Festschrift for Ludwig von Bertalanffy, Gordon and Breach Science Publishers, New York, especially Kamaryt, J., From science to metascience and philosophy, pp. 75–100. The influence of von Bertalanffy's Theoretical Biology (1932) on Woodger and Needham remains to be assessed.
  36. Needham became the Dunn Reader in Biochemistry in January 1933, when the previous holder, J. B. S. Haldane (1890–1964) resigned on becoming Professor of Genetics at University College, London. However, after 1926, when he became Director of the John Innes Horticulture Institute, Haldane's interests had shifted increasingly toward genetics. Needham's excitement at finally becoming the official "son" of his beloved professor, F. G. Hopkins, was conveyed in a letter to Woodger on Jan. 10, 1933, Needham's Archive, Box 2.
  37. Woodger to Needham, Jan. 25, 1930 (11 pp. letter), Needham's Archive.
  38. *Ibid.*
  39. *Ibid.*
  40. Woodger, J. H., 1931, The concept of "organism" and the relation between embryology and genetics, Q. Rev. Biol. 5:1–22, 483–463; *ibid.*, 1932, 6:178–207.
  41. Peters, R. A., 1930, "Proteins and Cell Organization," Trans. Faraday Soc. 26:797.
  42. Woodger to Needham, April 12, 1930, Needham's Archive, Box 3.
  43. Woodger to Needham, June 2, 1930, Needham's Archive, Box 3: "I was disappointed in your review of my book in Mind, because you seem to have misunderstood my intentions to a greater extent than I should have expected. How appallingly difficult it is for human beings to communicate with one another." Needham's review was published in Mind, 1930, 39:221–226; Woodger's letter to the editor of Mind, G. E. Moore, complaining about Needham's review appeared in Mind 1930, 39:403–405. Woodger's review of Needham's *The Sceptical Biologist* appeared in Mind, 1930 39:244–246.
  44. Woodger's letter to the editor of Mind. The reference was to Lewis, C. I., 1929, *Mind and the World Order*, Scribner's, New York.
  45. Woodger to Needham, Aug. 1, 1930, Needham's Archive, Box 3.
  46. *Ibid.*
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  48. For further details see Abir-Am, 1987. For a retrospective view of the discovery of the organizer and its impact see Needham, J., 1968, Organizer phenomena after four decades: A retrospect, in: 1968, *Haldane and Modern Biology* (K. Dronamraju, ed.), Johns Hopkins University Press, Baltimore, pp. 227–267. See also Medawar, P. B., 1965, A biological retrospect, Nature 207:1327–1331. For a contemporary view of approaching biological organization with physicochemical methods, see Watson, D. L., 1931, Biological organization, Q. Rev. Biol. 5:145–166.
  49. Jeffreys, H., 1931, *Scientific Inference*, Cambridge University Press, Cambridge.
  50. Woodger to Needham, July 7, 1931, Needham's Archive, Box 3.
  51. Woodger to Needham, Jan. 8, 1932, Needham's Archive, Box 3. The first meeting of the Bio-theoretical Gathering took place on Aug. 13–17, 1932; the negotiations over its organization started in April 1932; see details in Abir-Am, 1987.
  52. See Abir-Am, P. G., 1985, Recasting the disciplinary order in science: A deconstruction of rhetoric on "biology and physics" at two International Congresses in 1931, *Humanity and Society* 9:388–427.
  53. *Ibid.*, pp. 396–402.
  54. *Ibid.*

55. Needham's preprint, no date [1931], 4 pp. single spaced, Needham's Archive, Box 3.
56. *Ibid.* On Whitehead's philosophy of science see Plamondon, A. L., 1979, *Whitehead's Organic Philosophy of Science*, SUNY Press, Albany; Leclerc, I., 1975, *Whitehead's Metaphysics*, Indiana University Press, Bloomington; Lowe, V., 1962, *Understanding Whitehead*, Johns Hopkins University Press, Baltimore. See also Needham, J., 1941. Of the five books by Whitehead that Needham discussed as being influential on himself, only *Science and the Modern World* and *Process and Reality* had been published by 1931.
57. Needham [1931], p. 1.
58. Needham, J., 1962, Frederick Gowland Hopkins, *Perspect. Biol. Med.* **6**:2–46; based on a lecture at the Hopkins Centenary at Cambridge University in 1961.
59. Needham, 1925. Some of the views that Needham recalled as part of Hopkins' lectures to medical students in 1922 were originally outlined in Hopkins' Presidential Address to the physiological section of the British Association for the Advancement of Science in 1913; see Hopkins, F. G., 1913, The dynamic side of biochemistry, *Nature* **92**:213–223.
60. Needham [1931], p. 2.
61. See Driesch, H., 1929, *The Science and Philosophy of the Organism*, London.
62. Needham, [1931], p. 3.
63. *Ibid.*
64. *Ibid.* Needham's rhetoric typically combined socialist and religious metaphors. His colorful passages include the following: "If, arriving in front of the heavily fortified living cell, we simply accept the fact of its high organization as a primary datum, we do no more than sit down before it, and dig ourselves in, but if, advancing boldly to the walls, we blow loud blasts upon the trumpets of mathematical physics,—I will not prophesy that what happened at Jericho will happen again, but the odds are heavily in favor of it."
65. For details see Abir-Am, 1987.
66. Needham [1931], p. 4.
67. See "The assessment of interdisciplinary research in the 1930s: The Rockefeller Foundation on Physico-Chemical Morphology, 1988, *Minerva* **26**:153–176; and Abir-Am, P., 1982, The discourse on physical power and biological knowledge in the 1930s: A reappraisal of the Rockefeller Foundation's policy in molecular biology, *Soc. Stud. Sci.* **12**:341–382; Abir-Am, P., 1984, Beyond deterministic sociology and apologetic history: Reassessing the impact of research policy upon new scientific disciplines, *Soc. Stud. Sci.* **14**:252–263.
68. See "The assessment of interdisciplinary research in the 1930s: The Rockefeller Foundation on Physico-Chemical Morphology", 1988, *Minerva* **26**:153–176.
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## Chapter 9

# Induction and the Origins of Developmental Genetics

SCOTT F. GILBERT

If ever a history of ideas in developmental genetics were to be written . . . it would no doubt include as one of its most important chapters an account of the intellectual role that “inductive interaction” between the fields of genetics and embryology has played in the analysis of developmental mechanisms and their genetic control in higher organisms

Salome Gluecksohn-Waelsch (1981)

. . . the outsider sees most of the game.  
Conrad Hal Waddington (1968)

## 1. Introduction: The Problem of Synthesis

At the turn of the past century, the field of heredity included embryology, regeneration, and genetics. Discussions of genetics necessarily entailed a theory of development, and any theory of development had to show why eggs of different species developed in different ways. Thus, the theories of William Keith Brooks (1) or August Weismann (2) did not distinguish between genetics and embryology. The developmental mechanics of His, Roux, and Driesch likewise contained explicit genetic components whereby the hereditary determinants (thought to reside within either the cytoplasm or the nucleus) were seen to direct the processes of organ formation and cell differentiation.

The split between genetics and embryology emerged gradually, largely through the investigations of Thomas Hunt Morgan (3). Whereas most American and German experimental embryologists followed Boveri in thinking that the nucleus was the site of the hereditary determinants, Morgan was convinced that these determinants lay in the cytoplasm. Morgan had collaborated with Driesch on a project that involved the removal of cytoplasm from the uncleaved ctenophore egg (4). The results of such operations were defective embryos. Morgan declared that there was “no escape from the conclusion that in the protoplasm and not in the nucleus lies the differentiating power of the early stages of development.” However, in 1905, his close friend E. B. Wilson and his

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former graduate student Nettie Stevens both provided evidence that the nucleus did indeed contain the determinants of genetics and development. They both correlated the XX chromosome composition with female animals and the XO or XY chromosome complement with male animals (5). If this were true, then the nucleus determined the sex of the individual.

Morgan responded by investigating a parthenogenetic species of aphids, eventually correlating chromosome number and sex. However, he interpreted his results as still being consistent with the cytoplasm having the controlling role in development (6). However, by 1910, Morgan had found mutations in *Drosophila* that could be best interpreted as segregating with the X chromosome. Although he initially resisted this interpretation, he eventually came to see the genes as physically linked on the chromosomes. What had begun as an investigation as to whether the nucleus or the cytoplasm controlled development ended in the founding of the gene theory.

Immediately after 1911, genetics arose as a discipline within experimental embryology, but it soon evolved its own techniques, favored organisms, rules of evidence, and specialized vocabulary which separated it from the rest of embryology. Eventually, it acquired its own sources of funding and new journals (7,8). In his 1926 book, *The Theory of the Gene*, Morgan (9) formalized the split by declaring that genetics dealt exclusively with the transmission of hereditary traits, while embryology concerned the expression of those traits. He claimed that “the sorting out of characters in successive generations can be explained without reference to the way in which the gene affects the developmental process,” and that much confusion had arisen “from confusing the problems of genetics with those of development.” Genetics and embryology began to go their separate ways.

But Morgan remained an embryologist, publishing *Experimental Embryology* the year after *The Theory of the Gene*. When he left Columbia University to head the biology division at the California Institute of Technology, he returned to study the problems of ascidian development. Thus, when Morgan published *Embryology and Genetics* in 1934, many biologists hoped that it would reunite these disciplines. This was not to be the case. It was more a joint textbook than an attempt to resynthesize the field. The synthesis would be left for others to create. In 1939, Richard B. Goldschmidt and Ernest E. Just published their respective attempts to unify the fields. Goldschmidt would have had embryology subsumed under genetics, while Just saw genetics as a rather minor subset of embryology (7). At the same time, at least three other researchers, Salome Gluecksohn-Schoenheimer (later S. Gluecksohn-Waelsch; 1907–), Conrad Hal Waddington (1905–1975), and Boris Ephrussi (1901–1979), were attempting more balanced syntheses of the two disciplines. Burian, Gayon, and Zallen (Chapter 10, this volume) have written an account of Ephrussi’s synthesis of embryology and genetics. This chapter will focus on the work of Gluecksohn-Schoenheimer and Waddington.

This discussion is framed by two questions: First, what were the conceptual foundations of developmental genetics and how did they come into existence? Second, how did developmental biologists learn about the operon model of microbial gene regulation, which was soon to become the major paradigm of developmental genetics? Evidence will be presented that the conceptual founda-

tions of developmental genetics originated with researchers who were trained in experimental embryology but who met with frustration in their attempts to solve questions of development using embryological techniques. In particular, much of what we call developmental genetics today was framed by Salome Gluecksohn-Schoenheimer and C. H. Waddington, two researchers who had been interested in “the organizer problem.” Gluecksohn-Schoenheimer focused her research on mutations to elucidate normal developmental pathways. Waddington’s focus on embryonic cell competence caused him to propose the notion of canalization and its application in genetic assimilation. Later, this same phenomenon of competence caused him to focus on the cytoplasmic regulation of the genome and predisposed him to see the operon as a model for embryonic induction.

## 2. Salome Gluecksohn-Schoenheimer and the Path from Experimental Embryology to Developmental Genetics

### 2.1. Early Work on the T-Locus: The Search for Inducer Genes

The path from experimental embryology to developmental genetics—from the Freiburg laboratory of Hans Spemann to the Columbia University of Dunn and Dobzhansky—was first traveled by Salome Gluecksohn-Schoenheimer. Having studied chemistry and zoology at Königsberg and Berlin, she asked Spemann to be his graduate student in 1928. She does not consider it a happy partnership. “Our first meeting made it quite clear that we were not meant for each other, but I suppose Spemann did not have enough courage to turn me down outright.” Spemann, whom Gluecksohn-Waelsch recalled as being prejudiced against the women working in his laboratory, would not let her work on the exciting projects involving the organizer. Instead, he gave her “a rather boring descriptive study of limb development” for her Ph.D. dissertation research (10).

Spemann, like many other embryologists of his day, had no interest in the new science of genetics. However, although he did not believe that genes played a major role in embryonic development (11; see also Holtfreter, Chapter 6 in this volume), two members of his laboratory did perceive that genetics had some critical things to say concerning how organisms developed. One of these was Spemann’s assistant, Viktor Hamburger. Hamburger supervised Gluecksohn-Schoenheimer’s thesis and “was the only one who provided us students with some introduction to the principles of genetics.” The second person was Conrad Hal Waddington, a Cambridge graduate student who came to Germany in 1932 to study organizer phenomena and to learn the techniques of tissue grafting. He became one of Gluecksohn-Schoenheimer’s closest friends, and the two of them had several discussions concerning the possible roles of genes in development. Gluecksohn-Schoenheimer decided that when she completed her dissertation, she would attempt to uncover the roles that genes played in the development of the embryo.

But neither Hamburger, who was studying the innervation of embryonic limbs, nor Waddington, who was trying to isolate the molecules responsible for organizer function, was truly a geneticist. To study animal genetics in Germany meant studying in Richard Goldschmidt’s laboratory at the Kaiser Wilhelm

Institute in Berlin-Dahlem. So in 1932, as she finished her dissertation, Gluecksohn-Schoenheimer went to see the “Lieber Gott von Dahlem.” She recounts that she was unable to see him, however, because Goldschmidt’s assistant, Curt Stern, told her that it would be useless teaching genetics to a Jewish woman because of her poor professional prospects. She did not see Goldschmidt until years later when, fleeing the Nazis, he came to New York City (11).

In 1933, Gluecksohn-Schoenheimer and her husband, the noted biochemist Rudolph Schoenheimer, fled to America. Hamburger and Stern left the same year, eventually followed by Goldschmidt in 1936. While her husband had an appointment in Columbia University’s College of Physicians and Surgeons, Salome Gluecksohn-Schoenheimer worked as a research fellow in the laboratory of Samuel Detwiler. This was an obvious place to work since Detwiler was interested in those problems of limb innervation which the Freiburg laboratory had helped identify. This employment did not last long. Gluecksohn-Schoenheimer recollects that she met geneticist Leslie C. Dunn at a dinner party where he told her of his recent work on mutations of vertebrate development. Dunn had obtained a mouse strain with a dominant mutation (*T*; *Brachyury*) for short tails. Work by Dunn and his graduate student Paul Chesley had shown that the heterozygous (*T/+*) condition resulted in a shortened tail due to a constriction in the embryonic tail. Homozygous embryos died at 11 days in utero with the posterior half of their body missing. Chesley’s work pointed to an earlier defect in the notochord as being responsible for the lack of a posterior neural tube and other axial structures. In short, it appeared that the *T* mutation was involved in axial determination. It was even possible that the wild-type *T* gene controlled the posterior inducer substance of the notochord, itself. But the project was incomplete. Paul Chesley had committed suicide.

The project was ideal for Gluecksohn-Schoenheimer. Forbidden by Spemann to work on the central problems of axial determination in amphibians, she could now study them in mice. Unable to get genetic training in Germany, she could now learn the most modern genetics in the very birthplace of the gene theory. Moreover, she found that she might be working on one of the most important genes—a gene responsible for the posterior organizing substance of the mammalian embryo. Gluecksohn-Schoenheimer accepted Dunn’s invitation to work in his laboratory, even though it meant working without pay for a year.

The first papers on the *T*-locus mutants make it clear that Gluecksohn-Schoenheimer interpreted these phenotypes as being caused by a genetic defect in the induction of the posterior neural tube by the notochord. In 1938, she concluded (12) that “our data do not give conclusive evidence for conceiving the malformations of the neural tube as secondary to the disorders of the notochord, but they point in this direction.” By 1940, Gluecksohn-Schoenheimer was able to state more assuredly (13) that “in the heterozygous *Brachy* (*T+*) mouse the notochord in the posterior region of the embryo is defective and as a result the *Brachy* phenotype develops.”

In doing these studies on the *T*-locus, Gluecksohn-Schoenheimer made a virtue out of necessity and founded the first version of developmental genetics. Unable to manipulate the mammalian embryo inside the uterus and placenta, she had looked to nature’s own experiments. In the introduction to her first paper

on the tailless mice (14), Gluecksohn-Schoenheimer presented the first programmatic statement of developmental genetics, distinguished the activities of the "developmental geneticist" from those of the "experimental embryologist," and gave a rationale for the emergence of developmental genetics out of experimental embryology.

First, one could not study mammalian development as one had studied amphibian embryogenesis. "It is not possible yet to use transplantation, isolation, or vital staining methods on mammalian embryos as they have been used on amphibian embryos." Quoting Spemann (15) in the negative sense, she wrote, "For the present, however, the experimenter is not able 'to alter the course of events at a chosen point in a chosen manner and draw conclusions on their relations from the resulting changes'." Spemann's dictum for experimental embryology would not hold for the study of mammalian development. Gluecksohn-Schoenheimer's first paper on the subject began, then, in the context of Spemann's studies, but found his methods unusable.

Second, instead of manipulating the embryo and seeing its affect on the phenotype, Gluecksohn-Schoenheimer proposed to look at the phenotypes produced by mutant genes and relate them back to their embryological causes. "A mutation that causes a certain malformation as the result of a developmental disturbance carries out an 'experiment' in the embryo by interfering with the normal development at a certain point. By studying the details of the disturbed development it may be possible to learn something about the results of the 'experiment' carried out by the gene." In this attitude, she was closer to Boveri and Goldschmidt than to Spemann. Moreover, this program bridged the gap (as both Boveri and Goldschmidt tried to do) between genetics and embryology. Most American embryologists in the late 1930s did not think that genes acted during the early stages of development (7,8,16).

Third, Gluecksohn-Schoenheimer declared that this type of research was to be done by a new type of scientist, the developmental geneticist.

While the experimental embryologist carries out a certain experiment and then studies its results, the developmental geneticist first has to study the course of the development (that is, the results of the developmental disturbance) and can then sometimes draw conclusions on the nature of the "experiment" carried out by the gene.

In this three-paragraph introduction, Gluecksohn-Schoenheimer had moved from experimental embryology to developmental genetics. To be sure, there was something like developmental genetics before Gluecksohn-Schoenheimer coined the expression. The subjects of Goldschmidt's "physiological genetics" would certainly fit into this category. But Goldschmidt refused to give credence to Morgan's theory of individualized genes and held to his own hypothesis that mutant phenotypes were caused by the timing of gene activity. In Gluecksohn-Schoenheimer, however, we see the merging of Spemann's embryological concepts (induction, regulation, etc.) with the particulate gene theory of the Morgan school—the merging of Freiburg and Columbia. The work of Landauer and Dunn on the Creeper mutation of chicks also precedes that of Gluecksohn-Schoenheimer, but the original analysis of Creeper was primarily physiological rather than developmental. The embryology of the Creeper mutants (17) was not

accomplished by Landauer until 1944. With Gluecksohn-Schoenheimer, however, we have a programmatic statement for developmental genetics as a discrete science with its own methodology.

## 2.2. The Genetics of Induction

Between 1938 and 1949, Gluecksohn-Schoenheimer pursued a research program explicitly linking embryonic organizers and specific genes in the mouse. The first series of these investigations looked at the interactions between dominant and recessive mutations in the T-complex. The dominant T-mutation, as we have seen, was interpreted as affecting the notochord's ability to induce the neural tube. The recessive *t*-alleles, now referred to as "haplotypes," moreover, were interpreted as being involved in more general mesoderm-forming processes. In *t<sup>o</sup>/t<sup>o</sup>* embryos, the mesoderm failed to form at all, while in *T/t<sup>o</sup>* embryos, the mesoderm and notochord of the posterior regions were seen to be defective. At the time, these mutant genes, however, were considered to be alleles at the same locus. How could one allele affect just the chorda-mesoderm while the other allele affects the other mesodermal areas? The answer to this genetic quandry was to be found in the embryology of the mouse (18).

The effect of the two alleles *T* and *t<sup>o</sup>* on notochord and mesoderm might suggest that the two alleles act on two different structures. However, if considered from the embryological point of view, the notochord and mesoderm of the mouse have the same origin, namely in the tissue of the wall of the primitive gut.

This brought the problem back to what had been thought of as the mammalian equivalent of the dorsal blastopore lip. Indeed, Gluecksohn-Schoenheimer was trying to do with mutants what Spemann and the Mangolds had done by transplantations. As she would summarize in 1949, "The study of this material makes it very likely that in mammals the notochord plays a role in processes of early organization similar to that of the notochord in amphibians as analyzed with the techniques of experimental embryology" (19). Moreover, Gluecksohn-Schoenheimer thought that she could do with the T-locus what Spemann's group and the Cambridge laboratory of Needham and Waddington could not do: Find the inducer molecule, itself. Gluecksohn-Waelsch (20) would later write, "It was therefore hoped that the identification of the mode of action of T-locus genes—and the nature of their gene products—might provide leads toward the molecular analysis of normal inductive mechanisms."

The T-locus alleles were not the only mutations that appeared to control induction. The phenotypes caused by another, closely linked, mutation, Kinky (now abbreviated *Fu<sup>Ki</sup>*) were interpreted in terms derived directly from Spemann's work on amphibian embryonic regulation. Homozygous mutants of Kinky were found to have duplications of their dorsal axis, sometimes forming twin embryos (21).

Their striking resemblance to the double-monsters obtained by constriction experiments of amphibian embryos at the two-cell stage led to the suggestion that an "organizer" region analogous to that identified experimentally in amphibians existed in mammalian embryos and that its normal functioning was severely affected in *Fu<sup>Ki</sup>/Fu<sup>Ki</sup>* embryos. . . . There is no doubt that all these interpretations of mutational effects on the

developmental mechanism were strongly influenced by the orientation of the particular investigators and their view of development as depending on a series of inductive interactions.

Gluecksohn-Schoenheimer interpreted all three genes (*T*, *t<sup>o</sup>*, and *Fu<sup>Ki</sup>*) as disturbing “specific organizer relationships.” She interpreted the action of the Kinky gene as causing constrictions analogous to those done experimentally by Spemann and Holtfreter on salamander eggs (22). These famous studies had shown that the constriction of the egg down the medial plane caused the formation of two organizers, each of which formed embryonic axes, thereby creating twin larvae. Constriction in the frontal plane, however, caused the formation of one normal larva and one *Bauchstück*, an amorphous tissue mass consisting chiefly of endoderm and blood cells. Partial constrictions, moreover, caused conjoined larvae, an observation that Spemann had related to mammalian teratology (23).

According to Gluecksohn-Schoenheimer, the Kinky mutants had an inducing mesoderm that was divided in two, just like Spemann’s and Holtfreter’s constricted embryos. The duplicated axes formed when this constriction was in the median plane and the *Bauchstück*-like mass seen in several of the Kinky embryos also “might well be the result of a frontal constriction.”

Gluecksohn-Schoenheimer was aware of her integrating embryology and genetics. She announced that her research on the Kinky gene “was undertaken both from the point of view of the embryologist interested in the causal analysis of development and that of the geneticist interested in the analysis of gene effects” (24). Linking organizers to genes meant linking embryology to genetics.

During this investigation of axial development, other tailless or short-tailed mutant mice were found. One of these tailless mutants was due to the *Sd/Sd* genotype that also caused the lack of kidneys. Gluecksohn-Schoenheimer wrote that when confronted with such cases, the developmental geneticist must reverse the order of the experimental embryologist and work backward from effect to cause. Why weren’t there any kidneys in these mice? She demonstrated that the ureteric bud normally grew into the area of the metanephrogenic mesenchyme. When that occurred, the ureter continued to grow and branch, and the mesenchyme condensed into tubules. In the *Sd/Sd* mutant, however, the ureteric bud failed to reach the mesenchyme and no kidney was formed. In *Sd/+* heterozygotes, some tips of the short ureteric bud did find their way into the metanephrogenic mesenchyme, and a small kidney resulted. “These findings,” wrote Gluecksohn-Schoenheimer (25), “indicate strongly the existence of an inductive relationship between the ureter and kidney—such as has been shown experimentally to exist in other vertebrates, the chick, for example.”

In Gluecksohn-Schoenheimer’s work during this period, there is a reciprocity between genetics and embryology. Genetics could be used to analyze development in areas where experimental techniques had not yet been perfected. Embryology could identify the effects of these genes whose functions were necessary for construction of the embryo. These early embryonic abnormalities “represent the end result of a chain of events at the beginning of which stands the gene. The analysis of the action of the gene is our ultimate goal” (26).

This programmatic statement of 1945 differs from that of 1938. In 1938, the developmental geneticist was content to draw conclusions on the nature of the

“experiment” performed by the genes. Now, the further charge was to understand the nature of gene action. But Gluecksohn-Schoenheimer’s goal would have to await the techniques of molecular biology. She did not start analyzing gene activity until the mid-1970s when she turned the direction of her laboratory from morphological mutations in mice to analysis of the biochemical defects caused by the deletion of a specific portion of mouse chromosome 7. In Salome Gluecksohn-Schoenheimer’s research on developmentally lethal genes, we see the enormous role that experimental embryology, especially Spemann’s constriction and transplant experiments, had in the propounding of developmental genetics.

At the same time, Gluecksohn-Schoenheimer’s friend and colleague C. H. Waddington would also meet with frustration in his attempts to analyze the organizer. He, too, would turn to genetics as a way of approaching the problem of induction, and he would bring to the fruitfly the same procedure that Gluecksohn-Schoenheimer was employing to study mouse development. Waddington, however, was more theoretically inclined than Gluecksohn-Schoenheimer and was eager to reunify genetics and embryology not only with each other, but with evolutionary biology, as well. While Gluecksohn-Waelsch’s focus narrowed in on specific regions of mouse chromosomes 7 and 17, Waddington’s focus became increasingly wide. We shall see that Waddington’s studies served as a bridge linking the induction of organs in vertebrate embryos to the induction of enzymes in *Escherichia coli*.

