The biology of the cell surface / by Ernest Everett Just.

Contributors

Just, Ernest Everett, 1883-1941.

Publication/Creation

London : The Technical Press, [1939], ©1939.

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THE BIOLOGY
OF
THE CELL SURFACE

E. E. JUST

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THE CELL SURFACE

By

ERNEST EVERETT JUST

Natur hat weder Kern
Noch Schale,
Alles ist sie mit einemmale—GOETHE

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To the memory of

My Mother
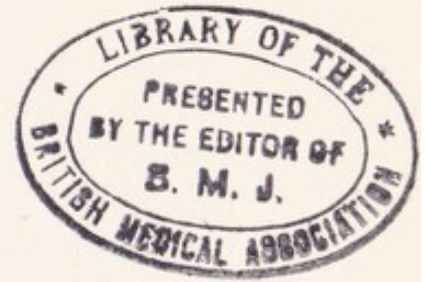
Preface

$\mathcal{W}$HEN I BEGAN THE WRITING OF THIS BOOK, I INTENDED merely to set forth my views on some biological problems which I had studied during a period of more than twenty-five years of research on the eggs of marine animals. I had thought my task easy; that it would be a simple matter to present my ideas and findings against the clearly delineated back-drop of general biological theories and on the basis of well-established fact. Merely to hint at the theories and to indicate briefly the factual evidence would suffice, I thought, to make myself clear to my colleagues. But in thinking thus, I had deluded myself; soon I realized that the back-drop had to be cleared in order to show its contours; that instead of a substantial platform I had only pieces of material that had first to be ordered and put together before they could serve me. This work had to be done, I found, before I had a stage on which I could present my own ideas and let them speak their parts in my interpretation of the drama of life unfolding before our eyes. It thus became imperative for me to examine and to appraise hypothesis and factual evidence and to define first every problem the discussion of which I had projected for my book.

Thus my book developed from a mere statement of my views for biologists engaged in a narrowly restricted field of investigation into a thoroughgoing presentation and discussion of biological problems for a wider audience. To all those who look with interest upon the manifestations of life in animals and in man, who desire to know more, and more

exactly what answers to their questions concerning life biology can give, this book would speak. Biologists in other fields, students in biology and related sciences and all who have general interest, I have endeavored to address in the following pages.

I have the strong feeling that many thoughtful and serious men in engineering, practical physics and chemistry and laymen outside of these professions do not know what modern biology is; even medical men often wonder what this biology of to-day offers. They have genuine interest in animal life and give much reflection to biological questions; yet present day biology leaves them untouched. For such readers text-books offer little. What the professional biologist denominates as biology—taxonomy, palaeontology, ecology, physiology, morphology, embryology, phyto- and zoo-geography, as well as genetics, biometry and the like, with their various subdivisions, each of which is again subdivided, as, for instance, cytology with its separate domains ruled by karyologists, "protoplasmatikers," "Golgiologists" and "mitochondriacs" etc., etc.,—needs to be brought into relation with the world outside biological institutes and recording offices. To the uninitiated this biological regimentation inspires awe because it connotes the abstruse too far removed from everyday life.

Even the most abstract truth needs to be expressed with simplicity and clearness and thus relate itself to everyday human experience. Complexity of expression is often a sign of incomplete knowledge and certainly it is not a *sine qua non* of learning, though there be those who consider profound and erudite that which they can never understand. However cloistered biology may be as a scientific research, as the science of life and having appeal to all men it should make itself articulate beyond its cloistered walls. I have endeavored in writing this book to express myself with such clearness that even the uninitiated

can follow my argument at least in the main. At the same time I bring the reader at once into the arena of conflicting biological thought, for only thus, I think, can he realize the status of the science to-day. I trust that what to biologists are well-known facts I have presented in a manner that elicits their interest.

Together with a definition of the general problems, my own work as well as my ideas are presented. The outcome of these is my theory which I set forth in a more explicit manner than heretofore.

The conception upon which the book is built, though latent in my earlier researches, did not come fully awake until 1930 while I was enjoying the hospitality of the Kaiser-Wilhelm-Institut für Biologie at Berlin-Dahlem. There I fell under the inspiration of Adolf von Harnack's personality. I like to feel that my work was influenced by the rich experience of personal contact with him.

The studies which gave rise to my conception were made during some twenty years, largely at the Marine Biological Laboratory, Woods Hole, Mass.; some few were made at the Zoological Station at Naples. For the support of many of these researches I am indebted to the late Mr. Julius Rosenwald, whose friendship I esteemed. However, this book could not have been finished but for the spontaneous and sympathetic understanding of my work shown by Dr. F. W. Keppel, President of the Carnegie Corporation. A grant from this corporation made possible a year's study necessary to complete the work. In the task of writing a book understandable also to non-biologists, I have further been sustained and encouraged by many friends, biologists, medical men, and others outside of these fields.

The book may be divided into three parts. Part I, comprising Introduction, Life and Experiment, Protoplasmic System, Ectoplasm, General Properties of the Ectoplasm and Water, though dealing primarily with the animal egg,

embodies principles which concern the fundamental organization of any living thing. Part II, including the Fertilization-process, the Fertilization-reaction, Parthenogenesis, Cell-division and Cleavage and Differentiation, discusses in particular the problems that refer directly to animal eggs in their earliest stages of development. Part III, embracing chapters on the Chromosomes and Ectoplasm, Ectoplasm and Evolution, and Conclusion, has to do with more or less theoretical discussions. Throughout the whole treatment, the principle that the cell is the biological unit is kept in mind. In particular, structure and function of the ectoplasm are emphasized; upon these my theory of the state of being alive is in large measure grounded.

Concerning the literature cited, I should point out that I have made no attempt to refer to all of the many original papers which I have studied and abstracted. Instead, with each revision of the manuscript I have reduced the number of titles in the bibliography. Nevertheless, the list of titles retained is ample for the argument set forth.

<div align="right">E. E. J.</div>

PARIS, FRANCE,
November, 1938.

Contents

Introduction

$\mathcal{T}$HE REALM OF LIVING THINGS BEING A PART OF NATURE
is contiguous to the non-living world. Living things have
material composition, are made up finally of units, mole-
cules, atoms, and electrons, as surely as any non-living
matter. Like all forms in nature they have chemical struc-
ture and physical properties, are physico-chemical systems.
As such they obey the laws of physics and chemistry.
Would one deny this fact, one would thereby deny the
possibility of any scientific investigation of living things.
No matter what beliefs we entertain, the noblest and purest,
concerning life as something apart from physical and chemi-
cal phenomena, we can not with the mental equipment
which we now possess reach any estimate of living things as
apart from the remainder of the physico-chemical world.

But although any living thing, being matter, is a physico-
chemical system, it differs from matter which constitutes
the non-living. This difference exists and would continue
to exist were some chemist at this moment to succeed to
synthesize out of non-living matter a living thing. The
analysis of living things reveals that they are composed of
no peculiar chemical elements—instead, they are made up
of those most commonly occurring. The difference can not
then be attributed to the elements. To be sure, certain
complex compounds, as proteins, carbohydrates and lipins
(fats and fat-like substances)—themselves compounded
mainly of the commonly occurring elements, carbon, hydro-
gen and oxygen, and never of rare elements—are peculiar

to living matter; but the synthesis of protein-like bodies, of sugar and of fat as well as the synthesis of thyroxin (a compound in the internal secretion of the thyroid gland), of products of other internal secretions and of vitamins must dissipate whatever belief may have lingered on since Wöhler's classic synthesis of urea (more than a hundred years ago) that some unknown vital principle sets apart the chemistry of living things from that of non-living.

And yet there is a difference which expresses itself in the chemical make-up of the living thing. It is its organiza-tion.[1] The difference with respect to chemistry thus lies in the peculiar combination of compounds which together make a heterogeneous system. This acts as a unit-struc-ture, whose behavior or manifestations are those of a single thing and not the sum-total of the multitudinous chemical components in an agglomerate mass.

Living matter has an organization peculiar to itself. Nowhere except in the living world does matter exhibit this organization. Life, even in the simplest animal or plant, so far as we know, never exists apart from it. Resting above and conditioned by non-living matter, life perhaps arose through the chance combination of the compounds which compose it. But who knows? A living thing is not only structure but structure in motion. As static, it reveals the superlative combination of compounds of matter; as a moving event, it presents the most intricate time-pattern in nature. Life is exquisitely a time-thing, like music. And beyond the plane of life, out of infinite time may have come that harmony of motion which endowed the combination of compounds with life.

Clearly then, the state of being alive reposing in com-binations, in the order in which the constituents are

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[1] *Dujardin, 1835; Brücke, 1861; Allman, 1879; Verworn, 1899; Moore, 1921.*

assembled both in space and in time, its investigation is limited. The direct analysis of the state of being alive must never go below the order of organization which characterizes life; it must confine itself to the combination of compounds in the life-unit, never descending to single compounds and, therefore, certainly never below these. Whereas physical science has to do finally with ultimate units of matter, the scope of biological research embraces the behavior of more heterogeneous combinations of these units. The physicist aims at the least, the indivisible, particle of matter. The study of the state of being alive is confined to that organization which is peculiar to it. It may be that life can never be written as a formula because it may be a physics and chemistry in a new dimension which, though superimposed upon the now known physics and chemistry, lies in an infinity which the human mind can not ever embrace—as a tone which theoretically we know exists but which the human ear can never hear. But be this as it may, life as an event lies in a combination of chemical stuffs exhibiting physical properties; and it is in this combination, i.e., its behavior and activities, and in it alone that we can seek life. A living thing represents in its unit of structure and behavior the highest order of complexity in nature. All this implies that the method employed in the investigation of a living thing can not be identical with that used in physical sciences.

With right we glorify refined and precise measurements—great accomplishments in the physical sciences rest upon them. The desire to extend their use to embrace the science of living things is understandable; nevertheless, quantitative measurements can never be used in biology to the extent that they are used in physics.

To be sure biology also employs measurements. He who studies the living animal or plant often has recourse, most of the time entirely unconsciously, to the estimation of

weight and size. The classification of living things, the earliest scientific treatment of life, relies upon measurements. The anatomist, be he interested in bones or in chromosomes, reckons size and number. These kinds of simple and direct measuring have a definite place in biology. Similarly have those relating to the functions of the living thing. No one questions the value of measurement for the analysis of the gases in human blood. He who would be foolhardy enough to deny the dependence of biology upon physics and chemistry would do well to ponder the history of the research on animal respiration, beginning with Lavoisier, whose work stands as a new starting point for both chemistry and biology. The final proof that the blood carries most of its contained oxygen in chemical rather than in physical union constitutes one of the most brilliant chapters in biology to read which quickens the pulse. The measurements on temperature, on pressure, of electrical conditions, of light, of the chemical breaking down of complex foods and the building up of these, the photosynthetic process in green plants without which human life would become immediately impossible—these and many others show that biology comprises a welter of physico-chemical measurements, a fact to occasion no astonishment since living things are physico-chemical entities.

The measurements employed in physical science which do not apply to living things concern states of matter below that level of organization which characterizes a living thing. True, matter in the living state as all matter is compounded of electrons. The high and enviable excellence of modern physics rests upon the beautiful and enthralling analysis of the ultimate unit-structure of the least particle of which all nature is composed. However, the fact that life as far as we know exists as a composite, and only as such, renders pure physical analysis, i.e., into electrons, inapplicable to the state of being alive. The fact that life dissolves

even when it is resolved only into compounds means, in the present state of our knowledge of electrons, an insuperable obstacle to an analysis of life into electrons. This would still be true were we suddenly to discover a peculiar compound which we could define as the compound of the state —or event—of being alive. As matter, a living thing may be analyzed with the means utilized in the resolution of non-living matter; but the moment we end the living state we render impossible any direct analysis of life. Clearly: if life exists only in a super-compound-state, contingent upon aggregation, analysis of its compounds being useless, analysis of molecular and atomic structure becomes equally futile. A quantitative biology that does not recognize this fact is doomed to failure.

Without needlessly elaborating, I do nevertheless wish to make myself understood. It would be shamefully unfair and ungrateful of any biologist even to appear to discount the researches in chemistry upon which modern biology so largely rests. The greatest biological investigator of our time, Pasteur, was a chemist. His work indicates the extent to which chemistry may carry biology. The work of Emil Fischer, of Albrecht Kossel and of a host of other chemists has fructified and fortified biology. Still the fact remains: the exact analysis of the compounds which comprise a living thing is only analysis of the compounds and by destroying life, such analysis fails to reveal the secret, the goal of all biology, the answer to the question, what is life?

Here one may interpose a question, an inquiry concerning the postulated life-molecule.[1] In earlier times one thought of life as reposing in a peculiar molecule, as the biogen-molecule. Chemical researches have however so far failed to reveal the life-molecule. Instead they show that living

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[1] *See Hopkins, 1912. See also, Minot, 1896.*

matter is composed of proteins, carbohydrates and lipins together with water, electrolytes and gases. It would be necessary, unless life resides only in protein, and this has not yet been proved, to obtain evidence to indicate that all these constituents named as components of living matter in some way combine as a single molecule. Further, with respect to proteins, carbohydrates and fats, we have no sufficient ground to warrant the conclusion that they exist in living matter in the same form which they have when they are isolated. This is especially true of the proteins; all of them known to us outside the living substance are probably changed in their nature chemically and physically by the methods of isolation. In the chemist's test-tube the organic constituents of the living substance may represent merely end-products of something in the living. In this, however, lies no reason for supporting the theory of a biogen-molecule; rather should we try to refine our methods of isolation, so that the results allow a more direct conclusion. If one says that the biogen-molecule depends upon a certain milieu—if, for example, being protein-like it depends upon fat and fat-like substances, sugars, various electrolytes and water—we need the postulate that the life-molecule is totally unlike molecules with which physical science has to do. I have no wish to quarrel with words; either the term molecule used in the expression, life-molecule, carries the connotation ordinarily assigned it by physical scientists or it embodies a non-physical conception. As a concept consonant with physical science it stands open to serious doubt; as a non-physical postulate it defeats its own purpose.

What is said of the biogen-molecule may be said of the gene-molecule, the hypothetical unit of which the chromosomes are assumed to be built. Like the former it too appears incapable of self-maintenance—it grows by what it feeds upon, the cellular matrix in which it lies; and more, never stands apart from other genes in the same chromo-

some. Since, according to the gene-theory, it represents in the body of the organism in which it exists a character which another gene (or genes) in another chromosome (or other chromosomes) may represent, it appears to act not singly but as part of the chromosome-structure and of the whole chromosome-garniture. We know that life exists in size below that recognizable by the microscope; but we possess no proof that life consists of a single gene. Much has been said recently of the size of the gene; but we should recall that size does not condition molecularity. Molecules vary in size. In addition, many investigators have become cautious in speaking of the gene as a molecule, preferring the idea that it may be several molecules.

The most potent objection to the gene-molecule- (or molecules) concept as the unit of life lies in the fact that the gene-theory fails to explain how a single cell, the egg, becomes a complex animal. A detailed discussion of this point is given beyond. Here it suffices to say that a particle (or all the particles together) which is only in part engaged in the unfolding of life during its course from egg to adult can not constitute life itself.[1] The conception of a hypothetical life-molecule is barren and indicates again the limitation of the quantitative method of analyzing life below the level where life is. The investigator of the living state can and must use physics and chemistry since the living state is a zone in nature and his method of investigation can parallel that of the physical scientist inasmuch as he finally comes to the unit-organization of life. Below this he can not go, for life is the harmonious organization of events, the resultant of a communion of structures and reactions.

In general, the organization of living matter, that is, of protoplasm, appears as consisting of two components, a nuclear and a cytoplasmic. Although most often these

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[1] *But cf. Jennings, 1936.*

are set off as two distinct regions, as a sphere (nucleus) within a sphere (cytoplasm), this sharp differentiation is not invariable. For several reasons, as will be shown beyond, much of modern biological investigation has centered upon the nuclear component as though it were indeed the kernel of life. Not only has the cytoplasmic component been relatively neglected but also have those protoplasmic systems which lack sharply defined and set-off nuclei received scant attention, although a bacterium whose protoplasmic organization fails to show a discrete nucleus is a living system. Because of the rapid rise of genetics, hegemony in the protoplasmic organization has been ascribed to the chromosomal structure of the nucleus and the cytoplasm has been subordinated as though it be a mere protective and nutritive shell. It is no part of the purpose of this book to minimize the achievements of genetics and the investigations on chromosome-structure, all outgrowths of descriptive studies on protoplasmic organization. Instead, inasmuch as life, as we know it so far, resides in the whole system, the pages which follow aim to show how far life-processes are related to the dual and reciprocal components, nuclear and cytoplasmic structure.

Investigations on cytoplasm have had largely to do with the formed bodies, the so-called cytoplasmic inclusions; only to a very small degree have they dealt with the cytoplasm itself and its differentiation into an inner and an outer region, endoplasm and ectoplasm. Only in a general way has the rôle of the ectoplasm, the surface cytoplasm, been hinted at; thoroughgoing descriptions of it, comparable to those of the nucleus, have not been made, doubtless because of the greater difficulties of observing it. None the less should the ectoplasm, as part of the protoplasmic system, be assigned a rôle in vital manifestations. An examination of its properties and behavior in life-processes

is all the more imperative because it has been so grossly neglected.

The play of environmental forces demands the continuous adjustment, balance and discrimination of the living thing. How this self-regulation comes about constitutes a great problem in biology. Not less capable is the power of self-differentiation exhibited by the living system. Against and with the outside world, repelling and responding, it makes itself anew, exhibits a series of events in sequence and order ever the same. Self-regulation and self-differentiation are fundamental expressions of the organization of living matter.

As an integral part of the protoplasmic structure the ectoplasm can not be divorced from this. The protoplasmic organization however much a composite of many parts—nucleus with its structures and cytoplasm with its regions—acts as a unit. To conceive it as such is to approach an understanding of those series of moving events that we call life. The ectoplasm, standing between the protoplasmic system's inner substance and the outside world, reacts first to environmental stimuli and thus conditions the responses of the whole system. Its rapidly occurring, highly active structural changes portray self-regulation and self-differentiation. Thus by its location and by its peculiar attributes, the ectoplasm becomes the most tangible expression of life-processes.

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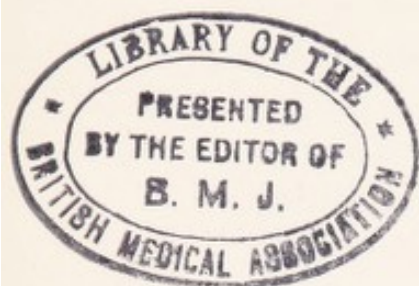
Life and Experiment

THE METHOD EMPLOYED IN THE INVESTIGATION OF THE living thing, the foregoing statements clearly indicate, must be different from that in physical sciences: the nature of the thing investigated determines the method of inquiry. Description is the method of most use in biology. It plays a larger rôle here than in physics not because experimental biology is a younger science than physics, as one often avers, but because of the heterogeneity and complexity of the composite life-unit. The more heterogeneous and complex an object, its parts undergoing multitudinous kaleidoscopic changes, the more important description becomes. And when this object is minute, its changes evanescent, accurate description is imperative. By description the objects may be defined and the changes recorded in more and more closely set stages.

This statement, however, by no means implies that experiment has no place in the study of the state of being alive. Both description and experiment are utilized by all the natural sciences—the extent to which each is used being determined by the state of the matter investigated. Just as the complex organization of living matter demands the larger employment of description, so it prescribes experimental method. The present chapter aims to evaluate the place of experiment in the study of a living thing in order to clear the ground for the discussion of the problems with which this book deals. Thus I present my conception of the rôle of experiment in

biology, a conception which resulted from years of experience and which by determining more and more my mode of experimenting had a considerable influence on the formation of my theory of the state of being alive. In order to set my conception off from that maintained by a substantial number of biologists, I begin by defining their point of view.

I refer to the so-called "physico-chemical" school of biology. Specific example is often the best of definitions. I could give many examples from the writings of the "physico-chemical" biologists to define their position; one will suffice. Says Loeb[1]:

> The physical researches of the last ten years have put the atomistic theory of matter and electricity on a definite and in all probability permanent basis. We know the exact number of molecules in a given mass of any substance whose molecular weight is known to us, and we know the exact charge of a single electron. This permits us to state as the ultimate aim of the physical sciences the visualization of all phenomena in terms of groupings and displacements of ultimate particles, and since there is no discontinuity between the matter constituting the living and non-living world the goal of biology can be expressed in the same way.

It is often said that in their thinking biologists are the most mechanistic of scientists, holding fast to concepts that the most mechanistic astronomers even have abandoned. Indeed, many biologists besides Loeb believed—and many even now believe—that biology as a science must finally be concerned with the ultimate particles, that life as well as all physical phenomena can be interpreted on the mechanistic basis of the motion of particles in a system that is rigidly ordered as to time and space. But so far any attempt at "the visualization of all phenomena in terms of groupings and displacements of ultimate particles" has

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[1] *Loeb, 1916.*

failed for biology. And it is to be doubted that any such "visualization" can ever succeed. We do not know that there is no discontinuity between the non-living and the living world and we certainly possess no evidence for the postulate that living phenomena can be expressed in "terms of groupings and displacements of ultimate particles."

