Intercellular Activities in Vertebrate Development

Problems of embryonic organization are being attacked at subcellular, cellular, and supracellular levels.

Jane Oppenheimer

It is a historically fascinating but in some ways scientifically worrisome phenomenon that one of the principal problems currently under study by investigators of embryology is very much the same as one posed by Aristotle nearly 2000 years ago. Aristotle asked, in a passage that has lately been frequently quoted: "How, then, are the other parts [of the embryo] formed? Either they are all formed simultaneously—heart, lung, liver, eye, and the rest of them—or successively. . . . As for simultaneous formation of the parts, our senses tell us plainly that this does not happen: some of the parts are clearly to be seen in the embryo while others are not. . . . Since one part, then, comes earlier and another later, is it the case that A fashions B and that it is there on account of B which is next to it, or is it rather the case that B is formed after A?"

Is it the case that A fashions B and that it is there on account of B which is next to it, or is it rather the case that B is formed after A? This is the problem of becoming; how does what is one thing become what is apparently another? *Does* it become, or was it arrived the whole time, only finally, like Mephistopheles in the melodrama, tossing off its dark cape and domino to reveal its original self?

Aristotle approached the problem in terms of organs: "I mean, for instance," he wrote in continuation of the passage quoted above, "not that the heart, once it is formed, fashions the liver, and then the liver fashions something else; but that the one is formed after the other [just as a man is formed after a child], not by it." By placing his emphasis on organs, he recognized organization (though he did not call it by name) as the primary clue to development and hence to the nature of the organism. Our very word *organism*, although in common use only since the 19th century, reflects the importance of the concept of organization in terms of organs which has dominated biology since the time of Aristotle.

Today the problems of development are attacked primarily at a cellular and subcellular level. Biologists of the mid-20th century believe that cells are the organs—to use the word in Aristotle's original sense of instrument—of development of the differentiating embryo. The most important aspect of development for the embryologist to study is the organization of the embryo. But since this has proved elusive, many investigators of development, though not all, have contented themselves with studying the primary elements of the embryo, the cells, and in turn their constituent parts.

The trends of thinking of embryologists are in some ways less highly organized than the morphogenetic activities of embryos, and it is hardly possible, therefore, to classify and neatly outline all the various methods currently being employed by embryologists to investigate all the varied methods employed by embryos to achieve their ends of making highly varied adults. The new tools of biological, biochemical, chemical, and physical sciences are being freely applied to embryological problems; the techniques of electron microscopy, of immunology and serology, of radiobiology, to name but a few, are being vigorously pursued in embryological laboratories. But even if it were desirable to list all the investigations currently exploiting such techniques, their results could not be—or at least have not been as yet—comprehended in a single scheme of development which answers satisfactorily for all the questions of how the organization characteristic of the multicellular adult develops by means of cellular and subcellular activities out of the organization of the single-celled fertilized egg. (The development of the organization of the prefertilized egg, perhaps the most important developmental phase of all, is virtually uninvestigated.) Therefore, in order to narrow the scope of the present article to at least partially comprehensible dimensions, I shall confine this discussion to one or two problems of cellular activity in vertebrate development that have been under attack during the past quarter century and that still are today, in investigations that attempt to elucidate the most important unifying principle of development to have been discovered during the present century.

Around twenty-five years ago, Hans Spemann's Silliman Lectures first appeared in print, summing up the work of the previous quarter century on embryonic induction, a specific kind of cellular activity. What was the embryological setting when Spemann published his book? Wilhelm Roux had at the end of the 19th century postulated two possible alternative relationships between developing embryonic parts: (i) either they influenced their neighboring parts during development or (ii) they did not. It seemed at the outset very simple to choose between these alternatives, and he outlined an experimental program which would enable embryologists to do so. His own experiments were rarely adequate to answer the questions he framed, but the questions were nonetheless often appropriate ones, and Spemann and Harrison and their followers developed the techniques of transplantation and of explantation to investigate Roux's problems and their own which grew out of them.

One of the important first results of experimental embryology was that in which at the beginning of this century Spemann demonstrated a dramatic interaction between neighboring cell layers in the production of a complex structure, the vertebrate eye. A lateral outgrowth of the brain, the optic cup, which later forms part of the eyeball, was shown, by defect and transplantation experiments, to induce the overlying ectoderm, by touching it, to form a lens. In some experiments the ectoderm, which would normally have formed a lens, failed to do so in the absence of such contact; in others, flank ectoderm grafted over the optic cup at an appro-

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priate time was able to form a lens, which it would not have done in its original position in the absence of contact with the optic cup. The ectoderm forms the lens in a very simple way: a circular patch of cells thickens, then separates off to lie below the rest of the ectoderm, which becomes skin. Enclosed by the iris, the lens rudiment will later elaborate the peculiar fibers which will become crystal clear for the transmission and focusing of light rays.

The lens-inducing effect is a general phenomenon, later found to occur in other vertebrate forms—certainly in birds, possibly in fish and mammals. The first demonstration of its occurrence in amphibians was, however, of prime importance in providing evidence for the development of the concept of embryonic induction.