### **3. The Concrescence of Genetics and Development: C. H. Waddington**

#### **3.1. The Scientist as Pluripotent Migratory Cell**

Conrad Hal Waddington identified himself as a student of “diachronic biology,” a science of “embryology-genetics-evolution which again form a group whose interconnections are obvious and unavoidable” (27). That Waddington did not see these three disciplines as distinct entities is reflected in his peripatetic training as a biologist. After graduating with a first-class degree in geology from Cambridge University in 1926, he began pursuing graduate work in paleontology. His thesis work involved analyzing ammonites, a group of extinct cephalopods that, he would later claim, “forces on one’s attention the Whiteheadian point that the organisms undergoing the process of evolution are themselves processes. . . . The whole developmental process is preserved so that one cannot avoid examining it.” Waddington admitted to being very much influenced by Whitehead during his last 2 years as an undergraduate (28), and his research in paleontology was partly funded by a philosophy studentship. This work in paleontology did not lead to a Ph.D. He had met Gregory Bateson, and the friendship between these men caused Waddington’s interest to move from paleontology toward genetics. However, Waddington felt that it was not possible to earn a living as a geneticist in Britain during the 1920s, so he looked elsewhere (29).

In 1929, Dame Honor Fell, director of the Strangeways Laboratory, was told about a “bright young paleontologist (who also had a scholarship in philosophy)

who had been reading Spemann's papers and wondered if the organ culture method developed here could be used for the experimental study of avian and mammalian embryos" (30). Indeed, she was impressed with Waddington's ideas and soon Waddington began working at the Strangeways Laboratory (31). By 1930, at the International Congress of Experimental Cytology in Amsterdam, Waddington was able to present his first results on culturing chick embryos. Another participant at this congress, Richard Goldschmidt, invited Waddington to come to Germany. Waddington got the funds to work in Germany, but he decided to work with Otto Mangold "for the purpose of learning the technique of amphibian operations" (32), rather than pursue genetics in Goldschmidt's laboratory. Mangold, a former graduate student of Spemann who was now his collaborator, was working on the problems of neural induction in amphibians. Waddington would adopt this set of problems for himself. From 1932 to 1938, Waddington attempted to clarify the nature of amphibian neural induction and to transfer the techniques of amphibian experimental embryology to the study of chick development.

### 3.2. The Chemistry of the Organizer: Sterols and Sterility

Waddington's work on chick embryos was marked by enormous success. Using his technique of growing avian embryos *in vitro*, he was able to manipulate and transplant different embryonic regions from one early chick embryo to another. He discovered that the elongation of the primitive streak was directed by the underlying hypoblast, and he referred to this process as an induction. He further showed that the formation of the chorda-mesoderm was directed by Hensen's node and thus reasoned that this most anterior portion of the primitive streak was analogous to the dorsal lip of the amphibian blastopore. His photographs of twin chick embryos resulting from the transplanting of an exogenous node beneath the ectoderm gave testimony to his being able to demonstrate induction in a warm-blooded animal.

Meanwhile, research into the chemical nature of the amphibian organizer substance was becoming more and more frustrating. In 1933, Waddington and Joseph and Dorothy Needham showed that the activity of the newt organizer could be duplicated by ether extracts of adult newts. This ether extract could turn presumptive epidermis into neural tissue. Since it did not specify the regional properties of the neural tissue (i.e., brain or spinal chord), Waddington referred to it as the "evocator" (33). (The other molecules that would then specify the regional properties of the neural tube were called the "individuators.") The properties of this lipid fraction suggested that the evocator was a sterol, and, indeed, both natural and artificial steroid hormones were found to induce neural tubes.

This initially caused great excitement, and Waddington and Joseph Needham spent over 3 years biochemically characterizing the active agent in the ether extracts. If the natural inducer substance turned out to be a sterol, Waddington and Needham would have linked embryology to two of the most exciting areas of contemporary physiology and biochemistry—steroid synthesis and reproductive endocrinology. Such a conclusion would also have been helpful in retaining

an active sponsor for their research, as the Rockefeller Foundation was keenly interested in sterol biochemistry and was already funding the technician for Waddington and Needham's research. A sterol inducer made good sense, and Needham outlined these reasons in his 1936 volume *Order and Life* (34). Sterols had been shown to provide the biochemical framework of (1) both the male and female sex hormones, (2) cancer-producing hydrocarbons, (3) vitamin D, and (4) pharmacologically active cardiac glucosides. Moreover, (5) Emil Witschi, one of the pioneering researchers on sexual development, had shown that sterols might be stored in eggs.

However, there was a problem. Some of the neural-inducing hormones were so unlike one another that there seemed to be no structural specificity. As Waddington and Needham end their discussion to one of their papers in 1935 (35);

Dodds has metaphorically spoken of these synthetic substances as skeleton keys, which can unlock several doors. . . . Here the skeleton key is so unlike the householder's latchkey that one wonders whether the house has been entered through the back-door, or, in an even more unorthodox manner, through a window.

During the same year, Waddington also published two papers on the competence of ectoderm to respond to neural and lens inducers. But the pivotal paper showing the central importance of competence was not to appear until 1936. This paper, by Waddington, Joseph Needham, and Jean Brachet (36), concerned the activation of the evocator substance. In this paper they retreated from the notion of a specific evocator substance released from the chorda-mesoderm which induces the ectoderm above it to become neural. Rather, they produce evidence that the evocator "substance, which is present throughout the whole embryo, is liberated or activated in one particular region, the organization center, by reason of a gradient system." The first evidence for this came from Holtfreter's 1933 experiments wherein he demonstrated that noninducing regions of the amphibian gastrula could acquire the ability to induce when the cells were killed. Thus, noninducing tissue contained the active inducing factor, in "some form in which it could not exert its normal influence." Julian Huxley (79) had speculated that the dorsal lip "organization center" was merely a region of extremely high metabolic activity. It was this quantitative difference, rather than any chemically qualitative ones, which distinguished it.

This speculation fit into the paradigm of C. M. Child's axial gradients which was very influential during the 1930s. Watanabe and Child (80) had recently proposed that gradients of respiratory rates might have profound effects in the determination of embryonic cell fate. To this end, "An attempt was made to raise the respiratory rate of isolated fragments of ectoderm by the use of dyes [methylene blue and cresyl blue] which act as respiratory catalysts, and the treated ectoderm was then tested for inducing capacity." The answer was ambiguous. First, the tissues incubated in methylene blue dye gave obvious neural induction, but a sensitive manometer showed no difference in the respiration rate between the inducing tissues and the noninducing ones. The researchers dismissed as "ridiculous" the notion that methylene blue resembled the natural evocator. Rather, they interpreted their findings as showing that the evocator was present in the competent tissue in an inactive state. A substance could cause neural induction either by releasing the natural evocator from its inhibitor or by

resembling the evocator itself. This allowed them to draw a distinction between the chemicals they thought resembled the evocator (such as sterols) and those chemicals which nonspecifically release the natural sterol inducer from its inhibitor (such as methylene blue) (37).

By 1938, the chemical identification of the evocator substance was still stalled. Waddington showed that estrone, a natural estrogen, seemed to be among the most potent inducers of neural tissue in amphibians, but the biochemists had not come any closer to finding the natural inducing molecule. As Yoxen (38) has shown, the project to characterize the organizer molecule had by this time become untenable because of the nonspecificity of inducers. Moreover, the departmental politics at Cambridge University stopped the Rockefeller Foundation from continuing its support of the project (39). That same year, using Rockefeller Foundation support, Waddington traveled to the United States. Here he worked with L. C. Dunn at Columbia University and renewed his acquaintance with Salome Gluecksohn-Schoenheimer. He made trips from New York City to visit Ross Harrison at Yale, Curt Stern in Rochester, and Streeter and Metz at Johns Hopkins. In January 1939, Waddington began a productive 3-month stay at the California Institute of Technology. In this extremely active genetics group (including Columbia University transplants Sturtevant and Dobzhansky), Waddington started applying to *Drosophila* the same type of developmental genetics that Gluecksohn-Schoenheimer had recently pioneered on mice. He analyzed 24 alleles known to cause deformation of the wing, and he showed their earliest visible deviations from wild type while they were in the pupa. The wing, he said (40), "appears favorable for investigations on the developmental action of genes."

### 3.3. Genes as Organizers

Not everybody believed that the "developmental action of genes" was important or that it even existed. In 1924, Spemann alerted his fellow embryologists to the threat posed by geneticists who felt that the genes explained all of development. "They have cast their eye on us, on *Entwicklungsmechanik*," he wrote (16). In 1937, Ross Harrison gave a lecture at the zoological sciences section of the American Association for the Advancement of Science, warning the geneticists to stay on their own turf. He warned of the dire consequences of "this threatened invasion" caused by the "wanderlust" of geneticists. Embryologists such as Spemann, Harrison, and Lillie ignored genetics, saying that development could not be controlled by genes that were the same in every cell type, while geneticists such as Goldschmidt laid claim to development, certain that the ontogeny of the embryo could be viewed as an epiphenomenon of gene expression (7).

Aside from Waddington, Gluecksohn-Schoenheimer, and Dunn, only a few others were actually studying the "developmental action of genes" at this time. Goldschmidt certainly was, but his version of genetics, summarized in *Physiological Genetics* (81), was not the genetics of the Morgan school. Boris Ephrussi was another researcher actively studying the developmental action of genes. As detailed by Burian, Gayon, and Zallen (Chapter 10, this volume), Ephrussi (41) undertook experiments on *Drosophila* eye pigments "to lay a bridge between causal embryology and genetics." Ephrussi's techniques were those of the embry-

ologist (transplantation) rather than those of the geneticist (breeding), and he explicitly looked at these phenomena in terms of development, using the term "inducer" to refer to the substance made by the wild-type cinnabar gene.

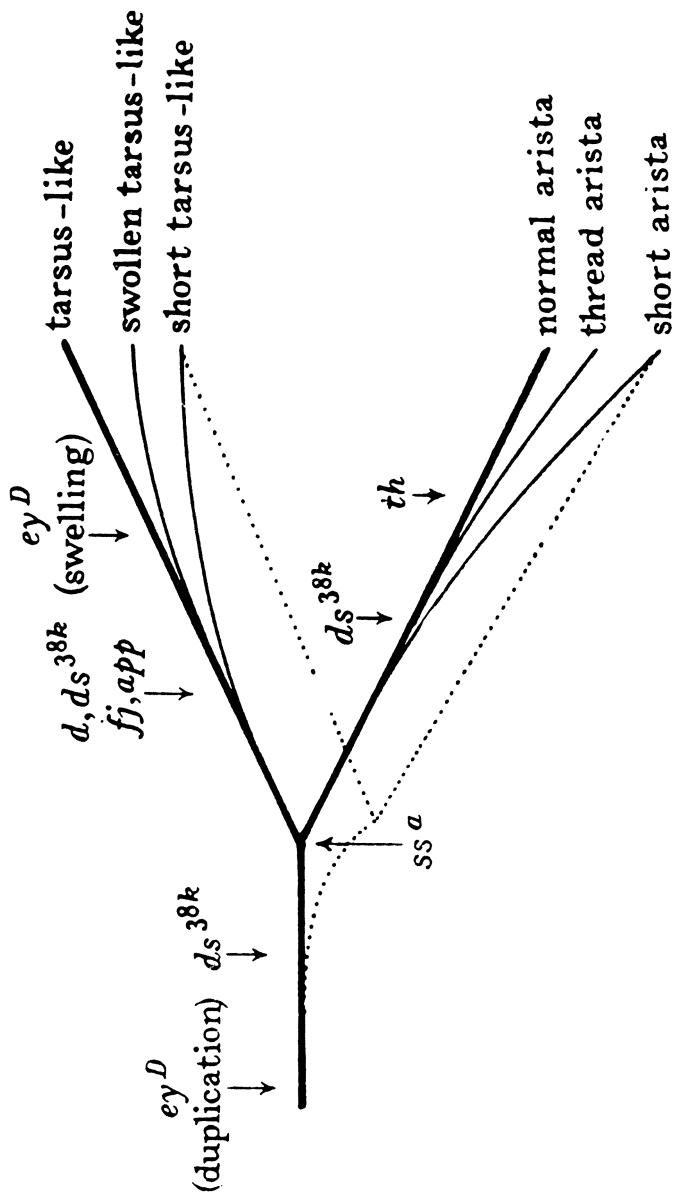
We have no lack of information concerning Waddington's views on this subject. Between 1938 and 1940, Waddington wrote two textbooks and two review articles concerning the developmental action of the genes. The first of these was in his textbook *An Introduction to Modern Genetics* (43). This was quite a presumptive undertaking for a 33-year-old, paleontologically trained embryologist who had yet to publish his first paper in genetics! The second review was written for *The School Science Review*. Another article, bearing the provocative title "Genes as Evocators in Development," was delivered at the first Growth Symposium in 1939 and published in the supplementary edition of *Growth* (42). Finally, Waddington's synthesis of genetics and embryology is given full treatment in *Organisers and Genes* (1940) (27).

One of the major themes in these reviews is the formal equivalence of organizers and genes. Since Waddington viewed development as a succession of distinct alternative conditions, "it can best be symbolized as a system of branching paths. The characteristics of each path will depend upon the developmental potencies of the tissue, that is to say, they will be under the control of the genes. We may also expect to find genes which act in a way formally like that of evocators, in that they control the choice of alternatives. Genes of this sort are in fact known. One good example is aristopedia in *Drosophila melanogaster* . . ." (42). Thus, Waddington developed lists of analogies. The gastrula ectoderm had the alternatives of becoming either epidermis or nerve tissue. The evocator acted to make it nerve, and without this inducer, it would become skin. In like manner, he argued, the anterior imaginal tissue of the *Drosophila* larva could become either leg or antenna. The wild-type *aristopedia* gene acted to make this tissue develop into antenna. In its absence (as when this gene mutates), the same tissue would develop into leg structures. Thus, the gene was acting as an organizer. Similarly, the genes that controlled the sexual phenotype of animal gonads and the color of *Drosophila* eyes also functioned as organizers. The male or female phenotype was determined from originally bipotential gonads. The choice of one of the two alternative paths was seen to be directed either by internal genes or by the external environment (where he mentions the case of *Bonellia*) (43). Ephrussi's studies on eye pigmentation were also interpreted in this manner. The eyes could become red or cinnabar colored. In the presence of the wild-type gene, the eyes become red. If this gene is not present, the alternative phenotype is chosen (44).

The ectoderm of the amphibian gastrula has two alternative methods of change open to it; it may become epidermis or if the evocator is added to it, it may become neural tissue. The case is exactly parallel to that of the pigment system of *Drosophila* at one of its branch points.

Thus, Waddington was able to speak (45) of "evocator genes which shunt development along one or the other of the possible developmental branches." This is illustrated in Fig. 1.

To Waddington, the embryologist and the geneticist were studying the same phenomenon (46).



**Figure 1.** Branch-track diagram showing the fate of antennal imaginal tissue in *Drosophila*. The wild-type aristopedia (*ss<sup>a</sup>*) gene would cause the cell fate to become antenna. However, the mutant allele at this locus would cause the cell fate to travel down the other path to become leg. Other mutations could act to modify these new conditions. [From Waddington (27).] For a more detailed discussion of Waddington's bifurcation diagrams, see Gilbert (66).

The similarity between the theoretical schemes we have arrived at on embryological and genetic grounds is immediately apparent. In embryonic development we are confronted with alternative modes of development, the choice between which is taken in reference to an external stimulus, in inductive development or to an internal one, in mosaic development. In considering the effects of genes, we find alternatives the choice between which may be taken in response to diffusible substances, as in the *Drosophila* eye colours, or apparently in response to internal factors as in aristopedia. It is clear that we have merely followed two different methods of approach to the same phenomena, and that the two schemes are in fact identical.

Since “both methods of approach to the study of development formulate the main problems in the same kind of way,” Waddington hoped (47) that “genetics and embryology can collaborate in finding the answers.” Indeed, Waddington begins *Organisers and Genes* by declaring a truce between the geneticists and the embryologists. “A coherent theory of development cannot be founded on the known properties of genes; in fact, it seems much more hopeful to try to fit our somewhat scanty knowledge of the developmental action of genes into a framework founded in the first instance on the direct experimental study of development” (48). Both the geneticist and the embryologist had valid research programs and a great deal more work to do in order to explain development.

### 3.4. The Primacy of Competence and the Foundations of Genetic Assimilation

Spemann’s 1938 Silliman Lecture series *Embryonic Development and Induction*, although far more complete than Waddington’s *Organisers and Genes*, spent hardly any time on the concept of the competence of the tissues to respond to induction. Waddington, however, devotes more space to this concept than to induction, itself. There are several reasons for this. One is that Waddington wrote, not of induction, but of the “evocator-competence system.” Second, the role of the evocator had been shown to be nonspecific. Many things could induce, but only certain tissues could respond to the inducer. This competence to respond in certain ways was linked directly to gene action (49).

The evocator is merely a differential; it is the competence which is responsible for the details of the developmental process and thus of the kinds of tissues produced. Since it is the genes which control the character of the animal and its tissues, it must in general be genes which determine the properties of the competence.

Waddington’s idea of “competence” differed from the analogous German term *Reaktionsfähigkeit*, which implies a passive ability to respond to the stimulus given it. For Waddington (50), competence is actively achieved by “a complex of reactions between substances which form an unstable mixture, which may at certain times have two or more alternative modes of change.” Thus, competence is manifest in an unstable (i.e., multipotential) system which an inducer can push to one equilibrium or another.

This view that genes control competence and that inducers merely push an unstable system into one of two alternative equilibria leads to the notion that competence, rather than induction per se is the central aspect of determination. This view had several important consequences. First, it explained the notions of

potency and segregation, so dear to the American embryologists, for once a tissue acquired its competence, the stimulus that induces it could be changed (51).

The transition from typical inductive development to cases of double assurance show[s] that the processes occurring in mosaic development, where no inducer is involved, are of the same general nature as those involved in competence; only in the former the factor which decides which mode of development will be followed by a piece of tissue is already present within it, instead of having to diffuse in from the surroundings.

Since the competence could be achieved independently from an evocator, and since different evocators could induce the same determinative process, a given competent tissue could transfer its ability to respond from one inducer stimulus to another. These evocators could be intracellular (as in mosaic development), intercellular (as in inductive development), or even environmental (as in the case of *Bonellia* sex determination). This will form the basis of Waddington's concept of genetic assimilation. Here, a tissue that is competent to respond to environmental evocators becomes competent to respond to internal evocators as well. In other words, an environmentally induced response can become an embryologically induced response. Waddington uses as an example the formation of calluses on the underside of the ostrich. Ostriches are born with the calluses already in place and thus appear to be a case of Lamarckian evolution wherein the adult skin would form calluses upon abrasion and the offspring would inherit such properly positioned calluses. Waddington was able to explain this by invoking a transfer of competence from an external inducer to an internal inducer. First, the belly skin of the ostrich has to be able to develop calluses in response to friction. This is a genetic competence that this region of skin has acquired through natural selection. Then, during evolution, the skin—which has already obtained the competence to form calluses—becomes able to respond to another, internal, inducer. The skin is thereby able to form calluses through embryonic induction. What had appeared to be a case of Lamarckian “inheritance of acquired characteristics through use” can be explained by development and natural selection (52).

Presumably its skin, like that of most other animals, would react directly to external pressure and rubbing by becoming thicker . . . This capacity to react must itself be dependent upon genes. . . . It may then not be too difficult for a gene mutation to occur which will modify some other nearby area of the embryo in such a way that it takes over the function of external pressure, interacting with the skin so as to “pull the trigger” and set off the development of the callousities.

This genetic assimilation hypothesis, based on the ability of competent tissue to switch evocators, operated according to strictly Darwinian natural selection and could also, wrote Waddington, “provide a plausable account of the result in terms of orthodox genetic and embryological mechanisms” (53).

The genetic assimilation hypothesis, based on competence transferring its triggering from one inducer to another, was modified as biology became increasingly molecular. Waddington would later use it to account for the most “Lamarckian” of molecular phenomena then known, adaptive enzymes in yeast and *E. coli* (54). Here, an “inducer” molecule would influence gene activity by interacting with cytoplasmic components that could activate or suppress specific genes in the cell. Although Waddington did not have anything to do with the discovery of the operon, he followed this work closely and brought it into

discussions of developmental biology. He clearly saw the bacterial operons as models for the elements of embryonic induction. But in order to appreciate how bacterial operons might be used to model embryonic blastomeres, he had to believe two general concepts that were not generally held at this time. First was a view of organisms and cells as “systems” of interacting parts. While Waddington would eventually expand this notion into a full-fledged cybernetics of development, his earlier views of systems come from Whitehead’s philosophy. Second, he had to believe that the cytoplasm activated the genome. This went against the grain of genetics, which held the nucleus to control the cytoplasm (see notes 7,8). Yet this would be crucial in his analysis of induction and provides the link between the embryological induction of organs and the microbial induction of enzymes.

### 3.5. Concrescence, Chreods, and Canalization

Waddington was a member of the Cambridge-based Biotheoretical Gathering, which was trying to provide a molecular basis for embryology. This group was self-consciously grounded in dialectical materialism, organicism, and a belief that the process philosophy of Alfred North Whitehead was important for studying emergent processes such as evolution and development (55–57). To be sure, different members of this group placed different import on each of these characteristics, and it is also probable that each of them interpreted dialectical materialism differently and saw different parts of Whitehead’s philosophy as being important. Joseph Needham, for example, placed Whitehead’s work in the context of dialectical materialism, the latter being (58) “a theory of transformations of the way in which the qualitatively new arises, of the nature of change in the world.” But to Waddington, Whitehead (“to whose writings I paid much more attention during the last two years of my undergraduate career than I did to textbooks in the subjects on which I was going to take my exams”) had superseded dialectical materialism with a fuller view of nature (28,59).

But what did Waddington take from Whitehead’s philosophy? Undoubtedly, the notion that there were no “things” except in their interrelationships was extremely important to Waddington (60), and he notes this in his autobiographical sketch (61).

As far as scientific practice is concerned, the lessons to be learned from Whitehead were not so much derived from his discussion of experiences, but rather from his replacement of “things” by processes which have an individual character which depends upon the “concrescence” into a unity of very many relations with other processes.

Indeed, Waddington stressed the role that concrescence had played in his biological thought. “In the late thirties I began developing the notion that the process of becoming (say) a nerve cell should be regarded as the result of a large number of genes, which interact together to form a unified ‘concrescence’.” He also claimed that this view motivated his initial work in *Drosophila*. Waddington retrospectively linked his appreciation of concrescence to his work on competence. Moreover, he appreciated the formal similarity of his masked evocator hypothesis to that of Jacob and Monod’s operon (62).

Again a few years earlier it came to be apparent that the "gene concrescence" itself undergoes a process of change; at one embryonic period a given concrescence is in a phase of "competence" and may be switched to one or another of a small number of alternative pathways of further change—but the competence later disappears. . . .

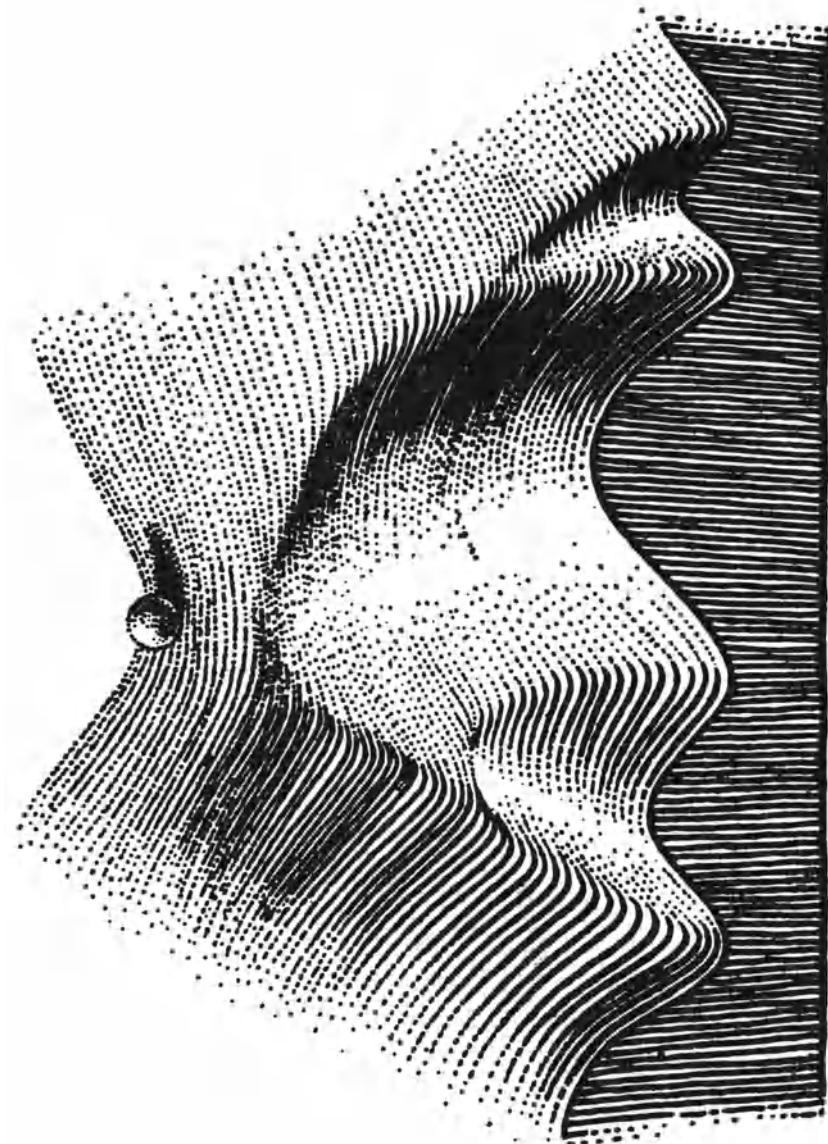
We showed that, in these terms, the specificity resides in the cells that react to induction—we called it "the masked evocator." This is very similar to the situation discovered by Jacob and Monod many years later in bacteria, where again the specific repressor molecules are internal to the cells which react to enzyme-inducing substances.