Nowadays, even for the physicist, Loeb's statement is too extreme. The evidence of physics does not yet permit such a view as to the finality of its concepts. In addition, before physical principles can be utilized profitably in biology, they must be sharply defined and accepted by physicists themselves.[1] Moreover, even before the advent of the relativity- and the quantum-theory, physicists were not agreed that mechanics constitutes all of physics. Here a statement written forty years ago by the biologist Whitman[2] is apt:

> While biology is certainly indebted to physics for some of its metaphysics, it is to the credit of physics to have made it clear that mechanism, indispensable as are its methods, affords no fundamental explanation of anything. As Karl Pearson has so well said, the mystery of life is "no less or no greater because a dance of organic corpuscles is at bottom a dance of inorganic atoms." What dances and why it dances is not explained by reducing size to the lowest limit of divisibility, and just as little by the assumption of ultra-physical causes. This is no criticism—no disparagement; it is only a confession of ignorance. The ultimate mystery is beyond reach of both mechanism and vitalism; let pretension be dropped, and approximation to truth be closer on both sides.

Further, as we have seen in the preceding chapter, our investigations of the living thing as such end with its disintegration. The moment that any such far-going physico-

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[1] *Watson, 1931.*
[2] *Whitman, 1895.*

chemical analysis, as that postulated by Loeb, is applied, the living thing disappears and only a mere agglomerate of parts remains. The better this analysis proceeds and the greater its yield, the more completely does life vanish from the investigated living matter. The state of being alive is like a snowflake on a window-pane which disappears under the warm touch of an inquisitive child.

This objection to the so-called physico-chemical point of view—i.e., that the goal of biology is the reduction of living matter to ultimate particles—is far more potent than those mentioned before. Certainly, it would still remain were the physicists able to state their concepts of the ultimate particles of matter in final terms. Though compounded of the same elements found elsewhere in nature and showing physical properties because of this composition, though amenable to physico-chemical laws, the living thing is refractory to analysis into ultimate particles.

Here Heisenberg may be quoted to show how we can conceive the particular realm of living things as part of the natural world and the natural sciences:

> If for instance one thinks of the problems which are connected with the existence of living organisms, one will suppose, from the point of view of modern physics, that the powers which act in the organisms limit themselves in a like manner that is rationally exactly perceptible, from the purely physical laws, as, for example, thermodynamics limits itself from classic mechanics. . . . The building of natural science therefore most probably can not become a continuous unit, so that simply by following the prescribed way one can come from one point in it to all the other rooms of the building. Rather, the building is made up of single parts, each of which though standing in manifold relations to the others, nevertheless is a unit that is in itself complete. The step from already completed parts of the building to a newly discovered one or to one to be constructed demands always a mental action which can not be performed simply by developing farther that which already exists.[1]

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[1] *Heisenberg, 1934.*

The physico-chemical biologists, however, do not picture natural science as Heisenberg does. Rather, they visualize the problems of the living organism as occupying a room in a single apartment of the building of physics, that of mechanics. Their view of physics, far more restricted than that of physicists themselves, has therefore obfuscated both the methodology and the philosophy of biology. This is shown by their loose usage of the terms, mechanism, mechanical, mechanistic and even machine, as though these be interchangeable.

In biology the term, mechanistic, is used as the antipode of vitalistic. Since practise has legitimatized this usage, there may be little reason to quarrel with it. Nevertheless I wish to point out that the physico-chemical school of biologists has erred in elevating the term, mechanistic, beyond the meaning assigned it by physicists. By their own doctrine the term should have the connotation assigned it by physics. The true antipode of mechanistic is non-mechanistic. The term, non-mechanistic, by no means implies vitalism. Not every physicist who opposes the mechanistic conception deems it necessary to support a non-physical, super-natural concept. Rather, he holds that the behavior of the ultimate particles of matter is not rigidly determined, perfectly predictable. Logically, those biologists who conceive vital processes as phenomena to be interpreted by physics, should adhere to concepts of physics. The physico-chemical biology should take cognizance of the fact that physics has grown beyond "classical physics."

Physico-chemical analysis into ultimate particles and the hypotheses derived from such work establish the fact of the existence of similarities between living and non-living. By virtue of its peculiar organization in space as well as in time, however, the living thing occupies a level in the natural world above that of chemical compounds. From this organization spring those characteristics by which we

commonly distinguish a living thing from a non-living; both the organization and these characteristics should claim more attention from those—be they biologists, mathematicians, physicists or chemists—who study the living state, than they have up to now received. Having agreed that there exists no chemistry peculiar to living things and that physical properties are possessed by the living and by the non-living as well, we have remaining the task of evaluating the differences.

It is not implied that only similarities have been studied and never the differences of the two regions. Nevertheless, those differences which set apart a living thing from a non-living should be studied more extensively as such. I can not see how they can be investigated by physico-chemical methods in the sense of Loeb, that is, by resolution into ultimate particles, by methods suitable for pure compounds used in the chemist's laboratory, or by any other that does not maintain the integrity of the living state. Biology should develop its theories by a method of work adapted to the peculiarities of the living thing and therefore quite distinct from those used in pure physics and pure chemistry. This statement does not imply that we should discard entirely for biology the use of physical and chemical means. Surely, no one would set himself against the use of the microscope or any other most refined apparatus, and of reagents, drugs, dyes, etc., the common equipment in the study of the living thing. Biologists count, measure and weigh and seek to detect cause and effect. But whatever means we employ should be adapted to that particular level which the living thing occupies in the natural world.

In an investigation which aims to explain the state of being alive, the first prerequisite is the appreciation of the limits which circumscribe this state. In the utilization of physico-chemical means, then, we need to recognize the extent to which we change the living state; and, if we go

beyond it, we must realize this fact. For this reason, the most definite knowledge of the manifestations of the normal living state becomes necessary. Experiments in biology, then, fall into three categories:

1. Experiments on non-living systems.
2. Experiments on killed living systems.
3. Experiments on living systems.

Number 1 includes experiments that furnish comparisons between living and non-living systems and that elucidate the result of cellular activity which expresses itself in secretion and excretion. Here belong physical models and artificial systems, as for example, semi-permeable membranes, suspensions of soap in water—used to imitate cleavage-patterns of the egg—colloidal solutions, passive iron wire—employed to illustrate nerve-conduction—etc. Also, here belong chemical analyses of substances that are produced by the living cell, as for example, hormones and digestive ferments and excretions, as urine, etc.

Number 2 includes experiments on the chemical composition of living matter, killed in the process of analysis. Though not experimental in the strict sense of the word, all histological and cytological studies on fixed tissues and cells are included in this category. When properly used, fixation gives a faithful picture of the living and as such has great value for the study of it.

Number 3 embraces experiments on the living system. These are of two kinds: those in which the normality remains unchanged and those in which it is altered. By means of the former, valuable data are collected by which indicia and criteria are established for supplementing and extending as well as interpreting observations and descriptions of vital processes such as respiration, etc. Such experiments are also of incalculable value for medicine. By the second type of experiments in this category we often succeed in making evident the rôles of various factors

by comparing their behavior in the altered living with that in the normal.

This review makes it at once clear that experimenting in biology requires not only knowledge of physics and chemistry but also, and in no less degree, that of the normal living organism, the fundamental object of biological investigation. The following discussion centers around experiments on eggs. However, it applies also to work on other types of cells.

The investigator must possess familiarity with the biological system whose investigation he undertakes. The chemist demands pure standardized materials for his work and the physicist is at pains to be sure that his apparatus always performs perfectly; neither is satisfied with experiments contaminated by sources of error that can be eliminated. Just because of the reason that biological systems are not to be compared to "chemically pure" substances and that their performance is not always one hundred per cent. perfect, the condition of the living thing studied should be as fully known as possible. However, a chemist who will use only purest chemicals will often in biological work use cells or organisms in poor or even moribund condition; the physicist who in the physical laboratory demands perfection in apparatus, frequently is content with whatever cell or organism given him for biological research, no matter how it behaves. Unfortunately, it is all too true of many biologists, even of many old-fashioned ones, that they investigate living systems which are not in optimum condition. In the haste to make experiments, many find no time to learn the optimum condition of the system under study. Some do not care and are content to report results that vary from day to day, while others would not be able to distinguish a really normal egg or other living structure from an abnormal one. Indeed, it would seem by the manner in which many

treat the organisms on which they work, "material" as they call them, that they prefer their living systems in a debased and degraded condition.

Surely, of all biologists the investigator who experiments on eggs needs to exercise most scrupulous care in order to be sure that the cells under investigation are in optimum condition. In the investigation of a system of organs, an organ or even a tissue, like nerve or muscle, although one can never be absolutely certain that all cells present are normal, the high number of cells that compose such a structure guarantees a high number of normally reacting cells and thus increases the probability of a normal response. In the case of eggs, one deals with structures which are single units, individuals that show each its own state of normality or abnormality. Furthermore, whilst it is true that out of each egg arise cell-complexes—tissues, organs, and organ-systems—these differ so much in degree of activity, in time of origin and in relation to each other in space, that the determination of the normality of the developing egg, made up at a given stage of a number of cells equivalent to that in a given tissue with which comparison is made, is more difficult than in this tissue. The developing egg is normal only if all these different complexes present are normal. But this necessity of careful investigation of the grade of normality in eggs has one important compensation which makes the study of eggs so fruitful for biology. The behavior in laboratory experiments of tissues, e.g., nerve, muscle, or tissues in culture, removed from an organism may not at all be the same as that in the intact organism. That countless investigators have obtained the same laboratory results speaks on the one side for the excellence of laboratory procedures, on the other for the wonderful capacity of tissues removed from an animal's body to withstand experimental treat-

ment. Whilst the doubt as to the identity in behavior of excised and of intact tissues does not deny the value of studies on the former for laboratory exercises, it should be borne in mind that only rarely and with great difficulty can these tissues be experimented upon *in situ* (in their normal surroundings). There is doubt that results from experiments made on tissues *in vitro* (excised) could be obtained from such experiments made on tissues *in situ*. With the egg the situation is different. We can speak with more certainty concerning its normality for in many cases nature gives us the opportunity easily to study it "*in situ.*" This advantage of a study *in situ* is also offered by many protozoan cells. The control of normality in them is even less difficult than in eggs, because they lack, even those with most complex life-history, the potential diversity of the egg-cell realized as development unfolds; the protozoan cell reduplicates itself only. This difference, however, though showing the greater difficulty in the study of eggs, indicates the greater possibilities of such study. Valuable as studies on Protozoa doubtless are for both biology and medicine, they lack direct bearing on the grand problem of biology, how out of a single egg arises the complex multi-cellular organism. Only in the egg and its development can we hope to trace to its source the pattern of structure, and to resolve into its motif the harmonious behavior which characterizes the many-celled animals.

If the condition of the eggs is not taken into account, the results obtained by the use of sub-normal eggs in experiments may be due wholly or in part to the poor physiological condition of the eggs. Thus, the failure of sea-urchin's eggs that are freed of their jelly, fully to separate their vitelline membrane after fertilization, as they normally do, does not mean that the experimental removal of jelly renders membrane-separation impossible but only that the

eggs are in a bad condition brought about by the injurious action of the agent employed to remove the jelly.[1] The physiological condition of all eggs known to me can be impaired by exposure to low temperatures. Indeed, since low temperature (like high) is an experimental means, to experiment on eggs from animals which have been kept in the ice-chest in order to delay shedding, is equivalent to compounding experimental procedures whose effects may be complementary or antagonistic. Thus, I would never think of exposing eggs of *Platynereis*, for example, which had been kept over night in the cold room (at 5°C.) to ultraviolet light because the low temperature alone gives the effect of polyploidy to obtain which I use the ultra-violet radiation.[2]

From a series of experiments made on the capacity of a sea-urchin's eggs to develop without spermatozoa, it was concluded that development can be caused by immersing the eggs in sea-water which had been charged with a substance liberated by others of the same species. This conclusion was unwarranted because, as I found, the results obtained are solely due to evaporation of the sea-water and not to the presence of substances originating from the eggs.[3] Another series of experiments was used to prove that this subtance liberated by the eggs and held to be the cause of their development, had been isolated. But I found that sea-water treated by one or by all of the reagents employed for precipitating the alleged substance was if anything more effective for causing development than sea-water containing the egg-substance. Thus the development was due not to the isolated (precipitated) sub-

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[1] *For discussion of this subject, see Just, 1928c. Derbès, 1847 knew not only that jelly surrounds the sea-urchin's egg but also that its absence does not impair the egg's development.*

[2] *Just, 1929e.*

[3] *Just, 1928a.*

stance liberated by the eggs, but to increased salinity of the sea-water.[1]

Few authors who quote Wilson's famous experiments on the eggs of the marine mollusc, *Patella*, I think, realize that Wilson never succeeded in obtaining normal fertilization.[2] It is not improbable that the results he obtained, upon which he based far-reaching conclusions which have been generally accepted, were in part due to the abnormality invoked by the artificial aid with which he induced fertilization. To induce cross-fertilization (fertilization of eggs with non-specific spermatozoa) often one has recourse to artificial aid, as changes in alkalinity or temperature of the sea-water. Such changes however bring about variations in the development of the egg (for instance of the sea-urchin) also if these develop from straight fertilization (fertilization of eggs with specific spermatozoa). This fact makes it clear that results obtained after cross-fertilization can not be ascribed to the influence of the non-specific spermatozoon or its chromosomes unless it is shown that the effect differs from that obtained in straight fertilized eggs that were subjected to the same changes in the medium by which the cross-fertilization was induced. Since the experiments on cross-fertilization that have been made were not controlled in this way, they allow no conclusion as to the action of the foreign sperm-nucleus.[3]

Even under constant external conditions the sea-urchin's larval skeleton will vary, as most exact study of normal straight fertilized eggs has shown.[4] Thus the extent to

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[1] *Just, 1929d.*

[2] *Wilson, 1904.*

[3] *It must therefore astonish us that Morgan (1932) not only accepts these experiments on cross-fertilization but also in a wide-sweeping statement categorically declares them to be proof for action of the genes on the cytoplasm.*

[4] *Tennent, 1910.*

which eggs in optimum physiological condition vary must also be known in order that differences discovered can be truly attributed to the experimental means.

These few examples show how careful we must be in drawing conclusions from experiments in biology. The highly irritable systems that we investigate demand a most exact control of the experimental factors. If only one of these is left aside in our evaluation of the obtained results, our conclusion may be wholly erroneous, especially if the factor induced a change at the beginning of the treatment. We must know whether we have before us normal or already changed, abnormal, eggs. We must control the effect of our means, even of those that are only aids, by following their effect singly before we use them together in one experimental setting. And above all must we be wholly familiar with the normal condition and normal development of the eggs we use, since only on this basis are we able to recognize abnormalities and thus the effect or effects of our experimental treatments.

The simplest and most generally adopted criterion for the eggs' normality lies in estimating the number of them that develop; one hundred per cent. development means optimum condition. Eggs that are laid already fertilized, and especially those in brood pouches or in capsules almost always develop normally. Also where the eggs and spermatozoa are deposited and fertilization ensues in the laboratory as in nature, the chances for normal development are enhanced. It is with those eggs whose deposition is induced by the experimenter and which are artificially inseminated and especially with those that are removed from the ovaries that most difficulties are encountered. In many cases such eggs develop, if at all, only in small numbers. They are only useful for studies on development if the cause for the low numbers of development can be discovered and the percentage increased. There are cases,

however, where eggs removed from the animal yield one hundred per cent. development. Here it is necessary to know that the quality of development parallels that of eggs normally laid under natural conditions. By comparing the development of eggs in their normal environment, of those naturally shed in the laboratory and of those removed from the animal, one can decide concerning the normality of the last named.

In rare cases, where always some immature eggs incapable of development are present, one may never obtain large numbers of developing eggs, yet those that develop are normal. Similarly, at the end of a breeding season animals frequently give, among those eggs that develop perfectly, some that fail to develop because they have passed the time of optimum condition for fertilization. For such eggs we can not use the criterion of the percentage of development to detect their condition; they can only then be considered to be wholly normal, if their development is normal at every stage.

Now the necessity of following the egg throughout its complete development can be obviated. I was able to establish definite criteria and simple physiological indicia of the optimum condition of eggs. These signs tell us within the first minutes after fertilization whether the eggs are in optimum condition and even indicate whether the eggs will be normal throughout their development. The signs inhere in the reactions following the mixing of eggs and spermatozoa and are in a measure as specific as the given gametes themselves. For any given egg, they appear differently when the egg is abnormal. For the eggs of a common sea-urchin, *Arbacia*, I found that their optimum condition, whether they are normally shed, induced to be shed by artificial means, or removed from the ovaries, can be determined within three minutes after insemination by the rate and quality of membrane-separation; by the

rapidity with which unfertilized eggs separate fully their membranes while in distilled water; by the rate at which fertilized eggs form extra-ovates when in hypotonic sea-water; and by the failure of heavily inseminated eggs to show polyspermy. Whenever these initial reactions are qualitatively poor, the development of the eggs is below normal.[1] These criteria I find hold for three other species of sea-urchins at Naples.[2] Also, for the egg of *Nereis* the quality of the striking ectoplasmic changes subsequent to fertilization constitutes an index of the condition of the egg and the quality of its later development.[3]

Further I found criteria which in later stages of development indicate whether this will be normal in its whole course. For example, in eggs of *Nereis* and *Platynereis*, the behavior of the oil drops is a reliable index of normality; one may be sure that swimming forms which possess one oil-drop and only one in each of the four gut cells can develop through metamorphosis to the adult stage and that no other will.[4] Doubtless other criteria can be found for every stage of the development. Every one such will prove valuable in extending our knowledge as to what is a good egg. In all cases however, where such criteria are not known, the whole course of development must be always followed before it can be said that development is normal.

It is clear that the condition of eggs largely depends upon that of the animals from which they come. Eggs normally shed from animals in a good state are preferable to those shed by weak animals and by animals in abnormal conditions. In every experiment comparison with and control by the normal, untreated cell or organism is obviously absolutely necessary. The quality of the

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[1] *Just, 1928c.*
[2] *Just, 1929c.*
[3] *Just, 1915a.*
[4] *Just, 1922g.*

investigated system determines the experimental results to such a high degree that the value of the investigation depends upon recognizing and appreciating this quality of the object.

The most exact knowledge of the normal form and form-changes of the living thing to be investigated is thus the prerequisite for present-day attack of biological problems. On the strengthening of our knowledge of these rests all progress of modern biological research, no matter how grandly physical, chemical, or mathematical it is.

For a long time to come biology will need accurate description and exact observation. The necessity for confirming the classic and exact studies still remains; it is imperative that these be extended. The demand for filling in gaps persists. Where minute details are wanting, they must be supplied. Wherever uncertainty or doubt obtrudes concerning a descriptive datum, this should as far as possible be removed. However much we desire quantitative instead of qualitative studies in biology, however much more we estimate elaborate experimental studies involving knowledge and skill in the use of physics and chemistry and mathematics, however much we yearn to place biology in the same category with the more exact sciences, we can not abandon purely descriptive work. I do not mean, I repeat, that biology stands irreconcilably apart from the other natural sciences. But I see no omen to indicate that all biological phenomena are capable of quantitative treatment.[1] By chance to-morrow or it may be in the very instant of this writing by some great discovery made in total ignorance of the morphological substratum of biology, someone might be able to appreciate the secret of life in its entirety. But it is just as likely that this biological millenium may never come. And I for my

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[1] *Cf. Mellor, 1922, on the use of mathematics in science.*

part believe that fully to embrace this faith in a chance discovery would anaesthetize activity and the will to seek, while life lasts, the mystery of life.

That we are far from having completely exhausted the possibilities of description in biology is clearly shown by the fact that no one of the grand phenomena treated in this book has been adequately defined by description that fulfils our demands for exactness.

My own observations having early taught me how rapidly changes ensue in the living egg, I have studied stages in development not of minutes' but of seconds' duration. Microscopy by extending the range of our vision increases our powers of observation. But even if there should be revealed to us the ultimate space-pattern, there would still remain the problem of the changes of this pattern in time; a gap between the beginning and the end of an event would persist. Just as with the aid of the various kinds of microscopes[1] we uncover the minutest space, so must we register most minute changes in time: we must clock the fleeting changes whose sum total is the living thing.

Studies of forms and form-changes must continue as long as there are organisms and their processes, eggs and their development, still unexplored. Since not all work ever done in this field is wholly dependable and perfect, there is all the more reason why the modern student experimenting on eggs must acquaint himself directly with the pattern which he aims to explain. Naturally, in that earlier period in the history of the study of eggs the new findings, as fertilization, chromosome behavior, etc., excited and stimulated the discoverers to exuberant expressions that were often in error because they came from work which though honestly well done was not always sufficient to justify the conclusions drawn from it. Work like that of Boveri which

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[1] *Note the recently invented electron-supermicroscope.*

has given a great impetus to biology, but which nevertheless does not satisfy our demand for exactness and completeness, should most certainly be repeated on a more extensive scale.

Even to-day exactness is often not carried as far as we should demand. Above I gave examples of incompletely controlled experimental work. Where living cells are treated statistically, the larger the number used, the more valuable are the conclusions drawn. Few workers would care to publish results like some of Morgan's who in eleven experiments had 1, 10, 4, 8, 8, 3, 3, 1, 2, 2, 4 eggs, and in other experiments had no eggs, yet spoke of percentage of development.[1]

Certainly, modern technique offers its advantageous apparatus also to biology for increasing exactness in observation and experiment. These modern achievements on the other hand have served in devaluating morphological knowledge, and impressed by technical progress many an investigator regards experimentation as a virtue in itself, a reward of its own.