It soon became apparent that other inductive systems have important roles to play during the course of amphibian development. Even more dramatic than the demonstration that an optic cup can induce flank skin grafted over it to form a lens was the demonstration that a circumscribed portion of the young amphibian embryo, the so-called organizer, which is located above the dorsal lip of the gastrula's blastopore, could when transplanted into another gastrula induce the formation of a more or less whole new embryo (Fig. 1). The organizer region comprises the primordia of the dorsal axial tissues (notochord, bilateral rows of skeletal muscles, head mesoderm). In the course of its inward movements during gastrulation, this region comes to underlay part of the ectoderm, which as a result of its contact with the underlaying layer becomes a self-delineated area, the neural plate. The cells of the plate elongate, then form a canal whose walls meet and separate from the skin to form a tube. This is the segregated primordium of the central nervous system, which will undergo manifold processes of growth and change of form to become the brain and spinal cord; its tissue will differentiate all the highly varied cell types which carry on the complex functions of nervous action and reaction and integration. It is thus an important problem for the embryologist to try to ascertain what stimulus from the underlying axial tissue layer impels the future neural plate ectoderm to carry out the chain of events which leads to the production of the elaborate central nervous system.

Leaving aside for a moment the specific issue, to which we shall return, of the nature of the inductive stimulus, we see that these experiments not only raised specific questions, as good experiments should, but that they also answered some general ones, as not all experimental results always do. They replied unequivocally in the affirmative, for some systems within vertebrate development at least, to Roux's question as to whether embryonic parts can influence other parts during development, and thus also to Aristotle's question as to whether A "is there on account of B which is next to it." Thus they were of the greatest theoretical significance, since they gave incontrovertible validity to the principle of progressive differentiation, which holds that each step in development is an outcome of the steps immediately preceding it and a necessary condition for those which are to follow it.

While the principle of induction does not explain all developmental processes in all organisms, innumerable experiments have shown its wide applicability to many of them. The emergence of not only vertebrate lens and vertebrate nervous system but of many other organs of the vertebrate embryo can be understood only in terms of induction. While the differentiation of some organs in some invertebrates is accomplished independently of induction, inductive proc-

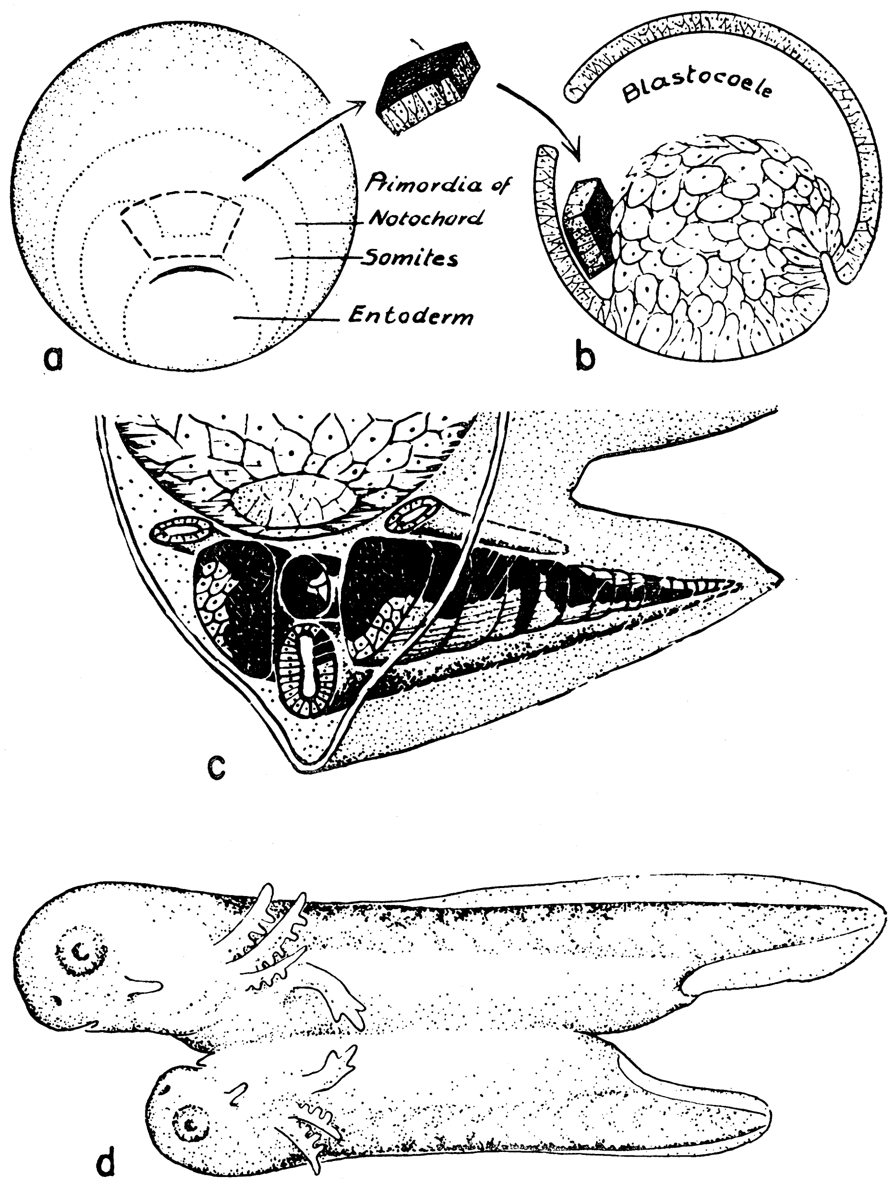

Fig. 1. Diagram of the transplantation of a piece of the amphibian upper blastoporal lip into another gastrula (*a, b*) and the structures self-differentiated and induced by the graft (*c, d*). In *c*, the tissues derived from the graft are shown in black, and the induced tissues, in white. The graft would probably be somewhat larger than shown if it were to induce an embryo as complete as that delineated in *d*. [From J. Holtfreter and V. Hamburger in *Analysis of Development*, B. H. Willier, P. A. Weiss, V. Hamburger, Eds. (Saunders, Philadelphia, 1955), p. 244, Fig. 82, reproduced by permission of Professor Holtfreter, the senior editor, and the publisher]