Waddington claimed that these were not merely retrospective glosses placed on his earlier work. Rather, "I tried to put the Whiteheadian outlook to actual use in particular experimental situations." Waddington wrote that he used the neologism *chread* (or *creode*) as a substitute for the Whiteheadian term "concrescence." Before using "chread" he had no shorthand way for saying "a stabilized or buffered pathway of change." This concept was also depicted by a visual image that Waddington called "The Epigenetic Landscape" and is shown in Fig. 2.

The epigenetic landscape was a way of depicting the branching patterns of development and at the same time portraying the different stabilities of these pathways by depth and contours. This concept is first seen in the Introduction to Modern Genetics (63) as a representation of development "not as a branching line on a plane but by branching valleys on a surface." The depth and contours of the "geological model represent probability, so that the valley bottom is really a representative of an equilibrium." The molding of these contours would be accomplished by various genes, "and genes like [the *Drosophila* eye pigment gene] *vermillion* which have their effects at certain branching points are like intrusive masses that can direct the course of the developmental process down a side valley."

Waddington likened the establishment of cell fate to a ball rolling down the valleys of the epigenetic landscape. At certain times (as in competent tissue), two downhill paths are possible, and the presence of an evocator could deflect the tissue into one or the other of these paths. Regulation took place only when the path of development took the embryo into a valley with "gently sloping sides." However, as development proceeded, the originally wide, gently sloping valley branches into subdivisions having far steeper sides. Here, regulation cannot take place.

Implicit in this model is the notion of canalization. (Indeed, Waddington quotes *Organisers and Genes* when he mentions canalization in his 1956 book *Principles of Embryology*, even though canalization is not explicitly mentioned in the earlier work.) Canalization is made explicit shortly afterward, though, in 1941, when Waddington states (64) that "developmental reactions . . . are in general canalized. That is to say, they are adjusted so as to bring about one end result regardless of minor variations in conditions during the course of the reaction." Canalization can be considered a buffering of the developmental pathways such that minor mutations would not greatly affect the course of development. Canalization would limit variations in development such that "if wild animals of almost any species are collected, they will usually be found to be 'as like as peas in a pod'." The canalized paths were themselves thought to be selected by natural selection. Waddington related this to genetic assimilation, stating that once a developmental path had been canalized (as in the ostrich's



**Figure 2.** Representation of the epigenetic landscape. The ball represents cell fate. The valleys are the different fates the cell might roll into. At the beginning of its journey, development is plastic, and a cell can become many fates. However, as development proceeds, certain decisions cannot be reversed. [From Waddington (77).] [For more details on the use of these epigenetic landscapes to synthesize genetics, embryology, and evolution, see Gilbert (66).]

ability to form a callus by friction), mutations would be able to switch development into that path.

Once within a canal, though, it is difficult to get out. Thus, canalization is not unlike the current notion of “developmental constraints.” Indeed, it was Waddington who suggested (65) in 1938 that all vertebrates were constrained to have a notochord since that transitory organ induced the neural plate, and who similarly claimed that birds (and presumably humans) had to have nonfunctional pronephric kidneys since they would give rise to the Wolffian ducts.

Waddington did not tell his readers how he derived the term and concept of canalization. Looking at the pictures of the epigenetic landscape (especially John Piper’s rendering of it on the frontispiece of *Organisers and Genes*), one would think that it followed from this visual image (66). However, it, too, probably came from Whitehead’s *Process and Reality*, where Whitehead discusses “canalisation” as well as concrescence. In fact, Whitehead’s use of canalization would be difficult for an embryologist to miss, for the term is used in discussing the development of the animal body and the emergence of ordered mentality. “Apart from canalisation,” wrote Whitehead, “depth of originality would spell disaster for the animal body,” for life is a passage from physical order to random mental processes to “canalised mental originality.” For Whitehead, canalization provided both the limits and the magnification of the creative urge, allowing things begun to come to completion (67). Thus we see that Waddington’s idiosyncratic approach to development—concrescence, canalization, and genetic assimilation—arose from his placing fundamental emphasis on competence (rather than on induction) and in his placing these observations in the context of a Whiteheadian philosophy of organismal change. In the next section, I attempt to show that his analysis of competence was, itself, a product of his Whiteheadian philosophy.

### 3.6. Systems in Dialogue

Whitehead was a philosopher of systems in the process of becoming. Indeed, the first three elements of his philosophy were the concepts of system, process, and the creative advance into novelty (68). For Whitehead, no thing existed except in relation to other things, and these nexuses were always changing, allowing new nexuses to form. All relationships were in the process of becoming, and all things were linked within systems. These were very useful concepts for biologists who were engaged in studying process, interrelationships, and the emergence of new forms. Many biologists in the Biotheoretical Gathering were deeply influenced by these notions (69).

This certainly seems to be the case in *Organisers and Genes*, which reads like a Whiteheadian primer on embryology. Throughout this book, Waddington stressed “interrelationships,” “causal networks,” and “interconnections.” (All these terms can be found on the first page!) He sought (70) to identify—not the inducer—but the “causal network underlying this particular process of differentiation” and hoped “to know the whole complex system of actions and interactions which constitute the differentiation.”

This tendency to think in terms of process, system, and interaction also distinguished his approach to induction from those of most other investigators (71). In 1940 he was not talking so much about the inducer as he was about “the evocator—competence reaction.” Similarly, he was not so much concerned with the action of genes as with “the system of tracks and their genetic control.” For Waddington, the parts of the embryo were always in dialogue. The evocator was nothing without the competent responding tissue, and the responding tissue was nothing without the evocator. They were linked in a system. Moreover, Waddington interpreted the results of embryology to show that these components interacted to affect development. Waddington looked upon an organism as a developing system and claimed that natural selection worked on aspects of development. Evolution was accomplished through heritable changes in an organism’s development. He credited this idea to his Whiteheadian outlook, and called his paper on evolutionary topics (1941) “the evolution of developing systems.”

The relationship between inducer and competent tissue paralleled that of the genes and the cytoplasm. The genes and the cytoplasm were in continual dialogue, and in Whiteheadian fashion, Waddington claimed (72), “Neither cytoplasm nor nucleus can be disregarded: In fact the most important subject to discuss is how they affect each other.” Moreover, just as Waddington saw the competent tissue as having primacy over the inducer, so he appears to give the primacy of the “interacting system of nucleus and cytoplasm” to the cytoplasm. This empowerment of cytoplasm marks a major difference between him and most geneticists, just as his empowerment of competent tissue separated him from most embryologists. In both cases, he “championed” the view of the “passive” partner. The inducer was thought to call forth a response from the competent induced tissue, and genes were believed to control the cytoplasm. Genes may be equivalent to organizers, but in both cases, what mattered was the responsive partner. The reasoning, too, may be similar. In induction, the competent tissue can be induced by several inducers. The specificity is in the tissue that is competent to be induced. Similarly, within the cell, all the genes are the same. The specificity resides within the cytoplasm.

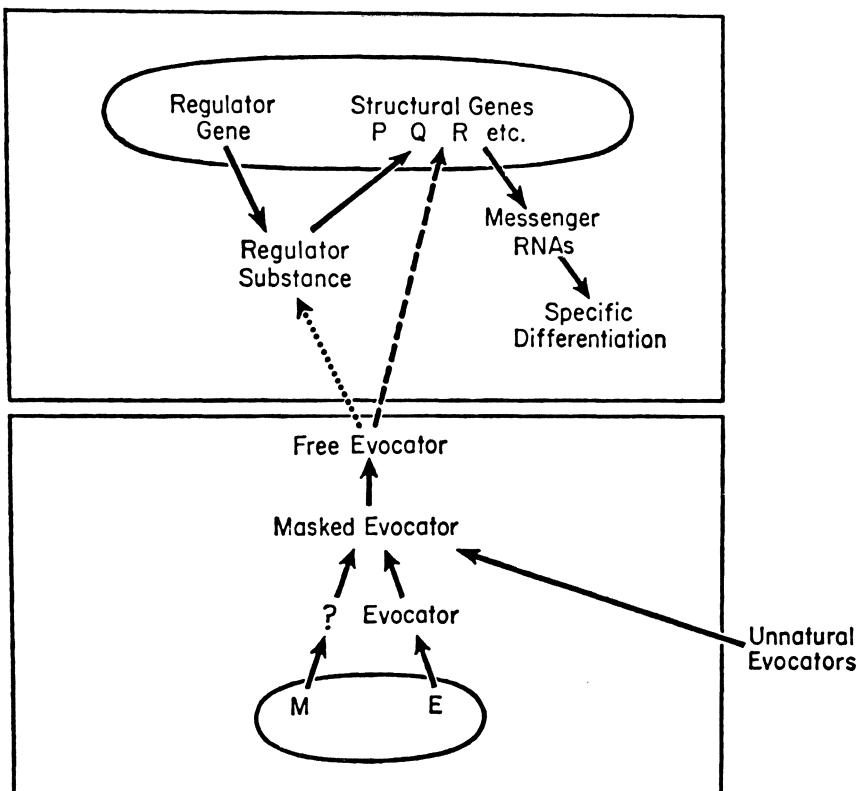
In his 1956 book *Principles of Embryology*, Waddington provided a provocative title to his chapter on developmental genetics: “The Activation of Genes by the Cytoplasm.” The normal “genetic” roles have been reversed. What was Waddington’s evidence for this reversal? Within this chapter, he presented four modes in which the cytoplasm had been seen to control which genes were expressed by the which cell. First, in mosaic eggs, the cytoplasm of the cell obviously controlled the genes expressed by its nucleus. Second, in induction, a diffusible substance is thought to affect “the interacting system of nucleus and cytoplasm” in the competent cell. Third, in *Drosophila*, the chromosome puffs, believed to represent active genes, were different depending on the cell they were in. Fourth, Sonneborn’s studies on *Paramecium* had demonstrated that “it is the condition of the cytoplasm which determines which of the loci shall be in operation.” This demonstration of the cytoplasmic control of G-antigen synthesis was particularly important, since Waddington called it “a clean-cut example of the activation, by different types of cytoplasm, of different specifically corresponding genes” (73).

Waddington saw the *Paramecium* experiments as being important to embryology, even though *Paramecium* was a unicellular protist (74). The fact that cytoplasmic control of nuclear genes “occurs, not in different parts of the same body but in various members of unicellular organisms does not make the phenomenon any the less relevant to the normal processes of development.” Thus, in 1956, Waddington was already using microorganisms as models for the cellular differentiation of multicellular organisms, and he states explicitly (75) that in order to study differentiation, one has to consider “the genetics of microorganisms as well as higher forms.”

Waddington’s 1956 model of cell differentiation starts from the premise of a system wherein the cytoplasm controls differentiation by interacting either with the genes directly or with the immediate product of the genes (76). The question then became how the constitution of the cytoplasm changed such that one canalized system would be followed rather than another. He builds a model wherein the genes compete for raw materials within the cell. He likens this to animals competing for limited food reserves and points out that slight changes in the initial conditions can effect a great difference in the final state. The evidence for this model comes from microorganisms, specifically from Spiegelman’s studies on adaptive enzyme production in yeast. If the yeast were grown on two substrates for which it originally lacked the appropriate degradative enzymes, they would gradually synthesize those enzymes. However, if those yeast cells were starved for nitrogen, the two proteins would enter into competition for the small nitrogen reserve. Moreover, when one of them is synthesized, its synthesis appears to be autocatalytic.

Thus, Waddington can bring his reader back to the original problem of induction and competence. In evocation, the environmental influence (the evocator) acts on the cytoplasm of the competent cell, “causing it to adopt one or the other of the alternative paths of development open to it. The fact that the reacting tissue retains its own specific characteristics . . . shows that the developmental paths are under genetic control and that evocation involves the differential activation of a particular set of genes” (77). The problem of induction therefore centers around the mechanism by which crucial changes in the responding cells’ cytoplasm allow one reaction to outcompete its rivals.

From the references in *Principles of Embryology*, we know that Waddington was already very much aware of Monod’s work on adaptive enzymes in *E. coli*. When Jacob and Monod published their researches on the operon model in 1961, they had a ready audience in Waddington. Waddington’s *New Patterns in Genetics and Development* was written that same year, and he began his book by showing the relevance of Jacob and Monod’s “repressor-operator system” to embryology. *New Patterns in Genetics and Development* was Waddington’s revision of *Organisers and Genes* on its twenty-first anniversary. Like the earlier book, it attempted to integrate genetics and embryology. But genetics had become molecular genetics, while embryology had not made many significant advances in those 21 years. Waddington conscientiously attempted to make the operon his basic paradigm of differentiation (78), and he explicitly linked the “induction” of bacterial enzymes with the “induction” of the neural tube (Fig. 3). Within a year of its being publicized, the operon model was being used as a paradigmatic model for cell differentiation.



**Figure 3.** Eukaryotic embryonic induction modeled on the lac operon of Jacob and Monod. Evocator substance from one cell (below) could be freed to diffuse into neighboring cell and either directly activate a series of genes or interfere with a repressor of the transcription of those genes. [From Waddington (78).]

In the work of C. H. Waddington from 1938 to 1962, we see the question of differentiation taken from the embryonic induction of vertebrate organs to the molecular induction of microbial enzymes. The steps can be summarized as follows:

Waddington's Whiteheadian outlook caused him to see dialectical interactions, rather than vectoral influences, between the nucleus and cytoplasm and between the inducer and the responding competent tissue. The lack of specificity in the inducer and the lack of gene specificity in the nucleus caused him to emphasize the roles of the cytoplasm in the nucleocytoplasmic system and the roles of the competent tissue in the inductive system. The emphasis on competence led to the concept of genetic assimilation wherein the inducer is changed from external to internal. Both the old and new inducers were thought of as small molecules coming from outside the cell, which were able to switch the genes of a competent cell from one canalized pathway to another. This led to Waddington's being ready to use adaptive enzymes of microorganisms as instructive analogs of eukaryotic cellular differentiation.

The pathway forged by Waddington could not have been made by a person who was purely a geneticist or purely an embryologist. Waddington's goal of synthesizing genetics, embryology, and evolution was critical for his ability to connect the disparate studies, and he was concerned that the success of molecular biology might overshadow embryological studies. He ends *New Patterns in Genetics and Development*:

I should like to see the present fashion for molecular genetics diluted by the diversion of rather more attention to fundamental embryology. Genetics has had its breakthrough, and those who want quick results can probably get them most easily by exploiting this. But the next breakthrough we need, to round off our understanding of fundamental biological processes, is an embryological breakthrough. Let us hope that we get it soon.

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11. Interview with S. Gluecksohn-Waelsch, Oct. 31, 1988. Judaism was not a casual concern to Gluecksohn-Schoenheimer. She recounts that in the 1920s she was a Socialist-Zionist, and that she entered biology in order to have something useful to teach on a kibbutz in Eretz Israel.
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- sivity in the early mouse embryo. But it did not appear that T-gene expression was confined to the mesoderm (see Bennett, D., 1975, The T-locus of the mouse, *Cell* **6**:441–454). In 1990, Herrmann and his collaborators (*Nature* **343**:617, 657) cloned the T-gene and correlated the expression of this gene with embryonic lethality. The expression of this gene was found only in the early mesoderm cells and the epithelium that gives rise to them. Eventually, the T-gene is expressed only in the notochord. These data were interpreted to indicate that the T-gene plays a direct role in mesoderm formation and in the morphogenesis of the notochord. Yanagisawa (1990, *Jap. J. Genet.* **65**:287–297) similarly hypothesizes that the T-locus products are involved in inducing the chordamesoderm.
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  46. *Ibid.*, p. 92.
  47. *Ibid.*, p. 184.
  48. *Ibid.*, p. 3. This insistence on placing the mechanism of gene action in the context of embryonic development continued to be a theme in Waddington's work. In *Principles of Embryology*, Macmillan, New York (1956), he would claim that "whatever the immediate operations of the genes turn out to be, they most certainly belong to the category of developmental processes and thus belong to the province of embryology." The problem of gene activity "is essentially an embryological problem."
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71. Ross Granville Harrison may have had a similar approach to induction–competence. In his 1933 review, “Some difficulties with the determination problem” (*Am. Naturalist* **67**:306–321), Harrison wrote that no one factor determines a tissue to the exclusion of other factors. Waddington paraphrased this when he wrote (*Organisers and Genes*, p. 4) that “No ‘stimulus,’ no simple cause, is itself an adequate explanation of anything.”
72. Waddington, C. H., 1956, *Principles of Embryology*, Macmillan, New York, p. 348.
73. *Ibid.*, p. 360.
74. There is some historical irony here. In 1896, E. B. Wilson used protist models of differentiation to show that the nucleus dominated the cytoplasm. (For details see Ref. 8, and Sapp, Chapter 11, this volume).
75. Waddington, 1956, p. 350.
76. Waddington postulated intermediates between the genes of the nucleus and the cytoplasmic proteins they produce. These intermediates were necessitated by the findings that cell cytoplasm could still synthesize new proteins even after their nuclei had been centrifuged away. He speculated that these intermediates might be plasmagenes (i.e., copies of genes residing within the cytoplasm) and even mentioned the possibility of their being RNA (*Principles of Embryology*, pp. 355, 406). For further discussions of plasmagenes, see Burian et al., Chapter 10, and Sapp, Chapter 11, this volume. See also Waddington's and Spiegelman's respective articles in: *Growth in Relation to Differentiation and Morphogenesis* (Society of Experimental Biology, Symposium 2) (J. F. Danielli and R. Brown, eds.), Cambridge University Press, 1948, pp. 145–154, 286–325.
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## Chapter 10

# Boris Ephrussi and the Synthesis of Genetics and Embryology

RICHARD M. BURIAN, JEAN GAYON, and DORIS T. ZALLEN

### 1. Introduction

The career of Boris Ephrussi (1901–1979) presents a series of fascinating perspectives on the intellectual and sociological difficulties that plagued those who wished to reconcile genetics and embryology during the middle of this century (2–8). Ephrussi was born in a suburb of Moscow, but spent much of his working career in France, with important periods in the United States. He made major contributions to the rapprochement between genetics and developmental biology. His work, which we believe has been undervalued both by historians of biology and by subsequent generations of biologists, is worth studying for at least three reasons:

1. Throughout his long career, he sought an adequate causal analysis of differentiation and development. Relatively early, he saw the need to accomplish this task by uniting the findings of genetics with those of embryology. To this end, he employed a great variety of experimental organisms and techniques and explored numerous conceptual and theoretical models. Accordingly, the study of his work provides considerable insight into the shifts in theory and technique that affected various attempts to come to grips with the problems of differentiation, development, and morphogenesis while maintaining consistency—and serious contact—with genetics.
2. Because he worked in both European and American settings and maintained extremely rich contacts with workers in numerous disciplines on both sides of the Atlantic, his career sheds light on the various integrative efforts—and tensions—that characterize the relationship between embryology and genetics during the middle of the century. He was intellectually central to a number of key debates, a proponent of an integrative

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view of the organism that, at times, left him at odds with his colleagues in genetics, a prime mover in the institutionalization of genetics in France, and closely involved with institutional developments elsewhere that reconfigured the map of biology.

3. Ephrussi and his co-workers pioneered a variety of experimental approaches to the analysis of the roles of nuclear and cytoplasmic factors in differentiation. They helped shape the transition from Mendelian transmission genetics to molecular genetics and influenced the transition from embryology to developmental biology.

This chapter surveys Ephrussi's career, focusing largely on his concerns with determination, differentiation, and morphogenesis. These concerns provide an Ariadne's thread for understanding his work, helping make sense of his successes and his failures. We will discuss Ephrussi's scientific life chronologically, for it divides conveniently into four fairly distinct phases, but we will concentrate on certain thematic constancies in anticipation of the synthetic discussion with which this chapter concludes.

## 2. Early Embryological Studies

The first period covers the work Ephrussi conducted between 1920, when he arrived in France as a student, and 1934, when he left for a year in T. H. Morgan's laboratory at the California Institute of Technology. During this period, Ephrussi worked on a great variety of projects and organisms under the tutelage of the embryologist Emmanuel Fauré-Frémiel. Much of his effort over the decade 1923–1933 went into two thesis projects, one on the early development of sea urchin eggs, and the other on tissue culture (9,10). The first of these included pioneering studies of cytochemical changes during the development of the sea urchin egg, carried out in part in collaboration with Louis Rapkine. The primary focus of this work, however, concerned the changing temperature coefficients at different stages of the mitotic cycle in cells from sea urchin and *Ascaris* embryos. This work eventually yielded a technique for synchronizing cell divisions experimentally. Work on his second thesis, on tissue culture (11), convinced Ephrussi that the specificity of cell types, fixed during the course of ontogeny, was determined by intrinsic factors, probably chromosomal genes.

During this period, Ephrussi concentrated on characterizing the mechanisms and the physicochemical correlates of determination and differentiation. The organization and distribution of his projects indicate that he was seeking clues to the control of cellular processes. Three examples support this description:

1. An early paper (12) concerned the mitochondria of *Drosophila melanogaster* and *Drosophila simulans*. Sturtevant (13) had shown that crosses between these two species yielded only one sex; reciprocal crosses showed that the sex of the offspring varied with the species of the female parent, thus suggesting cytoplasmic effects on the nuclear genome. Motivated by Sturtevant's argument that factor(s) determining differential viability by sex are located in the egg cytoplasm and by Fauré-Frémiel's work on mitochondria (14), Ephrussi examined the morphology

- of mitochondria in eggs and nurse cells. He found no differences in morphology or distribution of mitochondria and concluded that “a purely morphological study cannot pretend to be complete, but if one proceeds on the basis of such a study alone, one may deny that the mitochondria are the basis of any genetic differences between the two species” (12, p. 780).
2. A series of papers concerned the growth of fibroblasts *in vitro* and their occasional transformation into macrophages (15,16). These studies sought to demonstrate that growth and regeneration rates of these cultures were regulated in part by the local chemistry. This work characterized some of the factors affecting differential growth and transformation of genetically and nutritionally identical cells and sought to prove that the macrophages found in *in vitro* fibroblast cultures were transformed fibroblasts. These papers also illustrate Ephrussi’s concern to obtain quantitative results. A typical example is the demonstration (15) that, after a sheet of cells starved *in vitro* has been cut, cells grow at a different rate in regenerating segments than elsewhere in the culture.
  3. Two papers on the fate of explanted cells and organs from brachyuryc mice (17,18) mark the transition to the next phase of Ephrussi’s career. *Brachyury* (*T*) is a dominant, lethal when homozygous. Visible defects in embryonic morphology allow the identification of homozygous *T* embryos by the eighth day. Since these embryos survive for 2 days after the defects are first seen, one could explant tissues and whole organs (in particular, the heart) before the embryo was lost. Ephrussi showed that the *brachyury* mutations did not prevent cell proliferation, long-term survival and functioning of the heart, or the differentiation of cartilage. The mutation affected different cells differently, but it involved at least some correlative effects on other tissues or organs beyond those in which the defect was visible. Ephrussi favored the hypothesis, which he considered unproven, that the lethality was a consequence of “disturbance of the normal correlations” (17, p. 203; see also Gilbert, Chapter 9, this volume). These studies opened up a research problem: how did the same gene act differently in different tissues?

The early work sketched here illustrates the preoccupations that became central in Ephrussi’s career and scientific style. He began with broad zoological interests focused on differentiation, development, and physicochemical mechanisms of cell function, but he was not satisfied with classical descriptive embryology. To get at underlying causes, he sought speculative models (mitochondria are the locus of the cytoplasmic differences between *Drosophila* species; the lethal effect of the *brachyury* gene is caused by disruption of correlations) that could be submitted to rigorous, preferably quantitative, tests. He was skeptical of morphological findings until they could be integrated into a physiological account of biological processes.

Up to this point, genetics entered Ephrussi’s work only tangentially. His interest in that field arose from his growing conviction that intrinsic factors, seemingly located on chromosomes in nearly all cases (as the Morgan school claimed), determined cell capacities or competences.

### 3. Beadle and Ephrussi on *Drosophila* Hormones

The second phase of Ephrussi's career starts with his fellowship in Morgan's laboratory at the California Institute of Technology. There, although he published little transmission genetic work (3, pp. 392–393), he became thoroughly familiar with the techniques and theory of Mendelian genetics. The outstanding effort of this period was his collaboration with Beadle and others, in which he sought to unify genetics and embryology by application of embryological techniques to the genetic organism *par excellence*, *Drosophila melanogaster*. The aim of this work was to analyze the precise role of the genes in at least one ontogenetic pathway by working backward from a well-defined phenotype as far as possible toward the genes (3, pp. 389–400; 7, Chapter 5, especially pp. 132–133; 19, pp. 2–3).

The project originated in Sturtevant's discovery, using gynandromorphs (20), that the phenotypic effect of the vermilion (eye color) mutant of *Drosophila* could be suppressed by the presence of wild-type tissue elsewhere in the organism. With Sturtevant's encouragement, Ephrussi and Beadle decided to trace the pathway by means of which the vermilion and related genes affected or determined eye color (21). By transplanting mutant primordia (imaginal disks) fated to become eyes (but also other organs) into wild-type larvae (22), they were able to demonstrate that two distinct diffusible substances were manufactured or controlled by wild-type alleles at the vermilion and cinnabar loci, and that these diffusible substances controlled distinct steps in the formation of the brown component of normal *Drosophila* eye pigment.