Experiment for experiment's sake, ever a dangerous philosophy, becomes exceedingly baneful for biology. True, through it valuable knowledge may be gained for mapping out unknown terrain. On the other hand, by it the accumulation of data may become bewildering because it relates to topographical details impossible of reduction on a scale of value for other explorers. Moreover, such refined plotting of points often has no relation to the whole field—indeed, often the field as such is lost sight of and only unrelated minutiae remain. The main purpose of an experiment in biology should be the explanation of the naturally occurring phenomena. Here we encounter a difficulty not met with in physical science.

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[1] *Morgan, 1905.*

Animal cells are ready-made. We do not devise them; nor can we have them according to specifications which we ourselves set up. If, as some do, we regard living cells as machines, we appreciate that they are ready-made; we do not make them as physicists make their apparatus to prove a theory or test an hypothesis. Still less do we know them and their variables. We do not use them to prove theories; rather, either we elaborate theories from our observations on them, or, having set up a theory, attempt to establish it by experiment. In either case we seek to know the "machine" and not by machine-making to devise hypothesis or establish theory.

A cell is never a tool. Nor is it an instrument on which to whet one's physics and chemistry. Living matter is never an excuse and living phenomenon never an opportunity for the display of the investigator's physico-chemical knowledge. If we use an apparatus in order to determine oxidation in an inanimate system or devise a sensitive instrument to measure light, the apparatus or the instrument is a tool; but if one determines oxidation in a cell, the oxidation-determination is the tool; if one measures light produced by a cell, the measurement is the tool. In neither case is the cell a tool unless one frankly wants to compare its sensitivity for oxidation or light production with that of a man-made machine which of course is an entirely different reason from that of investigating the properties named as peculiarities of a living thing. The physicist devises a tool with which to measure a given change or to test an hypothesis; the biologist tries to measure changes in the cell—and the measurements are then the tool by which he evaluates the properties of the cell or tests his postulates of what these properties are.

I think that we can agree that in the experimental study of the egg's development the aim is the analysis of the stages found in nature through which the egg passes to

attain the normal adult condition. By experiment we attempt to ascertain the meaning of normally occurring stages which are co-existing and successive, co-ordinate and subordinate. We seek to unravel the tangle of the processes or, from another point of view, to stem the rush of developmental events in order to determine what in them is cause and what effect. The picture that the normal developmental process gives us must we trace in its every line and shadow and by experiment aim to elucidate their meaning.

Hence, the method for the study of living systems is this: to extend to the utmost a purely biological attack—that is, to know qualitatively every process; to mark its beginning and its end and in its duration to map out most closely set phases; to chart its course. This appreciation of the normal life-processes will set the goal which we strive to achieve by means of experimental attack. No matter how far the experimental methods change normal condition and behavior, always should results obtained with them have bearing on the normal. The greater the extent of the change, the more surely must we realize that it is a change. Conclusions drawn from greatly altered states concerning the normal need most careful scrutiny. Death-changes induced by experiment should be appreciated as such and be considered as revealing the living condition, which they extinguish, in no other way except by extinguishing it.

Those experiments which alter a normal process least have to-day especially great value in the study of the egg and its development. Few investigators nowadays, I think, subscribe to the naive but seriously meant comparison once made by an eminent authority in biology, namely, that the experimenter on an egg seeks to know its development by wrecking it, as one wrecks a train for understanding its mechanism; he might also have said, as a young child breaks up a watch to see the wheels go around. The days

of experimental embryology as a punitive expedition against the egg, let us hope, have passed. Instead comes the time of nice, exact, carefully graded treatment, wherever possible reversible in its action, for the purpose of analyzing development in ever more closely set stages, after this has been mapped out in the perfectly normal egg with meticulous care and scrupulous cleanliness. The most minute space-time organization of the living system makes the instrument on whose strings play the processes by action and interaction. By experiment we here slightly exaggerate, there lightly fret the tones out of which the harmony of the living state arises.

3

The Protoplasmic System

$\mathcal{A}$s already stated, the protoplasmic system does not always reveal itself as consisting of one nucleus and surrounding cytoplasmic mass, though this type is very widespread in occurrence. There are living things in which a discrete nucleus can not be discerned and on the other side we know formations which show many nuclei embedded in one continuous cytoplasmic mass. Thus, we may distinguish three types of the protoplasmic system according to the extent to which the nuclear substance is differentiated. A classification of the types of the protoplasmic system could also be made on the basis of the degree of the differentiation of the ectoplasm. But inasmuch as nuclear differentiation has up to now been more thoroughly studied, it is chosen here as the basis for a classification.

Some bacteria and blue-green algae have been reported as non-nuclear organisms. In them nuclear substance may, however, be deposited as granules so finely subdivided that they escape detection.[1] Indeed, in some cases whilst no formed nuclei or nuclear areas could be located, the presence

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[1] *The presence of nucleic acid in bacteria has been reported. Schaffer, Folkoff and S. Bayne-Jones (1922) extracted from 500 grams of dehydrated bacteria grown on a synthetic medium free from purine and pyrimidine compounds (constituents of nucleic acid) a non-hygroscopic, protein-free, powder which they consider a nucleic acid, though it contained no pentose, the sugar of plant nucleic acid. In the opinion of W. Jones and Folkoff (1922) the substance is a nucleic acid.*

of granules has been demonstrated, which respond to reagents as does chromatin (a substance found in nuclei). In others, by means of tests with dyes, chemical reagents, etc., investigators have demonstrated areas which respond as do nuclei in larger cells. Such systems are said to possess diffuse nuclei.

So much is clear: there are living forms which show no discrete nuclei. This fact indicates that in the organization of protoplasm the presence of a discrete nucleus is not indispensable.

The blue-green algae—bacteria are very closely related to them—evoke special interest because they probably represent the first forms of plant-life that arose on the earth. If it be established that there exist non-nucleated blue-green algae, this might mean that in the emergence of life, nucleus and cytoplasm were undifferentiated and only subsequently in the course of evolution became separated.

Doubtless there exists living matter of such minute size that it can not be seen with the highest powers of the microscope. Whether or not such protoplasmic systems possess the organization exhibited by cells seen under the microscope remains to be learned. In a discussion of the visible form of organisms it would be futile to include invisible forms. Nor can ultra-filterable viruses be included. In addition to the fact that they are of submicroscopic size is the question whether they be living things or only the product of living things. If they be living, they may despite their minute size possess the nucleo-cytoplasmic organization of visible cells; or, lacking such they may prove to be the most elementary protoplasmic system— protoplasmic ground-substance without clearly differentiated regions.

Many organisms consist of a mass of cytoplasm containing two or more nuclei. These may be low forms of life, Protozoa, like the slime-mould or the multi-nucleated

Opalina. Also among the higher animals certain sheets of protoplasm as the binding or sustaining (connective) tissue or the heart muscles of vertebrates show innumerable nuclei throughout their extent without interposed cell-boundaries. Such multi-nuclear protoplasmic systems are called syncytia.

The existence of syncytial protoplasmic systems has given rise to theories opposed to the so-called cell-theory which postulates that the cell is the unit of structure and of function. Rohde's views[1] concerning the preeminence of syncytia may be dismissed because they have little foundation in fact. Similarly, Studnicka's arguments[2] as to the living nature of cellular formations, fibres, etc., which come to be extra-cellular, do not warrant discussion here. The argument set up by Whitman[3] and by Sedgwick,[4] that the cell-theory of development is inadequate, out of which have grown the so-called organismal and organism-as-a-whole conceptions merits more attention although it too has slight basis in fact and is indeed almost wholly academic.

These conceptions originated as protests against the extremist's point of view that the individual cell is the end-all and be-all of life even in complex organisms; that through exact knowledge of single cells one could win an explanation of the organism as a unit. To-day we appreciate the fact that a mere agglomerate of cells equal in number to those that constitute an organism is not an organism. That organisms are units and act as such, be they composed of single cells, as Protozoa, or of myriads of cells, as the higher animals, no one will deny. The organismal or organism-as-a-whole conceptions have ren-

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[1] *Rohde, 1923.*
[2] *Studnicka, 1934.*
[3] *Whitman, 1893.*
[4] *Sedgwick, 1892.*

dered some service therefore in emphasizing this fact so universally accepted. Enough is known concerning the difference in behavior between cells when *en rapport* with others that make up the intact organism and those having been isolated therefrom, concerning the development of eggs and the significance of the orderly sequence in time and in space when specific embryonic cells are set off and concerning the pathological growths, as tumors, to warrant the clear conclusion that cells alone and as members of a unit-system exhibit different behaviors. However, no sufficient reason exists for assigning to cellular differentiation, the integration of cell with cell, or the specificity of organisms which resides only in cells and their products—all well accepted biological truisms—the new terms of organismal or organism-as-a-whole.[1] Moreover, the principle of unity—the expression of integration, differentiation and of specificity both in form and behavior—which distinguishes an organism from a mere mass of like cells, ought not be elevated to the position of an abstract principle divorced from the concrete physical basis on which living things are organized.[2]

An organism possessing no formed nucleus represents one, and a mass of cytoplasm containing many nuclei represents the other extreme type of protoplasmic systems. If we assume that nuclear structure (and therefore from it the discrete nucleus) is a derivative of the ground-substance, the various types of protoplasmic systems can be reconciled: the single nucleus of a simple cell is then the compounding of nuclear bodies evident in non-nuclear

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[1] *In view of the claims of priority pressed by several writers it may be worth-while to note that Descartes (1662) conceived the organism as a whole. See Delage's (1895) discussion of "organicisme."*

[2] *See Woodger, 1929, 1930 and 1931, for recent discussion of the organism-as-a-whole point of view.*

forms like bacteria; the compounding of nuclei themselves (a result easily obtained experimentally in normally mono-nuclear cells) leads to the multi-nucleate, syncytial or polyenergid condition. To a consideration of that proto-plasmic system which is most widespread in occurrence and which reveals itself as a simple membrane-enclosed cell with a single nucleus, I now turn.

For most organisms in both the animal and the plant kingdom this cell is the basic structural unit, no matter how complex or how large such an animal or plant may be. A whale and a giant red-wood tree as well as minute forms like a vinegar eel and a thread of green alga are patterns of cells. Animals and plants also exist as single cells, which within their boundary complete their life-cycles, exhibiting the vital activities shown by the more complexly organized individuals. These activities are perfect; diges-tion, respiration, conduction, contraction, reproduction, in a unicellular organism are as complete, though not so complex, as in man. These individuals in their organiza-tion and behavior reveal the single cell as an independent unit. At first one is amazed that microscopic creatures like the slipper-animalcule and the diatom, a plant of micro-scopic size, can carry on the functions of more highly organized forms of life; they seem at first to be imitations of the latter. But observation teaches that the millions of cells which make up the body of a multicellular animal or plant have lost in capacity: no one cell in them can perform all the functions to the same degree that a single-celled individual can carry out.

This loss of capacity is compensated for by an emphasis on some one function, as shown, for example, in cells in the human body. In it some parts, which together make up the nervous system, take over the business of conduction which is developed to a high degree. Other parts, the muscles, emphasize contraction; still others, respiration,

digestion, excretion, reproduction. Thus we speak of the various systems—nervous, muscular, respiratory, etc. Each system is made up of organs; each organ is a combination of different tissues; each tissue is a collection of cells of the same origin and usually of the same form.

Out of a single cell, the egg, emerges all this complexity of organization found in the adult human being or in most other multicellular organisms, animal or plant. The human egg during its development forms tissues, these aggregate to form organs, and the organs are united into systems. In an animal's origin, as well as in its definite adult structure, therefore, the cell is the unit of organization. This fact, together with that of the existence of unicellular animals and plants, shows that a large part of the world of living things is organized on the basis of cell-structure.

The history of the multicellular organism as it develops from the egg, a single cell, to the adult is very much like a synopsis of the history of the whole world of multicellular organisms; this has most probably evolved from a single-cell ancestor. Thus biologists classify animals and plants beginning with the single-cell individuals and ascending through grades of increasing complexity. Among animals, for example, the Protozoa stand first; next are the sponges, animals possessed of tissues only; above the sponges stand animals whose tissues are organized into organs and finally come animals with systems of organs. From the point of view of the history, both of the individual multicellular organism and of the world of multicellular organisms, the cell is the unit of the state of being alive.

That this cell must be regarded as a unit becomes further evident if we consider the fact that nucleus without cytoplasm or cytoplasm without nucleus is incapable of living. It is true that pieces of cytoplasm devoid of nuclei, as the red blood corpuscles of mammals, occur normally and live for a time; but these are specialized structures incapable of

the full complement of living functions. One can cut off pieces of a single-cell animal; if these pieces be without nuclei they die, whereas a piece containing the nucleus or pieces containing portions of the nucleus are capable of growth to the full size of the normal creature. In turn, nuclei deprived of their cytoplasm do not live. The unit of living matter in these cases is thus shown to reside in the nucleo-cytoplasmic organization.

We therefore adopt the point of view that the protoplasmic system is the unit of life. For the majority of animals and for all their eggs the protoplasmic system reveals itself as comprised of a single nucleus enclosed by cytoplasm—ground-substance containing suspended matter, the cytoplasmic inclusions—and the whole enclosed by a membrane. Such cells exhibit variations in several directions.

Cells may show extreme size-variations. They range in size from one micron in length, discernible only under the highest microscopical magnification, to the sporozoon parasite found in the digestive tract of Crustacea which measures 16 mm. in length, or to the unfertilized egg of the ostrich, 105 mm. in diameter, or that of a shark, 220 mm. in diameter, or to the nerve-cells in the human spinal cord, which may be one meter in length. Organization of the cell thus does not depend upon size. We may imagine that the skein of life is greatly contracted in some while it is more diffuse in others. The maintenance of the cell-size that is characteristic of a species still remains an unsettled problem. Maintenance of a definite and specific size is a revelation of the self-regulative capacity of the protoplasmic system.

Cells also show most varied shapes and forms; some are almost perfect geometrical figures—spheres, cylinders, cones; others are irregular or of forms not easily reducible to conventional geometrical figures. Often when in groups

they show the effect of compression; thus cells which, were they free, would be spheres, become polyhedral. The varied shapes of cells depend upon many diverse factors in addition to compression, such as presence of a supporting frame-work, as in Foraminifera, the greater or lesser fluidity of the cytoplasm, the structure of the limiting surface, and most of all the little understood intrinsic structure of the cell itself.

Cells, like all matter, are chemical in composition and display physical properties. Their form, structure, and morphology, however varied, are molecular in make-up. Hence, as we have seen, there is, in this respect, no difference between living and non-living matter. All the protoplasms known to us have in common a certain basic chemical structure. Since living things are part of the natural world it is not astonishing that they are composed chiefly of those chemical elements which are most frequently found in the world of non-living things: Carbon (C), nitrogen (N), oxygen (O), hydrogen (H), sulphur (S), phosphorus (P), iron (Fe), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and chlorine (Cl). In some cells are found other elements as lithium, barium and strontium; copper and zinc; fluorine, arsenic, bromine and iodine. Thus the difference between non-living and living systems does not reside in the basic elements of which they are composed. A difference obtains in the compounds into which these elements enter.

The elements, C, H, N, O, combined build up the protein molecules; out of C, O, H carbohydrates are formed; and C, O, H makes up most lipins; other lipins in addition contain N or P. These are the "organic" compounds of the living substance and exist in nature only in living substance; non-living things do not contain proteins or the oils found in animals and plants; sugars and starches (carbohydrates) occur naturally only in animal and plant

tissues. Thus, not the elements as such characterize the structure of living matter but the peculiar combination of elements which constitute the protein, the lipin and the carbohydrate molecules of living matter.

These compounds are suspended and dissolved in an aqueous medium containing electrolytes as Na, K, Ca, Mg, Cl, etc., with oxygen and carbon-dioxide. The whole mass of protoplasm—organic compounds, electrolytes, water and gases—it seems, maintains a fairly constant slightly alkaline reaction.

Each of the three organic compounds is built up of simpler molecules strung together: the proteins are strings of amino acids formed in some such manner as the following:

$$C_4H_9.CH(NH_2).CO.NH.CH_2.CO.NH.CH_2.COOH$$
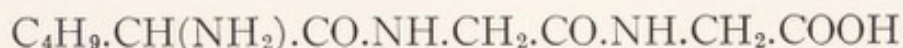
which is a poly-amino-acid or tripeptide of leucin:

$$CH_3CH.CH_2.CH(NH_2)COOH$$

and two molecules of glycin:

$$CH_3CH_2.NH_2.COOH$$

The more complex proteins show comparable linkage.[1]

The lipins, as shown by the chemical structure of a simple fat, are combinations of three molecules of fatty acid and one of glycerol, as:

$$CH_3.CH_2.CH_2.COOH + CH_2.OH$$
$$CH_3.CH_2.CH_2.COOH + CHOH$$
$$CH_3.CH_2.CH_2.COOH + CH_2.OH$$

with the loss of three molecules of water.

Monosaccharides (simple sugars), disaccharides (secondary sugars) and polysaccharides (starches and the like)

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[1] *With the aid of the ultra-centrifuge and by means of x-ray-spectroscopy valuable information is to-day being gathered concerning the protein-molecule.*

comprise the carbohydrates. Simple sugars like dextrose have the formula, $C_6.H_{12}.O_6$, secondary sugars $2(C_6H_{12}O_6) - H_2O$, or $C_{12}H_{22}O_{11}$, whilst more complex carbohydrates are only more molecules of simple sugars piled up.

Whereas plants because of their photosynthetic power can in sunlight or in artificial light produce sugars from CO_2 and H_2O in the presence of the green substance, chlorophyl, and from sugar thus formed synthesize lipins and proteins when N is present, most animals ingest food as protein, carbohydrate and lipin. Before utilization by the animal organism, these compounds must be broken down, i.e., digested, into their component parts: proteins to amino acids, carbohydrates to dextrose and laevulose and lipins to fatty acids and glycerol. These fractions are built up again by the organism into the more complex molecules. Special significance is attached to the synthesis of amino acids, for the proteins thus formed by the organism are peculiar to it and not to the organism which furnished them; thus herbivorous animals, cattle for example, convert the plant proteins of their food into proteins peculiar to beef.

The break-down of these compounds is accomplished by enzymes, chemical bodies which act much as catalysts— i.e., by accelerating chemical changes. Enzymes show a high degree of specificity. For proteins, there exist the enzymes like pepsin found in the human stomach and trypsinogen, produced by the pancreas; for the starches is the enzyme, amylase; for the sugars, sucrose, maltose, lactose, the enzymes are sucrase, maltase, and lactase respectively; for the lipins, lipase. The break-down is one in which water is used, a so-called hydrolytic cleavage. Enzymes also synthesize the end-products of digestion into proteins, carbohydrates and lipins in which case water is lost. Some enzyme-reactions are reversible: thus, the reaction lipin plus lipase = fatty acid plus glycerol, or

fatty acid plus lipase = lipin—depending upon the amount of water present.[1]

The chemical make-up of the three organic constituents of protoplasm renders easy their conversion into different compounds; the chains are broken up and when the links are reformed they show a different relation to each other. The proteins have the greatest lability in this respect. From the 21 amino acids which compose them it is theoretically possible to derive millions of different kinds of proteins.

This is one reason why the proteins are regarded as responsible for specificity; all species now in the world can doubtless be distinguished from each other on the basis of the chemical constitution of their proteins. But it should be borne in mind that protoplasm never exists entirely free of carbohydrates and lipins; that from sugars plants make protein; that sugar as a pentose or as hexose is an essential constituent of nucleic acid; that lipins are the greatest source of energy and are the great binding substance; that lipins if not present as such in the nucleus probably give rise to the phosphatized portion of the chemical substance, nucleo-protein, found in the nucleus; and finally, that much of the protein in cells does not exist as simple protein but as a conjugant with lipins or lipin-derived phosphate. Protoplasm is not a compound but a complex of compounds of these three organic constituents together with other constituents, water, gases, salts.

Water is the most abundant compound in protoplasm. Roughly, two-thirds of an animal's body is water; in some cases it may amount to more than 90 per cent. of the body weight. Even water-poor cells, as the enamel of the teeth and spermatozoa, still possess a great deal of water. Active protoplasm both liberates and utilizes CO_2 and O_2. Dis-

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[1] *See Bradley and others.*

solved in the water are several inorganic salts, chiefly chlorides, carbonates, phosphates of sodium, potassium, calcium, etc.

The researches on the analysis of carbohydrates and on the synthesis of sugar, on the analysis of proteins (especially nucleo-proteins) stand in the fore-front of biochemical achievements. And yet, there remains much to be learned of the chemical make-up of the cell. Farther and more refined analysis may furnish a clue to a better understanding of living matter as a complex of compounds. Up to now chemical analysis tells us only what is found in the living thing after it is killed. The very nature of chemical analysis demands isolation of the protoplasmic components as separate chemical entities and thereby sets up an obstacle in the search for the chemical constitution of the living state. To know even with absolute exactness the chemistry of each compound in the once living cell does not guarantee that we know how these are organized in life. Moreover, living organization is dynamic whereas the application of chemical analysis by necessity demands destruction of the very space-time structure which is the changing organization characteristic of life. No single datum now at hand warrants the assumption that the chemistry of the cell in the chemist's test tube is that of the living cell. Indeed, the evidence points to the contrary.