esses have been demonstrated to be responsible for the development of some organs in a number of invertebrates also. Embryologists agree that the exact character of the inductive mechanisms may differ in detail at different stages in the forerunners of different organs. But the discovery of the general occurrence of the phenomenon was one of the most momentous discoveries of 20th-century biology and was a factor of great importance leading to the recognition of the central position of problems of development in 20th-century biological thought and investigation.

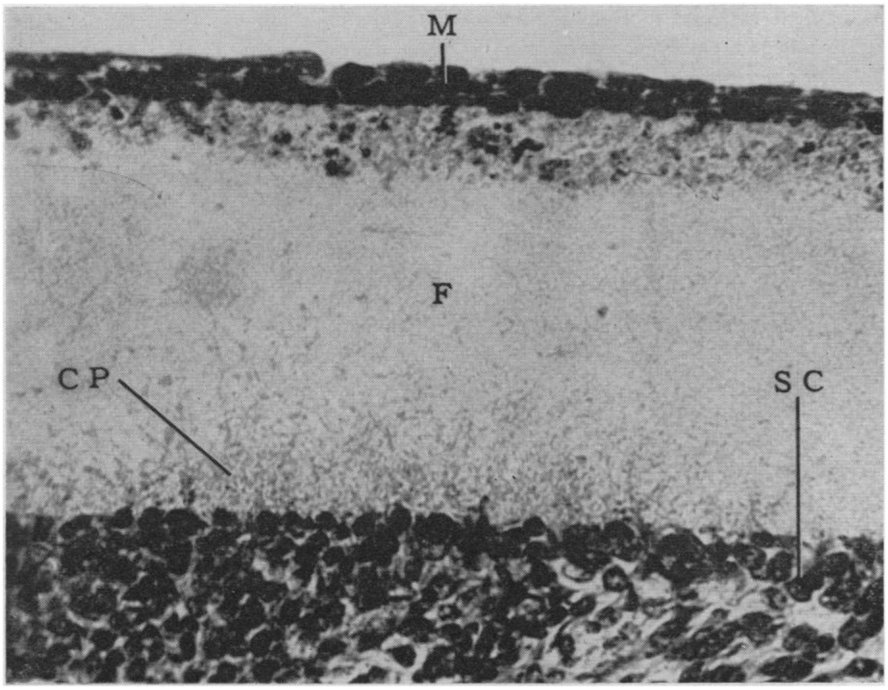

Fig. 2. Section through a culture in which mouse spinal cord (*SC*) has failed to induce tubule formation from mouse kidney-forming mesenchyme (*M*) through a filter (*F*), although cytoplasmic processes (*CP*) have visibly penetrated the filter. Weak inductions were produced through filters 20 to 65 μ in thickness but were largely eliminated when filters 80 μ in thickness were used. [From C. Grobstein, *Exptl. Cell Research* **13**, 579, Fig. 5 (1957); reproduced by permission of the author, editors, and the publisher]