The techniques and issues involved in this work are not those of classical Mendelian genetics. On the contrary, although the experimental protocols required genetically defined stocks, they did not involve direct use of genetic crosses. The techniques employed were those of experimental embryology—specifically, analysis of alterations in development brought about by explanting and implanting imaginal disks. The issues did not turn on the distribution or the mode of transmission of the relevant genes, but on the ways in which those genes acted.

Against this background, three conclusions informed Ephrussi's analysis: (1) the substances controlled by the genes were responsible for very specific reactions, (2) the substances acted at sites distant from those where they were produced, and (3) they were not species specific. These facts led Ephrussi to interpret the diffusible substances as hormones or hormone-like substances (23), even after Beadle and Tatum's work suggested that they are enzymes (24,25).

### 4. Ephrussi and Slonimski on Cytoplasmic Inheritance of Respiratory Competence in Yeast

The third phase of Ephrussi's career begins with his return to France at the end of World War II. During the early 1940s, his work slowed by his status as a refugee in the United States, Ephrussi began to take plasmagene models of differentiation and regulation seriously. These came to play a central role in his work.

Why plasmagenes? Why the emphasis on regulation? And how are these two related? These are complex questions calling for complex answers. After a few introductory comments, we turn to the experimental work and then to an analysis of the issues involved.

Of particular interest is Ephrussi's treatment of the role of cytoplasmic entities—especially the roles they might play in differentiation, determination, and heredity. Unlike mainstream American geneticists, Ephrussi was deeply preoccupied with questions concerning mechanisms of differentiation and physiological control of cellular properties. Unlike many embryologists, he was thoroughly convinced that the potentialities of the organism are controlled by an integrated system involving both the cytoplasm and the nuclear genes—that morphology, for example, is not controlled by an independent cytoplasmic system of heredity (26–30). Having been trained in France between the world wars, he was keenly aware of the problems of special interest in that country, including the interplay between infection and heredity and the concerns with cytoplasmic regulation of cellular physiology in the traditions of Claude Bernard and the Pasteur Institute. Finally, perhaps influenced by the skepticism of French positivists toward theoretical models, he was strongly inclined both to produce theoretical models of biological mechanisms and to stand back far enough from those models to submit them to rigorous (and, where possible, quantitative) tests.

This constellation of intellectual style and focal problems helps explain the ontogeny of Ephrussi's career and, indeed, the development of genetics in France after World War II. Ephrussi's role in the development of the discipline rests in part on his institutional influence: shortly after returning to France in 1945, he became that country's first Professor of Genetics (at the Sorbonne) and Director of the Centre National de la Recherche Scientifique's (CNRS) new Institute of Genetics (4). He thus played a decisive role in setting both the research agenda and the genetics curriculum in France.

The primary experimental organism employed in the postwar period was baker's yeast, *Saccharomyces cerevisiae*. Soon after returning to France, Ephrussi began genetic study of yeast, modeled on Beadle and Tatum's work on *Neurospora*. Mutagenesis experiments soon produced startling results, leading the research in a very different direction. Ephrussi found irreversible loss of respiratory competence (recognizable by "petite" colony morphology) in all progeny of yeast exposed to a sufficient dose of acriflavin (31,32).

Since yeast can grow via strictly fermentative (anaerobic) pathways, the mutation was not lethal. Moreover, one could isolate haploid progeny, grow clones, and perform outcrosses to obtain and analyze diploid progeny. The system was, therefore, well suited to genetic analysis. Biochemical studies (mainly by P. Slonimski and co-workers) revealed that a whole assembly of respiratory enzymes (including cytochrome oxidase), later shown to be bound to sedimentable particles, was lost. Intensive genetic analysis showed that the respiratory deficiency was inherited cytoplasmically and that genes for the component enzymes involved could not be separated by standard genetic crossing techniques.

Here was a clear case of cytoplasmic inheritance. Equally interesting were the facts that the mutations in question went in a particular direction (to loss of

respiratory competence) and involved switching of cell type and alteration of fundamental organismic properties. These were phenomena that called for genetic analysis and analysis of the developmental pathways involved. Furthermore, since baker's yeast was normally a facultative anaerobe, these studies could shed light on the regulation of this crucial physiological process. Given Ephrussi's interests, presented earlier, particularly the interest in dual systems of inheritance and the regulation of cellular states, it is not surprising that he spent 15 years analyzing the complex phenomena revealed in these experiments (33–35).

One particular finding deserves brief mention. By 1950, nuclear mutations had been discovered with a biochemical phenotype similar to that of the cytoplasmic defect. The genetics of the subsequent studies are too complex to explore here; in effect, Ephrussi and co-workers had shown by 1952 that the presence of the correct allele of a nuclear gene was required for proper functioning of the cytoplasmic particles required for respiration (28, pp. 38–42, 100) (35,36). Thus the details of an extremely complex nucleocytoplasmic interaction became a central focus of interest.

The concept that plasmagenes control the differentiation of cells (37–39) was one of the hypotheses available for development and testing. To understand the relevant background, one should recall the status of studies of cell differentiation and of enzyme synthesis in the late 1940s and early 1950s. The two problems were connected by the common belief that changes in the patterns of protein synthesis cause differentiation. Although it was widely recognized that genes somehow control the specificity of proteins, it was also widely held that control of the patterns of synthesis and quantities of product were cytoplasmically mediated.

Here is a rough statement of the hypotheses at stake. According to one popular model of protein synthesis, the conformation of a generalized peptide precursor or substrate molecule could be altered by its cytoplasmic surroundings, including the presence of genes or gene products. Thus, differentiation might occur by means of "substrate competition," i.e., competition in the cytoplasm between numerous copies of a gene or gene product to become the dominant cause of substrate reformation (40). A successful gene would exclude competitors for the same substrate and, by positive feedback and autocatalysis, come to be (or to have produced) the dominant protein product of the cell. Self-reproducing plasmagenes (i.e., cytoplasmic copies of a gene) could multiply at a high rate to bring about such an effect, thus causing differentiation.

Plasmagenes would belong to the larger class of self-reproducing cytoplasmic particles. Such particles exhibit genetic continuity—i.e., they can be produced only from precursor particles of the same sort. Cellular entities "endowed with genetic continuity" were important candidates for the status of plasmagenes or bearers of plasmagenes. Mitochondria, chloroplasts, kinetosomes, viruses, and a great variety of other cellular entities were among the entities in question (41–44). The bulk of the evidence from Ephrussi's laboratory was consistent with the obvious conclusion: respiratory competence depended on cytoplasmic particles endowed with genetic continuity which either were or (because a linked complex of enzymes was involved), more likely, contained plasmagenes.

Ephrussi's own attitude to such claims is strikingly cautious. It is well summarized in the "General Discussion" of *Nucleo-cytoplasmic Relations in Micro-organisms* (29). There he distances himself from the plasmagene hypothesis by arguing that all self-reproducing units in a cell, whether nuclear or cytoplasmic, are interdependent. Accordingly, although various "cytoplasmic mechanisms [involving self-reproducing, genetically continuous, cytoplasmic entities, including plasmagenes] . . . could be an instrument of somatic differentiation, none of them can be its primary factor" (29, p. 101, original italicized). Indeed,

We may . . . speak of autoreproduction of the integrated unit which is the cell, but it would be more correct not to speak of autoreproducing particles [which require the integrated cellular context to reproduce]: in view of their unique role in the process of identical perpetuation, I would rather speak of them as conservative units of the cell (29, p. 107) (45–48).

A further facet of this cautious attitude is Ephrussi's hesitation to identify mitochondria with the particles "endowed with genetic continuity" required for respiratory function. This hesitation is, in some respects, difficult to understand, though both Caspari and Slonimski have suggested in interviews that Ephrussi had previously been criticized for a hasty identification and was therefore twice shy. Still, from his earliest days, Ephrussi considered mitochondria to be genetically continuous (49) and plausible candidates for determining cell states. His resistance to such identifications illustrates, at least partly, general skepticism about the bearing of morphological evidence on physiological claims.

The techniques and issues involved were closely intertwined with those employed at that very time in the Pasteur Institute by Jacob, Lwoff, Monod, and Wollman, and their co-workers. To fully appreciate the role of Ephrussi and those working around him as founders of national and institutional traditions in genetics, still of central importance in France, it is necessary to understand the relationships between Ephrussi and his colleagues at the CNRS and the leading figures at the Institut Pasteur. These matters are of intense interest, deserving exploration on another occasion. However that may be, both in the Pasteur and in the laboratories at the CNRS, the traditions established during the late 1940s and the 1950s continue to this day. Ephrussi's own laboratory, for example, is still directed by Piotr Slonimski and is one of the leading sites internationally for the study of mitochondrial genetics.

## 5. Somatic Cell Genetics and the Return to Tissue Culture

The beginning of the final phase of Ephrussi's career is marked by his move to Western Reserve University (Cleveland, Ohio) in 1962. By then, the primary focus of his research was the study of intraspecific and interspecific somatic cell hybrids. In interspecific hybrids (where the cytoplasms and nuclei of two cells from different species fuse), differential chromosome loss is common. This allows researchers to locate genes on particular chromosomes. As is well known, the use of mouse–human cell hybrids (in which different hybrid cell lines lose different human chromosomes) was developed extensively in many laboratories (including Ephrussi's) and has become a central tool of human genetics. Various

extensions of the techniques involved are still in active use and, indeed, play a crucial role in the analysis of chromosome organization and gene regulation in eukaryotic cells. Similarly, the current use of fusions between malignant cell lines and cells producing specific immunological products to produce monoclonal antibodies is based on techniques employed in Ephrussi's and other laboratories in the 1960s and 1970s. The best review of the work pursued by Ephrussi and his colleagues, and, indeed, of the state of the field as of that date, is Ephrussi's *Hybridization of Somatic Cells* (50).

As we saw in Section 4, Ephrussi's interest in the regulation of respiration in yeast was instrumental; his underlying purpose was to shed light on

cell differentiation and cell heredity in higher forms. . . . In our search of the nature and seat of these phenomena in the Metazoan cell, we turned our attention to micro-organisms because we were unable to solve the problem by direct analysis of somatic cells. Our reasoning [about differentiation is] . . . by analogy (29, p. 99; cf. parallel remarks in 30, pp. 257 ff.).

At the beginning of this text, he observed that

what is needed is direct genetic analysis of somatic cells, for the assumed functional equivalence of [nuclei of] irreversibly differentiated somatic cells, however plausible, is only an hypothesis. Crosses between such cells being impossible, only nuclear transplantation from one somatic cell to another, or grafting of fragments of cytoplasm, could provide the required information; such experiments however will have to await the development of adequate technical devices. In the meantime, the closest approximation to the evidence we would like to have is provided by the study of lower forms which propagate by vegetative reproduction and possess no isolated germ line (29, p. 5) (51,52).

In consequence, even while the work on yeast flourished, Ephrussi sought to develop a system allowing a more refined treatment of the questions he had pursued in his studies of brachyuric mice and of *Drosophila* with Beadle—a system that would allow genetic knowledge to be combined with examination of the underlying details of the regulatory processes entering into differentiation. We cannot discuss here various false starts and transitional studies; it is clear, however, that he was strongly interested in renewed employment of tissue culture techniques for the study of somatic cells from roughly 1953 forward (53).

By a stroke of luck, the necessary technique was placed in his hands shortly before his move to the United States. The work in question was conducted by G. Barski, S. Sorieul, and F. Cornefert at the Institut Gustave Roussy near Paris. They achieved hybridization between two karyotypically distinct lineages of mouse cells (54). Knowing of Ephrussi's interests, but uncertain how best to exploit and develop their findings for his purposes, Barski contacted Ephrussi. After some delicate negotiations, Sorieul joined Ephrussi's laboratory to develop the new technique in that setting. The immediate collaboration fell short of expectations, remaining restricted to karyotypic markers (55); nonetheless, it set a major program of research in motion.

Somatic cell hybridization was the key to genetic analysis without dependence on sexual processes. The use of hybrids between cells of different genetic constitutions made a direct attack on the problem of differentiation possible. Ephrussi's remarks at one of the first international meetings on genetic variation in somatic cells (1965) reveals the Ariadne's thread guiding his program of research:

The field which, to me, has the greatest appeal and which, I am sure, can take immediate advantage of the hybridization techniques, is that of gene expression in development and differentiation.

I am sure we all believe today that the phenomenon of differentiation has its roots in events concerned with the regulation of the activities of genes or of gene controlled processes. If am sure also that you are aware of the fact that we know very little about regulation in somatic cells of multicellular organisms, and that, in spite of this (or because of this?) the direct extrapolation from the regulatory mechanisms in bacteria to those of higher animals is regrettably fashionable. Be this as it may, hybridization of somatic cells at last can provide some facts (56, p. 58).

The original cell hybrids could be recognized only by karyotypic markers. Ephrussi immediately sought to increase the power of the technique in a variety of ways. His laboratory and others (57,58) soon found that morphological, enzymatic, and biochemical markers could be used, either alone or in combination with karyotypic markers, to determine whether hybridization had occurred. Use of biochemical markers had the advantage of rapidity and simplicity. Such systems also were made to yield two particular advantages: First, the establishment of regimes that provided hybrid cells with a selective advantage—for example, allowing cell hybrids to overgrow parental strains. In fact, in many instances, the parental strains cannot grow at all in the selective medium (Littlefield's medium). Second, in consequence, it became possible to quantify various phenomena, most simply the proportion of the cellular population in which hybridization occurred. Together, these provided a useful tool for searching for ways to increase the incidence of hybrid formation.

We focus on the three lines of research that Ephrussi considered most crucial. These concerned the formal genetics of higher organisms, the study of differentiation, and the study of malignancy. Ephrussi held that unless the somatic cell hybridization techniques yielded dramatic advances on all these fronts (and perhaps others), they would have failed to live up to their promise.

We will say very little about formal genetics, which was not Ephrussi's central interest (59). Somatic cell hybridization allowed one to locate genes for particular enzymes (e.g., thymidine kinase) on particular chromosomes or chromosome arms (60) and provided an armamentarium of ever more sophisticated technical tools for zeroing in on the chromosomal location of genes essential for maintenance of particular cell functions or for the production of biochemically identifiable products. Although Ephrussi had "little taste for formal genetics" of this sort, he considered it "an absolutely essential prerequisite to the attack on any other problem involving genetic mechanisms" (50, p. 31) and equally "essential for the understanding of any other problem of cell biology, such as that of cell differentiation which . . . still represents a major unsolved problem" (50, p. 49).

To help analyze this problem, Ephrussi and his co-workers utilized an important distinction (for which he claims credit) between household and luxury functions (50, Chapter 4). "Household" functions were required for the metabolic maintenance of virtually all cells; they could not be used to distinguish one cell type from another and hence were of little help in explaining differentiation. In contrast, "luxury" functions were distinct in different cell lineages and organs; some cells (such as erythrocytes and melanocytes) devoted a preponderance of the cell's metabolic activity to such functions. Thus, analysis of the control of luxury functions and the conditions under which the commit-

ment to luxury functions was made or reversed was of central interest in studies of differentiation.

A particularly striking finding was that fusions between more and less differentiated cells typically extinguished the luxury functions of the more differentiated cells (61). Sometimes, e.g., in fusions with melanocytes, loss of chromosomes derived from the less differentiated strain in the resultant hybrid cells restored the lost functions (62). Weiss and Chaplain obtained similar results, though more difficult to interpret, for intraspecific markers of hepatic cell function (63). Similar findings were made in other laboratories.

Ephrussi initially interpreted these findings in terms of repressor mechanisms like those known in bacteria (64), but recognized that at least four or five other models were compatible with the available evidence (50, pp. 95 ff.). For our purposes, what is important is that he seriously entertained the hypothesis that such cases provided evidence of “an extraordinary stability of the differentiated state itself” since the capacity to resume the luxury function remains present, though deactivated, in the hybrid cells (50, p. 97). Using terminology borrowed from Abercrombie (65), Ephrussi maintained that the “epigenotype” of differentiated cells—“that part of the total genome which, under appropriate conditions, can be expressed in a given cell type to the exclusion of those limited to other cell types” (50, p. 53)—was retained in the face of the phenotypic dedifferentiation that often resulted from hybridization (66). Thus, different cell types express different epigenotypes, and the question of regulation can be put in terms of the control of epigenotype expression. We will return to this theme later.

The fact that parallel results were obtained in many laboratories with both intraspecific and interspecific cell fusions made it seem likely that the fundamental regulatory controls for particular luxury functions are not species specific. Thus the coherence of differentiated function—and the similarity of the signals that could extinguish or restore such functions in cells from widely divergent organisms—had a crucial role in Ephrussi’s interpretation of the outcome of these experiments.

As indicated in note 66, a major concern in interpreting these results was the extent to which the regulatory apparatus of transformed or malignant cell lineages differed from that of normal cells. This is only one of several reasons why Ephrussi considered the exploration of malignancy by use of somatic cell hybrids to be intellectually and practically urgent. Indeed, a number of fundamental biological issues were at stake. The interpretation of Ephrussi’s views on these matters is complicated by the fact that they were colored by unfortunate personal interactions with Henry Harris, who held competing views (67). Harris and Klein’s results (68), showing that chromosome loss could result in increase of malignancy, persuaded Ephrussi (in spite of his tentative earlier claims to the contrary, cf. note 69) that malignancy might be a recessive rather than a dominant trait. The issue turned on interpretation of the fact, parallel to that discussed earlier, that in those cases where malignancy was suppressed in cell hybrids, it could be recovered by loss of chromosomes originating from the parental line with lower tumorigenicity. Nonetheless, Ephrussi favored in interpretation in terms of epigenetic rather than genetic changes, parallel to the one he developed for the extinction of luxury functions.

In our concluding interpretive section, we will consider the importance of this sort of epigenetic interpretation as one of the guiding threads in understand-

ing Ephrussi's career and briefly relate it to the state of the interactions between the developmental and genetic perspectives in molecular genetics at the beginning of the 1970s, when the transition from embryology to developmental biology was decisively underway.

## 6. Coda and Conclusion

### 6.1. Coda: Common Themes in Ephrussi's Career

One of the striking features of Ephrussi's career is the frequency with which he employed new organisms and techniques in his research. At various times he worked with marine invertebrates, protozoa, *Drosophila*, yeast, mice, hydra, *Ascaris*, and tissue-cultured cells; although he was emphatically not a biochemist, he clearly sought out techniques (including biochemical ones) suitable for analyzing the genetic and epigenetic basis for determination, differentiation, and (to a lesser extent) morphogenesis in all these various organisms. He often played with new organisms even while pursuing one of the main programs of research listed earlier. (Thus, he tried to turn hydra into an experimental vehicle during at least three distinct periods of his career.) Furthermore, he oversaw a complex of activities in his laboratory that typically included work on four or five organisms at a time. For example, in a grant application submitted to the Rockefeller Foundation in August 1956, he lists 10 major experimental programs in his laboratory, utilizing five distinct types of experimental organisms (70).

Underlying this diversity, however, are some common themes. Throughout his life he retained the embryologists' concern—gained as a student of Fauré-Frémiel—for determination of the fate of cells and tissues. This explains his commitment to shaping various organisms and techniques into vehicles for addressing fundamental questions about cellular determination, differentiation, and organismic development. Although we have divided his career into fairly distinct periods, his enduring preoccupation with these underlying themes provides a key to understanding the roles that he played, his successes, and his failures. The unity of his career turns on his attempt to understand the control of determination, differentiation, and development (71)—both the competences of the cell (which, presumably, were specified mainly by its genes) and the regulatory devices that determine the combination of products manufactured under genetic and epigenetic control. Over time, as new techniques became available and as he exploited new organisms, he sharpened his basic questions, moving, in general, from the level of organisms and organ systems to the cellular and subcellular levels.

The single most important change affecting his work was, of course, acquisition of the tools and techniques of genetics. The methods of that discipline offered him a vehicle for the production of speculative hypotheses that could be rigorously tested and subjected to quantitative investigation. The combination of the themes and techniques of genetics and embryology is a central feature of his scientific style—a feature that helps account for his remarkable success in developing three major programs of research involving diverse organisms and techniques into international importance.

He retained the embryologists' respect for the integrity of the organism; neither nucleus nor cytoplasm alone could account for determination and differentiation—the role of each in interaction with the other had to be teased out. And teasing out required the use of analytical techniques by which opposing detailed mechanistic hypotheses, however speculative, were developed to the point that they allowed rigorous, ideally quantitative, testing.

Consider, for instance, how he approached the issue of correlation of functions. From his earliest embryological papers, the mode of action by means of which a product at one place affected a process at another was of crucial importance to him. This is apparent in his study of the effects of the brachyury mutation, in his preference for a hormonal over an enzymatic interpretation of the diffusible substances that he and Beadle discovered in *Drosophila*, and in his probing of cytoplasmic and epigenetic pathways for control of function in somatic cell hybrids.

A related, yet underappreciated, aspect of Ephrussi's approach is also best understood as a carryover from his embryological training. This is the degree to which he required satisfactory solutions of biological problems to reflect both structure and function. He was never truly at home in biochemistry (72). Although the lateness with which he came to biochemical techniques has something to do with this, we suspect that there is a deeper reason as well; until nearly the end of his career, biochemistry did not connect its enzymatic analyses with the structures of the cell. For Ephrussi, any interpretation of the cell that treated it as a mere bag of enzymes was utterly unpersuasive. Traditional biochemistry, while perhaps not this extreme, was an inadequate tool; it did not provide a structure-function analysis of the sort he required, of a sort ancestral to those that are now the daily fare of molecular biology. For example, in his laboratory's work on yeast respiratory enzymes, it was not sufficient to understand which enzymes were gained or lost, nor even the genetic controls underlying such gain or loss. For Ephrussi, before that system could be considered adequately characterized it was also necessary to connect such information with knowledge of the binding of the enzymes to membranes, the interaction of soluble enzymes with nuclear products, the mechanisms by which those interactions were controlled, and the interrelationships between the nuclear mutations affecting the particles that produced the respiratory enzymes (or on which they functioned).

An example of the importance of the treatment of the cell as an integrated whole—of the integration of nuclear and cytoplasmic determinants of cellular behavior—can be found in his treatment of somatic cell hybrids. In that work he resisted a strictly genetic or biochemical analysis of such properties as the gain or loss of contact inhibition on grounds that one needed an account of the role of the cell membrane in sending back signals that modulated the action of the hybrid genome. In setting up his discussion of the epigenotype, he employs an explicitly embryological perspective:

. . . what is inherited is not the differentiated state as such, but the ability to undergo a certain type of differentiation, or, if you prefer, the commitment to a certain course, to the exclusion of all others. This commitment is the result of what embryologists call "determination," and it is the determined state, and not the differentiated state that is inherited (50, p. 52).

Furthermore, even at this stage, he saw the regulatory question in terms of the broadest range of models, among which he personally favored cytoplasmic

ones and those involving the cell membrane (73). After objecting that most of the hypotheses proposed to explain regulation "are concerned with differentiation rather than with determination; and that much of what is considered as a cause of differentiation is the effect of the still completely mysterious and elusive act of determination" (50, p. 55), Ephrussi argued that

if what Hershey (74) calls the unwritten dogma is correct (i.e., "the inference that all three-dimensional structure is encoded in nucleotide sequences"), the establishment of different epigenotypes in the course of development must be coded for [in] nuclear DNA because the whole program of development is transmitted from generation to generation. But I also think that whether the functional restriction of the total information, which results in different epigenotypes of different cell lineages, is due to a change in the chromosomes themselves (as it seems to be in the inactivation of one of the Xs in mammalian females, for example . . .) or is only a reflection of a change elsewhere in the cell (say, in the cell membrane) is an entirely separate and largely unresolved question worthy of very serious consideration . . ." (50, p. 555, note omitted).

To put the unity of Ephrussi's style in slightly hyperbolic terms, consider how many of the critical experiments performed in his laboratory employed the embryological technique of explantation or a close analog. The technique was obviously central in the work on the *brachyury* mutation and in his studies of *Drosophila* eye pigments. What is less obvious is its close relationship to the yeast work (75) and to the work on somatic cell hybrids. In the case of yeast, the analysis of respiratory function required analysis of the fate of respiratory competent or incompetent cells in colonies of the other type; furthermore, one of the reasons for the uncertainty about the connection between mitochondria and the hypothetical particles responsible for respiratory competence was the inability to carry out the analog of an explantation experiment that showed decisively that mitochondria bore the responsibility. In the case of somatic cell hybrids, the mouse-human hybrid cells in which human chromosomes were differentially lost functioned as an approximate equivalent to a system for explanting one or a few human chromosomes into a mouse cell.

A full biography would take up many issues that we cannot treat here. Among these are the effects of changes of technique and organism on his work; his sometimes ambivalent relationship to the arrival of biochemical and molecular tools of analysis; the probing skepticism with which he devised, coopted, and tested conceptual and theoretical models; his skepticism about the exclusive role of nuclear genes in determination; his skepticism about the interrelationships between morphological structures and units of function (76).

## 6.2. Conclusion: Interactions among Disciplines; Morals from a Career

Embryology has recently gone through a transformation marked by a change of label: many of its traditional problems are being treated within what is now called developmental biology. The first stages of the transition in question are mirrored in, and were partly influenced by, the later phases of Ephrussi's career.

Although Ephrussi was working as a geneticist, as he came to grips with new disciplines, techniques, and organisms he remained preoccupied with embryological questions. As one of the founders of physiological genetics, interested above all in gene action and its control, he helped bring that discipline into the

new world of molecular genetics. He is one of the few leading figures during this period to have retained, and even insisted on, a traditional embryological perspective while making this transition. We believe that it is partly for this reason that he chose not to work with prokaryotic systems—they were unsuitable for answering his questions (77). He did so on grounds of principle—he believed that work with prokaryotes could not answer the classical questions concerning determination and differentiation of eukaryotes, that work with prokaryotic systems could not take adequate account of the integration of the cell or of the regulation of metazoan development. His choices in these matters (which were clearly self-conscious) affected the nature of his influence on genetics and on developmental biology and forced him to accept certain technical limitations in his own program of research.