These shortcomings of chemical analysis of the living thing have served to focus attention upon its physical attributes. Many biologists, therefore, seek to determine the physical diagnostics of non-living and living on the one hand, and of the living before and after it is killed, on the other.

The presence of water in large amount confers upon protoplasm certain physical properties; similarly, the remaining substances entering into the composition of the cell are responsible for other such properties. Recently,

a great deal of attention has been given to the physical properties of the protoplasmic system. A new and flourishing branch of biology, bio-physics, stresses this aspect of the study of life. Some of the more general of these physical properties may be mentioned.

Protoplasm is a liquid capable of flow. This fluidity can be demonstrated in several ways. First, direct observation shows that in many cells the cytoplasm streams.[1] In Amoebae, in many egg-cells, etc., currents can be discerned. Cells which fail to reveal the presence of such currents often show the cytoplasmic granules in intense Brownian movement. Again the fact that in many cells it is possible by very slight centrifugal force to shift and to separate the cytoplasmic inclusions according to their specific weights, demonstrates the liquid nature of protoplasm. Also, one may compress eggs, draw them out into long strands or otherwise treat them as if they were elastic bags of water.[2]

The cell-liquid and the various formed bodies suspended in it reveal that protoplasm is both a solution and a suspension, somewhat like milk, for example, which is a solution of water, electrolytes, proteins, sugar, and lipins in which globules are suspended. Protoplasm being largely made up of protein and complex carbohydrates in the colloidal state, is itself in this state. A colloid (literally glue-like[3]) is matter in the state which can not or can only with difficulty pass through an animal membrane; whereas crystalloids, like cane sugar and common salt, can very readily pass through such membranes. Protoplasm exhib-

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[1] *Cf. Goette, 1875, who not only very clearly observed protoplasmic streaming in an egg but also proffered an interesting hypothesis as to its significance.*

[2] *Just, 1928a.*

[3] *Armstrong, 1927, p. 656, points out the bad usage of the term, colloid.*

its many properties characteristic of this state of matter. This is not to say that the colloid state is peculiar to organic compounds, such as proteins and carbohydrates; the most thoroughly investigated colloids are those of inorganic substances. It is to be emphasized that the term, colloid, applies to a state or condition only and not to a kind of matter different from crystalloid. Since we know that protoplasm is in the colloid state, it should not astonish us that it behaves as other matter in this state.[1]

The specific gravity of the total of the cell-contents is usually greater than that of water. But where much fat is present, as in some fish-eggs, the specific gravity of the cell is lower than that of sea-water, as shown by the fact that such eggs are found floating on the surface of the sea. In some eggs the specific gravity increases after fertilization, a change undoubtedly due to loss of water.

The refractive index of a cell is a physical property deserving attention. It depends upon the ground-substance and the cytoplasmic inclusions. With the state of aggregation of these latter, the shape and the water-content of the cell, the refractive index of the cell varies. The influence of these factors is shown during the process of cell-division when the cell's refractive index changes with the dispersion of the inclusions, loss of water and change of form of the cell.

Another physical property of the protoplasmic system much studied in recent years is its viscosity. The significance attached to such studies has, in my judgment, been grossly exaggerated. Nothing up to now indicates that viscosity has the importance for the state of being alive assigned it by the various investigators who, with different methods, and even with the same method, obtain widely diverging results and announce conflicting opinions. The

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[1] *See Svedberg, 1925, and Heilbrunn, 1928.*

problem of protoplasmic viscosity, with special reference to animal eggs, can be dismissed in a few words.

The statement made above that the cell contains a high percentage of water, does not imply that the cell-contents flow as freely as water or that they always maintain the same degree of flow. Before the present vogue of measuring the viscosity of protoplasm it was shown that the cell-contents have a higher viscosity than water and that this viscosity varies from time to time in a particular cell and differs in different cells. One thinks at once of correlating viscosity with the water-content of a cell: the more water, the less viscosity and vice-versa. To an extent this is true, but the situation is not so simple. Take the case of eggs actively going through the process of division. During each cleavage-cycle, substances move back and forth between nucleus and cytoplasm, between cytoplasm and cytoplasmic inclusions and between egg and external medium. Only in the case of the last named exchange, obviously, does the cell lose water; in the first and second, water moves from place to place within the protoplasmic system. Now since, as the older observations have abundantly shown, changes in viscosity parallel the cell's rhythmical activity in division, it becomes necessary in measuring viscosity-changes to appreciate the fact that water does not shift between nucleus (and structures associated with it) and cytoplasm only; one must also recognize the shift between cytoplasmic inclusions and cytoplasm, especially if one estimates the change in viscosity on the basis of the movement of these inclusions, for example, by means of centrifugal force. Since yolk-spheres undergo physical changes during cell-division, one can not in measurements of protoplasmic viscosity assume them as unchanging in deriving conclusions concerning the viscosity of the cytoplasm. Also, in experimentally treated eggs, one can only draw conclusions as to the effect of the

means if one knows definitely that this is on the cytoplasm alone, or on one or another of the inclusions or on the whole of the cell-contents. Finally, the effect of experimental treatment should be known to be reversible, and coagulative death-changes should not be confused with what is denominated as the normal viscosity-changes in normal and viable cells. Until the viscosity studies have been refined, changes in this physical property cannot be regarded as an infallible sign of the state of being alive.

A leading characteristic of biological researches of the last twenty-five years lies in the emphasis which came to be placed upon the investigation of the physical properties of protoplasm—an emphasis so great that it has virtually created another department in an already heavily departmentalized science. Everywhere nowadays bio-physics, the physical chemistry of the cell, and the colloid chemistry of protoplasm are in evidence. Many an interesting new datum concerning electrical conductivity, hydrogen-ion-concentration, temperature-coefficients, etc., has been accumulated, whilst the older conceptions of osmotic pressure, surface tension and the like have been refined by more exact mathematical treatment. If from all these studies far less has come than had been hoped, one benefit of them deserves emphasis. No matter how refined a study on a physical property of the living substance may be, its value for the understanding of vital activities is determined by its relation to these vital activities and by its correlation with known form-changes and normal processes in viable cells. Modern studies in bio-physics and the like have served to indicate anew how necessary is adequate knowledge of the visible structure and the form-changes of cells, and how much we still need to extend this knowledge. What is called the morphology of a cell—its visible form and its visible form-changes—still remains the basis of biological investigation both for its own sake and as the

basis of an attack by bio-physicists, bio-colloid-chemists, bio-physical chemists, by which may possibly come an elucidation of the problem of vital manifestations. I therefore turn to a description of the cell.

Under low power of the microscope, a living unfertilized egg of the sea-worm, *Platynereis*, mounted in a few drops of sea-water, at first glance appears as a greenish-yellow sphere everywhere crowded with smaller spheres except near the centre and at the periphery beneath the well marked egg-membrane; the whole presents a pebbled or shagreen effect. A second glance, especially if the egg is brought into focus so that one obtains an optical section of it, gives clearly the regions first noted. The large clear area near the center is the nucleus, sharply set off from the remainder of the cell by its transparency and apparent homogeneity. Outside of it, closely crowded against the nuclear boundary are innumerable spherules with some twenty or more refringent globules (oil drops) interspersed. Among these spherules and globules minute bright granules are scattered. Under higher magnification still smaller granules may be discerned. Spherules, globules and granules lie in the endoplasm or inner cytoplasmic region. Beyond it is a clear band, the ectoplasm, or outer region of the cytoplasm. In this egg the ectoplasm is crossed by fine radial lines; these appear to reach the egg-membrane, called the vitelline membrane, though actually they end in the plasma-membrane which is difficult to discern and which lies underneath the vitelline membrane.

If we fix this egg quickly with some chemical reagent which preserves it in a state closely resembling the living, and cut it up into thin slices, we easily discern structures seen with more difficulty in the living cell. Figure 1 is of a stained slice from such a fixed egg. In the nucleus, granules of various sizes and two chromosomes are shown. These bodies lie in a faintly granular grayish-blue field

enclosed by the nuclear membrane. The cytoplasm is sharply differentiated into two regions: the endoplasm, crowded with yolk spheres, oil drops and granules, the smaller of which are mitochondria; and the ectoplasm with its radially disposed lines which extend to the plasma-
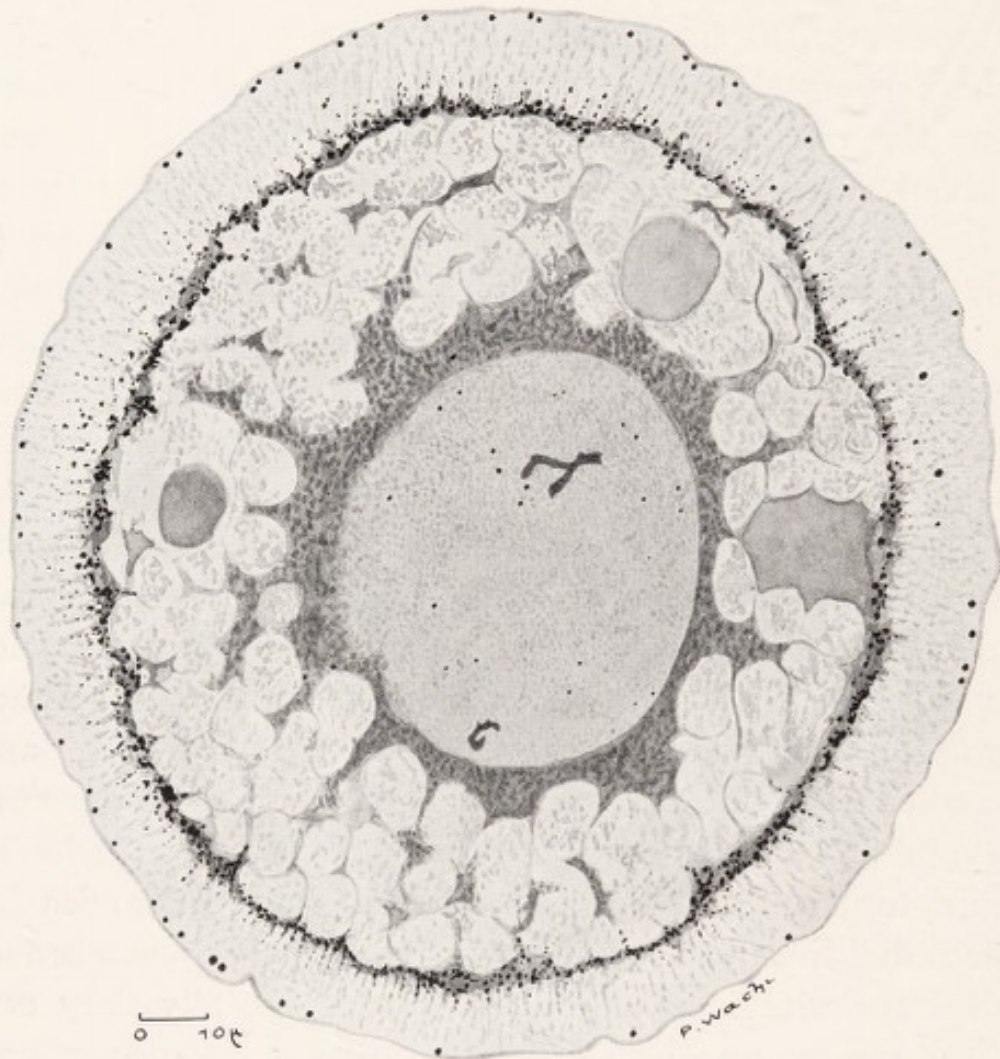
FIG. 1.—Section of an unfertilized egg of *Platynereis megalops*. Surrounding the large nucleus, germinal vesicle, is the cytoplasm, which is clearly marked off into two zones, the endoplasm and the ectoplasm.

membrane beneath the vitelline membrane. The egg of *Arbacia*, a sea urchin, is similar to that of *Platynereis* except that the whole egg including the nucleus and the endoplasmic bodies is smaller, the ectoplasm a mere line, and the vitelline membrane an extremely fine film. In

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all cells nucleo-cytoplasmic organization is visible and ecto-endoplasmic differentiation is expressed.

Nuclei may vary in size, but their size is generally constant for a given species of cell, though this varies during the process by which the cell reduplicates itself. In eggs it also varies during the end-stages of ripening, as will be shown later.

The nucleus also shows diversity in form. Nuclei though most frequently spherical or oval or tending toward these two forms, may be quite irregular as those found in white cells of human blood, for example. The amoeboid, irregularly form-changing nuclei in the spinning gland cells of butterflies have often been described. Again, during the process of division an otherwise spherical or oval nucleus may be very irregular due to incomplete or slow fusion of the separate chromosomes into one nucleus. This is clearly shown in nuclei of some eggs, as those of the thread worm, *Ascaris*, the water-flea, *Cyclops*. In these the nucleus may be regarded as a composite of many smaller nuclei, each containing one chromosome. Although this condition is not met with in all animal cells, it may be regarded as a fundamental condition of nuclear structure.

That the chromosomes are the best known structures in the cell is due in part to the fact that they can be so easily studied because of their affinity for colors, which gave them their name. In the living cell[1] they often appear as refringent drops of the same shape and approximately the same size as when fixed and artificially stained with one or another dye. A constant number of chromosomes is characteristic for the cells of a given species of animal. This number is best ascertained in fixed and stained cells, though it can be made out in some living cells, while they

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[1] *Flemming, 1879, Peremeschko, 1879.*

are in stages of nuclear division for then the chromosomes are most easily visible.

The chromosomes also appear as strands of condensed structures lying in the more fluid substance of the nucleus, the so-called karyolymph. When the nucleus is at rest the chromosome-material appears as granules and is designated chromatin. How far this appearance coincides with the real state of the chromosome-material has yet to be determined. Since the chromosomes display regularity in structure and behavior during the process of nuclear division when they are visible and, after fading from view, appear again as bodies of the same number, form and character, it lies close at hand to conceive that they maintain their organization also when they are seen as granules. Theoretically, for the chromosome-theory of heredity, which will be discussed later, it is also simpler to conceive that the chromosomes always maintain their form and never become disorganized by breaking up into granules.

The rapidity with which the nucleus carries out its changes might be considered sufficient for the maintenance of integrity during both the resting and the dividing stage. But the fact that the nucleus remains separated from the cytoplasm may be due to an interposed membrane. Though such a membrane can be demonstrated in many cells, its presence generally in cells has been denied. It is further not at all clear how this membrane arises. As with the presence or absence of the nuclear membrane, so with the linin, a net-work of material in the fixed nucleus: some hold that it is a real structure, others that it is merely an artefact.

There is present in the nucleus a nucleolus (in some cases there are several nucleoli) which may be a double structure. Its function is not known. Indeed, one can not be sure of its origin or fully follow its history from one cell generation to the next.

The chemistry of the nucleus is known from studies made especially on blood cells and spermatozoa in which the nuclear material is rich in comparison with the cytoplasmic. Since in the spermatozoa the nuclei (sperm heads) are chromatin in the most condensed state known, their chemisty is practically the chemistry of chromatin. The following is the chemical constitution of an animal's cell-nucleus (spermhead of the white fish):

$$C_{96}.H_{184}.N_{54}.O_{22}(C_{43}H_{57}N_{15}P_4O_{30})4 - 24H_2O$$

The nucleus essentially is made up of a protein conjugated with a sugar-containing compound which through the presence of phosphoric acid becomes an acid, nucleic acid. The presence of phosphorus suggests that although lipin may not be present as such in the nucleus, it is necessary for the formation of nucleic acid. Nucleo-protein contains protein and carbohydrates which probably come together through the mediation of phosphorus-containing lipin. In the light of this suggestion the synthesis of nucleo-protein represents the highest chemical activity of the living substance since we see here synthesized the three important substances that distinguish living from non-living matter chemically: protein, carbohydrates and fats. For this reason I consider it an approach to the understanding of chemical activities in living matter to study nucleo-protein (and especially nuclein) with respect to its rate and duration of formation and its amount.

In dividing cells the intact or so-called resting nucleus becomes active and exhibits changes after which it divides. However, the nucleus is not to be thought of as a body implanted in the cytoplasm with no relation to it except during synchronous division. Since in the so-called resting stage the nucleus continually increases in size to the moment in which the activities leading to its division set in we assume that it takes up material from the cytoplasm—

substances in solution. Indeed, with respect to the nucleo-cytoplasmic relations, the so-called resting stage of the nucleus is as important as the stage of division.

During the stages of nuclear division there also occur changes in the cytoplasm, as changes in refraction, in dispersion of granules, changes within the granules themselves. The fact that these changes can be correlated with the stages of nuclear behavior suggests that chemical syntheses and analyses in the living cell are in part at least controlled by the movement of substances in solution to and from the cytoplasm out of and into the nucleus. To this point I return later.

As has been said above, the cytoplasm is composed of an inner core immediately surrounding the nucleus and, external to this, an ectoplasmic region extending to the cell-membrane. In this thus differentiated area of extra-nuclear protoplasm formed bodies of different size are suspended. By determining what in this extra-nuclear mixture of fluid and suspended bodies is indispensable to the life of the cell, we may derive a definition of cytoplasm.

The suspended bodies are chiefly: droplets of oil, yolk, small formations known as Golgi-bodies, granules called mitochondria and chromidia; further, starch, crystals of various kinds, secretion-granules, excretory products. But these cytoplasmic inclusions are not to be taken for the cytoplasm itself, as is strongly indicated by the following facts. First, animal cells vary with respect to the content of cytoplasmic inclusions: not all cells contain them all and some even under high power of the microscope appear to contain none. Second, in many cases the inclusions occupy no constant position in cells: thus yolk generally present in eggs is variously distributed. Third, and most important, portions of eggs devoid of all cytoplasmic inclusions having been fertilized develop as do whole eggs with all inclusions present. Consider the egg of the marine worm, *Chaetop-*

terus, after it has been centrifuged with force sufficient to stratify its inclusions according to the specific gravity of each: between the zone of oil-drops, the lightest constituents of the cytoplasm, and the yolk-spheres massed at the opposite pole, lies a clear zone which under high power of the microscope and with bright-field illumination appears optically empty; with the dark-field or after proper fixation this zone appears to be made up of extremely fine granules. This clear area if cut away, even though it be but a small fraction of the egg, will develop if fertilized with almost the same pattern and tempo of development as the whole egg. This is likewise true of eggs of sea-urchins. The conclusion is that of the extra-nuclear substances the only part absolutely necessary for life is the clear inclusion-free menstruum. I therefore define as cytoplasm this menstruum only. In the following pages I shall adhere strictly to this definition.

Despite the fact that the cytoplasmic inclusions are not essential to cytoplasm, they have undoubted functions and merit here brief notice. In the following treatment, I speak mostly of egg-cells.

The lipins (fats and fatty substances), discussed above, are present in eggs largely as oil and in yolk (lecithin). The oil (fluid fat) in eggs exists as drops in various degrees of emulsification. Whilst it is easy to observe oil drops in some eggs in which they are relatively large—as in the egg of the sea-bass,—it is often difficult to observe them in some other eggs where they are in a state of fine division. In the unfertilized egg of *Nereis* one can count the twenty to twenty-two oil drops present and can as development ensues after fertilization follow the gradual reduction by coalescence of this number to four. In the living larval worm one can further watch the emulsification of these into finer and finer drops which eventually disappear—as further shown by micro-chemical tests on both living and killed

larvae. This observation teaches us one function of the oil in this egg-cell: it is a reserve food-material utilized only in the period before the young worm actively begins to ingest food. A gram of fat, as is well known, is richer in energy than a gram of carbohydrate or of protein.

Lipin plays a rôle both in the water-holding power of cells and in the cells' immiscibility with the surrounding medium. It exists in the cell in other forms than oil drops. So, cholesterol is said to be found in the membranes of some eggs; the human red blood corpuscle likewise contains it. A closely related compound, ergosterol, is nowadays much discussed because of its significance in vitamin-chemistry. Fat is also associated with pigments and thus may play a part in oxidation-processes. Yolk is another form in which lipin occurs in egg-cells.

Yolk in eggs appears in manifold chemical and physical forms. The identification of yolk-bodies in eggs rests largely upon staining reactions. Spherules in the cytoplasm which are stained dark blue or black with iron haematoxylin are usually assumed to be yolk. If finally they come to lie in the endoderm cells one concludes that they are food material and presumably yolk. Even so, these spherules show striking differences. Take, for example, the yolk spheres in the eggs of two closely related marine worms, *Nereis* and *Platynereis*. In the former egg the yolk spheres are homogeneously blackened by haematoxylin; in the latter every yolk sphere is a delicately reticular structure staining faintly with the haematoxylin. In each case these bodies come to lie in the four cells which compose the gut.

At the beginning of the process of incubation, yolk constitutes the greatest bulk of the chick's egg, whereas at the time of hatching the yolk has largely disappeared. This fact means, as we shall see in a later chapter, that yolk has been converted into cytoplasmic substance. In the

same way, of course, the growing animal after having been hatched from the egg manufactures its peculiar kind of protoplasm from the food which it takes in. So also yolk is used up in the development of other large eggs, as those of fishes and amphibians; in smaller eggs, however, development proceeds to the stage of hatching without the utilization of yolk. For example: in the *Nereis* egg, yolk is not used during cleavage by the blastomeres; it goes into the four macromeres which form the gut. A sea-urchin egg, in which the yolk has been displaced by centrifugal force, develops perfectly. And yolk-free fragments of marine eggs develop normally as stated above.