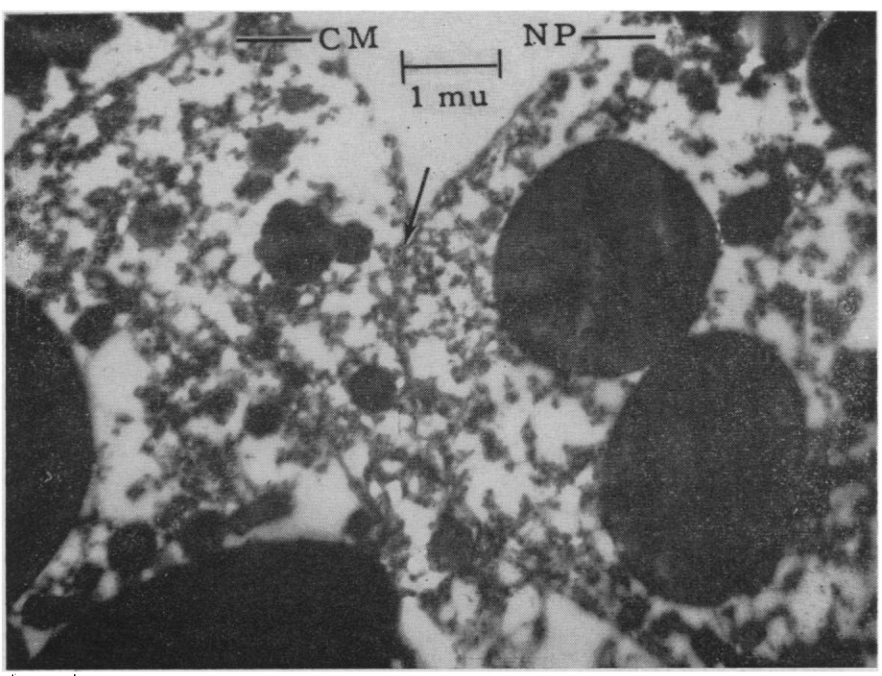

Fig. 3. Electronmicrograph showing process of neural plate cell (*NP*) in contact with process of chordamesoderm cell (*CM*) in an early neurula stage of *Xenopus*. The arrow points to the region of contact. [From R. M. Eakin and F. E. Lehmann, *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* **150**, 187, Fig. 13 (1957); reproduced by permission of Professor Eakin, the editors, and the publisher]

To return to the specific question as to the nature of the stimulus by which one group of cells affects another during induction, it may be said at the outset that it has not yet been answered unequivocally for any single system, although the failure to arrive at a solution has surely not been for lack of trying, especially in the case of the induction of the amphibian nervous system, the problem on which the greatest amount of experimental attention has been expended.

At least one reason for the lack of final success in solving the problem was the adherence of embryologists to currently fashionable ways of thinking, which encouraged them to hope too early for too simple a chemical answer to too complicated an embryological, and hence an organizational, problem. Already in the early 1930's, chemical attitudes began to be assumed by investigators of embryonic organization. It was found at that time that killed organizer cells and also killed nonorganizer cells could induce the development of an amphibian nervous system, and next that tissues, sometimes treated and sometimes not, taken from varied organs of varied organisms, invertebrate as well as vertebrate, could cause inductions in the amphibian egg. Biochemistry was less advanced in the 1930's than in the 1950's—in fact the attempt to find *the* substance that could perform the miracle of organizing an embryo may have been one of the spurs to its rapid progress—and it seemed less naive then than now to ask: What is *the* substance that acts as *the* organizer? When Harrison gave his lecture at the Harvard Tercentenary in 1936 he could quote as quite appropriate the couplet from *Faust* in which Faust's pedantic student sang, "What used to be organized Now we can crystallize."

An early shattering of hopes resulted from the claims that a great diversity of chemical substances could act as inductors. Among the many substances demonstrated to produce inductions of sorts were a number of possible physiological significance; these included steroid hydrocarbons, fatty acids, proteins, including nucleoproteins, and nucleic acids. Indeed, embryology rendered another great service to biochemistry by calling attention to the possible significance of ribonucleic acids in protein formation through Jean Brachet's histochemical studies on the localization of ribonucleic acid–rich particles in various regions of the amphibian embryo.

In view of the multiplicity of agents demonstrated to be able to induce the formation of the amphibian nervous system one might suppose that Harrison's successors delivering lectures today would find the quotation from *Faust* less apposite. Yet there are many for whose articles even now Goethe's lines might form a fitting rubric. It was perhaps historically inevitable that investigators in the early 1930's should have attempted to pinpoint a specific chemical agent as "the organizer"; while it may be equally inevitable, it is puzzling that the search for one, or for a few such agents, continues almost as vigorously today. This attitude is somewhat questionable from the biochemical point of view, since the biochemists themselves now seek to characterize the activities of cells in terms of metabolic systems rather than of special substances per se. Making the jump, however, from organizer to chemical substance is even less excusable on the strictly biological side, since "the organizer" is by definition a whole district of living cells and not at all any kind of substance extractable from this district. Embryologists who keep in mind the complexities inherent in the processes of organizing the development of an embryo are well aware of the fallacy of attributing the control of such organization to the presence of a single omnipotent chemical substance as such.

The search for chemical substances able to influence differentiation in the amphibian has altered in some respects, in that some of the effective agents now being isolated seem to induce ectodermal and others mesodermal structures, some anterior and others posterior ones. A merit of this change in attitude is that it is no longer generally considered to be a single magic substance that induces a nervous system or even organizes a whole embryo, but rather a number of substances that can affect cellular differentiation, at least in vitro. Nonetheless the significance of the varied agents currently under investigation remains a matter of some confusion.

The agents studied are being isolated not from the embryo itself but from fractions of highly complex organs or tissues, such as mammalian liver or kidney. Other sources of effective fractions —bone marrow, whole blood, blood serum, plasma, homogenized 9-day chick embryos—are also hardly to be described as physiologically or biochemically simple or homogeneous. While no evidence has as yet been adduced to prove that agents corresponding to those in the effective fractions of liver, kidney, bone marrow, and so forth actually exist in the developing embryo, one group of experiments has suggested that an effective agent may be produced by amphibian chordamesoderm cells growing in tissue culture. It has been claimed that this agent is ribonucleic acid, and the evidence points to the fact that at least some of the effective agents extracted from the complex organs and tissues under analysis may be nucleoproteins or nucleic acids. Many investigators consider this line of investigation to be one of the most promising currently being followed, and it is to be hoped that it will lead to further clarification of inductive mechanisms.