The most crucial choice was his insistence on the importance of the problem of determination and his skepticism about the direct applicability of the models developed in prokaryotes to that problem. In consequence, it was necessary to search for ways of attacking the problem directly, which meant working with eukaryotes (or eukaryotic cells) and distancing oneself from the aphorism attributed to Monod that “what is true of *E. coli* is true of the elephant” (78). Like Ephrussi (and, indeed, under his influence), Monod had begun his major research program on enzymatic adaptation with the hope of “understanding how cells with identical genomes may become differentiated, [may acquire] the property of manufacturing new, or at least, different, specific patterns or configurations” (79, p. 224). But unlike Ephrussi, Monod came to believe that the problem could be simplified in such a way that an analysis of regulation of the states of prokaryotic cells yielded the basis for a general resolution of such problems. This helps account for his choice to remain with bacteria and for the extreme patience with which he developed the biochemical tools for characterizing the steps involved in galactose metabolism and its regulation. Indeed, for some time Monod tried to persuade Slonimski that cytoplasmic inheritance in yeast was too complex and too messy to yield valuable results (80)—results that would uncover the abstract principles or the detailed mechanisms of regulation.

At the time, Monod was right, of course, in that it proved harder to use eukaryotes to shed light on cellular regulation and determination processes than it was to analyze regulatory mechanisms in prokaryotes. Given the then-available techniques, which did not include any means like those subsequently developed for direct characterization of the genome and its transcripts, one might argue that Ephrussi was reaching beyond the limits of what could be done in seeking a subcellular, even molecular, account of the controls of cellular determination.

This is the threshold on which Ephrussi stood toward the close of his career, a threshold that, we suspect, he knew he could not cross. His ambivalence is clear in the way in which he closes the introduction of his book on somatic cell genetics:

The combined use of these facts and techniques [of somatic cell genetics] permits today  
the production of practically any hybrid one wishes to have for any purpose.

And the upshot of it is that, like real molecular biologists, we now receive with fear  
each new issue of P.N.A.S. (50, p. 30).

Ephrussi's ambivalence had at least two roots. One was that he was no longer comfortable with the techniques involved (81). But there is surely a second root as well: it was slowly becoming clear that the new technologies of DNA biochem-

istry—the innovations that would lead to identification of particular mRNAs, molecular cloning, and the like—would surpass the tools of classical physiological/biochemical genetics, even when applied to somatic cells, as vehicles for solving the problems he had kept in front of geneticists for nearly three decades. And it was too late for him to make those tools his own, too late to incorporate them into yet one more major program of research in pursuit of the questions that had dominated his career. He was, after all, already 70 years old.

The successes of the postwar years were, however, remarkable. Aside from the crucial role he played in establishing the institutional basis of genetic research and the curricular basis for the teaching of genetics at the university level in France and his importance in binding together the work of the leaders of competing disciplines on two continents, he developed two major programs of research, employing radically different techniques and organisms, uniting the problematics of different disciplines. He insisted that the concerns of the embryologist (transformed into a developmental biologist) belong near the center of molecular biology at a time when this view was unpopular. His commitment to the problem of determination drove him to switch from system to system, organism to organism, technique to technique, developing them on a large scale so that he and others could exploit them to the limit for the purpose of analyzing regulatory processes in eukaryotes. His insistence on the need to deal directly with the problems of regulation and determination in eukaryotes helped keep those problems from being bypassed in the enthusiasm engendered by the operon and the ease of conducting molecular biology by working with bacteria.

Although Ephrussi did not succeed in producing a system that allowed the problem of determination to be resolved in full molecular detail, he can be credited with keeping it in the foreground in various disciplinary communities and developing tools without which it could not have been directly confronted. By insisting on treating the cell and the organism as integrated wholes, by requiring explanations of regulatory mechanisms to take account of nucleocytoplasmic relations and to unite structure with function and membrane mechanisms with genetic information, he helped to set themes that are central to developmental genetics and cell and developmental biology at the present moment. Indeed, the difficulty we now encounter in establishing clear disciplinary boundaries among workers in these fields is, in part, the legacy of his work.

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7. Sapp, J., 1987, *Beyond the Gene: Cytoplasmic Inheritance and the Struggle for Authority in Genetics*, Oxford University Press, New York, especially Chapter 5, “Boris Ephrussi, Nucleo-cytoplasmic Relations, and the Institutional Strategy of French Genetics.”
8. Gans, M., and Slonimski, P., 1985, Boris Ephrussi, 1901–1979, *Universalia 1980*, Encyclopaedia Universalis France, Editeur, Paris, pp. 548–549.
9. The following brief characterizations of Ephrussi's theses parallel those of (5).
10. Ephrussi, B., 1932, Contribution à l'analyse des premiers stades du développement de l'oeuf. Action de la température, Imprimerie de l'Académie, Paris; Ephrussi, B., 1932, Croissance et régénération dans les cultures des tissus, Masson, Paris.
11. Ephrussi's early work on tissue culture was greatly facilitated by a visit to the laboratory of A. Fischer in Berlin in 1927, supported by the Rockefeller Foundation.
12. Ephrussi, B., 1925, Sur le chondriome ovarien de *Drosophila melanogaster*, *C. R. Soc. Biol.* **92**:778–780.
13. Sturtevant, A., 1920, Genetic studies on *Drosophila simulans*. I. Introduction. Hybrids with *Drosophila melanogaster*, *Genetics* **5**:488–500.
14. Fauré-Frémiel, E., 1910, La continuité des mitochondries à travers les générations cellulaires et le rôle de ces éléments, *Anat. Anz.* **36**:186–191.
15. Ephrussi, B., 1931, Vitesse de croissance et vitesse de régénération des cultures de tissus *in vitro*, *C. R. Soc. Biol.* **106**:274–277.
16. Ephrussi, B., 1931, Action de l'extrait embryonnaire sur la vitesse de régénération des cultures de tissus, *C. R. Soc. Biol.* **106**:546–548; Ephrussi, B., 1931, Action du plasma et du sérum sanguin sur les macrophages formés *in vitro*, *C. R. Soc. Biol.* **106**:635–637; Ephrussi, B., 1931, Sur les facteurs limitant l'accroissement des cultures des tissus *in vitro*, *C. R. Acad. Sci.* **192**:1763–1765; Ephrussi, B., and Hughes, Y., 1930, Sur la transformation de fibroblastes en macrophages, *C. R. Soc. Biol.* **105**:697–699.
17. Ephrussi, B., 1933, Sur le facteur léthal des Souris brachyures, *C. R. Acad. Sci.* **197**:96–98.
18. Ephrussi, B., 1935, The behavior *in vitro* of tissues from lethal embryos, *J. Exp. Zool.* **70**:197–204.
19. Horowitz, N., 1990, George Wells Beadle (1903–1989), *Genetics* **124**:1–6.
20. Sturtevant, A. H., 1920, The vermilion gene and gynandromorphism, *Proc. Soc. Exp. Biol. Med.* **51**:325–327; Sturtevant, A. H., 1932, The use of mosaics in the study of the developmental effect of genes, *Proc. Sixth Int. Cong. Genet.* **1**:304–307.
21. Beadle, G., and Ephrussi, B., 1935, Transplantation in *Drosophila*, *Proc. Natl. Acad. Sci. USA* **21**:642–646; Beadle, G., and Ephrussi, B., 1935, Différenciation de la couleur cinnabar chez la Drosophile, *C. R. Acad. Sci.* **201**:620–621; Beadle, G., and Ephrussi, B., 1936, The differentiation of eye pigments in *Drosophila* as studied by transplantation, *Genetics* **21**:76–86; Beadle, G., and Ephrussi, B., 1936, Development of eye colors in *Drosophila*: Transplantation experiments with suppressor of vermilion, *Proc. Natl. Acad. Sci. USA* **22**:536–540; Beadle, G., and Ephrussi, B., 1937, Development of eye colors in *Drosophila*: Diffusible substances and their interrelations, *Genetics* **22**:76–86; Beadle, G., and Ephrussi, B., 1937, Development of eye colors in *Drosophila*: Pupal transplants and the influence of body fluid on vermilion, *Proc. Roy. Soc. Lond., B* **122**:98–105; Ephrussi, B., 1938, Aspects of the physiology of gene action, *Am. Nat.* **72**:5–23; Ephrussi, B., and Beadle, G., 1935, La transplantation des disques imaginaires chez la Drosophile, *C. R. Acad.*

- Sci.* **201**:98–100; Ephrussi, B., and Beadle, G., 1935, Sur les conditions de l'autodifférenciation des caractères mendéliens, *C. R. Acad. Sci.* **201**:1148–1150; Ephrussi, B., and Beadle, G., 1936, A technique for transplantation for *Drosophila*, *Am. Nat.* **70**:218–225; Ephrussi, B., and Beadle, G., 1937, Développement des couleurs des yeux chez les *Drosophila*: Influence des implants sur la couleur des yeux de l'hôte, *Bull. Biol. Fr. Belg.* **71**:54–74; Ephrussi, B., and Beadle, G., 1937, Development of eye color in *Drosophila*: Transplantation experiments on the interaction of vermilion with other eye colors, *Genetics* **22**:65–75.
22. As Horowitz (19) points out, Beadle and Ephrussi also tried, but failed, to grow imaginal disks in tissue culture for the purposes of their analysis.
  23. Ephrussi, B., 1942, Analysis of eye color differentiation in *Drosophila*, *Cold. Spring Harbor Symp. Quant. Biol.* **10**:40–48; Ephrussi, B., 1942, Chemistry of eye-color hormones of *Drosophila*, *Q. Rev. Biol.* **17**:327–338; Ephrussi, B., and Herold, J., 1944, Studies of eye pigments of *Drosophila*. I. Methods of extraction and quantitative estimation of the pigment components, *Genetics* **29**:148–175, especially p. 148.
  24. Lily Kay has pointed out (personal communication) that there is no early formal statement of the one gene–one enzyme hypothesis in Beadle's writings. The steps by which genes came to be interpreted as determining the structure of particular enzymes are far more complex than the usual casual historiography suggests; it is to be hoped that a careful study will soon be devoted to this topic. Horowitz (19) cites Beadle's first reasonably clear statement of the one gene–one enzyme hypothesis as occurring in Beadle (25).
  25. Beadle, G., 1945, Biochemical genetics, *Chem. Rev.* **37**:15–96.
  26. See, for example, Sapp (7) or (28). The latter describes the reasons, especially important in the French setting, which led such figures as André Lwoff to seek and expect to find a system of double control—two independent hereditary systems, one nuclear and one cytoplasmic—that accounted for the development of organisms. As many historians of biology have pointed out, well into the 1940s and 1950s it was still an issue whether genes control minor decorative and species-differentiating characters, but that a second, still unknown, cytoplasmic genetic system controlled cellular competence and the major features of the Bauplan. See also Gilbert (28) for a useful development of these themes in another context and (29, pp. 98–112) for Ephrussi's insistence, as of 1953, that the integrated character of the cell and the partial autonomy of cytoplasmic factors or systems in determining heredity made such distinctions on either side counterproductive. As (30, pp. 259–260) shows, however, Ephrussi took seriously models of “the cell as a whole” (p. 260) which treated cytoplasmic heredity as “specially concerned with fundamental cellular functions” (p. 259).
  27. Burian, R., and Gayon, J., 1991, Un évolutionniste Bernardien à l'Institut Pasteur? Morphologie des Ciliés et évolution physiologique dans l'oeuvre d'André Lwoff, in: *L'Institut Pasteur: Contribution à son histoire* (M. Morange, ed.), Editions de la Découverte, Paris (in press).
  28. Gilbert, S., 1988, Cellular politics: Ernest Everett Just, Richard B. Goldschmidt, and the attempt to reconcile embryology and genetics, in: *The American Development of Biology* (R. Rainger, K. Benson, and J. Maienschein, eds.), University of Pennsylvania Press, Philadelphia, pp. 311–346.
  29. Ephrussi, B., 1953, *Nucleo-cytoplasmic Relations in Micro-organisms: Their Bearing on Cell Heredity and Differentiation*, Oxford University Press, Oxford.
  30. Ephrussi, B., 1951, Remarks on cell heredity, in: *Genetics in the 20th Century* (L. C. Dunn, ed.) Macmillan, New York, pp. 241–262.
  31. Slonimski tells an ironic story regarding the choice of acriflavin: “[Ephrussi] picked up acriflavin as a mutagen for the following reason. [Werbitzki (32) had shown that when] he stained *Trypanosoma* with acriflavin . . . the dye was concentrated in a beautiful very bright orange colored spot in the cytoplasm. And he decided that this was the nucleus because the dye was concentrated in the nucleus. . . . Ephrussi deduced that if it was so strongly concentrated in the nucleus it would produce mutations. So that's why he picked acriflavin” (interview with R. M. B., Nov. 21, 1985). The irony, if this account is correct, is that, as Ephrussi explains (29, pp. 9–11), the stained body in Werbitzki's experiments turned out to be the kinetoplast, and the dye was showing the existence of DNA in a cytoplasmic organelle.
  32. Werbitzki, F. W., 1910, Über blepharoblastlose Trypanosomen, *Zentralbl. Bact.* **53**:303–315.
  33. The story to this point is reviewed in (29, Chapter 1, especially pp. 13–50). Among the original research papers and early reviews are (34); Ephrussi, B., and Hottinguer, H., 1950, Direct demonstration of the mutagenic action of euflavine on baker's yeast, *Nature* **166**:956; Ephrussi, B., and

- Hottinguer, H., 1951, On an unstable state of yeast, *Cold Spring Harbor Symp. Quant. Biol.* **16**:75–84; Ephrussi, B., Hottinguer, H., and Chimenes, A. M., 1949, Action de l'acriflavine sur les levures. I. La mutation "petite colonie," *Ann. Inst. Pasteur* **76**:351–364; Ephrussi, B., Hottinguer H., and Taylitzki, J., 1949, Action de l'acriflavine sur les levures. II. Étude génétique du mutant "petite colonie," *Ann. Inst. Pasteur* **76**:419–450; Ephrussi, B., L'Héritier, Ph., and Hottinguer, H., 1949, Action de l'acriflavine sur les levures. VI. Analyse quantitative de la transformation des populations, *Ann. Inst. Pasteur* **77**:64–83; Marcovich, H., 1951, Action de l'acriflavine sur les levures. VIII. Détermination du composant actif et étude de l'euflavine, *Ann. Inst. Pasteur* **81**:452–468; Slonimski, P., 1949, Action de l'acriflavine sur les levures. IV. Mode d'utilisation du glucose par les mutants "petite colonie," *Ann. Inst. Pasteur* **76**:510–530; Slonimski, P., 1952, Recherches sur la formation des enzymes respiratoires chez la levure, Thesis, Faculté des Sciences, Paris; Slonimski, P., and Ephrussi, B., 1949, Action de l'acriflavine sur les levures. V. Le système des cytochromes des mutants "petite colonie," *Ann. Inst. Pasteur* **77**:47–63; cf. also Ephrussi (29, 34).
34. Ephrussi, B., 1949, Action de l'acriflavine sur les levures, in: *Unités biologiques douées de continuité génétique*, Editions du Centre National du Recherches Scientifiques, Paris, pp. 165–180.
  35. Chen, S. Y., Ephrussi, B., and Hottinguer, H., 1950, Nature génétique des mutants à déficience respiratoire de la souche B-II de la levure de boulangerie, *Heredity* **4**:337–351.
  36. Ephrussi, B., Margerie-Hottinguer, H., and Roman, H., 1955, Suppressiveness: A new factor in the genetic determinism of the synthesis of respiratory enzymes in yeast, *Proc. Natl. Acad. Sci. USA* **41**:1065–1071.
  37. Cf., e.g., Wright, S., 1941, The physiology of the gene, *Physiol. Rev.* **21**:487–527; Wright (38); Spiegelman, S., 1946, Nuclear and cytoplasmic factors controlling enzymatic constitution, *Cold Spring Harbor Symp. Quant. Biol.* **11**:256–277; Sonneborn, T., 1949, Beyond the gene, *Am. Nat.* **37**:33–59.
  38. Wright, S., 1945, Genes as physiological agents, *Am. Nat.* **79**:289–303.
  39. Sonneborn, T., and Beale, G. H., 1949, Influence des gènes, plasmagénés, et du milieu dans le déterminisme des caractères antigéniques chez *Paramecium aurelia* (variété 4), in: *Unités biologiques douées de continuité génétique*, Editions du Centre National du Recherches Scientifiques, Paris, pp. 25–32.
  40. Around this time many important figures, including Beadle, Monod, and Spiegelman, for example, thought that genes might themselves prove to be enzymes and/or that some form of "instructional" alteration by substrate of protein conformation was the basis of enzyme specificity. Monod (79) surveys the reasons, as of 1947, for holding an instructional theory of enzyme formation (79, Sections II and V) and for treating enzyme formation in terms of substrate competition (79, Section IIIC). Cf. also Spiegelman, S., 1950, Modern aspects of enzymatic adaptation, in: *The Enzymes* (J. B. Sumner and K. Myrback, eds.), Academic Press, New York. It is worth noting that Wright and Delbrück proposed, independently, an alternative to the plasmagene hypothesis, namely, a hypothesis of self-perpetuating steady states in the cytoplasm. Cf. note 38 and Delbrück, M., 1949, Discussion [of Wright (39)], in: *Unités biologiques douées de continuité génétique*, Editions du Centre National du Recherches Scientifiques, Paris, pp. 33–35.
  41. A major conference, sponsored by the CNRS and devoted to such particles, was held in Paris in 1948; the proceedings were published as CNRS, 1949, *Unités biologiques douées de continuité génétique*, Editions du Centre National du Recherches Scientifiques, Paris. The conference was one of a series, sponsored by the CNRS and the Rockefeller Foundation [cf. Zallen (42)]. The context for understanding the great importance of this conference in the establishment of genetics in France is set in notes 4 and 43. For the notion of genetic continuity involved, cf. also notes 27, 34, and 44.
  42. Zallen, D. T., 1990, The Rockefeller Foundation and French Research, *Cahiers hist. CNRS* **5**: 35–58.
  43. Burian, R., 1990, La contribution française aux instruments de recherche dans le domaine de la génétique moléculaire, in *Histoire de la Génétique* (J-L. Fischer and W. Schneider, eds.), ARPEM, Paris, pp. 247–269.
  44. Lwoff, A., 1949, Les organites doués de continuité génétique chez les Protistes, in: *Unités biologiques douées de continuité génétique*, Editions du Centre National du Recherches Scientifiques, Paris, pp. 7–23.
  45. Nanney (46, pp. 15–18) dates the demise of the plasmagene hypothesis to the later 1950s, pointing

- especially to a conference organized by Ephrussi at Gif in 1957 and a conference in Gatlinburg in 1958. Cf. Lederberg, J., 1958, Genetic approaches to somatic cell variation, *J. Cell. Comp. Physiol.* **52**(Suppl. 1):383–401, Nanney (46), and Ephrussi (47).
46. Nanney, D. L., 1986 MS, *Leftovers* [an unpublished personal memoir].
  47. Ephrussi, B., 1958, The cytoplasm and somatic cell variation, *J. Cell. Comp. Physiol.* **52**(Suppl. 1):35–53.
  48. Nanney, D. L., 1958, Epigenetic control systems, *Proc. Natl. Acad. Sci. USA* **44**:712–717.
  49. Cf. Fauré-Frémiel (14) and Ephrussi (12) on *Drosophila* mitochondria. The laboratory evidence, e.g., differential centrifugation, suggested a connection with mitochondria from the beginning of the postwar studies. Even after obtaining evidence that under appropriate conditions the mitochondria of respiratory competent cells stained with indophenol blue, whereas those of respiratory incompetent cells did not, Ephrussi refused to pronounce the connection of mitochondria with the respiratory enzymes to be definitively established. As Ephrussi put it in 1952 (29, p. 35, italicized in the original): “The only conclusion we may draw today with a high probability of being right is that the normal yeast and the vegetative mutants differ by the presence in the former and the absence in the latter of cytoplasmic units endowed with genetic continuity and required for the synthesis of certain respiratory enzymes.” A slightly less cautious formulation is offered in Ephrussi (30, pp. 251–254).
  50. Ephrussi, B., 1972, *Hybridization of Somatic Cells*, Princeton University Press, Princeton, NJ.
  51. Throughout the 1950s, Ephrussi (47,52) continued to stress the need to study development and differentiation in metazoa rather than microorganisms. Note 52, denies the easy application of the findings of microbial genetics to the problems of development and differentiation in metazoa and points to the need for careful distinction between constancy of the genome in metazoan cell lineages and irreversibility of differentiation in those same lineages. Lederberg's comments on these two papers [Lederberg, J., 1956, Comments on gene–enzyme relationship, in: *Enzymes: Units of Biological Structure and Function* (O. Gaebler, ed.), Academic Press, New York, pp. 161–169, especially p. 162; (45), especially pp. 384 ff., with its discussion of the distinctions among “genetic,” “epigenetic,” and “epinucleic”] are pertinent and point to the value of studies employing tissue culture. Ephrussi's (47) is based in part on Nanney (48); cf. also Nanney (46), which describes the circumstances involved.
  52. Ephrussi, B., 1956, Enzymes in cellular differentiation, in: *Enzymes: Units of Biological Structure and Function* (O. H. Gaebler, ed.), Academic Press, New York, pp. 29–40.
  53. For this purpose Ephrussi spent much of 1959–1960 in the laboratory of Renato Dulbecco to learn modern tissue culture techniques. Of some importance to his subsequent use of somatic cell hybridization techniques is the use of virus-infected tissue cultures; cf. Ephrussi, B., and Temin, H., 1960, Infection of chick iris epithelium with the Rous sarcoma virus *in vitro*, *Virology* **11**: 547–552.
  54. Barski, G., Sorieul, S., and Cornefert, F., 1960, Production dans des cultures *in vitro* de deux souches cellulaires en association, de cellules de caractère “hybride,” *C. R. Acad. Sci.* **251**:1825–1827.
  55. Sorieul, S., and Ephrussi, B., 1961, Karyological demonstration of hybridization of mammalian cells *in vitro*, *Nature* **190**:653–654; Ephrussi, B., and Sorieul, S., 1962, Nouvelles observations sur l'hybridation “*in vitro*” de cellules de souris, *C. R. Acad. Sci.* **254**:181–182; Ephrussi, B., and Sorieul, S., 1962, Mating of somatic cells *in vitro*, in: *Approaches to the Genetic Analysis of Mammalian Cells* (D. J. Merchant and J. V. Neel, eds.), University of Michigan Press, Ann Arbor, pp. 81–97.
  56. Ephrussi, B., 1965, Introduction, in: *Genetic Variation in Somatic Cells* (J. Klein, ed.), Academic Press, New York, pp. 55–60.
  57. Littlefield, J., 1964, Selection of hybrids from mating of fibroblasts *in vitro* and their presumed recombinants, *Science* **145**:709–710.
  58. Ephrussi's collaborative work with R. Davidson and M. Weiss extended the available techniques considerably, as did that of H. Harris (Harris, H., 1970, *Cell Fusion*, Harvard University Press, Cambridge, MA) and T. Puck (e.g., Kao, F. T., and Puck, T., 1970, Genetics of somatic mammalian cells: Linkage studies with human–Chinese hamster cell hybrids, *Nature* **228**:329–332, and many others).
  59. Because mouse–human cell hybrids lose human chromosomes preferentially, they were an

- especially important tool in the formal genetics of humans. Ephrussi drew heavily on the results of Bodmer's and Ruddle's laboratories; e.g., Boone, C. M., and Ruddle, F. H., 1969, Interspecific hybridization between human and mouse somatic cells: Enzyme linkage studies, *Biochem. Genet.* **3**:119–136, and Miggiano, F., Nabholz, M., and Bodmer, W., 1969, Hybrids between human leukocytes and a mouse cell line: Production and characterization, in: *Heterospecific Genome Interaction*, The Wistar Institute Symp. Monog. No. 9 (V. Defendi, ed.), The Wistar Institute Press, Philadelphia, pp. 61–76.
60. Weiss, M., and Green, H., 1967, Human–mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes, *Proc. Natl. Acad. Sci. USA* **58**:1104–1111; Matsuya, Y., Green, H., and Basilico, C., 1968, Properties and uses of human–mouse hybrid cell lines, *Nature* **220**:1199–1202; Migeon, B. R., and Miller, C. S., 1968, Human–mouse somatic cell hybrids with single human chromosome (group E): Link with thymidine kinase activity, *Science* **162**:1005–1006.
  61. In all cases studied, the differentiated cells stemmed from a tumorigenic or malignant line of differentiated cells, such as melanocytes. One of the skeptical concerns that Ephrussi consistently raised about the interpretation of the results to be discussed here concerned the possible relevance of malignancy as a source of artifacts.
  62. Davidson, R. L., and Yamamoto, K., 1968, Regulation of melanin synthesis in mammalian cells, as studied by somatic hybridization. II. The level of regulation of 3,4-dihydroxyphenylalanine oxidase, *Proc. Natl. Acad. Sci. USA* **60**:894–901; Klebe, R., Chen, J. T., and Ruddle, F. H., 1970, Mapping of a human genetic regulator element by somatic cell genetic analysis, *Proc. Natl. Acad. Sci. USA* **66**:1220–1227.
  63. Weiss, M., and Chaplain, M., 1971, Expression of differentiated functions in hepatoma cell hybrids. III. Reexpression of tyrosine aminotransferase inducibility, *Proc. Natl. Acad. Sci. USA* **68**:3026–3030.
  64. Davidson, R. L., Ephrussi, B., and Yamamoto, K., 1968, Regulation of melanin synthesis in mammalian cells, as studied by somatic hybridization. I. Evidence for negative control, *J. Cell. Physiol.* **72**:115–127.
  65. Abercrombie, M., 1967, General review of the nature of differentiation, in: *Cell Differentiation* (A. V. S. De Reuk and J. Knight, eds.), Churchill, London, pp. 3–12.
  66. These views are importantly similar to that of Waddington, developed during the preceding 15 years or so, that cellular competence was the key feature to understand induction (in the sense of Spemann) of development. Cf. Gilbert (Chapter 9, this volume) for a useful discussion of Waddington's views on this topic.
  67. The complexities of the interactions with Harris are evident in the references to him in Ephrussi (50), especially Chapter 7 on the study of cancer.
  68. Harris, H., Miller, O. J., Klein, G., Worst, P., and Tachibana, T., 1969, Suppression of malignancy by cell fusion, *Nature* **223**:363–368; Klein, G., Bregula, U., Weiner, F., and Harris, H., 1971, The analysis of malignancy by cell fusion. I. Hybrids between tumour cells and L cell derivatives, *J. Cell. Sci.* **8**:659–672.
  69. Defendi, V., Ephrussi, B., Koprowski, H., and Yoshida, M. C., 1967, Properties of hybrids between polyoma-transformed and normal mouse cells, *Proc. Natl. Acad. Sci. USA* **57**:299–305.
  70. The application is in the Rockefeller Foundation Archives, Arch 2, Series 500D, NCSR-Genetics, Box R1050, unprocessed material. Ephrussi lists 23 distinct individuals who participated in the various projects. The organisms under study were yeast, *Pneumococcus*, basidiomycetes, *Podospora*, and *Chlamydomonas*. The work on *Pneumococcus*, carried out by his wife, Harriett Ephrussi-Taylor, concerned the role of DNA in bacterial transformation. At this point in our research, we do not know whether Boris Ephrussi was directly involved in this line of work.
  71. Slonimski and Weiss each suggested this unifying theme spontaneously in interviews.
  72. Again, Slonimski, Weiss, and many others have stressed this in interviews.
  73. Consider this quotation from (50, p. 54), remarkable for 1972: “[Recognizing that differentiation is often reversible], most people concerned agree, I think, that differences in epigenotype are not due to truly genetic changes (in the sense that gene mutations are). They therefore consider different epigenetic states as corresponding to different regulatory states resulting in the expression of different parts of the total genetic information in different cell types. However, there is no agreement as yet as to whether this is due to selective transcription of the genome; to selective destruction of messenger RNA; to selective transport of messenger into the cytoplasm; to

selective translation; or to selective gene amplification [a process which is gaining popularity although in so far as I am aware, it has been definitely demonstrated thus far only for the cistrons responsible for the synthesis of ribosomal RNA. . . .] I shall add that relatively little consideration is given to regulation at other levels: there is, however, increasing and, I think, justified interest in the regulatory role of the cell membrane." [Footnotes and references omitted.]