In the egg of *Nereis* the yolk spheres are polyphasic, i.e., they contain a protein-skein and lipin. If these eggs are placed in hypotonic sea-water, oil in minute drops escapes from the yolk-spheres, leaving a reticular and more watery medium, the whole enclosed by a strong membrane. The yolk spheres now resemble those present normally in the egg of *Platynereis*. On transfer to normal sea-water the oil re-enters the yolk-spheres and their original optical properties are restored.[1] The process is thus reversible. An observation like this shows how yolk may vary and that we can not satisfy ourselves with only a rough identification of it as that by staining. Certainly, what thus we identify as yolk is not a pure lipin.

The Golgi-bodies or Golgi-apparatus, commonly a net of fibres or a cluster of granules of varying form and size, have been described for almost every type of animal and plant cell. Their meaning and function are yet to be made clear. Undoubtedly in some cases what have been described as Golgi-bodies are drops of fat;[2] in other cases even vacuoles have been described as Golgi-apparatus.

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[1] *Just, 1926.*
[2] *Just, 1927a.*

Mitochondria are minute spheres, rods or filaments. In the egg of *Platynereis* pictured they are rods; while in that of the closely related *Nereis* they are spheres. In some cells they assume first one then the other form. It has been held that the mitochondria are phospholipins. However, because with the most careful cytological technique yet devised (that invented by Altmann), later workers[1] were unable to prove that mitochondria contain fats but, instead, could demonstrate their protein nature, doubt is thrown on the older view. Certainly in those marine eggs which I have studied on this point, the mitochodria do not reveal the specific gravity of fat; for, when the eggs are centrifuged, instead of lying in the zone next to the fat they take the place above the yolk.[2]

Chromidia are generally believed to be cytoplasmic granules derived from the nucleus. I may refer again to the egg of *Nereis:* in it one can observe granules which seem to come out of the nucleus, wander into the cytoplasm and come to lie at the cell-periphery in which position they can not be distinguished from mitochondria. Under various experimental conditions, as after ultra-violet radiation or by treatment with hypotonic sea-water, the number of such granules in the vicinity of the nucleus is increased. Often also with such experimental means chromosomes are eliminated which degenerate into granules not easily, if at all, distinguishable from chromidia and mitochondria. This degeneration of chromosomes is quite different from the degeneration of chromosomes in abnormal eggs which fail to develop; these latter can never be mistaken for chromidia or mitochondria because structurally they offer an entirely different aspect. On the basis of

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[1] *Bensley and Gersh, 1933.*

[2] *Bensley and Gersh are extremely cautious in their conclusions and it may be that even under the conditions of their experiments the lipin-fraction of the mitochondria was destroyed.*

my observations I venture the opinion that chromidia and mitochondria represent stages in the transformation of granules of nuclear origin.

Of granules other than mitochondria and chromidia found in eggs, pigment is most striking. Many eggs seen *en masse* have beautiful colors—various shades of red, orange, yellow or green. There are some species which have eggs of two distinct hues; thus individuals of the little marine worm, *Autolytus varians*, have either red or green eggs. The pigment of sea-urchin eggs has been shown to be a lipochrome.

Occasionally crystals can be demonstrated in eggs. Thus Meves has described fine slender bodies found in fertilized eggs of *Psammechinus* and I have found them also in fertilized eggs of *Echinarachnius*. That such formations are increased in cross-fertilized eggs[1] I do not doubt, but that they are solely due to the effect of the foreign spermatozoon is to be questioned in view of Meves' and my own observations.

Some of these cytoplasmic inclusions, as well as others, that are not here discussed as, for example, secretion-granules which leave the cell, are certainly temporary structures. Study of the history of the egg from its earliest formation, when its cytoplasm always appears homogeneous, through the stages during which it becomes laden with inclusions, teaches that inclusions appear in the cytoplasm during development. The building up of cytoplasmic inclusions is an indication that the egg takes in food-material from its surrounding medium. Indeed, in some eggs, as those of the flatworms, the yolk, elaborated by special gland-cells, is merely deposited around the egg.

In limiting the application of the term, cytoplasm, to the clear and almost homogeneous menstruum or ground-

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[1] *Tennent, 1920; Hibbard, 1922.*

substance and thereby dismissing the various inclusions suspended in it as necessary constitutents of cytoplasmic structure, we dismiss much of the work done on the chemistry of the cell as relevant to the chemistry of the cytoplasm inasmuch as chemical studies have been made so largely *en gros*—encompassing everything including food-material and effete matter which might be present in each of the thousands of cells which are often necessary for a single chemical determination. The chemistry of the cytoplasm, from my point of view, would embrace only the chemistry of the inclusion-free ground-substance. Since we do not yet possess such, we can only approximate it by considering that which remains after we have subtracted from what is known of the chemistry of the whole cell that which is known of the chemistry of the nucleus, of the various inclusions and of the cell-membrane. This so little light does not throw its beams far and we remain still much in the dark. Moreover, there is always the obstacle that what we call the chemistry of the whole cell is not that of living protoplasm; the isolation necessary for chemical study may change the cell-constituents. Micro-chemical studies on cell-inclusions are certainly valuable as are those on the inclusion-laden protoplasm. But there is the greatest need for such studies on the ground-substance itself.

The definition of cytoplasm here given makes mandatory a revaluation also of studies on the physical properties of protoplasm. Here, in order to know what properties inhere in the ground-substance, unless this be studied as such, the known properties of nucleus and cytoplasmic inclusions should be subtracted from those known for the whole cell. It is a curious fact that in that domain of physical chemistry which by its definition we should expect to encompass the study of the ground-substance workers have laid more stress on the whole cytoplasmic area and neglected the possibility of investigation of the ground-substance which

when isolated from, and free of, cytoplasmic inclusions is wholly viable. I refer to that branch of chemistry, colloid chemistry, so-called, where fact and fancy are dispersed in the medium of a naiveté which so often characterizes new adventures along narrow confines.

An egg like that of *Nereis* in which the formed bodies, oil-drops and yolk spheres, are visible when magnified only twenty times can not with respect to these bodies be considered by a colloid chemist as other than an extremely coarse suspension. It can not be regarded as a colloidal solution for a colloidal solution is usually defined as one in which the particles range in size from 1, 2 or 6 to 250 $\mu\mu$. The smallest are at the lowest limits of visibility with the ultra-microscope and the largest come just within range of microscopic observation. Even such eggs as sea-urchins', whose oil-drops, yolk-spheres and pigment granules are smaller than the oil-drops and yolk-spheres of the *Nereis* egg, can not be looked upon as colloidal solution for their inclusions are visible under relatively low power of the microscope and thus do not fall within the range of sizes of particles in a colloidal solution. But the ground-substance of these eggs is a colloidal solution since its particles, barely visible under the highest powers of the microscope, are more clearly revealed by dark-field illumination.

The definition of cytoplasm here given also means discarding theories of the morphological structure of cytoplasm since these, the alveolar, filar and granular theories, refer to the cytoplasm plus inclusions.

Even if we would define cytoplasm to embrace the cytoplasmic inclusions, the alveolar theory of cytoplasmic structure would be untenable. In the first place, although it is true that there are cells which show a vacuolated or foam structure, this is by no means all-pervading but is limited to certain cellular regions. Moreover, such structure is far from being demonstrable in all cells and the

experimental evidence indicates that it is non-essential to
the living cell-substance. Secondly, the alveolar structure
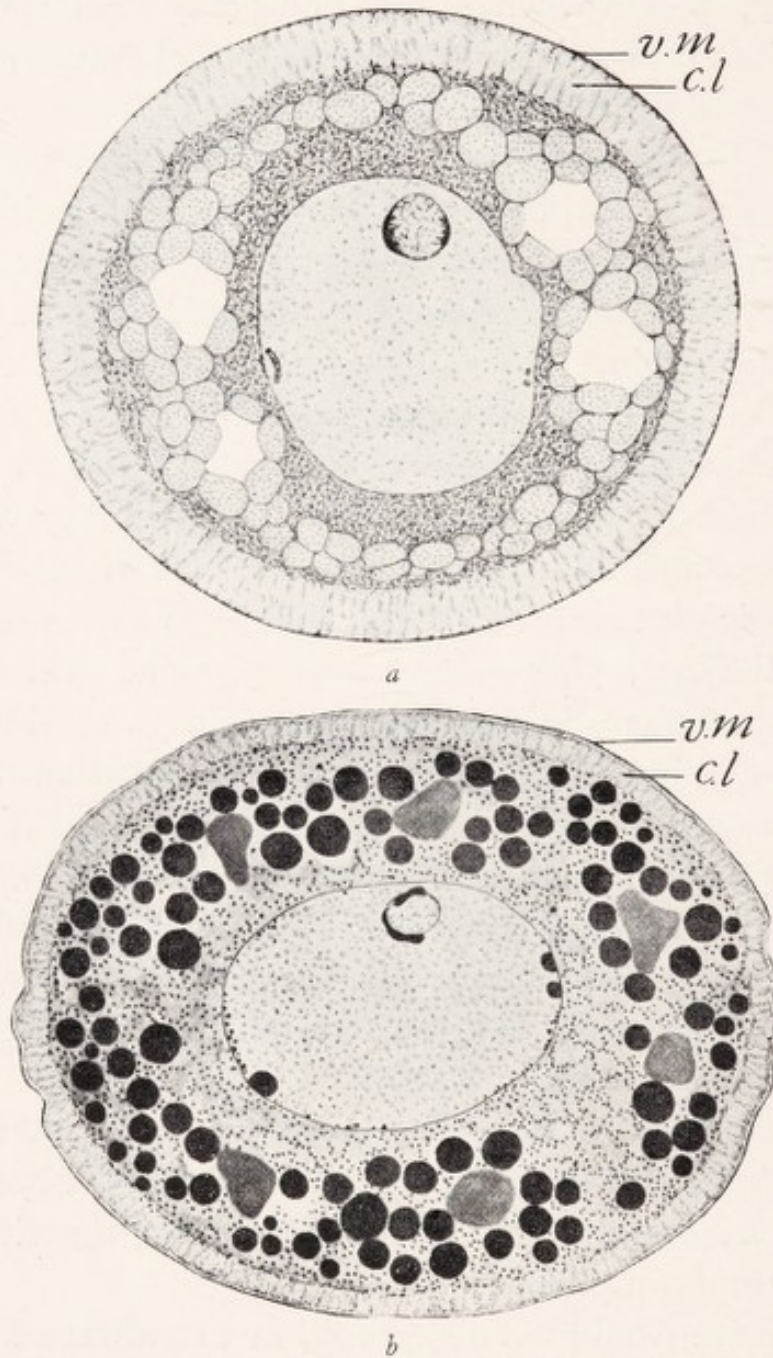
FIG. 2.—Two sections of the unfertilized egg of *Nereis limbata* (after Lillie).
a is badly fixed as shown by the partially dissolved yolk spheres and the wholly
dissolved oil drops which in *b* are intact. Compare *b* with the figure of the living
egg as shown in Fig. 22 (p. 158). v.m., vitelline membrane; c.l., ectoplasm.

described for fixed cells, as it often has been, is the result
of improper fixation. In such cells the cytoplasm appears
a mesh, empty holes occupying the greatest space, holes

which have been brought about through the action of the fixing solution in dissolving out fat, yolk or other cytoplasmic inclusions. Compare the pictures given, (Fig. 22) of a living, (Fig. 2a) of a poorly fixed, and (Fig. 2b) of a properly fixed egg. In Figs. 22 and 2b oil-drop and yolk-sphere are intact. When one bears in mind that Fig. 2b is from a very thin section, about one-thirtieth of the entire egg, and that the larger inclusions present can very easily be displaced in sectioning, the picture is all the more convincing: the resemblance to Fig. 22 is striking. But compare Fig. 2a—a mesh-work like a fish-net. From pictures inferior to this (Fig. 2a), wholly disregarding the structure seen in the living egg, Wilson derived his support of the alveolar theory of protoplasmic structure.[1] When one speaks of the physical basis of life and refers only to the morphology of the cell, one is misleading[2]—doubly so if one has in mind a dead cell showing mostly holes. True, protoplasm is the physical, i.e., the corporeal basis of life; also in the fixed state this basis is of interest. But we shall not understand it, if first we vacuolate it and then ascribe to the holes essential meaning without knowledge even as to the significance of the substances which we replaced by holes. On the other hand, to say that the cytoplasm of the living cell is alveolar in structure because the holes of the dead cell were occupied by formed bodies is to give new meaning to the term, alveolus; I can not see how a droplet of fat or a spherical yolk-mass can be designated an alveolus. I have often pressed them out of an egg into sea-water where they remain intact. If they are moved by centrifugal force from one to another region of the cell, they do not leave empty spaces behind them. Hence, neither they nor the places they occupy in the fluid cytoplasm are alveoli.

[1] *Wilson, 1926; but cf. Mathews, 1906.*
[2] *Wilson, l.c.*

But let us for the moment dismiss badly fixed eggs, pictures of fenestrated or lacunar substance, also the empty spaces previously occupied by oil and yolk, and extend the meaning of the word alveoli to embrace such inclusions as oil-drops and yolk-bodies seen in the living egg as discrete spheres. How far does this assumption strengthen the alveolar theory of protoplasmic structure? Can we conclude that inasmuch as each "alveolus" (oil-drop or yolk-sphere) is laid down by the egg during its history, an egg in that stage before oil or yolk is deposited as easily visible discrete spheres has no structure? Shall we say that in those eggs which distribute their "alveoli" in such manner that some cells possess all the "alveoli" and others none, those cells which do not contain them are structureless?

The alveolar theory has found support from the fact that certain artificial mixtures resemble some kinds of proto-plasm. Witness emulsified oil. If we examine under the microscope a thin layer of mayonnaise—olive oil and vinegar plus egg-yolk, the emulsifying agent—we find that the oil is in drops and among them are scattered yolk-spheres. By carrying the process of emulsification further and further, we obtain smaller and smaller oil-drops and in this way make structures that resemble those of eggs with larger or smaller oil-drops. Such a film of mayonnaise when fixed by a proper agent for cytoplasmic fixation retains faithfully its appearance before fixation and simulates the picture of properly fixed egg-cytoplasm. The emulsification obtained by beating olive oil with vinegar or lemon-juice, without egg-yolk, though less permanent, bears also a close resemblance to the living cytoplasm of a living egg, say that of a star-fish. It has even been held that protoplasm is a foam-structure because of the resem-blance of some cellular structures to such foams prepared in the laboratory. As I see it, all that these and similar models tell is that the living cell can hold its oil in the form

of discrete drops of larger or smaller dimensions. They give us no information concerning the mode in which the living cytoplasm moulds the oil-drops—now coalescing them, now emulsifying them. They fail to reveal the value of such structure for life phenomena. Finally, for the alveolar theory, a yolk-sphere is as much an "alveolus" as an oil-drop; and yet although we do not know as much about yolk as we would wish, nevertheless, what knowledge we have is sufficient to warrant the conclusion that both physically and chemically a yolk-sphere is something quite different from an oil-drop; a yolk-sphere, unlike an oil-drop, is a combination of fat and protein.[1] If one adheres to the alveolar theory by denominating oil-drops and yolk-spheres "alveoli," one excludes from consideration the differences between these "alveoli," thus stressing their shape rather than their physico-chemical make-up.

The filar theory of cytoplasmic structure never has attracted many adherents, doubtless because of the limited occurrence of fibrils in cytoplasm. For the most part, when found, they are in fixed cells, in which, before fixation, they can not be seen.[2] The evidence for their presence in some other living cells is by no means convincing. Thus, the cytoplasm of outward flowing cell-processes appears to be filar—but this may be due to granules disposed in rows by the streaming of the medium that is forced through the orifice.[3] Mitochondria-granules may by close alignment appear as fibrils.

Altmann[4] suggested that granules in the cytoplasm are the elementary living substance. Both before and since his time others have put forth similar theories, often including more kinds of cellular granules than Altmann did.

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[1] *See also Konopacki, 1929.*
[2] *Lewis and Lewis, 1924.*
[3] *See Bütschli, 1890; also Just, 1928e.*
[4] *Altmann, 1890.*

Undoubtedly, Altmann's granules are mitochondria; as such they are only cell-inclusions. As to other granules described in this connection, one must remember that many granules in cells are purely secretory—that is, precursors of the cell's secretion, as the granules found in a salivary gland or in the pancreas—that some others are storage-stuffs, others, excretory products. In other words, granules are derivatives of cytoplasmic substances or expressions of cytoplasmic activity. That to which they owe their origin and not they themselves would seem more likely to be basic.

For the reasons given, I think that we may discard the alveolar, filar and granular theories of cytoplasmic structure. Oil-drops, yolk-spheres, threads and granules in the cytoplasm make the contents of a cell a gross suspension. Such a suspension as that of an egg of *Nereis* or of a sea-urchin does not represent the basic structure of life and its peculiar physical state does not offer any clue to the solution of the problem of vital activity.

Turning to the menstruum containing the formed bodies, the ground-substance, we find, as stated above, that it appears to be almost optically empty. If, however, one examines the hyaline region of a centrifuged egg of *Arbacia*, *Nereis* or *Chaetopterus*, with extreme care without pressure on the egg—for pressure induces abnormal formations—one can observe very fine granules as barely visible points. Thus, as also said above, the ground-substance is to be considered as a colloidal solution, for it has particles below the size seen under the ordinary microscope, but which can be seen under dark-field illumination. Until we know more of the physics and chemistry of the ground-substance, we must derive a conception of its structure by observing it in the living state and when properly fixed. The fact that well-fixed ground-substance so closely resembles the living makes fixation a supplementary aid in its investigation and permits us to draw conclusions concerning the living state

from the fixed. Processes too fleeting to be followed exactly in the living can be followed in their single steps in the fixed. By thus supplementing the study of the living by that of the fixed ground-substance, we may approach the understanding of the basic structure of cytoplasm. My confidence in the value of the study of properly fixed cells rests upon the fact that such study has confirmed my observations of the extremely delicate momentary changes in the ectoplasm of living cells. Without ever discounting the postulate that the living cell is the basis, control and aim of biological investigation, I have come to appreciate the value of proper fixation. But since the use of fixation has been and is questioned by many workers, I must for a moment dilate on this point.

When at the end of the last century Fischer[1] and Hardy[2] independently inveighed against fixation of protoplasm, neither made much impression. But the impression grew; to-day we discern a reaction of no mean proportion against the study of fixed cells. Some histologists adopt an apologetic air, offering their findings on fixed tissues in all but a furtive manner; others have given themselves over to the cinematograph or to the culture of living tissue removed from the animal.

Now without doubt conclusions as to living protoplasmic structure drawn from study of dead cells should ever be subjected to severest criticism. He who works with dead cells should never be under delusions that he has to do with other than a dead thing. I do not believe that any competent histologist takes any other view. And yet, there is often inference by those who hold lightly all work on fixed cells, whatever its purpose or conclusions, that the investigator of fixed, sectioned and stained cells forgets

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[1] *Fischer, 1899.*
[2] *Hardy, 1899.*

not only that he deals with dead matter but also that he subjects the corpse to some thirty separate treatments before he places sections of it under his microscope for investigation.

Hardy's paper nowadays hailed by so many as having infallible authority falls short in three directions[1]: First, Hardy studied the action of too few fixing solutions; second, he failed to make comparisons of the fixed with the living cells; and third, he used cells in bad condition.

Every student of histology knows that he can not assume that because he has successfully fixed cells of one tissue he can employ this same fixation for every other kind of cell. The technique of fixation has not passed very far beyond the trial and error stage and, therefore, every new type of cell encountered offers a new problem of fixation. Hardy should have used more agents and of a much more diversified constitution instead of holding so strictly to those of closely related composition.

Hardy made comparisons between the fixed cell (for example, gut cells of *Oniscus*, the so-called pill-bug)[2] and protein solutions and came to the conclusion that his fixing agents act upon the protoplasm as they do upon protein. This conclusion revealed nothing new. It would have been far more valuable had he made comparisons between the living and the fixed cells in order to ascertain how far was the deviation of the fixed from the normal. Further, Hardy investigated always cells of tissues and never free-living cells as Protozoa or eggs in their normal environment. This

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[1] *In his more thoroughgoing work Fischer contributed to our knowledge of microscopic technique with respect to the use of dyes.*

[2] *I may point to the fact that the choice of this object, gut cells of Oniscus, was most unfortunate, for several reasons. These cells, for instance, contain much chitin whose presence prevents uniformity of fixation. See further: Scheikewitsch, 1895; McMurrich, 1895; Conklin, 1897.*

fact warrants my third objection to his conclusions for two reasons: First, his cells were already in some state of *post mortem* changes of greater or lesser degree depending upon the time elapsing between their removal from the animal and their fixation—these changes being more rapid, and, therefore, a more serious source of error, for tissue-cells removed from the warm-blooded animals which he used. In the case of warm-blooded animals it would also make a great difference whether or not the animal from which the tissues were removed, was anaesthetized. Second, he made no allowance for the bulk of tissue used; that is, his conclusions would have been far more sound had he used a thin sheet of tissue made up of a layer of one or two cells instead of compact masses from the glands investigated. Not only were his cells in some degree of *post mortem* change, but also were they fixed unevenly and cut into sections of different thickness.