If, however, ribonucleic acids are as important for the synthesis of proteins as modern biochemistry suggests, it would be surprising if the suitable administration of ribonucleic acid or ribonucleoprotein to an embryo were not to result in some alteration of its development, provided it succeeded in entering its cells at all. But the fact that the alteration takes the form of making a new ner-

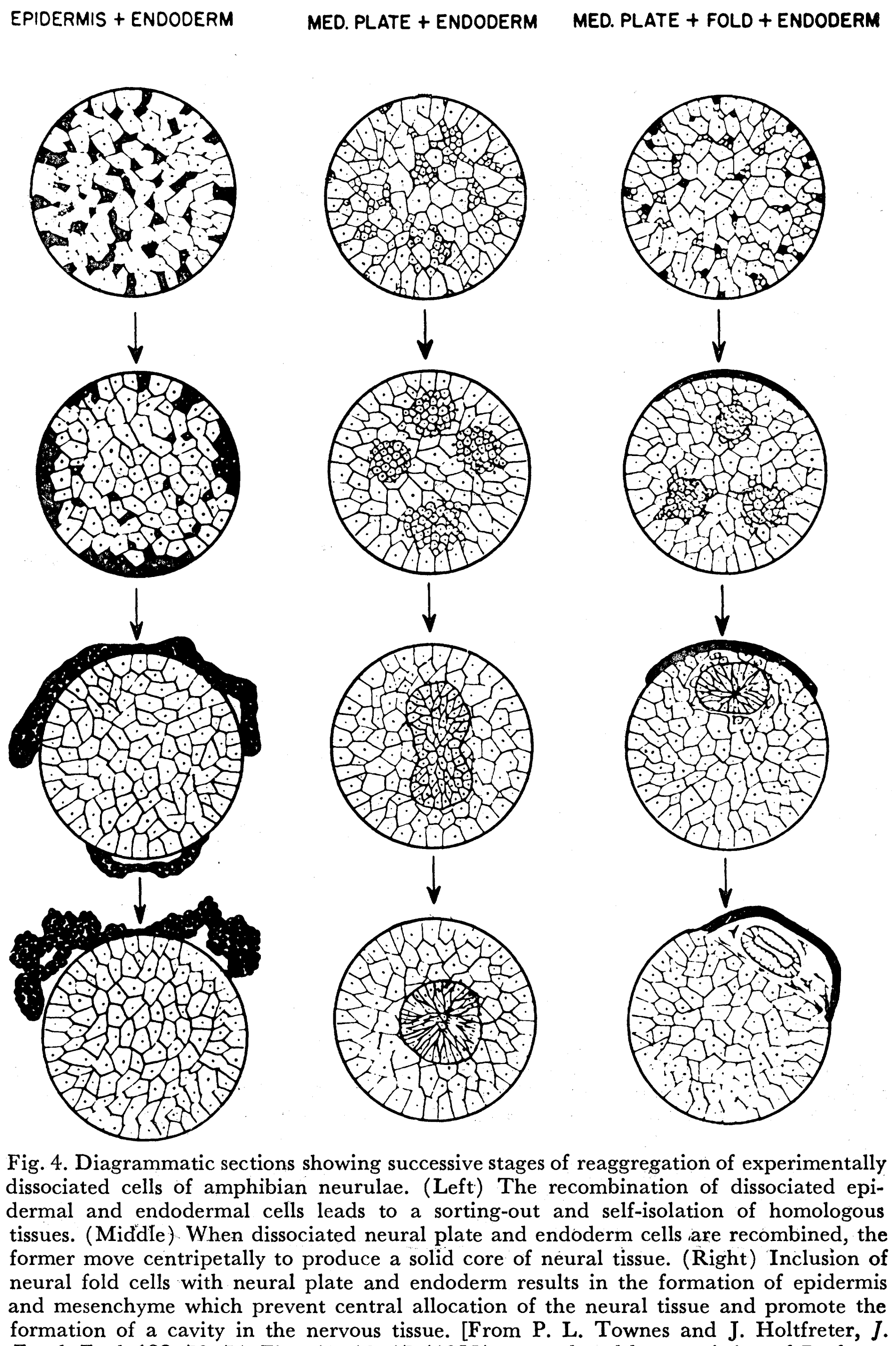

Fig. 4. Diagrammatic sections showing successive stages of reaggregation of experimentally dissociated cells of amphibian neurulae. (Left) The recombination of dissociated epidermal and endodermal cells leads to a sorting-out and self-isolation of homologous tissues. (Middle) When dissociated neural plate and endoderm cells are recombined, the former move centripetally to produce a solid core of neural tissue. (Right) Inclusion of neural fold cells with neural plate and endoderm results in the formation of epidermis and mesenchyme which prevent central allocation of the neural tissue and promote the formation of a cavity in the nervous tissue. [From P. L. Townes and J. Holtfreter, *J. Exptl. Zool.* **128**, 79, 74, Figs. 18, 16, 17 (1955); reproduced by permission of Professor Holtfreter, the editor, and the publisher]

vous system or a new embryonic axis is attributable as much to the responding cells as to the administered ribonucleic acid or ribonucleoprotein. The nature, biochemical or physiological, of the processes whereby the reacting cells respond to the administration of ribonucleic acid, ribonucleoprotein, or any other inducing agent is something that embryologists realize it is important to know but an area they have as yet not been able to investigate.