74. Ephrussi refers here to Hershey, A. D., 1970, Genes and hereditary characteristics, *Nature* **226**:697–700.
75. An indication of the extent to which embryological questions and explantation techniques were on Ephrussi's and Slonimski's minds at the beginning of the yeast work is that Slonimski turned to galactose metabolism in yeast in desperation after 2 years of technical failures with nuclear transplantation experiments in sea urchins—experiments of precisely the sort carried out in frogs by Briggs and King some 5 years later. Cf. Briggs, R., and King, J. T., 1952, Transplantation of living nuclei from blastula cells into enucleated frogs' eggs, *Proc. Natl. Acad. Sci. USA* **38**: 455–463.
76. Of great importance, too, are institutional factors. These concern not only the constraints imposed on the organization of work and the sources of funds, but also the disciplinary matrices in which Ephrussi worked both in France and in the United States. We shall address some of these issues in a future paper.
77. Ephrussi had ample opportunity to make such a change had he so desired. Monod and others urged him to work in the simpler prokaryotic systems that were readily available to him, both through Harriett Ephrussi-Taylor (who had worked on transformation in *E. coli* with Avery) and through Monod's laboratory. Furthermore, as Slonimski was in close contact with Monod's group at the Pasteur, there would have been no difficulty in importing any new microbial techniques into the laboratory.
78. Recall the quotation cited earlier: "I am sure also that you are aware of the fact that we know very little about regulation in somatic cells of multicellular organisms, and that, in spite of this (or because of this?) the direct extrapolation from the regulatory mechanisms in bacteria to those of higher animals is regrettably fashionable" (56, p. 55).
79. Monod, J., 1947, The phenomenon of enzymatic adaptation, *Growth Symp.* **11**:223–289.
80. Slonimski interview with R.M.B., Nov. 21, 1985, p. 22.
81. In an interview with R.M.B., May 1989, Mary Weiss draws a parallel between Ephrussi's position vis-à-vis the new biochemistry of DNA and RNA and his earlier reaction to biochemistry: "He admired it and he stood off from it. . . . [H]e became a champion of molecular biology because he was defending a cause. Pushing forward science. But he was not at ease with molecular biology. In addition, he realized that he was no longer a front runner, and that was extremely important to him, to be a front runner" (transcript, p. 24).

## Chapter 11

# Concepts of Organization

## The Leverage of Ciliate Protozoa

JAN SAPP

### 1. A Discourse on Exceptions

Biologists have long disputed the question of whether or not one can exploit Protista as technologies to better understand the organization of multicellular organisms. Some have argued that Protozoa must be understood and studied solely in their own terms, not as cells, but as organisms possessing an organization fundamentally different from that of Metazoa. Protozoa represent a world unto themselves having evolved in directions altogether divergent from the typical text-book cell: They are “noncellular” or acellular organisms. Others have argued, to the contrary, that in all its essential details a Protozoon is homologous to a Metazoan cell. Although the term “noncellular” may be used when they were studied entirely on their own, without reference to other forms of life, the term “unicellular” was perfectly applicable to Protozoa when they were compared with multicellular organisms. Still others adopted a middle ground, arguing that though Protozoa show similarities to the basic structure of cells, they have many morphological and physiological characteristics of their own which are not found generally in the cells of Metazoa. In recent years, the question of the uniqueness of Protozoa has arisen anew and moved to the center of controversy in reference to general mechanisms of development and evolution.

The social and intellectual stakes involved in these questions are evident in a book review by D. L. Nanney (1) of *Development and Evolution* (1983) in the *Journal of Protozoology*. Nanney, a ciliate protozoologist, asked readers why they might want to know about a book such as this. He prefaced his answer by lamenting that protozoology may be a dying discipline in the United States: membership of the Protozoological Society of America, established in an era of unprecedented growth in scientific research and teaching, had steadily decreased, “courses in protozoology have virtually disappeared from college catalogs,” and “positions for trained protozoologists, under the flag, are difficult to find.” Nanney encouraged those who had a partiality for protozoa to search for evidence for the value of their investigations wherever it could be found.

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To Nanney, protozoa present the same challenge to biologists as all organisms: to explain the relationship between informational structures at successive levels of organization and the emergence of higher orders of organization in both ontogeny and phylogeny. However, certain phenomena are more readily investigated in protozoa than in other organisms. One of the most important of these is the perpetuation of cortical organization. Studies of this phenomenon indicate that changes in cell structure can be perpetuated for hundreds of cell generations and through sexual reproduction free from the influence of genes or DNA. Among the 17 papers included in *Development and Evolution* one was by Joseph Frankel (2) devoted to studies of cortical inheritance in protozoa (though work done on protozoa was alluded to in several others). Frankel's inclusion in the "illustrious roster of investigators," Nanney wrote, constituted "a vote for the proposition that protozoa are not entirely a world apart" and have relevance for issues in modern biology: "Like a distinguished list of earlier protozoan workers, Frankel finds in protozoa a firm platform upon which to stand and exert leverage on the biological world view."

The counterweight to this leverage is considerable. The phenomenon of cortical inheritance conflicts with the view that development and evolution have their seat solely in DNA. As Nanney put it, it seems to "disturb the experimental and conceptual foundations of the modern consensus." This was not simply a provocative statement by an investigator defending his specialty from possible extinction, or the danger of theoretical irrelevance. To support his claim, Nanney quoted from John Maynard Smith. When explaining why he himself held a Weismannian and gene-centered view of evolution and when discussing the age-old question of the inheritance of characteristics acquired by adults, Maynard Smith (3) remarked:

There are a few well-established exceptions, of which the phenomenon of "cortical inheritance" in ciliates is perhaps the most important. Neo-Darwinists should not be allowed to forget these cases, because they constitute the only significant threat to our views. However, the overwhelming majority of inherited differences are caused by differences between chromosomal genes.

At first glance, one might think that this provocation from an esteemed neo-Darwinian evolutionist was just what was required to bring protozoa back to the center of modern biological discussions. But to Nanney (4), there was a serious problem underlying Maynard Smith's statement. The implication was that ciliated protozoa were exceptions; the phenomena reported in them had to be treated as special cases. That is, one could not use phenomena studied in ciliated protozoa for constructing general knowledge about development and evolution:

I must admit to being uncomfortable with this role proposed by Maynard Smith for the protozoa. I reject the view that ciliates in their cytotactic inertia employ unique mechanisms, and I see dangers in justifying ciliate studies on the grounds that their interesting phenomena are only uncomfortable exceptions to our general understanding. Students of the protozoa have been down this road before, in the doctrinal conflict between nuclear and cytoplasmic mechanisms in heredity and development. The nuclear-cytoplasmic impasse, along with much of the interest in ciliates, dissolved in an understanding of the nucleic basis of molecular specificity for all kinds of traits. Because of the artificiality of the dialectics, the protozoa were for a time given (perhaps undeserved) attention, but later they were subject to (perhaps undeserved) neglect.

This discourse on “exceptions” between protozoologist and neo-Darwinian evolutionist and Nanney’s remarks about the involvement of protozoan research in “artificial dialectics and doctrinal oppositions” require explanation. Throughout the twentieth century, protozoan research—as it pertained to problems of development and evolution—was carried out close to the margins of, and often in virtual conflict with, genetics orthodoxy and neo-Darwinian concepts. For most of the century, these problems were intermingled with debates about the relative importance of the nucleus and cytoplasm in development and heredity (5). In brief, the situation was as follows: While Mendelian geneticists in the United States were building up general laws concerning the behavior of chromosomal genes and warding off criticisms of their general synthesis, various instances of non-Mendelian inheritance were reported. Such cases were often grouped together under the rubric of cytoplasmic inheritance. However, the relatively few cases of non-Mendelian inheritance were often dismissed as “exceptions” by Mendelian geneticists and neo-Darwinian evolutionists who confined the scope of the phenomena to limited cases, and to specific groups of organisms. As the leader of the *Drosophila* school, T. H. Morgan, wrote in 1926, “Except for the rare case of plastid inheritance all known characters can be sufficiently accounted for by the presence of genes in chromosomes. In a word the cytoplasm may be ignored genetically” (6).

It was during the 1940s and 1950s, when microorganisms were domesticated for genetic use, that cytoplasmic inheritance came to the fore of genetic discussions in the United States. Investigations of ciliated protozoa played a leading role in bringing the cytoplasm to the attention of geneticists. Those supporting the generality of cytoplasmic inheritance accounted for most cases in terms of cytoplasmic genetic particles and bodies (chloroplasts, mitochondria, kinetosomes, centrosomes); in some cases no specific subcellular bodies were identified. On the other hand, those defending what was often referred to as the “nuclear monopoly” continued to classify cases of cytoplasmic inheritance as “exceptions” (7).

With hindsight, those advocating cytoplasmic genetic entities were correct in that geneticists now are quite comfortable with notions of mitochondria and chloroplast genomes, plasmids, and so forth. Such cases are no longer considered to be exceptions, and T. H. Morgan’s view that “the cytoplasm may be ignored genetically” no longer prevails. Only cases involving the cytoplasmic inheritance of cortical organization still carry the stigma of being “exceptions.” To understand how protozoological work came to be involved in debates over the relative importance of the nucleus and cytoplasm, over gene-centered and non-gene-centered views of development is to understand some of the central theoretical oppositions that have shaped thinking about development and heredity. To understand how certain classes of cytoplasmic inheritance lost the label of exceptions, whereas others still carry the stigma, is to understand one of the major revolutions that occurred in developmental biology. Discussions of the theoretical necessity to invoke extrachromosomal agents of development and heredity originate where they end: in protests and demonstrations against the theoretical structure proposed by August Weismann. And it is also there that we begin this brief overview.

## 2. Deconstructing the Germ Plasm

Many theories of heredity and development were proposed in the second half of the nineteenth century, but none were as influential as those of August Weismann. None created conceptual oppositions and moved scientists into the laboratory to the degree as did those of Weismann. With the exception of Weismann, none of the nineteenth-century theorists of heredity have followers today who carry their names. We can begin to understand the opposition Weismann constructed by a brief examination of the arguments in his celebrated book *The Germ Plasm: A Theory of Heredity* (1893). Weismann began by assuring his readers that his attempt at a theory of heredity was “no mere work of the imagination” (8). It provided a step on a gradual journey to a true theory of heredity. Though what exactly might be found at the end of the journey was not clear, one at least had a rough idea of what one was looking for, and the method of navigation was established: “Reason supported by observation will lead us to the goal.” Reason supported by observation also presumably decided the kind of answer one was looking for. In Weismann’s day the major choice was between epigenesis or preformation. Weismann based his argument on a theory of preformation. He did not propose, of course, that parts of the organisms were actually present in miniature in the germ plasm, rather that they were represented by vital units in the germ plasm (9). That is, he held the germ plasm to be composed of vital units which collectively carried all the primary constituents of the individual. He endowed each unit of the germ plasm with properties of growth and multiplication by fission.

Germ plasms were supposed to be “bodies of highly complex constitution, each containing all the primary constituents which are necessary to the formation of an organism” (10). Weismann had important allies. During the second half of the nineteenth century biology was dominated by theories of heredity that postulated morphological entities. Recognized as bearers of heredity, a large number of such bodies were thought to build up the individual somehow. The “gemmules” of Charles Darwin, the “pangenes” of Hugo de Vries, the “physiological units” of Herbert Spencer were highly thought of by biologists. There was agreement that all explanations of heredity must be understood in terms of such morphological entities. Weismann recognized this consensus to be important. In effect, it constituted the problematic which constrained the kinds of explanations sought. As he put it “we may now perhaps hope to succeed in recognising the probable explanations among the many possible ones” (11). It would be within the boundaries of preformationist principles that the truth would someday be formulated. For such preformationist or morphological theories of heredity, “life” was not problematic. Like de Vries before him, Weismann argued that making a theory of life was impossible at present but that it was possible to arrive at a satisfactory explanation of the phenomena of heredity if one simply took for granted the essential phenomena of life: nutrition, assimilation, and growth (12). “Life” as such was simply assumed and bestowed on the hereditary substance, or idioplasm, which was made up of “vital morphological units,” which Weismann named “biophors.”

Weismann developed his theory with two further assumptions. He reasoned that the special, organized, living hereditary substance of all multicellular

organisms, unlike the substance composing the perishable body of the individual, is transmitted from generation to generation. This was his theory of the continuity of the germ plasm. This conclusion led him to doubt the usually accepted view that variations acquired by the body could be transmitted to successive generations, "and further research, combined with experiments, tended more and more to strengthen my conviction that in point of fact no such transmission occurs" (13). The other assumption concerned the location of the special hereditary substance. Weismann located it not in the cell body (cytoplasm), but exclusively in the chromosomes of the nucleus. The reasons he offered for his belief that the nucleus alone was the bearer of hereditary qualities were echoed by leading American geneticists throughout the twentieth century. The first argument might be called the issue of the common denominator. In "higher organisms," the sperm cell is many hundred times smaller than the egg cell. Yet, Weismann (14) reasoned, "we know that the father's capacity for transmission is as great as the mother's." The nucleus provided a place for equal transmission of hereditary substance from both parents. Second, studies of fertilization seemed to indicate that the essential part of this process consisted of the union of the nuclei of the egg and sperm cells (15). Third, observations on cell division showed that the nuclear complex possessed a wonderfully exact apparatus for dividing the chromatin substance in a fixed, regular manner (16). Finally, Weismann appealed to the "economy of Nature": "This substance can hardly be stored up in two different places, seeing that a very complicated apparatus is required for its distribution: a double apparatus would certainly not have been formed by nature if a single one suffices for the purpose." "This statement that this substance is the hereditary substance can, therefore hardly be considered as an hypothesis any longer" (17). Weismann raised it to the value of a "fact."

But if the nucleus contained all the hereditary substance, by what means could it build up the cell body and the body of the complex organism? Weismann considered two possibilities: either the idiosyncrasy must be capable of exerting an emitting influence (molecular stimuli, or control of the cell by enzymatic action of the nucleus) or material particles must pass out of the nucleus into the cell body. He rejected the first alternative on the ground that it would have to take for granted the fundamental point of the matter requiring explanation. A theory based on an emanating influence, he believed would have to assume the presence in the cell body of organized structures or vital substances which would be stimulated by the nucleus. It was necessary to explain how the differentiated parts and structures of the cell body got there in the first place. Weismann found it difficult to believe that cell structures could be developed *de novo* by an emitting influence exercised by nucleus on the cell body. In his view, this amounted to *genetatio equivoca*: cell structures would have arisen by operation of an external influence on the given substance of the cell, just as would be the case in primordial generation. Certainly, one had to consider this as logically necessary at least once in organic evolution. But it was not necessary to assume that it occurred each time a new organism was developed from an egg. Weismann thus reasoned in favor of the second hypothesis, first formulated by de Vries: vital units pass into the body of the cell through the nuclear membrane and there form its parts and structures through the qualities of which they are the special

bearers (18). The migration of minute vital particles into the cell body had the advantage of "great simplicity and lucidity." Once constructed in the course of evolution, Weismann's vital units could only arise from others like themselves and could not be formed spontaneously:

This fact is confirmed by experience. Not only does a cell always arise from a cell, and a nucleus from a nucleus . . . but all the other constituents which occur in the cell body and determine its structure never arise, so far as we know, by generatio equivoca, or as de Vries expressed it "neogenetically." They are always produced by the division of similar structures already present. This is apparently true of the green chromatophores [chloroplasts] and the "vacuoles" of plant-cells, as well as the "sphere of attraction" of centrosome, which controls the division of the nucleus: the same must also hold good for those invisible vital units, the various kinds of biophors, which have arisen during the course of the earth's history by gradual adaptation to continually new conditions of life (19).

In Weismann's scheme, all cell structures originated from biophors migrating from the nucleus into the cytoplasm. He did not specify exactly when the first biophors constructed the cell, nor was he concerned with the actual process of morphogenesis or assimilation. He confessed that he knew no more about these issues than he did about the behavior of biophors in the cell body in response to those which migrated into it from the nucleus. He assumed that the invaders, or "immigrants," might struggle with those already present in the cell body and perhaps result in cannibalism: "Presumably a struggle of the parts occurs, in which the weaker are suppressed and serve as nutritive material for the stronger ones" (20). Although this was pure conjecture, Weismann insisted that one issue was certain: "The nature of the cell is really decided by elements of the nucleus."

Weismann's vital entities had to do more than eat, reproduce, immigrate, and struggle. They also had to find the right place at the right time in the course of ontogeny. To account for the orderly nature of development, Weismann did not ascribe conscious motivation to his entities. Instead, he attributed the orderly nature of development largely to an inherent order and hierarchy of the units in the idiospasm. This ordering was due to inheritance; it was an evolutionary problem. Weismann proposed that the complex structure of the germ plasm was organized into a series of hierarchical units each with a fixed position in the architecture of the germ plasm. Each was endowed with special properties: biophors, which determined which parts of cells lie close together in the germ plasm so as to form a higher unit, the determinant, a vital unit of a higher order. Determinants were held to be primary constituents of a whole cell or group of cells. They in turn were definitely localized so as to ensure that they reached the right cell and the right position in the course of ontogeny. Each determinant had a definite size and form and thereby formed a vital unit of the third degree, the *id*, which in turn had special qualities differing from those of its component determinants, which were in turn composed of biophors. The fundamental vital properties of growth, assimilation, and multiplication by division were also attributed to the *id* as to all vital units. Each idiospasm was thought to be composed of a number of *ids*.

Weismann assumed that "the changes in the *id* of the germ-plasm during ontogeny consisted merely in the gradual disintegration of the determinants into smaller and smaller groups, until finally only one kind of determinant is con-

tained in the cell viz. that which has to determine it" (21). Again, he appealed to the economy of nature to support his scheme: "Why should Nature, who always manages with economy, indulge in the luxury of providing all the cells of the body with the whole of the determinants of the germ-plasm if a single kind of them is sufficient?" (22). In an earlier essay Weismann had appealed to military organization to explain the efficiency of organismic organization:

The development of the nucleo-plasm during ontogeny may be to some extent compared to an army composed of corps, which are made up of divisions, and these of brigades, and so on. The whole army may be taken to represent the nucleoplasm of the germ-cell: the earliest cell-division . . . may be represented by the separation of the two corps, similarly formed but with different duties; and the following cell-divisions by the successive detachment of divisions, brigades, regiments, battalions, companies, etc.; and as the groups become simpler so does their sphere of action become limited (23).

In brief, Weismann held that the disintegration of the idiosyncrasy was due to a combination of three factors: (1) the inherited architecture of the germ plasm, in which each determinant has its fixed position; (2) the unequal rate of multiplication of the various determinants; and (3) the forces of attraction and repulsion, which are situated within each determinant and result from its specific nature as a special and independent vital unit.

### **3. Revolt from Morphological Theories: Protests and Demonstrations**

By the first decade of the twentieth century a new generation of "experimental embryologists" rejected the theoretical framework of such morphological theorists as Weismann. They sought to make embryology a branch of physiology. It was frequently claimed that morphological theories did not help to solve any of the fundamental problems of biology; they merely placed these problems beyond the reach of scientific investigation; they assumed the very thing of which physiological investigators sought knowledge: "life." It was claimed that no real evidence existed for these "anthropomorphic" units, and (as Weismann himself believed) if their existence were to be demonstrated one might despair of gaining any actual knowledge of life. C. M. Child summed up this attitude:

The hypothetical units in Weismann's theory are themselves organisms with all the essential characteristics of the organism that we know; they possess a definite constitution, they grow at the expense of nutritive material, they reproduce their kind. In other words, the problems of development, growth, reproduction and inheritance exist for each of them and the assumption of their existence does not bring one a step nearer to the solution of any of these problems. These theories are nothing more nor less than translations of the phenomena of life as we know them into terms of the activity of multitudes of invisible hypothetical organisms, and therefore contribute nothing in the way of real advance (24).

Experimental embryologists repeatedly argued that the problem of the organism as a whole was not an evolutionary problem ascribable to an inherited "architecture" of the germ plasm; it was a physiological problem. These protests were combined with many demonstrations contesting the validity of Weismann's scheme. Some of the most important experimental demonstrations were made

against two central tenets of Weismann's preformationist theory of development: (1) his assumption that the cells controlled themselves, i.e., that the fate of the cells is determined by forces situated within them, not by external forces, and (2) his proposed theory of the disintegration of the germ plasm during the course of development. The first, most widely known experimental demonstration of this kind was carried out by Hans Driesch (25). Driesch managed to separate the first two segmentation cells of a sea urchin egg and observed that each of them developed into a completely normal, though half-sized, larva. To Driesch, these results clearly disproved the existence of special regions in the germ that give rise to special organs. It was not necessary to assume that the cells of a developing organism occupied a predetermined position, as proposed by Weismann. Epigenetic theories were developed in direct conflict with Weismannian preformationism. In epigenetic theory, the organism was conceived as the product of the reactions between a particular kind of protoplasm and environmental factors. Individual cells represented the product of such reactions and their grouping with others to form complex organisms involved further reactions of the same sort.

Weismann himself had protested against the strength of these experimental demonstrations. He pointed out that Driesch's results conflicted with those of Wilhelm Roux, who, in an earlier experiment on frog's eggs, reported that if one of the cells at the two-cell stage of the embryo is killed by pricking it with a hot needle, then the other goes on to produce half an embryo. He claimed that Roux's experiments confirmed his theory and were conclusive, but that those of Driesch were not. He further suggested that his own careful reasoning was more reliable than the results of experiments:

Other than experimental methods may lead us to fundamental views, and an experiment may not always be the safest guide, although it may at first appear conclusive. Even Driesch himself doubts whether the above-mentioned experiments made by Roux are really conclusive, though in my opinion he is wrong in doing so: he asks, in fact, whether the uninjured segmentation-sphere of the frog would not behave exactly in the same manner as that of the sea-urchin if it could be actually isolated instead of remaining in close connection with the other injured sphere. Thus even the apparently incontrovertible result of this experiment may be doubted.

It seems to me that careful conclusions, drawn from the general facts of heredity, are far more reliable in this case than are the results of experiments, which, though extremely valuable and worthy of careful consideration are never perfectly definite and unquestionable. If what was said in support of the theory of determinants in the first chapter of this book be borne in mind, the conviction that ontogeny can only be explained by evolution [preformation], and not by epigenesis, seems to force itself upon us (26).

No doubt what Weismann has to say about the power of experiments does have to be borne in mind. However, his protests of Driesch's results were soon swamped by a wave of further experimental demonstrations. In subsequent years, the behavior of egg cells described by Driesch for sea urchins was observed in many different species. In the well-known aphorism of Driesch, the whole problem of development seemed to be brought into focus:

The relative position of a blastomere in the whole determines in general what develops from it; if its position be changed, it gives rise to something different. In other words, its prospective value is a function of its position (27).