True, there is bad and unreliable fixation of eggs. Nevertheless, it is erroneous to condemn all fixation. If I find that after fixation an egg very closely resembles the living I can draw conclusions on the basis of this resemblance especially since knowing that the cell is dead I take into consideration that its proteins are changed—coagulated, gelated, precipitated, and that still other changes have taken place. Instead of abandoning the study of fixed cells we should use proper fixation in order to check and extend observation on the living. Neither the cinematograph nor tissue culture will quite replace the good fixation of the cell for comparison with the living.

Although our ignorance of the ground-substance is profound and will remain so until we study it frankly as such,[1] isolated from all the coarse particles suspended in it, nevertheless we have at our disposal certain evidence which may

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[1] *Just, 1936b.*

form the basis for the extension of our knowledge. That the clear, nearly homogeneous, almost structureless ground-substance of an egg can (with a nucleus) develop, renders it at once amenable to all treatment given an entire egg. As it develops it exhibits the same form-changes. Here I restrict myself to the discussion of one well-known phe-
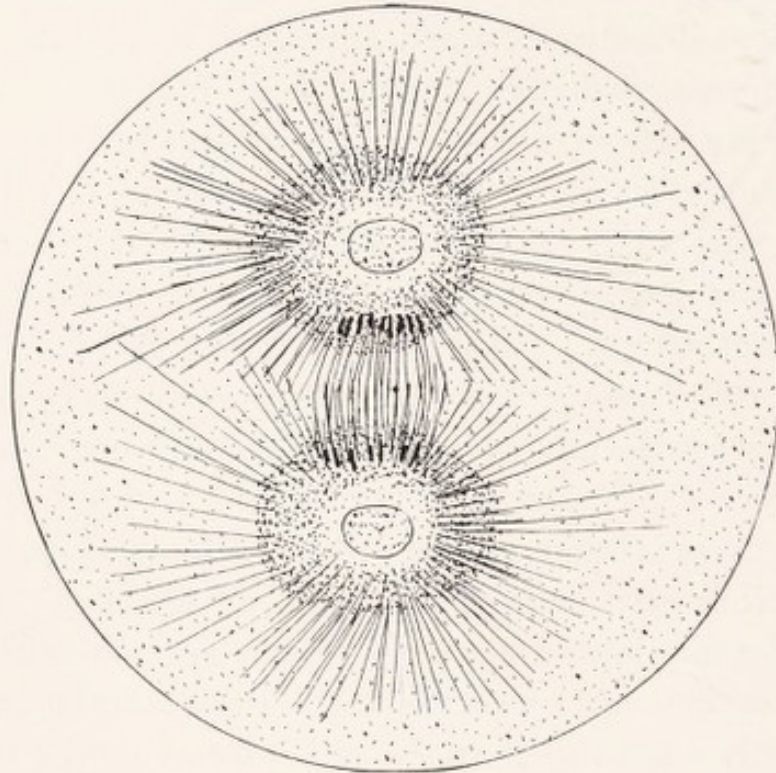
Fig. 3.—Boveri's classic figure of a mitotic spindle in the egg of *Echinus microtuberculatus*. But cf. the aster in Fig. 29b (p. 174) and the mitotic complex in Fig. 33d (p. 259).

nomenon, namely, the appearance of the radiating structures seen in so many cells during cell-division.

In many cells division of nucleus and of cytoplasm occur simultaneously. Division of the nucleus may be a very simple direct process, a constriction and separation of the constricted parts or a more complex and indirect process with the chromosomes going through a very orderly sequence of manoeuvres on a bundle that appears to be made up of threads. In this latter type of nuclear behavior there often appear in the cytoplasm (occasionally in the

nucleus) radiations from each polar end of a group of fibre-like structures, the so-called mitotic spindle. (Fig. 3.) The radiations center at a minute granule or at a larger vacuolar sphere-structure; in some cases both granule and sphere are present. The minute body is the centriole or centrosome and the sphere is the centrosphere; the radiations because of their disposition are called astral rays. Best seen in cells that possess granules, the rays are also demonstrable in cells which are granule-free. Further, cells which show no rays are often filled with granules. Therefore the rays do not depend upon the cell-inclusions. Seen in the living egg of *Platynereis*, for example, every astral radiation is a granule-free track whose greater width is about 3 μ. In the living *Arbacia* or *Echinarachnius* egg the rays have the same configuration. Nothing in the living or properly fixed egg appears as the stiff coarse "astral fibres" of poorly fixed eggs. The radiations are without doubt no fibres at all. It is often held that they are paths of flow and in living granule-rich cells they do certainly appear to be such —the flow causing a displacement and close packing of granules and thereby accentuating the radiate appearance. There is no unanimity of opinion concerning the nature either of the radiations or of the spindle-"threads" on which the chromosomes move. I share with others the idea that spindle-"threads" and astral radiations are both extremely fine delicately thin sheaths enclosing fluid material, that they are not in any sense fibres with contractile power. (See Fig. 29b and Fig. 33d.)

As an almost perfectly homogeneous and fluid system, cytoplasm could scarcely show differentiated pathways of flow even in extremely thin-walled tubes; less could it show contractile fibres. Either then cytoplasm is not structureless or it has power to elaborate structure sufficient to account for the ray-configuration of the astral system. In either case the cytoplasm is different before and after the

appearance of the rays. These rays thus indicate structure in the cytoplasm or at least the capacity of the cytoplasm to form structures.

So far I have dealt with the cytoplasm, the ground-substance outside of the nucleus. I should remind the reader that I consider the nucleus likewise as differentiated ground-substance. The formation of intra-nuclear spindles just referred to, the origin of centrosomes from nuclei, sometimes described, can be interpreted on the same basis as the formations in the cytoplasm. Nucleus and nuclear constituents are themselves conceived as expressions of the capacity of the ground-substance to form structures.

In its other derivative, the ectoplasm, the ground-substance likewise exhibits capacity for structural formations. Indeed, ectoplasmic structure in many cells, e.g., Protozoa, is a diagnostic characteristic. I wish to consider this peripheral ground-substance as it appears in animal cells. But first I must discuss the question of the cell-membrane, because it has obfuscated a proper appreciation of that part of the cell beneath the membrane, the ectoplasm.

The cell-membrane, like many another cell-structure, is a perennial subject for debate. The majority opinion is that cells are invested with membranes but the minority is as insistent that a membrane is neither present nor necessary. Of those in the camp of the majority, some hold that the cell-membrane is an endogenous structure, a living thing built by the cell; whilst others deny its life, considering it a sort of excretion; and still others assert that it is a formation induced by the cell's external medium. Opponents of the membrane's existence declare that since the cell-substance is in the colloid state, it is unnecessary to postulate the presence of a membrane: the mere boundary of the colloidal system is sufficient to preserve the cell's integrity. According to them the cell is somewhat like a drop of oil in water; just as the surface of the oil keeps the oil intact, so does the

surface of the more heterogeneous cell-substances prevent cellular disintegration. Also, are there those who unwittingly hold a foot in each camp.

Now, whenever there are so greatly divergent opinions, one suspects either insufficient factual basis, or confusion arising from too loose definition. I strongly suspect that the difficulty here arises out of the fact that investigators in their discussions are often not dealing with the same thing; much of the argument then is beside the point. The cellulose wall of a plant cell is something quite different from the surface of a Paramoecium as both observation and experiment show. Similarly, is it hazardous to compare the surface of the red blood corpuscle in human blood with that of human cells or even with the red blood cells of vertebrates other than mammals since the mammalian red blood corpuscle is not a true cell because it is devoid of a nucleus. Therefore, if the mammalian red blood corpuscle lacks a membrane this is no proof that true cells also lack such.

The first proposition is thus clear: in defining a cell-membrane we must be careful to distinguish between the various coverings enclosing cells, excluding, for example, discussion of such formations as cellulose walls around plant cells. The second proposition is, likewise, obvious: if we speak of cell-membranes, we must speak of cells; the presence or absence of a membrane on a piece of a cell like the mammalian red blood corpuscle does not come within the discussion. The issue concerns cells and cells only. Indeed, much of the work on mammalian red blood corpuscles should be used only most judiciously in making comparisons with true cells.

Thus restricted, the discussion on the cell-membrane centres around two questions: Is there a membrane around animal cells? And if present, is it a living part of the living system or an exogenous formation? If exogenous, I

presume that it is non-living. I think that the question of necessity or purpose is not involved. To say that one does not see how it is possible for a cell to remain intact without a membrane most certainly does not mean that it can not thus remain. Here I speak only of those cells that I know best, egg-cells, though I hold that the discussion can apply to some extent also to other animal cells which, like the eggs, are often held to be without membranes.

In the description given above I pointed out that on eggs, as, for example, that of *Platynereis*, two membranes are present; the outer or vitelline, and the inner or plasma-membrane. Whenever egg-membranes are discussed, the two membranes should be clearly distinguished. Furthermore, when the vitelline membrane is spoken of, it should be clearly stated whether the unfertilized or the fertilized egg is considered. Much of the disagreement concerning the nature and properties of egg-membranes is due to the confusing of the vitelline and the plasma membrane and to failure to appreciate the difference of the former before and after fertilization.

Generally, the essential difference between a vitelline and a plasma-membrane lies in the fact that the latter is continuous with the egg-cytoplasm whilst the former is discontinuous with it, set apart from it and separated by a space, the so-called perivitelline space. Using this simple distinction we meet, however, with the difficulty that in many eggs that membrane which after fertilization is thus so clearly set off as a vitelline membrane, before fertilization appears as a plasma-membrane, continuous with the cytoplasm. When after fertilization such a vitelline membrane separates from the egg, on the cytoplasmic surface of the egg appears a new plasma-membrane.

In observing one living egg I may be able to say in a moment that a membrane is present, it being so sharply differentiated; observing another species of egg, I may not

be so sure but doubt vanishes if I know the history of the egg. Take the unfertilized egg of the sea-urchin concerning the presence of whose vitelline membrane there has been much dispute. Knowing the history of this egg as it develops in the ovary I know that it arises by successive divisions, each egg everywhere bounded by a membrane except at its point of attachment to the ovarian wall. I know also that the egg is part of the parent whose cells arose by a long series of cell-divisions, of separations by cell-surfaces, from a fertilized egg. This history strongly indicates the presence of a membrane and the simple experiments made previously by others which I repeat and confirm strengthen the indications. What is true of this egg is true of all other eggs that I have studied: they possess membranes built by the egg, living structures and not adventitious depositions from the outside. In other eggs, those of worms, molluscs, ascidians as well as of other echinoderms, the presence of a membrane before fertilization can not be doubted. The fact that membranes become separated at a distance from the eggs after fertilization does not mean that the eggs are now without plasma-membranes for they at once form them anew. Careful observation can establish this as true.

It follows from these considerations that the membrane is neither a deposition nor a precipitation induced by the egg's surrounding medium. This is not to say that the egg-plasma never forms precipitation-membranes. On the contrary, the contents of an egg caused to burst by application of pressure may often show a congealed surface. But this death-change by no means constitutes evidence that the membrane on the living intact egg is a precipitation-membrane. A pared apple in time develops by dessication an outer tough coat but this is not the same structure as the removed skin. Nor does it follow on the definition of protoplasm "as a film-pervaded system," which is often set

forth, that the external covering of a cell is similar to or identical with such intracellular partitions.

The understanding of the cell-surface and of the capacities residing in the ground-substance is forwarded by appreciation of that component of the protoplasmic system, which I now discuss in detail: The ectoplasm.

4

The Ectoplasm

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HAECKEL,[1] DESCRIBING THE CELL-STRUCTURE OF sponges, first used the terms, exoplasm and endoplasm. He clearly distinguished two regions of the cytoplasm as follows:

> In the hyaline contractile ground-substance of the proto-plasm a varying mass of small dark granules is constantly embedded; these usually surround the nucleus. On the living flagellar cell, as long as it is active *in situ*, is a thin granule-free cortical sheath. Thus one can more or less clearly distinguish between a structureless outer cortical substance and a granular inner medullary substance. The outer cortical substance (exoplasma) is fully hyaline, some-what firmer, less watery, more strongly refractive and contains no granules; the inner medullary substance (endo-plasma) is granular, somewhat softer, more watery, less refractive (and contains the granules) and also now and then vacuolar. No matter how distinctly the regions some-times are set off from each other, they are never sharply separated but pass insensibly the one into the other without fixed limiting layer, much as is the case of hyaline cortex and granular medullary substance in the body of an infusorian.

Later Haeckel says[2] that through its variously changing surface formation, the exoplasm gives to the entire flagellar cell its characteristic form.

This clear description given by Haeckel may serve as the basis of our definition of the ectoplasm (exoplasm of Haeckel): the ectoplasm is the superficial region of the protoplasmic ground-substance set off from the remainder

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[1] *Haeckel, 1872.*
[2] *Haeckel, l.c.*

(both nuclear and cytoplasmic ground-substance) by differences in physical properties, in structure and in behavior; as part of the cytoplasm, as differentiated ground-substance, it is definitely a living part of the cell. The inner region of the cytoplasmic ground-substance plus its cytoplasmic inclusions—granules and the like—is defined as the endoplasm.

It is not to be supposed that ecto-endoplasmic differentiation in animal cells was unknown before Haeckel's time. Although not named as such, those regions were known to the older students of the cell.[1] Almost any original paper and text-book of theirs points out differences between the inner and the outer region of the extranuclear cell-contents. Thus, Henle in reports of his researches and Koelliker in his text-book on human histology gave clear descriptions of what subsequently were known as ectoplasm and endoplasm. Kidney, liver and intestinal cells were both described and figured to show these regions. The cells of the skin were favourite objects for such descriptions.

If one observes a section of the kidney taken from man or other vertebrate one notes that certain cells are striated and possessed of radial projections. Thus, these cells reveal an ectoplasmic differentiation.

The ectoplasmic area is less clearly marked off in the human liver cell. The manifold functions of the liver—the breaking down of red blood corpuscles, the formation of urea, the storage of sugar as glycogen, the mobilization of fat—are reflected in the many pictures of itself that the small polyhedral liver-cell can exhibit. It also appears differently in conditions of hunger and after rich nourishment. Nevertheless, in all its different appearances, the liver-cell shows a differentiation between inner and outer regions of the cytoplasm, as Koellicker observed.

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[1] *For example: Leydig, 1857; Kühne, 1864.*

The cylindrical cells of the human intestine, measuring 22–26 by 6 μ, show on the side presented to the intestinal lumen a brush border or *bordure en brosse*. Thus, in these cells as in those of the kidney and liver one notes an ecto-endoplasmic differentiation.

In the skin are cells which interlock by means of fine threads, the so-called intercellular bridges.[1] Cells of other tissues are connected similarly, as for example those in the retina of the eye. Though many claim that these connections are artefacts, the abundance of evidence from studies on various tissues from many different animals supports the view that these connections are normal cell-processes. As such they are evidence of ecto-endoplasmic differentiation.[2]

In view of the substantial body of convincing data showing the presence of intercellular connections, it becomes difficult to understand why many authors still deny their existence and assert that fixation creates them. But quite apart from the demonstration of these intercellular connections in well-fixed tissues exist clear cases, some of them among the earliest described, showing living cells in rich connections with each other. As will be shown later connections between cells of the developing egg have been long known and can be easily demonstrated. I think that almost no one to-day doubts that in the nerve-system the neurones are in contact with each other by processes which make the synaptic membranes.

The body-structure of higher animals, especially of vertebrates including man, embraces certain groups of cells notable for the presence of extra-cellular substances associated with and produced by them. These groups of cells comprise the binding or connective tissues of the body. Their products are fibres of various kinds, cartilage and

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[1] *See Schuberg, 1903, and later writers.*
[2] *Flemming, 1879, on living cells.*

bone. Studies on the development of fibres in connective tissue, on that of cartilage and of bone indicate that these structures which finally come to lie outside of the cell are products of the ectoplasm. Indeed, it has been postulated that connective tissue fibres even after deposition outside of the cell are living ectoplasm. Whether living or not, the intercellular substance of connective tissue and its derivatives (cartilage and bone) are to be regarded as extreme formations of ectoplasm, which have lost their organic connections with their cells of origin.

Intercellular fibres cast off by cells are to be distinguished from such fibres which are true cells, having nuclei. These, muscle and some nerve cells, are characterised by their great length and hence by a preponderance of surface to mass. They thus possess relatively more living ectoplasm as part of the cells than any other cells so far discussed. Muscle-cells are notable for their high degree of contractility and students of physiology regard this as a surface-phenomenon. Fixed preparations of smooth or plain muscle-cells (individual fibres) show longitudinal threads or fibrils whilst striated muscle-fibres (both those of the heart and of the skeletal or voluntary muscle) show in addition striations running at right angles to the length of the fibres. Much difference of opinion is expressed concerning the significance of these fibrils because studies both on the developing and the definitive muscle-fibres have yielded conflicting results and led to various interpretations. In addition, many investigators doubt the actual presence of fibrils in muscle-fibres inasmuch as they have been unable to see such in living cells. Although it would seem hazardous to venture an opinion in view of this uncertainty with respect both to the longitudinal threads described in fixed smooth muscle and to the additional cross-striations reported in striated (cardiac and skeletal) muscle as seen in fixed preparations, nevertheless, I may offer suggestions concern-

ing them.   These suggestions are consistent with the well-established findings of studies made on both types of muscle-cells by the method of tissue-culture devised by Harrison and with the fact that in the muscle-cell the ectoplasm is preponderant.   Also, they stand in accord with our knowledge of ectoplasmic structure in cells generally.

The smooth muscle-fibre, an elongated slender spindle-shaped cell, reveals itself in fixed preparations as possessed of fine longitudinal threads, fibrils or myofibrils.   Lewis and Lewis were unable with the use of either bright- or dark-field illumination to observe any fibrils in living smooth muscle-cells grown in tissue-culture.   According to them, large flat cells seldom contract whereas the elongated and band-like ones sometimes exhibit rhythmic contraction. These latter show ectoplasmic processes.

> Very much elongated overlapping processes may simulate fibrils when parallel to the long axis of the cell.   In many places the cells seem to spread out under considerable tension, and it appears to be along the lines of tension that the contractile substance coagulates into fibrils of various sizes.[1]

From what is known of the contractile power of ectoplasmic processes of cells generally in tissue-culture, it is safe to conclude that the contractility of the smooth muscle-cell is likewise inherent.   I suggest that the pictures of fibrils obtained in fixed preparations represent ectoplasmic processes somewhat altered by the action of the reagent employed to fix the cell.

Striated muscle differs from smooth by showing alternate light and dark bands traversing its long axis.   The exact structure of striated muscle remains in question. Since it begins its development as smooth muscle, it would seem most profitable to attempt an elucidation of its structure through study of its developmental stages.

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[1] *Lewis and Lewis, 1924.*

But in these studies is lack of agreement. According to one view the fibrils arise from cytoplasm which is itself of fibrillar structure; another view maintains that the fibrils arise from cytoplasmic inclusions. The living muscle-cell in tissue-culture often fails to show the structure seen in fixed preparations; according to many investigators cross striations do not appear until after the cell is fixed. I venture the following suggestion as to the cross-striations in the muscle-cell.

The young striated muscle-cell, like that of smooth muscle, possesses ectoplasmic prolongations. The prolongations arising first fuse at their outer tips and thus form a membrane so that this region of the ectoplasm has a palisade-like structure. Repetition of this process throughout the length of the cells forms fibrils (the membranes) crossed by ectoplasmic prolongations.

Beyond I shall show that the surface of the fertilized egg of the sea-urchin is made up of radial filaments covered by a thin membrane. My conception of the striated muscle-fibril has its origin in this fact. Since other cells than eggs show such projections, it is not too extreme to suggest that the embryonic muscle-cell also possesses such. The slender muscle-cells have the inherent capacity to build up successively striated fibrils by reconstitution of their surface. The sea-urchin's egg builds one such surface layer which remains inseparably bound to the egg-cell; the muscle-cell builds many such surfaces.

Also on other grounds my conception of the origin of the cross-striated muscle-fibril is not so far-fetched as it may seem. In the first place, the generally accepted doctrine of muscle-activity emphasizes the rôle of surface in the phenomenon of its contraction. The fact that in the contraction process, in one stage, oxygen is utilized, is evidence indicating a surface-reaction. Moreover, the shape of the muscle-fibre strongly supports the theory that

its activity is limited to the surface. On more general grounds, it may be pointed out that it is held that muscle-contraction, ciliary action and amoeboid movement are similar and have a common basis. Ectoplasmic threads may be considered as fine amoeboid processes, delicate pseudopodia; a cilium may be regarded as a modified pseudopodium. On the basis of my suggestion the striae of a cross-striated muscle can be regarded as threads of ectoplasmic substance, and muscle as a strand compounded of pseudopodial processes.