Furthermore, whether indeed any large molecule actually passes from inducing to responding cells remains another open question, and one which unfortunately tracer techniques have not as yet been sufficiently refined to answer unequivocally. One of the more recent pastimes of embryologists has therefore been to search for visible mechanisms whereby substances of large molecular size might pass from the inducer to the induced. In one series of experiments in which a different inductive system from that we have been describing is employed—namely, an effect of embryonic mouse spinal cord on mouse kidney tubule formation in tissue culture—it has been demonstrated that a filter of known pore size which fails to permit protoplasmic contact between inducer and induced (Fig. 2) still permits at least weak inductive effects. In another study, protoplasmic continuity between amphibian chordamesoderm and overlying ectoderm has been suggested (Fig. 3); this the investigators postulate as a possible pathway for the exchange of macromolecules, lipoid droplets, or even formed cytoplasmic elements.

But meantime, while the search for effective large molecules or molecular aggregates of fashionable composition continues, together with the search for their means of transfer by fashionable methods, some skeptics continue to worry about some experiments whose results seemed most unfashionable at the time of their first discovery. In the 1940's, an old experiment, the isolation in salt solution of amphibian gastrula ectoderm without chordamesoderm—an experiment which could theoretically have only one result, namely, no nervous system formation—suddenly had, when carried out on a different amphibian species, a totally different result. Nervous system was formed. Furthermore, these results were found to be modifiable by the simple expediency of changing the hydrogen ion concentration of the solution. (Back in the 1930's, by the way, when the first chemical studies were made, F. G. Fischer, a collaborator of Spemann, had demonstrated nucleic acid, among many other agents of a dissimilar nature, to be an inducer, and had felt it probable that the acid components of the nucleotides were the effective agents, although the organic acids he found effective were large molecules and he had no success with hydrochloric acid.) In the new experiments performed in the 1940's the use of distilled water instead of salt solution, the addition of alcohol to the medium, or the absence of calcium ions also resulted in the formation of nervous system by isolated ectoderm. The general significance of the new demonstration of nervous tissue differentiation by isolated ectoderm and the particular nature of the action of the environmental medium still remain to be satisfactorily interpreted. The experimental results were nevertheless of vast importance in that they proved the necessity of maintaining a skeptical attitude towards the conclusiveness of the results obtained with defined substances of complex nature as regards their inductive specificity. They pointed towards the fact that what determines the outcome of the experiment is not so much the nature of the applied chemical agents as the intracellular physiological mechanisms brought into play by the chemical stimulus, and thus they suggest the paramount importance of developing new methods, more adequate than the old, to investigate these mechanisms.

The fact that the embryologists who danced down the primrose way of biochemistry have failed to find within cells master molecular agents which govern the formation of embryos has not, however, perturbed some investigators who have meantime continued to study embryos at cellular and supracellular levels,

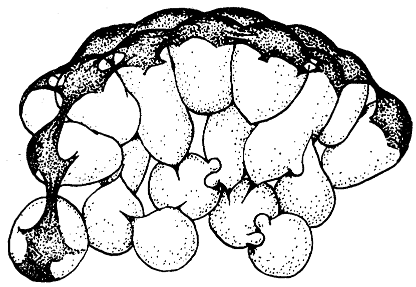

Fig. 5. Surface coat covering cells of the morula stage of *Amblystoma punctatum*. Filliform and knoblike processes can be seen interconnecting the blastomeres. [From J. Holtfreter, *J. Exptl. Zool.* **94**, 265, Fig. 1 (1943); reproduced by permission of the author, editor, and publisher]

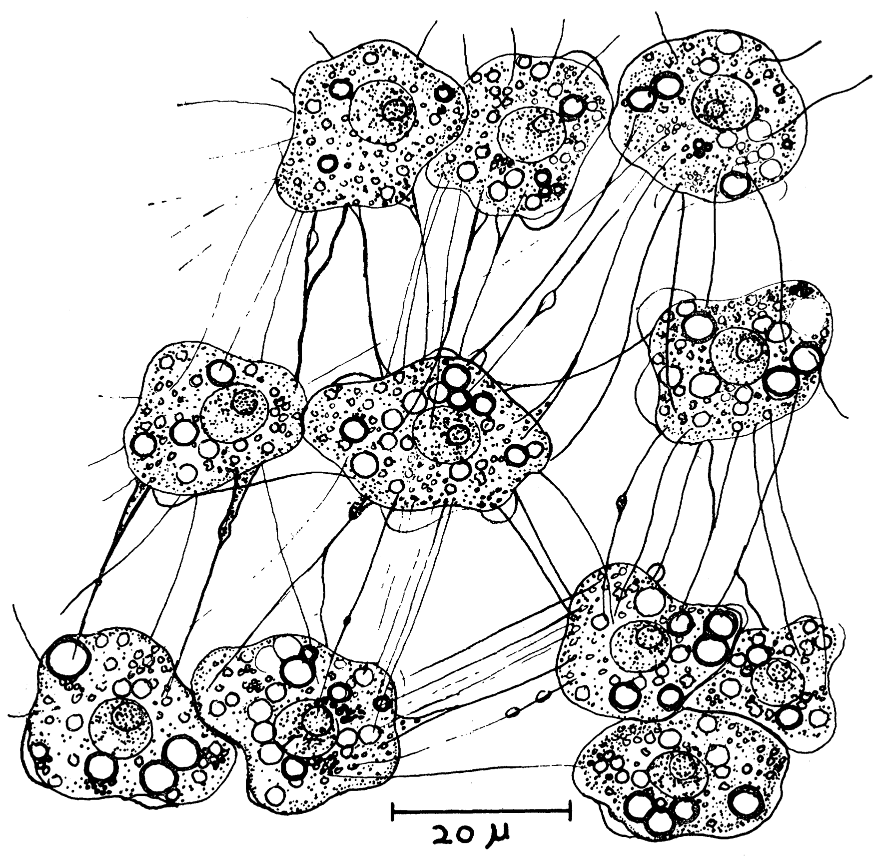

Fig. 6. Intercellular fibers joining living segmental plate cells at the definitive streak stage in the chick. [Courtesy of Nelson T. Spratt, Jr., University of Minnesota]