The principle of the “organism as a whole” became the central tenet of embryology; the capital problem was to find a basis for it. Both points—the regulative qualities of eggs and the functional equivalence of the blastomere nuclei—led many embryologists to localize the primary seat of differentiation in the cell cytoplasm.

#### **4. Weismannism under a New Banner: Geneticists versus Embryologists**

With the rise of Mendelian genetics the problem of accounting for the organism as a whole continued. New kinds of experimental demonstrations, based on cross-breeding analysis combined with cytological observations of the behavior of chromosomes, heightened interest in the cell nucleus. By 1915 the Morgan school had placed hereditary determinants (genes) on chromosomes and the Mendelian-chromosome theory was constructed. One might argue that there need not have been any conflict between gene theory and the physiological conception of the organism as a whole. They were simply ideas concerning different things: geneticists were concerned with the hereditary constitution of the germ cells and embryologists were concerned with the behavior of this protoplasm in certain environmental relations. However, there was no such simple convenient division of labor. On the contrary, there were serious conflicts over the theoretical and technical scope of the two disciplines (28). American geneticists repeatedly stated that one day they would be able to account for development in terms of the governmental control of chromosomal genes. In 1919 Morgan himself claimed that one could account for “the organism as a whole” in terms of “the collective interaction of genes” (29). European geneticists such as Wilhem Johannsen, who coined the central genetic terms “genotype,” “phenotype,” and “gene,” were careful to deny any relationship between gene theory and the “speculative” “morphological” and “historical” theory of Weismann (30). Nonetheless, the Mendelian-chromosome theory as developed by the Morgan school shared several characteristics with Weismannian theory. First, it was a particulate theory: second, it maintained the paradox of nuclear equivalence during cellular differentiation.

It was a particulate theory in that it rested on the notion that the germ plasm in the nucleus of the cell contained a host of determinants which were more less independent of each other and could perpetuate themselves unchanged. It was obvious to embryologists that regions, parts, and organs are localized and become different in the course of development according to an orderly pattern: that is, a progressive organization occurs. Embryologists continually repeated that interpretation of the order and control of hereditary potentialities in the individual organism was a stumbling block for particulate theories of heredity. It was often stated that those supporting particulate theories had to either deny or ignore the problem, or had to postulate a “supergene” or vitalistic “directive force” to control and order all the individual genes. With regard to resolving this problem there was no consensus among embryologists. Some, such as Driesch, came to assume that there was an Aristotelian “entelechy” acting as a directing guide in each organism (31). To most embryologists, organization was the prob-

lem of the origin and nature of a pattern underlying and determining where, when, and how the differences appear and in what they consist. Embryologists maintained that the pattern of morphogenesis was an epigenetic process involving interactions between cells and that morphogenesis was ultimately conditioned or constrained by a specific structural organization or polarity in the egg cytoplasm. Some, such as C. M. Child, who developed a gradient theory, sought a physiological basis for this structural organization. This view did not necessarily conflict with the chromosome theory of heredity except when geneticists attempted to interpret the individual as “the collective action of genes” alone.

Still others, such as Jacques Loeb, E. B. Conklin, and J. W. Jenkinson, sought an inherent, preformed, hereditary basis for this organization. They postulated a primordial physicochemical structure in the cytoplasm of the egg which provided a guide for genes and sufficed to determine the first steps in the differentiation of the organism. As Loeb stated, “. . . the unity of the organism is due to the fact that the egg (or rather its cytoplasm) is the future embryo upon which the Mendelian factors in the chromosomes can impress only individual characteristics, probably by giving rise to special hormones and enzymes” (32). This view directly conflicted with the claim that genes were the principal basis of developmental and evolutionary change. The implication of this theory was clear to embryologists: genetic inheritance only “topped off” the more fundamental organic features. Or, as Conklin put it: “We are vertebrates because our mothers were vertebrates” (33).

The second problem, the paradox of nuclear equivalence during cellular differentiation, was rooted in observations that chromosomes showed no visible changes from one somatic cell generation to the next. Yet, there were observable differences in the cytoplasm. By the mid-1920s the accepted view that no sorting out of chromosomal factors occurs during development. This was supported by cytological studies of chromosome behavior in cell division, as well as experimental embryology (demonstrations like those of Driesch above). As Morgan stated, “Each cell inherits the whole germ plasm” (34). As long as this remained part of gene theory, it seemed to many embryologists that the differentiation of cells during ontogeny was an environmental relationship mediated through the cytoplasm, not the nucleus. The view that cellular differentiation had its seat in the cytoplasm was pervasive among embryologists throughout the first half of the century. Ross Harrison expressed a common view among embryologists when he wrote in 1937:

The prestige of success enjoyed by the gene theory might easily become a hindrance to the understanding of development by directing our attention solely to the genome, whereas cell movements, differentiation and in fact all developmental processes are actually effected by the cytoplasm (35).

## 5. Plasmagene Theory

Discussions of cytoplasmic inheritance occupied a prominent place in genetic discourse in the 1940s and 1950s when microorganisms were domesticated for genetic use. The idea that cellular differentiation was controlled by cytoplasmic elements, as suggested by embryologists, was rehabilitated by a new

generation of geneticists. The importance of ciliated protozoa for studying these problems was promoted through the work of T. M. Sonneborn and his associates at Indiana University. After learning to control mating in *Paramecium* and thereby domesticating the organism for genetic use, Sonneborn investigated various strange and novel phenomena which challenged genetic orthodoxy. For the study of the role of the cytoplasm in heredity and development, *Paramecium* was an exceptional tool. At conjugation nuclei are exchanged between cells but the two cytoplasms do not normally mix. As a result, if paramecia differing in both cytoplasms and nuclei conjugate, their progeny will possess identical nuclei but diverse cytoplasm. It was possible, then, to obtain at will various combinations of different nuclei and cytoplasms and to study the role of both.

After World War II, several leading European biologists, including Boris Ephrussi, André Lwoff and Philippe L'Héritier, Jean Brachet, and C. D. Darlington, joined forces with Sonneborn to support the existence of cytoplasmic genetic entities (36). Genetic demonstrations of cytoplasmic inheritance began to be linked with long-neglected cytological studies of cytoplasmic bodies. Since the late nineteenth century, simple microscopic observations had suggested to cytologists that all sorts of cells contain in their cytoplasm a variety of particles endowed with physical continuity. These included centrioles, which in most animal and some plant cells could be seen at the poles of the mitotic spindles; blepharoplasts and kinetosomes, which could be found at the base of cilia and flagella of many microorganisms and of ciliated and flagellated cells of Metazoa; the kinetoplasts of trypanosomes; mitochondria and chondriosomes, ubiquitous elements apparently present in any living cell, whether of animal or plant origin; and several types of plastids (chloroplasts, leukoplasts), which assumed various functions in plant cells and in some flagellates. All these particles were often considered by cytologists to arise from preexisting elements of the same sort and many of them were held to be especially important for tissue differentiation. However, American geneticists had generally ignored such cytoplasmic bodies as there were few experimental demonstrations comparable to those made for chromosomes to suggest if and how they played a role in development and heredity.

During the 1940s and 1950s, all these cytoplasmic entities were grouped under the rubric "plasmagenes." Plasmagenes were generally thought to be self-reproducing genetic entities, varying in size from microscopically visible particles to submicroscopic particles of the same order of size as nuclear genes. Some of the larger plasmagenes, such as chloroplasts, were thought to contain plasmagenes within them. The chief theoretical importance of plasmagenes was their usefulness for accounting for cellular differentiation. Unlike the nuclear genes, they sorted out during cell division and offered a plausible mechanism to account for cellular differentiation in the face of nuclear equivalence. Inasmuch as plasmagene theory was based on self-reproducing morphological entities sorting out during cell division, it was similar to the theory proposed by Weismann for nuclear determinants. However, there were some striking differences. First, unlike Wiesmann's biophors, plasmagenes were not usually assumed to be immigrants from the nucleus. Instead, they were normally understood as self-duplicating, mutable particles that depended on nuclear genes for their maintenance or normal functioning, but not for their origin or for their

specificity. In other words, plasmagene theory was based on parallel evolution of both nuclear and cytoplasmic genetic elements. Second, the differential sorting out of plasmagenes during development was not attributed to the inherent nature of the elements themselves, as Weismann had proposed. Both the reproductive rates of plasmagenes and in some cases their nature could be directly influenced by environmental factors.

The work of Sonneborn and his colleagues on a “killer trait” in *Paramecium* provided the exemplar of the interaction of plasmagenes and the environment. Some strains of *Paramecium* produce a poison that killed *Paramecium* of certain other strains. The killer trait, first reported by Sonneborn in 1943, was reasoned to be due to a cytoplasmic genetic substance he called kappa. The relationship of kappa to the effects of environmental conditions was striking. The growth rate of *Paramecium* could be controlled by such environmental factors as nutrition and heat. If cells were kept in a medium where fission was rapid, they tended to multiply more rapidly than the cytoplasmic factor kappa, and finally, the large majority of cells ceased to be killers. In other words, the concentration of these factors with cells of a clone, and thus the character of the cell (killer, sensitive, resistant), could be controlled by environmental conditions. The behavior of kappa in relation to the organism as a whole was similar to the case of chloroplast behavior in certain flagellates. For example, when *Euglena mesnili* is cultivated in the dark, the rate of division of its chloroplasts is slowed down and this process results in the decrease of the average number of chloroplasts per organism. Eventually organisms arise that are devoid of chloroplasts.

The chloroplasts of *Euglena mesnili* and the kappa of *Paramecium* were models for plasmagenic behavior during development in higher organisms. However, those supporting cytoplasmic inheritance did not advocate just one kind of mechanism for understanding cellular differentiation: they were more eclectic. To them, evolution was much more opportunistic than Weismann assumed. Plasmagenes showed many different behaviors. Some cases of cytoplasmic inheritance involved interactions with the living environment, some involved complex interactions with the nucleus. Still others seemed to involve no genetic particles but, instead, were held to be due to self-perpetuating metabolic states. In a small book entitled *Nucleo-cytoplasmic Relations in Micro-organisms*, Boris Ephrussi, the leading proponent of cytoplasmic inheritance in France, summarized the possible mechanisms cytoplasmic inheritance offered for understanding cellular differentiation:

The non-living environment can induce changes of the concentration of Kappa particles and of antigenic type in *Paramecia*, and loss of cytoplasmic particles in yeast. . . . Lastly, we find that nucleus and cytoplasm affect each other's activity. The cytoplasmic particles of yeast are activated by a nuclear gene. In turn, in *Paramecia*, definite cytoplasmic states permit the expression of definite nuclear genes.

Here is a set of facts that ought to help explain development (37).

With regard to development, there is a third major difference, besides location in the cell and response to the environment, that distinguished plasmagenes from the biophors and determinants of Weismann. Plasmagenes ate, reproduced, mutated; they responded differentially to different environments; they interacted with each other in various modes of competition and cooperation. But they did not control order within the cell or organism. Plasmagene theorists did not believe that plasmagenes and nuclear genes alone could account for the orderly

nature of development. Plasmagenes were instrumental, not directive, in cellular differentiation.

Although plasmagenes and nuclear genes determined the nature of organs and parts, they did not control the orderly arrangement of parts within cells or complex organisms. Plasmagene theorists repeatedly stated that the cell was not “a bag of enzymes.” It was not made up simply of an anarchic assortment of particles struggling with each other. It was organized. To account for the orderliness within a cell or multicellular organism, plasmagene theorists often imposed an additional organization which itself was not determined by genetic particles. In 1951, Sonneborn phrased the problem in the following terms:

Perhaps it will be objected that there are some self-duplicating cytoplasmic elements which the nucleus cannot make. Then suppose these too can be cultivated *in vitro*. Is anyone willing to believe that, if all such self-duplicating components of the cell were thrown together in a test tube in the proper proportions with adequate food for their multiplication, a *Chilomonas* cell or any cell at all would result? Although the whole picture is admittedly imaginary, it makes the nature of the problem sharp and clear. If cells cannot be reconstituted in the way suggested, then it seems to me we are forced to admit that the molecular and particulate arrangement of the cellular materials, their organization into a working system, is itself a part of the genetic systems of the cell (38).

Ephrussi, an ex-embryologist, phrased the problem in embryological terms: “The fundamental anisotropy of the egg cytoplasm itself has a genetic basis.” Hence, he argued that “the fundamental problem of genetics in relation to development becomes that of the origin of the specific molecular pattern of the cytoplasm which confers to the egg its vectorial properties” (39). Although to Sonneborn and Ephrussi the organization of the cell was a genetic problem for the future, some protozoological observations and theorizing pertaining to this problem had already been assembled by André Lwoff.

In a small book, *Problems of Morphogenesis in Ciliates: The Kinetosomes in Development, Reproduction and Evolution*, Lwoff offered a far-reaching theory of the role of kinetosomes in development. Kinetosomes are visible granules, located at the base of each cilium of ciliated protozoa and ciliated cells of higher organisms. In brief, Lwoff wanted to show how observations of the life history of kinetosomes in ciliated protozoa visibly confirmed two theoretical conclusions of embryologists: development is in part controlled by cytoplasmic particles and the fundamental control of differentiation operates under the influence of a morphogenetic “field” (40). In Lwoff’s view, “the morphogenesis of a ciliate is essentially the multiplication, distribution, and organization of populations of kinetosomes and of the organelles which are the result of their activity.” His arguments regarding kinetosomes were based on investigations of the natural history and life cycle of ciliates which he and Edouard Chatton had carried out in the 1920s and 1930s. Lwoff acknowledged that the cytoplasm represented a very differentiated system with cortex, mitochondria, kinetosomes, and chloroplasts, all of which he claimed were endowed with genetic continuity. As Lwoff stated:

Cytoplasm is not just a collection of enzymes or a plastic and complaisant receptor passively submitted to the dictatorship of genes, but certainly contained self-reproducing bodies endowed with specificity (41).

Lwoff strove to develop a quasiorganismic theory of the nature and behavior of kinetosomes in line with plasmagene theory. Observations of hundreds of ciliates, Lwoff argued, indicated that kinetosomes are always formed from preexist-

ing kinetosomes. They were held to be able to multiply independently of the nucleus and to give rise to chains or rows of kinetosomes (kineties) along the cell surface or cortex. Even in organisms that are devoid of cilia during a long period of their life cycle, the kinetosomes were often seen to be organized forming what Chatton and associates (42) called "infraciliature." Kinetosomes moved and varied constantly, and Lwoff pointed out that one could follow their "life history" during different phases of the life history of the ciliate. Like other living cytoplasmic entities, the killer particles of *Paramecium*, and the viruses of plants, Lwoff argued, kinetosomes were sensitive to the environment of the "host" in which they lived. The metabolism of the host and other environmental conditions affected the relative rate at which these particles multiplied and in certain cases could lead to their disappearance. The metabolism of the cell, light, and temperature could cause variations in the relative multiplication rates of kinetosomes in a way similar to the behavior of chloroplasts in *Euglena*.

Kinetosomes not only possessed properties of growth and division and were responsible for the production of cilia, they were also endowed with another special property, which embryologists following Driesch called "prospective potencies." This regulative ability made them fundamental to the morphogenesis of protozoa: they were organized into different structures and systems according to their position in the cell. Kinetosomes were held to produce various products of their "metabolism": cilia, trichocysts (cylindrical rods that elongate toward the inside of the cell), and all sorts of fibers and organelles (43). The expression of kinetosome potentialities, their organization into specific patterns, and their division depend, Lwoff argued, on their position within the cell, that is, on their latitude and longitude. Viewing the kinetosomes as a model for visible plasmagenes, Lwoff looked at plasmagenes in ciliates as Driesch and Spemann had looked at cells in metazoans: "One plasmagene may possess many potencies and turn out different organelles according to its position and to the phase of the life cycle" (44).

Kinetosomes multiplied and then became organized and oriented along the cell cortex in orderly ways. But what was responsible for the orderly organization? The cortex is composed of linear arrays of a large number of fundamentally similar "ciliary units" arranged in a repeating pattern. A ciliary unit is a sophisticated structure that includes a kinetosome, cilium, a variety of subcortical fibers, and specialized membranes. At each level of organization observable with an optical microscope, the cortical pattern was remarkably constant and reproduced faithfully through a regular sequence of events during growth and fissions. Several protozoologists, most prominently Fauré-Frémiel and Vance Tartar, had argued that differentiation and morphogenesis in ciliates was essentially a cortical phenomenon. Tartar (45), for example, argued that "ciliates present us with a living fiber system having morphogenetic capacities." Fauré-Frémiel (46) compared the cortical system made up of rows of kinetosomes (kineties) to a crystalline network or to the complex mesh of a supramolecular structure of the crystalline type and suggested that the cortex or ciliary system commands morphogenesis. Lwoff, on the other hand, did not ascribe this power of organization to the inherent properties of kinetosomes themselves. Kinetosomes, he argued, did not control their own destinies. They did not command; they obeyed. Like the cells of multicellular organisms, the fate of kinetosomes in ciliates was

controlled by "some mysterious and powerful field of forces" (47). In Lwoff's view, Sinnott offered a good definition of the morphogenetic field that was adequate because it was very general: "A field is the sum of the reactions which an entire protoplasmic system makes with its external and internal environment, reactions which are determined by the specific physiological activities of the living material of which the organism is composed" (48).

To understand the nature of this field and the behavior of kinetosomes that submitted to it, Lwoff applied Paul Weiss's concept of "molecular ecology," according to which the cell is an organized mixed population of molecules and molecular groups (49). To Weiss, the spatial localization of parts of a cell resulted from the "response of organized elements to fields of organized (i.e., non-random) physical and chemical conditions." Weiss summarized his concept of "molecular ecology" in 10 propositions. All centered on the notion that the spatial organization of the contents of the cell and its constituent particulate elements required "a primordial system of spatially organized 'conditions' to set the frame for the later differential settlement of different members of the dispersed molecular species." In Lwoff's view, the cortex of the ciliate cell provided the spatially organized conditions to set the frame for later differentiation in the ciliate cell. It had the properties of a morphogenetic field (50).

Thus, Lwoff told the story of the behavior of kinetosomes and their organization in the cell in terms of Weiss's ideas of molecular ecology. The orderly nature of kinetosome organization in the cell and their differential behavior at different phases of the ciliate life cycle resulted in the first instance from their search for food (51).

It is clear that kinetosomes need some food not only to live but also to grow and to multiply, and probably some specific food. . . . at certain phases of the [ciliate] life cycle, kinetosomes of one region multiply whereas others do not. The specific food must therefore be concentrated in certain areas.

In their search for food the kinetosomes segregated into their appropriate ecological environments. The localization of the specific food ("existential and operational prerequisites") was in turn "controlled by their affinities [electronic or intermolecular forces] for some differentiated parts of the cortex or, in the last analysis, by the properties of the given molecule and the cortex" (52).

Although Lwoff recognized that his model for kinetosome behavior and organization was only a plausible suggestion, one issue was certain: "Differentiation in a ciliate is essentially a cortical phenomenon" (53). The question was to what extent data concerning ciliates could be extended to other organisms. This was as much an issue for Lwoff in 1950 as it is for Nanney and Maynard Smith today. Ciliates are noncellular organisms in the sense that they do not develop by compartmentalizing into differentiated cells. However, in Lwoff's opinion, this did not mean that the behavior of kinetosomes could not be used to understand the problem of morphogenesis in multicellular organisms. The different regions of ciliates were comparable to differentiated cells of complex organisms. Kinetosomes illustrate the notion of a morphogenetic field in a most spectacular way (54). Moreover, Lwoff pointed out that the importance of cell surface had been stressed by many embryologists: E. E. Just (1939) went so far as to consider that "in the entire animal kingdom, with the exception of mammals, the embryo

arises from the egg surface" (55). Therefore, it was entirely reasonable to compare morphogenetic factors in ciliates with Metazoa:

I should like to say that an orderly or organized assymetry, like that of an egg or of a ciliate, may only be the reflection of cortical properties. A constantly flowing endoplasm cannot be asymmetrical. The building blocks of different organelles may be asymmetrical; the organelles may be asymmetrical. But if we consider the ciliate as an organism, we reach the conclusion that organized asymmetry or simply organization can belong only to a more or less rigid, or more or less permanent, system, that is to say, to the cortex (56).

## 6. “Switching” Concepts and Metaphors

During the 1940s and 1950s evidence for cytoplasmic inheritance had found its meaning in attempts to resolve what was commonly referred to as the “developmental paradox”: how can cells with identical nuclear genes come to be differentiated? The postulation of plasmagenes, cytoplasmic self-reproducing bodies sorting out under the direction of the environment during development, represented one of the most widely discussed solutions to this dilemma. The nuclear genetic system was assumed to be responsible for the transmission of traits between sexual generations, while cell heredity or somatic cell differences were regulated by cytoplasmic genetic entities. Thus the cell was divided into two heredity systems, each with a different function.

The notion of a cytoplasmic basis of cellular differentiation was by no means universally upheld by geneticists during this period. Those who supported it offered two arguments in favor of their view. First, there was no reason to believe that nuclear differentiation ever occurred, whereas they did have demonstrations of cytoplasmic differentiation and they did offer plausible models. Second, they argued that it was clear that development was an environmentally directed phenomenon. Yet, geneticists knew no means to induce specific gene mutations. Indeed, genes were held to be largely immune from environmental influences and geneticists had abandoned ideas that environmentally directed gene mutations could occur. The most common assumption was that the persistent changes involved in differentiation had their seat in the cytoplasm. Ephrussi (57) phrased the dilemma for geneticists clearly:

Unless development involves a rather unlikely process of orderly and directed gene mutation, the differential must have its seat in the cytoplasm.

Defenders of the “nuclear monopoly” had failed to match these protests with actual demonstrations of their own to show if and how nuclear differentiation could occur. Indeed, instead of demonstrating alternative models based on nuclear differentiation, defenders of the “nuclear monopoly” had simply criticized purported demonstrations of cytoplasmic inheritance. They claimed that the evidence for many reported cases of cytoplasmic inheritance was not definitive, that explanations of the experimental results in terms of nuclear control were often possible, that some cytoplasmic particles such as kappa were symbionts and that others were rare exceptions, merely curiosities of little relevance for general mechanisms of development and heredity. However, during the late 1950s and early 1960s, this changed dramatically. Demonstrations of nuclear

differentiation began to appear. The argument for invoking a cytoplasmic basis for cellular differentiation began to be undermined, and dissent occurred among the ranks of cytoplasmic researchers themselves (58).

"It might appear," wrote Nanney in 1957, "that the dichotomy between germinal and somatic inheritance, between cytoplasmic and nuclear bases was after all a mistake, and that investigations may now converge with a unified perspective" (59). The showdown for cytoplasmic geneticists occurred in 1958 when Nanney, supported by cases of nuclear differentiation in multicellular organisms and his own studies of antigen specificity and mating-type determination in ciliated protozoa, protested against the "geographical basis" of classification of genetic mechanisms. Nanney argued convincingly that the perpetuation of metabolic stable states (systemic properties of cells), ultimately traceable to nuclear genes, could in principle account for many cases of "cytoplasmic inheritance." In other words, stable states could be transmitted through the cytoplasm but need not be determined by the cytoplasm. One could no longer make a distinction between mechanisms based on nuclear and cytoplasmic location. Endorsing Nanney's argument, Ephrussi wrote (60), "This has been a major source of confusion in the past, and it is not going to be easy to avoid in the future because we have all been trained to regard the problem of differentiation as a nucleus/cytoplasmic dilemma."

Studies of nuclear regulation and feedback mechanisms culminated with the lac operon system in bacteria put forward by Jacques Monod, François Jacob, and their colleagues. Their demonstrations that genes could be switched "on" and "off" in bacteria represented the pinnacle of what was often termed the "revolution" in developmental biology. As Sonneborn put it in 1964,

The current reorientation is really quite simple, involving only a change in the basic assumption of the role played by the genes. Formerly, it was assumed that the whole set of genes was active in every cell. Hence cells that have the same set of genes cannot become diverse by reason of direct genic action (61).

Cellular differences, even among cells with identical sets of genes, could be due to the activity of different genes in those identical sets of genes. One no longer had to think of all the genes being active all the time. This reorientation guided genetic research on cellular differentiation after 1960.

Technological metaphors quickly replaced social metaphors of the cellular regulation and genetic control. Models of cellular differentiation in terms of elementary organisms responding differentially to environmental influence and struggling with each other in various modes of conflict and cooperation were replaced by communication networks, circuitry, feedback loops, and information. "The cell," wrote Jacob and Monod, "must be visualized as a society of macromolecules, bound together by a complex system of communications regulating both their synthesis and their activity" (62). In the model they described in bacteria, enzyme synthesis is regulated at the molecular level by circuits of transmitters (regulatory genes) and receivers (operators) of cytoplasmic signals (repressors) which control the rate of messages sent from the nucleus. Jacob and Monod warned that one should remain cautious and "refrain from assuming that operons occur in higher forms until the necessary genetic and biochemical tests have been preformed" (63). Nonetheless, such rapid extrapolation of such mech-

anisms to other organisms occurred that by 1964, Sonneborn wrote that these studies in bacteria provided “the now dominant hypothesis—one might almost say the principle or article of faith—that cellular differentiation is ultimately traceable to and due to variable gene activity” (64).