This suggestion of mine concerning the origin of the muscle-fibre from repeated building of sheets of ectoplasm is interesting in the light of a passage from Lewis and Lewis in their "Behavior of Cells in Tissues"[1]:

> There are three conditions resembling fibrillae, which are often observed, not only in heart-muscle cells, but in smooth muscle, endothelium, and mesothelium as well; namely, a linear arrangement of long mitochondria, an overlapping of long, slender processes, and tension striations. The latter deserve most consideration, because they resemble closely the appearance of fibrillae in fixed cells. Migrating cells become more or less flattened out on the solid supports under considerable tension. The direction of this tension appears to be in line with the cell processes, as though the latter produced a pull on the ectoplasmic layer. The visible striae of various widths and lengths thus produced in the living cell are not permanent but may disappear and new ones may appear in line with new processes. The exact significance of these striations is uncertain. They may extend across the nucleus, indenting it or almost cutting it in two. They seem to be a phenomenon produced by tension and reversible when the tension is altered or relaxed. On fixation they may retain their identity and stain more deeply than the rest of cytoplasm, resembling myo-fibrillae. The latter occur however in fixed cells which do not show tension striae.

Certain nerve-cells are also notable for their fibre-like nature. Among these are the longest cells known. One

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[1] *Lewis and Lewis, l.c.*
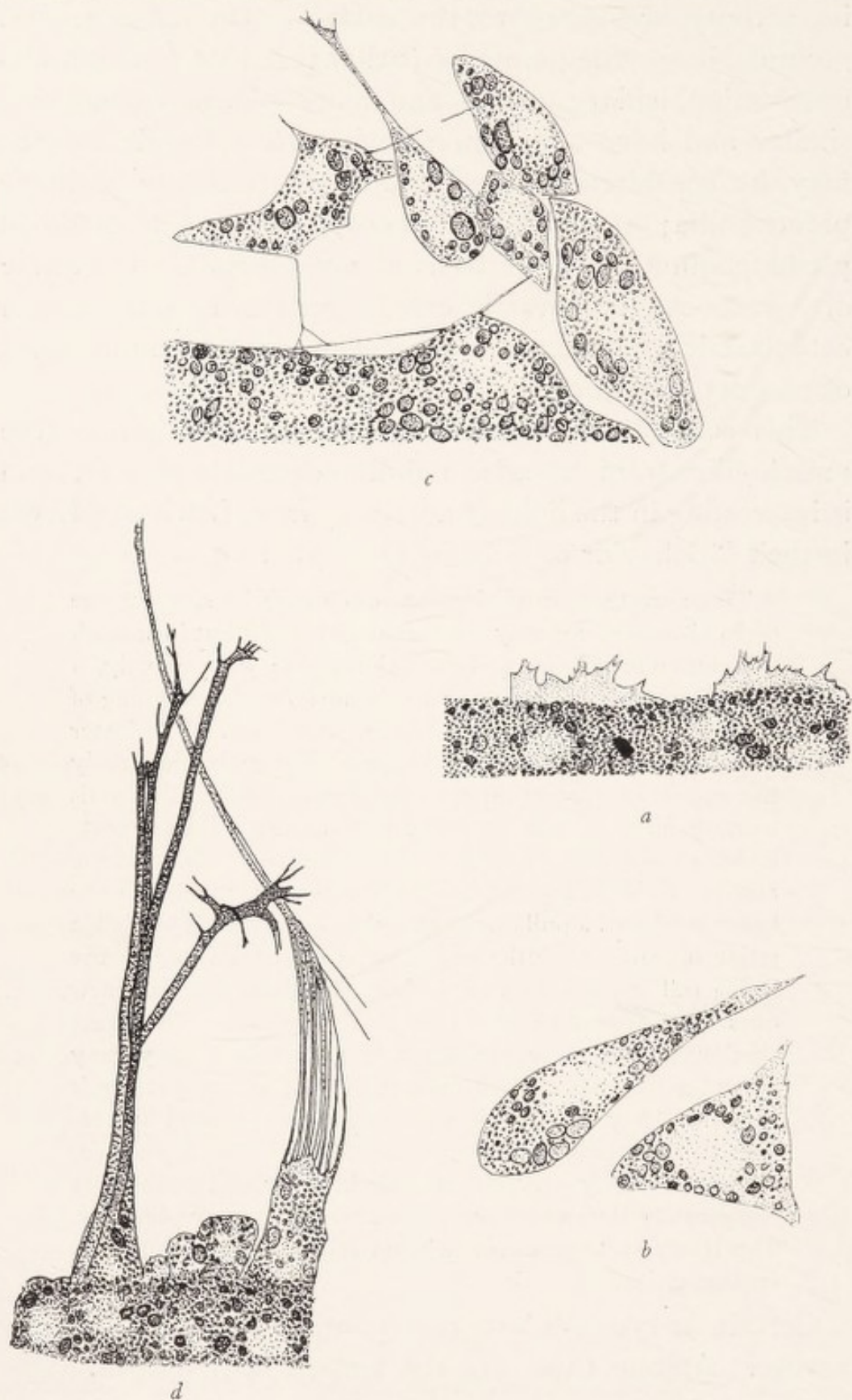
FIG. 4.—(after Harrison).   *a*, row of ectoderm cells showing amoeboid ectoplasm; *b*, *c* and *d* are protoplasmic processes in developing nerve cells.

cell and its process, for example, of the sciatic nerve in man which is made up of processes from nerve-cells in the spinal cord, may be easily a meter in length. What we speak of as a nerve is really a bundle of processes from nerve-cells. When, for instance, one makes experiments in the laboratory on excised nerves—the sciatic nerve of the frog is a favorite object for such—one uses only the ectoplasmic portions of cells. Therefore, conduction by nerves means conduction by ectoplasm. That the nerve-fibre is ectoplasm was fully demonstrated by Harrison's classic and epoch-making work on the origin of the nerve-fibre.

Harrison's researches while establishing the mode of origin of the nerve-fibre also furnish a beautiful demonstration of ecto-endoplasmic differentiation. Harrison, the originator of the now so widely used method of growing tissues outside of the body of an animal, had as his object the settlement of the much debated question of the mode of origin of the vertebrate nerve-fibre from the central nervous system. By growing young embryonic cells taken from the spinal cord of a frog embryo at a stage in which he knew that no nerve fibres had yet developed, he was able to prove that these cells extend pseudopodial processes which by farther growth become the nerve-fibre. Says Harrison[1]: (Fig. 4.)

> From the time when the tissue is implanted in the lymph it shows a tendency to spread out, and often broad laminae made up of a single layer of cells are found at the periphery of the mass, while individual cells may move off entirely by themselves. This is the case with both nervous and axial mesodermic tissue, as well as with pieces of ectoderm, though the latter more often roll themselves into complete spheres. One notable peculiarity that has frequently been observed is the formation of large round or oval openings in the flattened tissue, which may be surrounded by very narrow bands or rings of tissue with cells sometimes in single file. This phenomenon may possibly be due

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[1] *Harrison, 1910.*

to the mechanical action of the fibrin upon the implanted tissue, but the spreading out of the cells into thin sheets seems to result largely from the activities of the cells themselves. These activities, which are common to several tissues, in fact to all except the very inert yolk-laden endoderm and, perhaps, the notochord, may be referred to a form of protoplasmic movement having its seat in the hyaline ectoplasm found at the angles and sometimes at the borders of the cells. The movement cannot be observed clearly in the larger masses of cells on account of their opacity, but it may be seen very clearly in those cells which leave the main masses and wander off by themselves. These cells are irregular in shape, varying from unipolar form and having a varying amount of ectoplasm at their angles. The movement is amoeboid in character and results either in a change in shape of the cells or in their movement as a whole. Such cells are found usually in greatest numbers in preparations of the medullary cord, and from the cranial ganglia (branchial ectoderm), that gives rise by its movement to long fibres. Cells of the epidermis show their power of movement in somewhat different form. As has frequently been observed, the general tendency of isolated bits of epidermis is to round off into small vesicles, which, when left in water, may move about for days by means of their cilia. Within the lymph the same thing frequently takes place, although there is apparently greater resistance to the process of rolling up, and the cells may often remain together in the form of extensive sheets. Along the free border of these sheets of cells there often appears a fringe hyaline of protoplasm, which undergoes continuous amoeboid changes. In one case of this kind it was observed that the sheet of cells gradually spread out toward the side on which this fringe was placed. Since the work of Peters (1885–1889) it has been generally admitted that wound healing in the epidermis is primarily due to the movement, in part amoeboid, of the epithelial cells, so that it seems quite possible that in this fringe of hyaline protoplasm above described, we have one part of the mechanism by which the movement of cells in wound-healing is brought about. The most inert of all the tissues is the endoderm, which will remain for days in the lymph, practically unchanged, gorged with yolk and devoid of hyaline ectoplasm. The notochord is also very inactive, although large pieces of this structure may show after a

time the early stages of normal differentiation, unaccompanied, however, by growth, i.e., increase in length.

. . . The movement of the embryonic cells in the lymph clot is very distinct, and is due beyond doubt to the activities of the hyaline ectoplasm, which is accumulated especially at the angles of the cells. It there forms extremely fine filamentous pseudopodia, through the activity of which the cells may change their shape or move from place to place. The exact character of the movement is not the same in all kinds of cells and it varies greatly in intensity. Axial mesoderm and medullary cord yield cells that frequently wander for considerable distances by themselves; epidermis, when it does not roll up into bands or spheres, may form a hyaline fringe, and spread out considerably; pieces of the central nervous system and the primordia of the cranial ganglia give rise to the fibre-like structures described in the last section; the endoderm and notochord remain almost inert.

In passing, I should like to refer to two points which baffle students of tissue-culture. I offer an interpretation of them on the basis of my knowledge of ectoplasmic behavior in general.

The first concerns the observation so often noted that living cells in tissue-culture tend to assume a spindle-shape, especially when they migrate from the bit of tissue implanted in the culture-medium. There is no correlation between the original form of the cell and the spindle-form subsequently attained as we can conclude from the studies of many observers, who on various types of cells have confirmed Harrison's original observations. Two suggestions have been offered: that the spindle-form is a reversion to a less differentiated (i.e., embryonic) cellular condition and that it is the effect of the cell's new, and abnormal, environment. My suggestion is that the spindle-shape expresses gain in ectoplasm. I base this view on the fact that cells when isolated usually show relatively more active ectoplasm than when they are in contact and further on the observation made by Harrison that spindle-shaped cells become

extremely tenuous as they move along a delicate track, such as the thread of a spider's web, which behavior I interpret as a pseudopod extending in one direction.

Concerning the cause of migration, two views are maintained. According to one, the cells react positively to solids; according to the other, the cells move away from regions of too high acidity. My own interpretation is that cells in tissue-culture are no longer under the restraint imposed upon them when they stand in coordination with and subordination to their normal environment, i.e., other cells and tissue fluids. Thus their ectoplasmic activity is no longer ordered and controlled by the contact but is exaggerated especially in their free and exposed surfaces—that is, in the regions where they are not in contact with the cells of the bit of tissue implanted on the medium. Hence, they migrate away from the mass.

Suspended in the blood-plasma of vertebrates are red blood corpuscles. In mammals these are devoid of nuclei and therefore not true cells. All other vertebrates possess nucleated red blood cells. The structure of these latter has been worked out by Meves, who found that they have a well-marked ectoplasm.

The blood of vertebrates contains in addition to the red blood corpuscles the leucocytes or white blood cells. In human blood these cells are sub-divided into four kinds, cell-size, staining reaction of cytoplasmic granules, and nuclear form being the characters on which the sub-divisions are made. In a healthy adult individual there is a fairly constant number of each type of white blood-cell. This number is used as an index for pathological states—many diseases have each a characteristic "blood-picture" which is an important diagnostic aid. On these highly interesting cells ectoplasm can be easily demonstrated. It has largely been assumed that for phagocytic activity and movement of white blood cells the same theories hold that have been

postulated for these activities in free-living Amoebae. But even were there only one accepted theory of the cause of amoeboid movement in free-living Amoebae, there would still remain the necessity to prove that such a theory could apply to white blood cells in the blood-stream of warm-blooded animals. It would be more advantageous further to study the white blood-cells themselves. Since amoeboid movement and capacity for engulfing solid particles reside in the ectoplasm,[1] the study of primary importance for the elucidation of these phenomena exhibited by human white blood-cells relates to the ectoplasm of the various types of these cells and to each of them in their different physiological states in health and in disease.

This cursory review dealing mostly with vertebrate tissue-cells by no means pretends at exhaustion. Thoroughly to treat the subject of ecto-endoplasmic differentiation as found in cells of multi-cellular animals would demand a volume in itself. But the review shows that from sponges to man tissue-cells exhibit ecto-endoplasmic differentiation; that the ectoplasm gives rise to processes which interlock with other cells; that the ectoplasm may be cast off to form inter-cellular substance; that it enters largely into the formation of contractile cells (muscles); that it is the conductive material *par excellence* of nerve-cells and that cells, as amoeboid blood cells, in their locomotor capacity indicate ectoplasmic action.

Ecto-endoplasmic differentiation is by no means confined to the tissue-cells of multicellular animals. Very early in the history of the study of Protozoa, it was recognized that in these unicellular organisms the superficial cytoplasm is marked off structurally from the interior. Indeed, nowhere else in the animal kingdom is the ectoplasm so sharply defined by so rich and various sculpture.

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[1] *Metschnikoff on amoeboid cells of sponges, 1879.*

To Haeckel we owe the application of the term, exoplasm, to the superficial cytoplasm of the Protozoa. Writing in 1873 on the morphology of the Infusoria, he emphasized the differentiation of the protoplasmic body into a bright, firmer cortical substance and a granular, softer medullary substance, a differentiation found also in Amoeba as in many parenchyma cells of higher animals. This outer layer is further distinguished from the medullary portion of the cytoplasm through a lower water content and an independent contractility. Later authors, as Bütschli, Doflein and others, have merely reiterated Haeckel's original description, confirming it through their own observations.

The protozoan ectoplasm may be highly differentiated: for support and protection; for motility; for food-capture; and for oral and anal modifications. In it are located the contractile vacuoles. On the basis of their means of locomotion Protozoa are classified into four groups: those like the Amoeba, that move by means of pseudopodia; those that move by means of flagella; those like Paramoecium that move by means of cilia, and, in a negative way, those like the Sporozoa, to which the organism that causes malaria belongs, which are characterized at least during one period of their life-history by lack of locomotor-apparatus. In other words, the classification of the groups is built upon ectoplasmic differentiation.[1]

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[1] *Though this book concerns itself with the animal cell, I may note in passing that in many plant cells, especially in the higher, multi-cellular, organisms, the living cytoplasm is entirely superficial in location, the interior of the cell being a vacuole of non-living materials. Also, I may call attention to the Bacteria which possess a strongly marked ectoplasm, investigated especially by Gutstein (1926 and earlier). The ectoplasm of Bacteria has been much discussed in connection with their structure in relation to the valuable diagnostic aid for identifying these organisms furnished by the Gram stain. (See Schumacher 1928 and earlier.)*

While the ectoplasm of the Protozoa has been well known for years, that of eggs has not been generally recognized, albeit eggs from every phylum in the animal kingdom either have been described as showing ectoplasm or can be shown to possess such. Since here we are concerned primarily with eggs, it is necessary to review in detail the evidence with respect to their ectoplasmic differentiation.

The terms, exoplasm and endoplasm were first used by Haeckel to describe the outer and inner cytoplasmic regions of the sponge egg. In his monograph on the sponges referred to at the beginning of this chapter, he speaks of the hyaline exoderm (exoplasma) and a granular endoplasm sharply set off from it. Metschnikoff[1] in describing eggs of a sponge emphasized its changes as due to amoeboid processes of the surface-cytoplasm. Gatenby[2] has described the ectoplasm of the sponge egg as follows: (Fig. 5).

> Even in the youngest oocytes one may notice that at an early stage a clear ectoplasmic zone becomes differentiated from an inner or endoplasmic zone. The ectoplasmic zone contains few or no vacuoles, is smooth, and is often drawn out in the form of blunt pseudopodia or filamentous dendriform threads. The inner or endoplasmic zone is vacuolated completely and has a fine, frothy appearance; it is in this region that the cytoplasmic inclusions lie, granules in the ectoplasm of the oocyte being rare or never found. Occasionally, in preparations fixed in mixtures containing alcohol or acetic acid, the vacuoles collapse, and the egg comes to have a curious radiation of fibres around the nucleus (see Joergensen's figures 8a). Eggs treated with silver nitrate solution show the endoplasm browner than the ectoplasm. . . . In favourable cases not only young oocytes, but amoebocytes, may be seen to possess ectoplasmic and endoplasmic zones. As in the case of the older oocyte, the cytoplasmic inclusions lie in the endoplasm, while the pseudopodia consist mainly of ectoplasmic material.

[1] *Metschnikoff, l.c.*
[2] *Gatenby, 1919.*

During development of the egg, all the blastomeres in most cases come to have an equal portion of the ectoplasm.

The ectoplasm can be traced through cleavage up to the formation of a blastula, but it soon either becomes absorbed
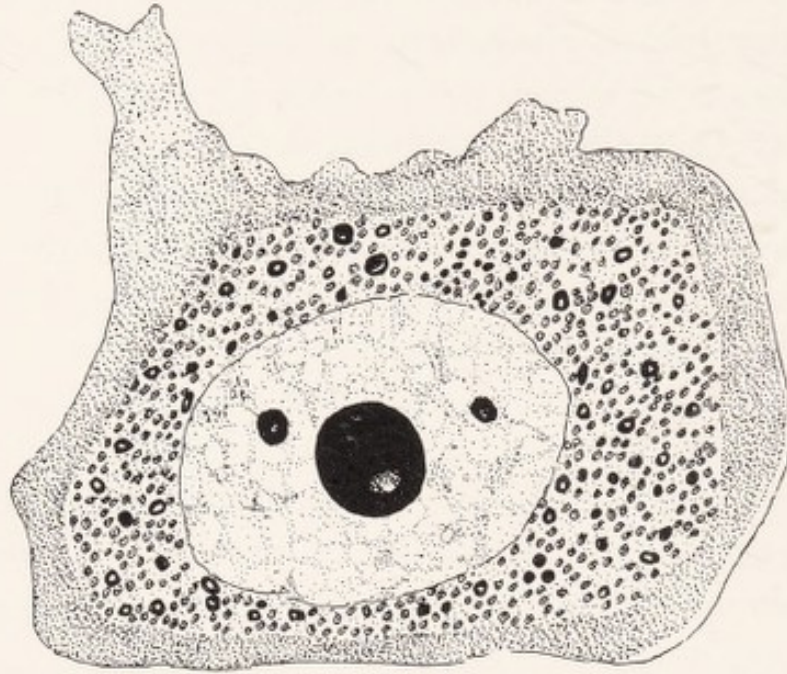
FIG. 5.—Egg of a sponge, *Grantia* (after Gatenby) showing amoeboid ectoplasm.

or is invaded by endoplasmic substance. . . . In a rare number of cases, it was found that in young blastulae the cells of one side had less ectoplasm than those elsewhere but in no example could I show that this inequality had any relationship to the formation of the flagellated and non-flagellated parts of the amphiblastula.
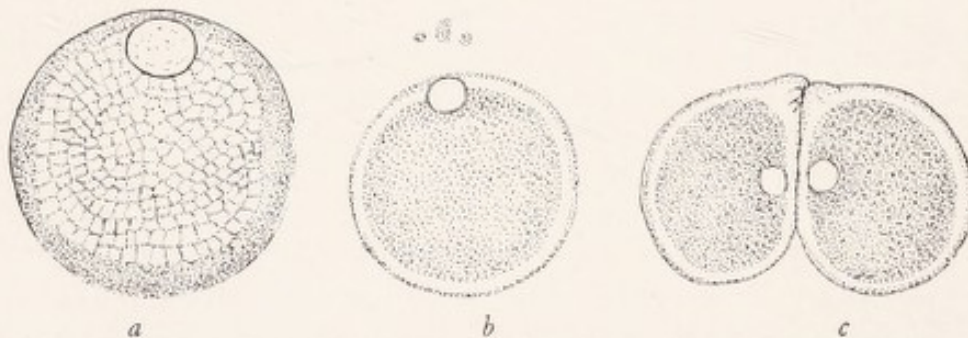
FIG. 6.—Eggs of medusae (after Metschnikoff). *a*, *Liriope mucronata; b*, and *c*, *Rathkea.*

In the cytoplasm of eggs of all classes of Coelenterates, including the Ctenophores, that is, the jelly fishes and their allies, ecto-endoplasmic differentiation has been frequently

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noted. (Fig. 6 and Fig. 7.) Kowalewsky[1] without naming the two regions gave a very clear description of them as found in several ctenophore ("comb-jelly") eggs. Subsequently, authors have confirmed Kowalewsky,[2] often only repeating the words of this great observer. Says Agassiz:

> The yolk mass of the eggs of *Idyia* (and all Ctenophorae) consists (after they are laid) of two layers, an inner yolk mass more or less fatty, made up of large, irregular spheres. This inner yolk mass is surrounded by a second thin, outer layer, finely granular. This outer layer and its enclosed central mass perform very different parts in the development of the embryo, and it is of the utmost importance to keep the changes these two layers undergo, clearly distinct, while following the development of the young Ctenophore. The outer layer as has been shown by Kowalewsky, is eminently the embryonic layer, while the inner mass acts as a mere nutritive yolk mass.