and from the laboratories of the latter are appearing results as dramatic as any which have ever been described for embryological experiments. Analysis involves resolution into elements, and the embryo's primary elements are cells. Embryos can now be physically as well as intellectually resolved into their cells, by treatment with trypsin or other dispersing agents, and the actions whereby such dispersed cells show affinities or disaffinities for each other, differing for different cell types and at different ages, are now being described (Fig. 4). The analysis of the factors responsible for such affinities and disaffinities has scarcely begun but promises fertile problems for investigation by modern immunological and other methods.

Finally, to conclude with the most important consideration of all, even in embryos such as those of the vertebrates, and in our amphibian example, in which induction and other cooperative cellular activities are essential components of progressive differentiation, these cellular actions are not isolated mechanisms but are components of the over-all processes used by the embryo to make its whole self. The significance of organizer action is that when the organizer is grafted, it induces not only a neural axis but a more or less whole embryo. In the heat of the press for the isolation of chemical agents this has sometimes been forgotten by embryologists, but never by embryos, and what becomes induced in any given situation in many cases seems to reflect at least a strong attempt of the reacting embryonic part to create a new whole.

A few investigators have studied agencies involved in the production and maintenance of wholeness by studying activities not only between but also over and above cells, which permit cells to conjoin their activities. Some visible entities have recently been described which might act in such capacities. In the amphibian embryo Holtfreter has demonstrated a supracellular coat (Fig. 5) which serves to coordinate the movements of the cells during gastrulation. Intercellular bridges, said to contain endoplasmic reticulum, have been demonstrated in the embryo of the chick (Fig. 6) at somewhat later stages than those we have been discussing for the amphibian. However, no open channels between early developing embryonic cells, such as those which connect synchronously developing cells of postembryonic stages—for instance, mammalian spermatids and spermatocytes (Fig. 7) and cnidoblast-forming interstitial cells of *Hydra*—have as yet been demonstrated.

But there are some embryologists who still believe that even if such bridges were to be demonstrated, and even if the composition of the molecules and molecular aggregates or formed bodies which might traverse them were known, the question still would remain open as to what organizes the cells to construct the bridges and to manufacture the agents which cross them and to create the necessary physical conditions to permit the development of wholeness in the aggregate of cells. Lest the reader misunderstand, this statement does not represent a retreat to the position of Driesch, who had to evoke a *deus ex machina* to explain the processes whose machinery transcended his understanding. It is rather an admission that if embryologists know that cells are important elements of the embryo, they are not yet able to define organization itself in physiological terms sufficiently precise to permit its analysis by physiological methods.

Embryos, like embryologists, are made up of atoms and molecules. What is not known about embryonic organization is how chemistry relates to structure, as was pointed out in the two summaries of the recent McCollum-Pratt Symposium on the Chemical Basis of Development. Yet much positive progress has been made in analytical embryology during the past half century. The most perceptive embryologists, such as Roux, Harrison, and Spemann in the past and Holtfreter, working at present, have been clever enough to isolate a few separate mechanisms which have been at least partially resolvable into simpler proc-