The reasons for the rapid and broad acceptance of this theory are complex and require detailed historical investigation. They would likely include the following. The metaphor of the cell society wired by communication systems certainly fits the larger social milieu of the 1960s. Second, part of the strength of the metaphor no doubt lies in the strength of the technologies to which it referred. Jacob and Monod repeatedly compared their regulatory elements to “the basic elements of electronic engineering, which could be organized into a variety of circuits fulfilling a variety of purposes” (65). Later, in his *Logic of Life*, Jacob suggested how this kind of model of cell organization emerged from technological developments:

With the development of electronics and the appearance of cybernetics, organization as such became an object of study by physics and technology. The requirements of war and industry led to the construction of automatic machines in which the complexity increased through successive integrations. In television sets, an anti-aircraft rocket or a computer, units are integrated which already result themselves from integration at a lower level. Each of these objects is a system of systems. In each of them, the interaction of the constituent parts underlies organization of the whole (66).

But it would be wrong to reduce the strength of this model solely to the triumph of electronic engineering and the extent to which it reflected the age of communication to which it belongs. A third reason for the strength of the metaphor lay in its ability to resolve the long-standing paradox of how certain cell lines with identical genes become differentiated. The operon scheme solved the problem of cellular differentiation without invoking environmentally directed gene mutations and without invoking a distinction between somatic heredity or development (cytoplasm) and sexual heredity (nucleus). It provided a model by which one could imagine changes in the nuclear genes that did not affect the actual structure of the genes. Environmentally directed development need not be incompatible with the notion of evolution in terms of gene mutations and natural selection. Inasmuch as it resolved “the nucleus/cytoplasm dilemma,” we can understand one of the main sources of strength for the operon model. Jacob and Monod were well aware of the theoretical stakes of their work: In one of their first synthetic accounts of their work in 1961, they wrote:

One point already seems to be quite clear: namely that biochemical differentiation (reversible or not) of cells carrying an identical genome, does not constitute a ‘paradox,’ as it appeared to do for many years to both embryologists and geneticists (67).

The most important aspect of the operon model for embryologists is the allowance it made for substances existing in the cytoplasm which are able to switch on or off, or to regulate the action of genes. It was hailed as providing the first pillar of the bridge long searched for, between cytoplasmic and nuclear action. If, for example, when the egg divides, some nuclei go into a region of cytoplasm containing substance A, certain genes will be put in action by that substance, while others will be repressed. Another cytoplasmic environment B may have another effect. The operon model thus provided a unified perspective

because it brought genetics and embryological theory together. The recognition that the nucleus was not functionally equivalent in all cells of the body led to the demise of the plasmagene theory. This did not mean, of course, that self-reproducing cytoplasmic particles played no role in development. It meant only that they did not have meaning for cellular differentiation because of their location in the cytoplasm. In subsequent years, when DNA was detected in the cytoplasm, theories of the cell in terms of quasiorganismic theories quickly remerged. However, the problematic was strikingly different. The location of these genes in the cytoplasm has their meaning today primarily in theories about symbiontism and the origin of the eukaryotic cell (68). However, with respect to the problem of differentiation and development, the central oppositions switched from a controversy over a nuclear monopoly to one over a molecular monopoly: DNA and an opposition to the model of cellular differentiation and organization offered by Monod and Jacob.

## 7. Supramolecular Structure and Morphogenetic Fields

Those geneticists, such as Sonneborn and Ephrussi, who had postulated a cytoplasmic basis for cellular differentiation during the 1940s and 1950s clearly recognized that variable gene activity and its regulation represented a solution to the “developmental paradox” without invoking plasmagenes. However, they denied that the organism was simply the ultimate epigenetic expression of information encoded in nucleotide sequences, as implied by the model put forward by Jacob and Monod. They persistently protested against the view that nuclear regulation and self-perpetuating cytoplasmic states based on models of “circuitry” ultimately traceable to the action of genes represented the exclusive or even the primary basis of cellular differentiation. Inasmuch as it could account for the kinds and relative amounts of proteins in differentiated cells, this model only replaced the plasmagene theory. Cytoplasmic geneticists had never believed that plasmagenes were the primary agents of cellular differentiation. They were instrumental, not directive, in cellular differentiation. Like embryologists, cytoplasmic geneticists had suggested that morphogenesis was conditioned or constrained by a specific structural organization as in the egg cytoplasm. Indeed, neither plasmagenes, nuclear regulatory genes, nor self-perpetuating metabolic states touched on the major and fundamental problem of morphogenesis: how different cells or parts of a cell come to be arranged in time and space. Ephrussi responded to Jacob and Monod’s claim “that biochemical differentiation . . . of cells carrying an identical genome, does not constitute a ‘paradox,’ as it appeared to do for many years to both embryologists and geneticists” as follows:

This statement tells us nothing about the nature of the primary causes responsible for the orderly, divergent biochemical differentiation of different cell lineages derived from a single egg (whether its mechanism be based on self-maintaining regulatory states or, for that matter, on any mechanism of differential gene activation or amplification). The real problem is that of the origin (seat) of the asymmetrical causes which bring about these asymmetrical effects (69).

In the 1950s Sonneborn, Lwoff, and other protozoological investigators had suggested that the asymmetrical organization or structure of the ciliated organ-

isms could not be causally determined by genes. This supramolecular structure, which, Lwoff had argued, manifested the properties of a morphogenetic field, was responsible for controlling when and where gene products became located and what they formed. Molecular biologists had demonstrated how genes control the specificity of proteins, and also that DNA occurred in such cytoplasmic organelles as chloroplasts and mitochondria. But they had never demonstrated how genes control the development of structures at the supramolecular and microscopically visible levels. During the 1960s and 1970s, the problems of how this structural organization and how the differences in organization between different cells of the same organism were determined became the central focus of protozoological investigations. Sonneborn phrased the problem of translating chemistry into organismic and cellular organization as follows:

"How are the chemical substances which the genes make, and the products of their interaction, translated into organised structures?" For the cytoplasm is more than a bag of chemicals. It is highly structured, even on the purely chemical level. The enzymes resulting from the action of the various genes often form systems that operate within millisecond speeds in ordered sequences: and this calls for their precise organization in ordered spatial sequences. On the grosser levels of visibility in the light microscope, the distinctive structures of diverse cell types are of course obvious and well known, and in recent years the fantastically powerful electron microscope has yielded much more insight into the structural organization of the cytoplasm. How is this organization, especially the differences in organization between different cells of the same organism, determined? (70).

Systematic protozoological research on the origin of this cellular organization was carried out in direct opposition to the doctrine of self-assembly, which was gaining ground among molecular biologists. The chief molecular biological assumption concerning control over cell structure was based on a belief in a transformation of disorder into order due to three factors: the physicochemical properties of gene-produced proteins, their random collision, and the ionic and molecular constitution of the cell "soup" (71). This view was strengthened by the demonstration, in viruses, that a linear genetic code could be translated into three-dimensional structure. The nucleic acid control of virus organization indicated that genes determined an amazing degree of precise nonrandom structural patterning. But ciliate protozoologists protested against extending this account to cellular organization, claiming that viruses did not have organismic and cellular status. Sonneborn (72) summarized the criticism as follows:

Yet a virus is far from a cell. . . . A virus does not grow and divide like a cell. Its nucleic acid replicates and its other structures are separately formed, the parts later coming together in the final organization. On the contrary, the integrity of nonrandom cell structure persists throughout growth and division which immediately suggests that the preexisting structure plays a decisive role that may not be explicable by mere random self-assembly of genic products.

Even some leading viral geneticists doubted that the organization of the cell could occur by self-assembling parts. Salvador Luria (73), for example, suggested that individual macromolecules did not have all the "knowledge written inside themselves" to make a cell any more than individual humans had all the information to make a complex culture. Cultures themselves possessed information that was not known by any of their individual members. Information also had to be

transmitted by cultural means: "You have to have cultural information in order to continue to make a complex product whose blue-print is not written out." In effect, Luria remarked that just as biological evolution is separate from cultural evolution, so is DNA evolution separate from the evolution of cellular organization. One could no more explain cellular organization in terms of genes than one could explain human cultural organization and evolution in terms of biology.

From the point of view of ciliate protozoologists, an explanation of cellular organization in terms of random collisions of gene products seemed to be as absurd as explaining cultural evolution in terms of random collision of individuals. Doubts as to the efficiency of so simple a hypothesis as self-assembly had long been proposed by investigators of ciliated protozoa. Although ciliates may not be more highly organized in any fundamental sense than many other cells, their complex patterns of organization were more readily observable with the optical microscope. The conspicuous, constant normal organizational features of the ciliates made them excellent tools for experimental analysis of the function of preexisting structure in genic action. The most impressive structural organization in the ciliates has already been mentioned: the complex structures that make up the cell surface, i.e., the "skeleton," ectoplasm, or cortex. The cortex is composed of linear arrays of a large number of fundamentally similar "ciliary units" arranged in a precise repeating pattern. At each level of organization observable within the limits of resolution of the optical microscope, the cortical pattern is remarkably constant and reproduces faithfully through a regular sequence of events during growth and fissions. It was difficult to imagine how this organization could arise *de novo* by purely random collisions of gene-produced proteins.

All the protozoological work pointed to the same conclusion: the guiding mechanism for the elaboration of formed parts was to be sought in the most solid portion of the cell, the ectoplasm. When in 1961 Vance Tartar posed the question "What are stentors good for?" he readily found his answer in the cytoarchitecture of the cell and the relevance of its study for the "great unsolved problem of organic form": "A cytoarchitecture which has repeatedly been postulated as necessary to explain the orderly development of eggs is visibly displayed in stentors and does in fact play a cardinal role in their morphogenesis" (74). Thus protozoologists divided the cell into two parts: genes and gene products on the one side and, on the other, a cell cortex or skeleton that seemed to manifest supramolecular properties.

Strictly speaking, however, the observations of protozoologists said nothing about the origin of the guidance mechanism. From a genetic point of view, the important point was whether the evolutionary changes in the cell cortex were due to genomic changes, independent cortical changes, or parallel series of independent but selectively correlated changes in genome and cortex. Did it represent a principle of inheritance that was relatively independent of genes? Did it also represent a basis upon which evolutionary change could occur? In other words, if the structure or organization were changed would the change be inherited? In principle, cellular organization could be recreated every time an egg is created or every time a ciliate emerges from a cyst. Nonetheless some primordial organization was often assumed. Sonneborn himself favored the

possibility of “a parallel, independent, and selectively correlated evolution of genome and cortex” (75). Though various observations and experiments in ciliated protozoa suggested to protozoologists that more than self-assembly was involved in the formation of cellular structure, one crucial step was lacking in making the demonstrations convincing. Genetic demonstrations were required to exclude the possible role of genes or genic action.

By the 1960s, when Sonneborn turned to this problem, he had developed sophisticated techniques for the analysis of *Paramecium aurelia*. Based on cross-breeding analysis of cortical differences and grafting experiments, he demonstrated that gross differences in cortical organization bred true to type through both sexual and asexual reproduction, free from both nuclear intervention and the control of the fluid part of the cytoplasm (76). The genetic basis, he concluded, was located in the cortex of the cell. In grafting experiments, Janine Beisson and Sonneborn inverted a small patch of ciliary units (sophisticated structures that includes kinetosome, cilium, a variety of subcortical fibers, and specialized membranes). They observed that the inverted patch grew during cell division until its rows extended full length along the body surface. Thereafter, progeny inherited the inverted row or rows. The only cells containing the inverted patch were those derived by fission from preexisting cells with the inverted patch, and it was shown genetically that neither the nucleus nor the free-flowing cytoplasm influenced the transmission of this characteristic. The theoretically important conclusion was that structural information could be maintained in, and transmitted by, supramolecular structures. The ciliate cortex of *Paramecium* seemed to carry information for its gross organization and transmitted the organization to progeny independently of genes:

Our observations on the role of existing structural patterns in the determination of new ones in the cortex of *P. aurelia* should at least focus attention on the information potential of existing structures and stimulate explorations, at every level, of the developmental and genetic roles of cytoplasmic organization (77).

These kinds of results have been, and continue to be, reproduced and extended by several of Sonneborn's former students and associates working on *Paramecium* and *Tetrahymena* (78). Sonneborn coined the term “cytotaxis” for the ordering and arranging of cell structure under the influence of the old. Based on studies of cortical inheritance, all investigators of ciliated protozoa agree with Sonneborn (79) that cell organization does not reside exclusively in gene-determined structures of polar molecules:

Without cytotaxis an isolated nucleus could not make a cell even if it had all the precursors, tools and machinery for making DNA and RNA and the cytoplasmic machinery for making polypeptides. Self-assembly of genic products can go only so far; to go the whole way, cytotaxis must be added on. Strong evidence now confirms the old dictum that only a cell can make a cell. Preexisting cortical structures would play a role in determining where some gene products go in the cell, how these combine and orient, and what they do.

This did not mean, of course, that genes did not play an important role in the determination of cellular structures. Reproduction of cortical structures is recognized to be typically very indirect, involving a complex series of events. The kinds and quantities of molecules directly and indirectly resulting from genic action are considered to be necessary, but not sufficient. The relations of genes

and supramolecular structures in the formation of new structures was expressed by Nanney (80) in the following figurative terms:

In an extreme polar interpretation, one might postulate that nucleic acids specify only proteins, which must be appropriate for cellular design, but not decisive. In this case the cellular architects (that is, preexisting structures) might be required to determine whether the eventual edifice constructed of the building blocks would be a railroad or a cathedral. I doubt the value of this extreme analogy, but some intermediate position may be more consonant with the larger biological realities than either extreme.

Cortical inheritance clearly fell outside neo-Weismannian conceptions on two counts: the characters in question are not due to particulate determinants and there is no clear distinction between the determinants of cell form (genotype) and the manifested form (phenotype) (81). Cells that acquire inverted ciliary rows could propagate such inversions. The stakes were clear from an evolutionary point of view. They challenged what A. D. Hershey (82) called "the unwritten dogma," according to which "biological evolution is solely the evolution of nucleotide sequences." From the point of view of developmental biology, the stakes were also clear. To those who were familiar with the work on cortical inheritance during the 1960s and early 1970s, these results were exemplary demonstrations that molecular models of cell differentiation in terms of gene regulation described for bacteria are not sufficient to account for the stability epigenetic changes in more complex organisms. One could not reduce cellular differentiation to programmed genic changes. When Boris Ephrussi discussed cortical inheritance and the genetic basis of morphogenesis in 1972, he prefaced his remarks by noting:

... while I have little doubt that an interpretation of stable epigenetic states based on models of "circuitry" of microbial type could be constructed, I feel to try to force it into such a scheme would be not only premature but definitely dangerous at this stage: in the field we are considering, the time has come to cease satisfying ourselves (as is still too often done with what may be mere analogies which, almost inevitably, result in too rigid adherence to what has become The Dogma) (83).

The phenomenon of cortical inheritance in ciliates was placed beyond doubt. During the 1970s and 1980s discussion centered around the scope and significance of the phenomenon and providing a plausible mechanism to account for it. In regard to the first issue, those who investigated cortical inheritance in Protozoa recognized that one had to be careful when extrapolating from Protozoa to multicellular organisms, just as one had to be careful when extrapolating from bacteria (which they considered to be not much more than a bag of enzymes) to more complex organisms. Indeed, there were few cases of experimental evidence supporting cortical inheritance in Metazoa. However, absence of evidence did not necessarily imply evidence of absence. Protozoologists generally attributed the lack of evidence not to a lack of correspondence, but to the technical difficulties of demonstrating such phenomena in Metazoa. Sonneborn himself had often emphasized the similarities between morphogenesis in Protozoa and Metazoa in his published papers throughout the 1960s and 1970s. The only major difference, he argued, was the units of organization: cells and tissues in Metazoa, and parts of the cortex of a single cell in the case of the ciliates. Embryological literature was replete with arguments for the importance of the cell cortex in cell-cell interactions. Cortical localization was also impor-

tant in the pattern of some mosaic eggs. The many parallels, Sonneborn argued, suggested that the roles of the cortex and the principles of their operation may be fundamentally alike in unicellular and multicellular animals. However, in drawing such parallels, Sonneborn realized he was trespassing on traditional taboos about the uniqueness of Protozoa:

Doubtless ciliate protozoologists would be pleased if their studies of morphogenesis proved to be of general significance. I regret to have to say, however, that many—very many—of them have not only largely isolated themselves from the rest of biology, but have stressed how different Ciliates are from all other organisms. This has been accepted by many other biologists. As a result they have virtually no interest in Ciliates (84).

In order to reach an audience for cortical inheritance, one had to do more than point to some similarities between the organisms and to the similarities between the problems to be solved. The interpretation of the mechanism through which spatial ordering is achieved in the organism would also have to be one which would be seen by developmental biologists working on Metazoa to be applicable to their work. To capture their interest one needed to fish for the solution to the mechanism of cortical inheritance using the same theoretical net used by those interested in pattern formation and spatial ordering in Metazoa. Developing an adequate and common conceptual framework for pattern formation remains one of the central problems in this domain.

Throughout the 1970s a major question was whether the phenomena of cortical inheritance could be explained in terms of the principles of molecular biology or whether new abstract principles had to be invoked. One of the traditional ways in which abstract principles could be understood was in terms of magnetic fields (85):

If cells draw on an extragenic source of information, a second abstraction must be invoked, another vital principle superimposed on the genotype. A likely candidate already exists in what is usually called cell polarity, which tradition places in the ectoplasm for good reason—it's a spatial principle and as such requires mystical language. Seemingly independent of the visible particles that respond to it, polarity pervades the cell much as a magnetic field pervades space without help from the iron filings that bring it to light. Biological fields are species specific, as seen in the various patterns and symmetries of growing things.

Not all biologists agreed that it was necessary to invoke new abstract principles to explain cell organization. Indeed, there was considerable disagreement about this even among those protozoologists who investigated cortical inheritance. Sonneborn himself sought to account for cortical inheritance within the theoretical framework of molecular biology. For Sonneborn the problem of organization was a problem of molecular structure. Preexisting structures played a direct role in providing a scaffolding or template through which building blocks were assembled (86). The determination of large-scale shape by fitting together pieces in a building-block or jigsaw-puzzle manner operated continuously from the scale of atoms and large molecules upward. According to this metaphor, the structural elements of the cortex simply provided the “scaffolding” for the insertion of new structures. As Nanney (87) put it, “Because of this morphogenetic role of preexisting structure, the cell has some of the properties of an organismic crystal.” Within this theoretical framework, the control of cell organization

"would be fully vested within the structural elements themselves plus the microenvironment under their localized influence" (88). The only truly fundamental difference between this model and that of self-assembly is that the cell would not have to be reconstructed anew each generation.

During the 1970s Joseph Frankel developed an alternative view: the genesis of large-scale order occurs outside the structural confines of molecular biological principles (89). This view was strengthened by the demonstration that large-scale structural inherited changes in the cortex could be transmitted in certain ciliates that lack visible cortical structure in the cyst state (90). Such cases indicated that the perpetuation of cellular organization does not always depend on preexisting visible structures. In some cases at least, the perpetuation of cellular organization is suggestive of an invisible force which is responsible for morphogenetic patterns. Based on these observations, Frankel argued that there may be at least two kinds of mechanisms operating to maintain cell structure: a local constraint involving microscopically visible cell structure acting as a scaffold and a more global level of pattern development based on morphogenetic gradient fields. Frankel also argued that cortically inherited differences were impermanent and that all inherited cortical aberrations eventually returned to a genetically determined "stability center" or genetically constrained "stability range."

The terms of Sonneborn's dialectic were nucleus versus cytoplasm, self-assembly versus inherited preexisting cell structure or order (91). Frankel, on the other hand, made a significant shift during the 1970s. He, too, pointed to the existence and importance of inheritance of cell surface organization in ciliates but tried to do so to a different audience and with a different emphasis from Sonneborn's. Frankel's focus was on the use of the inheritability of large-scale cell surface patterns in ciliates as a device for investigating the nature of morphogenetic fields. The fact that these fields could be clonally inherited in ciliates provided certain opportunities for studying their nature that are not available in other organisms—one could study endless cellular replicas in which a mutant gene can be expressed while the genotype remains constant and preexisting cortical framework retains its continuity. Frankel's concern with the mechanisms controlling morphogenetic fields was part of a new wave of interest in old, but unsolved, problems in developmental biology.

During the 1970s there was a revival of interest in pattern formation in multicellular organisms and a renewed concern with Driesch's fundamental concept that the development of a part is a function of its position in relation to the whole. This revival brought with it new debates concerning developmental constraints on evolutionary change and how genes act in controlling spatial patterns of developing organisms. The chief protagonists in this debate were Lewis Wolpert (92) and Brian Goodwin (93). In brief, Wolpert, who led the renewed interest in the problem of pattern formation, argued that "positional information" is universal, but that cellular interpretation of that "positional information" is controlled by local genomic activation. Under the scheme proposed by Wolpert, all fields would be similar and all cells would behave according to position and genome. Hence, for Wolpert, positional information provided no constraints for evolutionary change, since genes effectively specify pattern. Goodwin, on the other hand, challenged the notion of ultimate genic supremacy and argued that fields themselves, and the changes that occur in them, were

governed by “generative principles” embodied in the processes that set up fields. In Goodwin’s scheme, genes played only secondary roles in selecting which of the potential fields (prepatterns) were to be implemented. The “generative principles” governing fields provide developmental constraints on evolutionary change. This viewpoint, he argued, contradicts neo-Darwinian selection theory and the neo-Weismannian reduction of developmental patterns to the expression of the genome.

During the 1970s and 1980s Frankel began to ally work on ciliate morphology with the views of developmental biologists who began to account for pattern formation and morphogenetic fields in multicellular organisms. He argued that morphogenetic phenomena in Metazoa described in terms of “positional information” were just as evident in ciliates. Although he applied Wolpert’s notion of “positional information” to cellular morphogenesis, there was one fundamental difference between the observed phenomenon in ciliates and Wolpert’s view of the genomic control of fields. Within a single cell there are no “local genomes”; therefore, fields could not be controlled by local genomic activation. The present state of play is best summarized by Frankel himself:

Goodwin was quick to point out that this extension of positional information fundamentally challenges the notion of ultimate genomic supremacy. Thus my application of the idea of positional information has suffered (or enjoyed, depending on how one looks at it) the ironic fate of being supported by an opponent of the positional information concept (Goodwin) and being ignored and almost certainly disapproved of by the principal proponent of that concept (Wolpert) (94).

In a volume boldly entitled *Beyond Neo-Darwinism. An Introduction to the New Evolutionary Paradigm*, Goodwin (95) challenged what he called “genocentric biology,” which claims “that all aspects of organismic form are determined by the hereditary particulars encoded in DNA.” Such a view, he reasoned, would be logically valid if there existed a causal chain of determination from DNA to protein structure to all aspects of organismic biology. To refute this claim, Goodwin referred to the work on ciliated protozoa which showed that specific patterns of cortical morphology, such as reversed orientation of cilia in a kinety (row), is inherited by a mechanism that is relatively independent of DNA. In other words, the specific causes of morphology in these organisms do not come solely from genes. Goodwin used the leverage of phenomena observed in ciliated protozoa to move the theoretically important point that neither genes nor cytoplasm alone defines sufficient conditions as sources of constraint on biological form: “Reproduction in this large category of organisms,” he argued, “does not conform to Weismann’s scheme . . . , nor to any of its subsequent modifications” (96). For Goodwin, cortical inheritance was not an exception: it represented a rule. Yet he qualified his arguments based on ciliates with the comment, “Since the ciliate protozoa are often regarded as very much the bizarre, if not lunatic fringe of biology, let us now move to centre stage and consider reproduction and morphogenesis in *Drosophila*” (97).

## 8. Concluding Remarks

Exceptions do not exist in the sense that *Drosophila*, bacteria, and ciliates do. The judgments by which exceptions are constituted are historically variable.

However, although the notion of exceptions can never be an objective descriptive category, this is not to say that an “exception” is simply what some people whimsically choose to call an exception. For there is nothing at all whimsical about such kinds of judgments; they have their roots in deeper structures of belief which, in turn, are conditioned and constrained by the hierarchy of disciplines, problems, organisms, and techniques in biology.

Protozoa have had a controversial place in studies of development and heredity throughout the twentieth century. However, one cannot reduce the “exceptional” status of the phenomena reported in them to the nature of the organisms themselves nor to theories about their phylogenetic relations to other organisms. One has to understand the disciplinary relations of these organisms as laboratory technologies to other organismic technologies for constructing knowledge about heredity. Those producing the unorthodox results in the 1940s and 1950s has persistently argued that the relative lack of evidence for cytoplasmic inheritance compared to Mendelian inheritance was not a reflection of nature. It was due to the relative ease of investigating Mendelian phenomena with the techniques available and to a lack of willingness on the part of most geneticists to investigate and report non-Mendelian phenomena when they detected them. When cytoplasmic inheritance was observable, they argued, it was often due to the special technical attributes of the organisms that made possible investigation of the phenomena (98). During this period, cytoplasmic inheritance was not an exception because it was found in protozoa, it was an exception because it contradicted Mendelian genetic principles.

Today, the nuclear–cytoplasmic dispute is defunct. Inasmuch as mitochondrial and chloroplast inheritance are traced to cytoplasmic DNAs, they are no longer classified as “exceptions.” These cases lost their exceptional status the moment they conformed to the doctrines and metaphors of molecular biology and neo-Darwinism. The opposition today is no longer between nucleus and cytoplasm; the debate is no longer over a “nuclear monopoly,” but, instead, over a “molecular monopoly.” Only those phenomena which do not conform to molecular biological doctrines such as cortical inheritance in ciliated protozoa still carry the stigma of being “exceptions.” Cortical inheritance in ciliates continues to be allied with embryological concerns, and protests against reducing morphogenesis to the control of genes. Moreover, as André Lwoff (99) has recently argued, as a non-genic characteristic produced by interactions between the organism and environment, the organization of the cortex in ciliates also represents a striking example of the “heretic” and “condemned” concept of the inheritance of acquired characteristics.

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