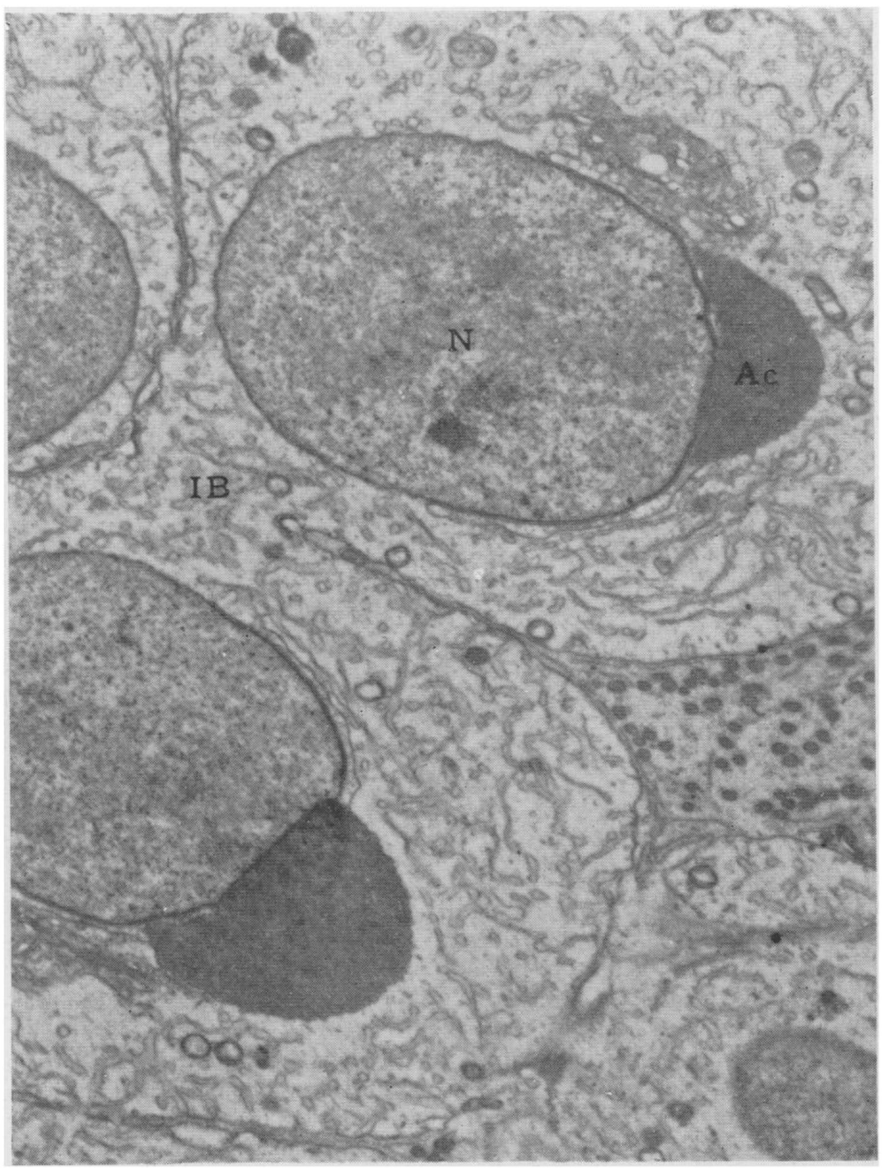

Fig. 7. Electronmicrograph showing intercellular bridge between two conjoined spermatids of the guinea pig. (*IB*) Intercellular bridge; (*Ac*) acrosome; (*N*) nucleus. [Courtesy of Don W. Fawcett, Cornell University Medical College]

esses. But the resolution into simpler processes has been partial, not complete, and the relation of the isolated mechanisms studied to the organization of the whole has thus far defied analysis and thus stands as a challenge to the embryologists of the future.

Wilhelm Roux was once asked by Emperor Franz Josef, who made a visit to his laboratory, how he made discoveries in experimental embryology. Roux replied that the investigator "must have a question in his mind, and then look for an appropriate method to force an unequivocal answer to it." Investigators have made great progress toward compelling an answer to the question raised by Aristotle, but the complete answer to it will never be known until a new Aristotle frames an equally cogent question or set of questions regarding the organization of the whole. Embryos are notoriously resistant to threats of force, and the new Aristotle, like the old, will surely be someone who, like Roux, like Harrison and Spemann, like Holtfreter, understands the living whole embryo sufficiently to deal with it on its own terms. Embryos are creative artists, and, like other artists, they create form. The difficulties that face whoever tries to explain their success have their counterparts in those confronting anyone who tries to account in specific terms for the greatness of any work of art. Knowledge of the molecular constitution of his pigments does not suffice to explain the genius of Leonardo. In embryology as in art, appreciation is probably more effective than atomizing as an introductory approach to the understanding of the genesis of form.

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Relationship between Stimulus and Response

The "shape" of the problem serves to clarify the disparity between graded and quantal response.

S. Loewe

In all sciences concerned with excitable biological systems, the task of quantifying the relationship between the excitatory stimulus and the biological response is complicated by the differences in excitability among the individuals studied. This article (*1*) tries to analyze the problems arising from this complication. As an almost uniquely suited proving ground for the analysis, the field of pharmacology has been chosen. This field is entirely devoted to the study of a chain of events that begins with the pharmacological stimulus, called "dose" (D), and ends with the ultimate response to this stimulus, called "effect" (E).

The practical importance of the carriers of the pharmacological stimulus, the "drugs," has directed the efforts in this field toward an especially ambitious goal—namely, that of arriving ultimately at a single numerical expression of potency (P), the stimulatory strength inherent in a drug. The greater the effect E elicited by a certain dose D, the higher the potency, and the greater the dose required to elicit a certain effect, the lower the potency:

$$P = f(E/D)$$

Hence, the student of potency sets out to measure the quantitative relationship between D and E. Very soon, however, he finds himself at a parting of the ways where one fork is marked "graded-response," the other, "quantal response." The road signs as well as the guidebooks may suggest that the two roads offer him an equal chance. Whether or not this conclusion is correct only a reliable road map will tell. Only a view of the *Gestalt* of the problem (*2*) will provide precise information on how closely akin graded and quantal responses are and on what role either of them plays in determining the dose-effect relationship and potency.

In such an endeavor, one must dispense with all and any procedures of transformation ingeniously introduced for biostatistical purposes—for example, with the use of metameters such as log D, E probit, and logit. Any such metameter (*3*) is a mathematical function of the magnitude "as measured," a function "used in calculations" "because of its convenience" (the quotations are from Gaddum, *4*) as a means of converting curvilinear into rectilinear rela-

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