Fol[3] also described ecto-endoplasmic differentiation in Coelenterate eggs, using the term, ectoplasm, instead of Haeckel's exoplasma. Perhaps the most striking ectoplasmic structure described in eggs is that found in the *Beroë*-egg noted first by Chun and later by Yatsu.[4] The ectoplasm in this egg is of a bright green colour, a fact that one
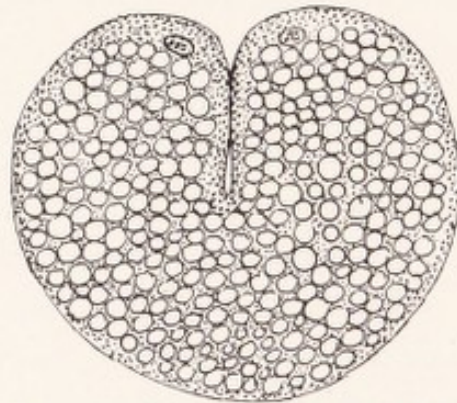
Fig. 7.—Egg of a ctenophore, *Escholtzia* (after Kowalewsky).

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[1] *Kowalewsky, 1866; 1873. See also Kowalewsky and Marion, 1883.*

[2] *Agassiz, 1874.*

[3] *Fol, 1873.*

[4] *Chun, 1880; Yatsu, 1907. For other observers on the ectoplasm of Coelenterate eggs see: Metschnikoff, 1886; Ziegler, 1898; Appelöf, 1900; Hargitt, 1904; Spek, 1926; Conklin, Carn. Publ., 103; Ciamician, 1879; Korotneff, 1880; Harm, 1903; Maas, 1908; Torrey, 1907; Komai, 1922.*

can easily confirm under low power of an ordinary microscope. Indeed, I have with the naked eye seen the green cytoplasm on this rather large egg; also have I found that under pressure the endoplasm, which Kowalewsky did not consider living substance, will stream out as the large egg ruptures leaving the green surface-cytoplasm intact.

The difference between the peripheral and the inner cytoplasm of eggs of flat-worms has been often observed,[1] though some authors fail to do so, and still others have expressed the opinion that the structural differentiation observed might be due to fixation.[2] Many flat-worm eggs present serious difficulties for study in the living state because they are laid in capsules each containing several eggs; removal of the eggs from the capsule may mean their injury. In addition, because of opacity, they do not always lend themselves to observation of their finer structure. Nevertheless, one may assert that eggs of turbellarians and trematodes and even of those of cestodes (tape-worms) exhibit a resolution of the cytoplasm into an endoplasmic and an ectoplasmic region, as Metschnikoff, Selenka, Lang, Pereyaslowzewa and Janicki, among others, have shown.

The thread-worms, nematodes, include *Ascaris*, a parasitic worm which has been the object of many researches and which will come up for reference frequently in this book. The unfertilized egg of *Ascaris* shows, according to Van Beneden and Meves, ectoplasmic differentiation; irregular in shape, after fertilization it becomes spherical having in the meantime secreted a substance which forms an enclosing shell. The eggs of free-living nematodes, closely allied to *Ascaris*, show a similar change;

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[1] *Metschnikoff, 1883; Selenka, 1883; Lang, 1884; Pereyaslowzewa, 1892; Warren, 1903; Janicki, 1907.*
[2] *Halkin, 1902.*

on fertilization, bubbles in the cytoplasm break through the surface which undergoes amoeboid changes, then the eggs attain a regular contour.[1] *Cerebratulus*, a long ribbon-like worm found in the sea, lays eggs in which the cytoplasmic granules in the endoplasm differ from those at the periphery.[2] This egg also displays remarkable amoeboid changes[3] which always speak for the presence of an ectoplasmic structure.

Eggs of the wheel animalcules or Rotifera according to several investigators show a distinct ectoplasm.[4]

In Yatsu's interesting account of the development of the egg of the mollusk-like animal, *Lingula*,[5] is a detailed description together
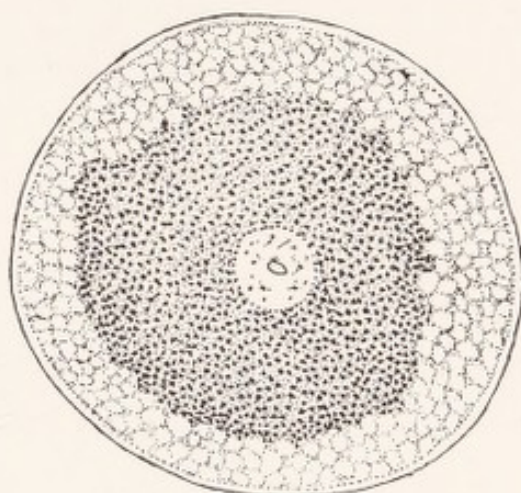
Fig. 8.—Egg of *Lingula anatina* (after Yatsu).

with pictures showing the surface-located cytoplasm set off from the endoplasm. (Fig. 8.) According to Prouho,[6] the egg of *Membranipora* possesses delicate projections which he figures.

In the unfertilized eggs of the echinoderms (starfishes, serpent starfishes, sea-urchins, sea-cucumbers, sea-lilies) the ectoplasm is well defined. Selenka[7] has described the changes which a sea-urchin egg undergoes before it reaches the stage when it is ready for fertilization. These

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[1] *Bütschli, 1873; also v. Erlanger, 1897 and later authors.*
[2] *Coe, 1899.*
[3] *Andrews, E. A., 1897; Wilson, C. B., 1899 and others.*
[4] *Zelinka, 1892; Storch, 1924 and others.*
[5] *Yatsu, 1902.*
[6] *Prouho, 1892.*
[7] *Selenka, 1878.*
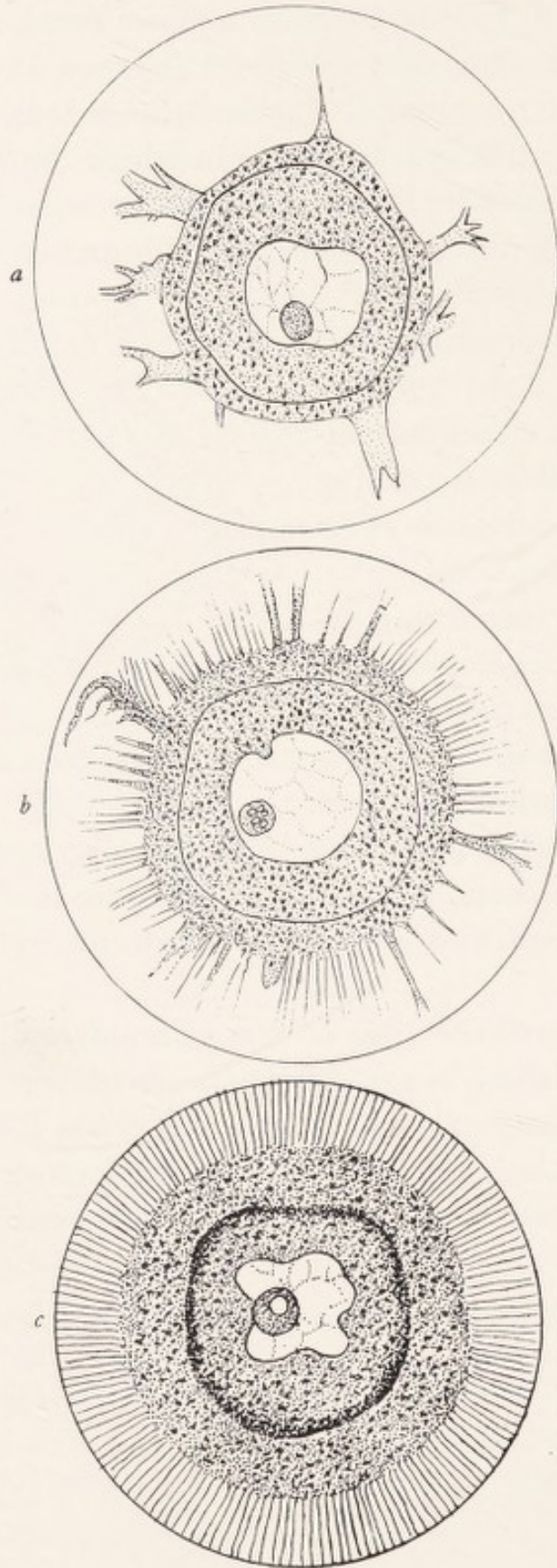
FIG. 9.—For descriptive legend see page 95.

changes largely concern the ectoplasm, as the appended figures (9) show. Though I have sought to obtain these stages in other species of sea-urchins, I have so far failed. In mature echinoderm eggs the ectoplasm, though difficult to observe, can be made out in the living egg as a homogeneous extremely narrow rim beneath the vitelline membrane. In fixed preparations it is only very faintly marked off from the endoplasm. After fertilization the ectoplasm stands out clearly because of the now present hyaline
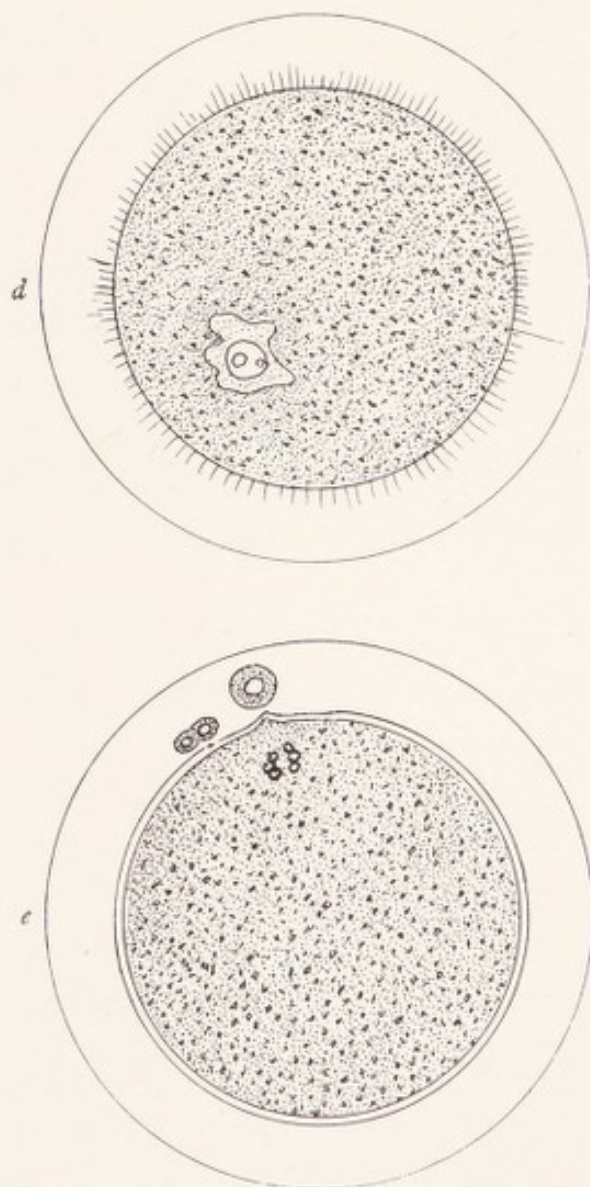
FIG. 9.—Development of the ectoplasm in the egg of *Toxopneustes variegatus* (after Selenka).

plasma-layer which though it has often been described[1] (Fig. 10) has never, as we shall see in the chapter on cell-division, been properly interpreted by those who have observed it, and generally been misunderstood by those who have on the basis of experiments on it made theories concerning its rôle. For eggs of the brittle starfishes and of the sea-cucumbers, Selenka has given good descriptions, pointing out that the layer is most pronounced in eggs of the brittle starfishes, less in those of sea-urchins and least in sea-cucumbers'. I find that in these as in the eggs of the starfish the ectoplasm can

Fig. 10.

Fig. 11.
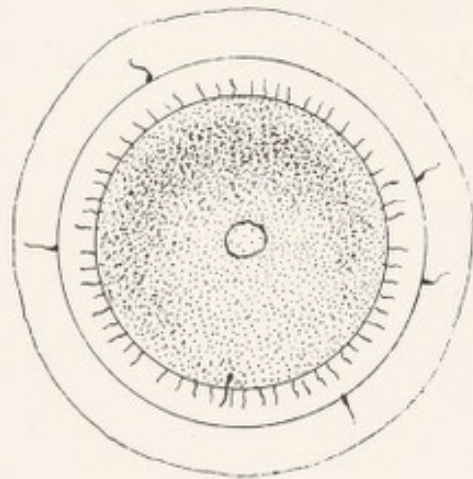
Fig. 10.—Egg of *Echinocyamus pusillus* (after Théel) to show structure of ectoplasm after full separation of vitelline membrane following penetration of a single spermatozoon.
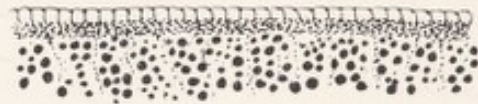
Fig. 11.—The ectoplasm of the egg of *Rhynchelmis limosella* (after Vejdovsky and Mrazek).

always be distinguished in sectioned eggs fixed with certain reagents for then minute granules appear; also the delicately radial structure is evident.

I turn now to the eggs of the segmented worms. Very strikingly does the ectoplasm differ from the endoplasm in the egg of *Rhynchelmis*[2] as pictured here (Fig. 11). But no less clearly or remarkably do the inner and outer regions in the egg of *Phascolosoma*[3] reveal themselves. The ecto-

[1] *Hertwig, 1876; Selenka, 1878; Fol, 1879; Ludwig, 1880; Selenka, 1883; Berthold, 1886; Théel, 1892; Hammar, 1896; Andrews, 1897; Ziegler, 1904; Meves, 1914.*

[2] *Vejdovsky, 1892; Vejdowsky and Mrazek, 1903.*

[3] *Gerould, 1907; Bergmann, 1903, on annelids.*

plasmic structure of the *Rhynchelmis* egg is similar to that recorded for the *Nereis*-egg by several workers beginning with Goette[1] and this in turn recalls Spengel's older descrip-tion for the egg of *Bonellia*.[2] Also the Chaetopterus-egg has an interesting ectoplasm,[3] so too that of *Thalassema*[4] (Fig. 12).

Among the great group of soft-bodied animals, the mollusks, including the clams, snails and ink or cuttle fishes, the following produce eggs in which the ecto-plasm has been observed to be marked off from the endoplasm:

Fig. 12.—Ectoplasm on the living egg of *Thallassema mellita* (after Lefevre).

Mactra[5] and *Mytilus*[6] (clams), various species of *Chiton*,[7]
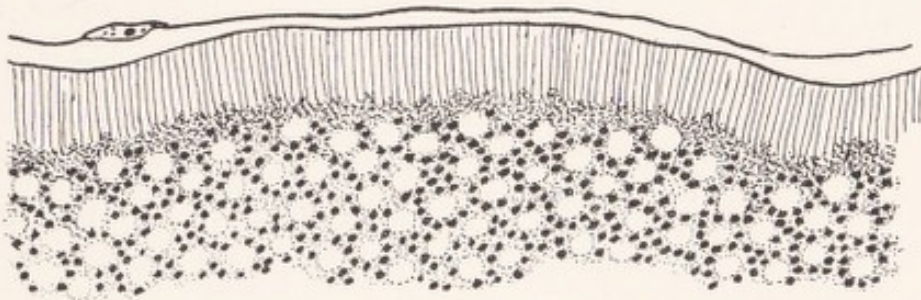
Fig. 13.—Ectoplasm of the egg of *Patella* (after Jörgensen).

Patella,[8] (a snail) (Fig. 13), *Dentalium*[9] (a scaphopod) and the cuttle-fishes (cephalopods).[10] In the eggs of *Mactra*

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[1] *Goette, 1882; v. Wistinghausen, 1891; Wilson, 1892; Hempel-mann, 1911; Lillie, F. R., 1912.*
[2] *Spengel, 1879.*
[3] *Lillie, F. R., 1906.*
[4] *Torrey, 1900; Lefevre, 1907.*
[5] *Kostanecki, 1904.*
[6] *Meves, 1915.*
[7] *Kowalewski, 1878.*
[8] *Patten, 1885; Jörgensen, 1913.*
[9] *Wilson, 1904.*
[10] *Bergmann, l.c.*

the outer sheath of cytoplasm contains large granules, whilst in that of *Mytilus* according to Meves' figures the ectoplasm in the fixed egg is a clear margin bounded by a homogeneous and apparently firmer sheath of cytoplasm beneath the very thin vitelline membrane. In figures of the eggs of *Chiton* also one distinguishes ectoplasm from endoplasm. In addition, my observations convince me that in the eggs of the razor clam, *Ensis*, and of the small clam, *Cumingia*, the outer region of the cytoplasm differs from the inner. The large ellipsoid eggs of cephalopods—ink-fishes and allies—show a disc of clear cytoplasm containing the nucleus at the upper more pointed end which is continuous with a very thin layer enclosing the inert yolk. Disc and layer constitute the active substance of the egg; in them development ensues; it is as though the cytoplasm in these eggs were all ectoplasm.

Among eggs of arthropods are those of insects,[1] whose ectoplasm is even more strongly marked than that in eggs of coelenterates. Beyond we shall note that the ectoplasm of these eggs has most interesting behavior. Appended is a picture from Heider showing the ecto-endoplasmic differentiation in the egg of a beetle (Fig. 14). Eggs of other arthropods clearly show ectoplasm.[2]

Among the lowest forms of Chordates, the Ascidians, we find that the beautifully transparent egg of *Phal-*

Fig. 14.—Egg of *Hydrophilus pisceus* (after Heider).

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[1] *Heider, 1889.*

[2] *Groom, 1894.*

lusia[1] reveals ecto-endoplasmic differentiation. The egg of *Amphioxus*[2] likewise has an outer sheath of cytoplasm marked off from the inner (Fig. 15). The ectoplasm of the latter egg as pictured by Sobotta recalls that already above described for the egg of *Nereis* or of the sea-urchin. That all other authors do not note cytoplasmic differentiation in the egg of *Amphioxus* one may attribute
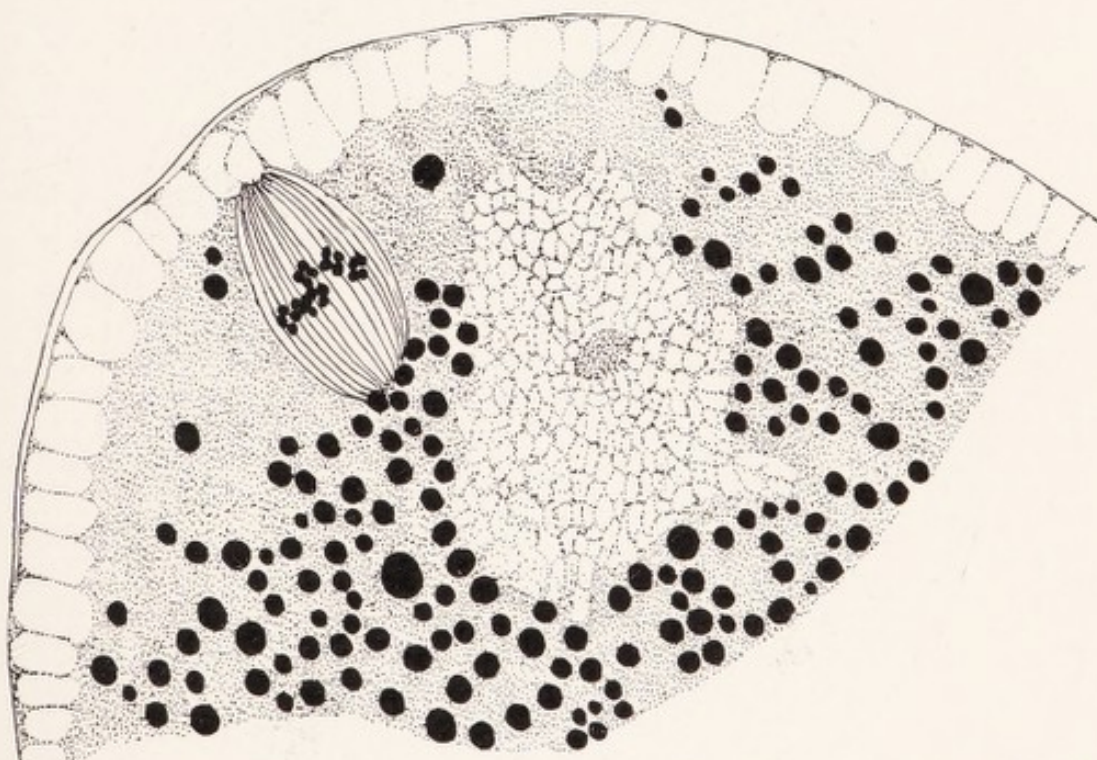
FIG. 15.—Ecto-endoplasmic differentiation in the egg of *Amphioxus* (after Cerfontaine).

either to oversight or to the quality of fixation. The separation of the vitelline membrane exemplified by this egg constitutes additional evidence for similarity of its ectoplasmic structure to that of the other eggs named. However, this membrane separation has seldom been observed because of the difficulty of securing the early fertilization-stages for observation.

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[1] *Meves, 1913.*
[2] *Sobotta, 1897; Cerfontaine, 1906.*

Ascidians and *Amphioxus* belong to the chordates without back-bone or vertebral column. Fishes, amphibians, reptiles, birds and mammals are chordates with a vertebral column. The lamprey is an example of the low vertebrate, having no true jaws to the mouth as other vertebrates have.
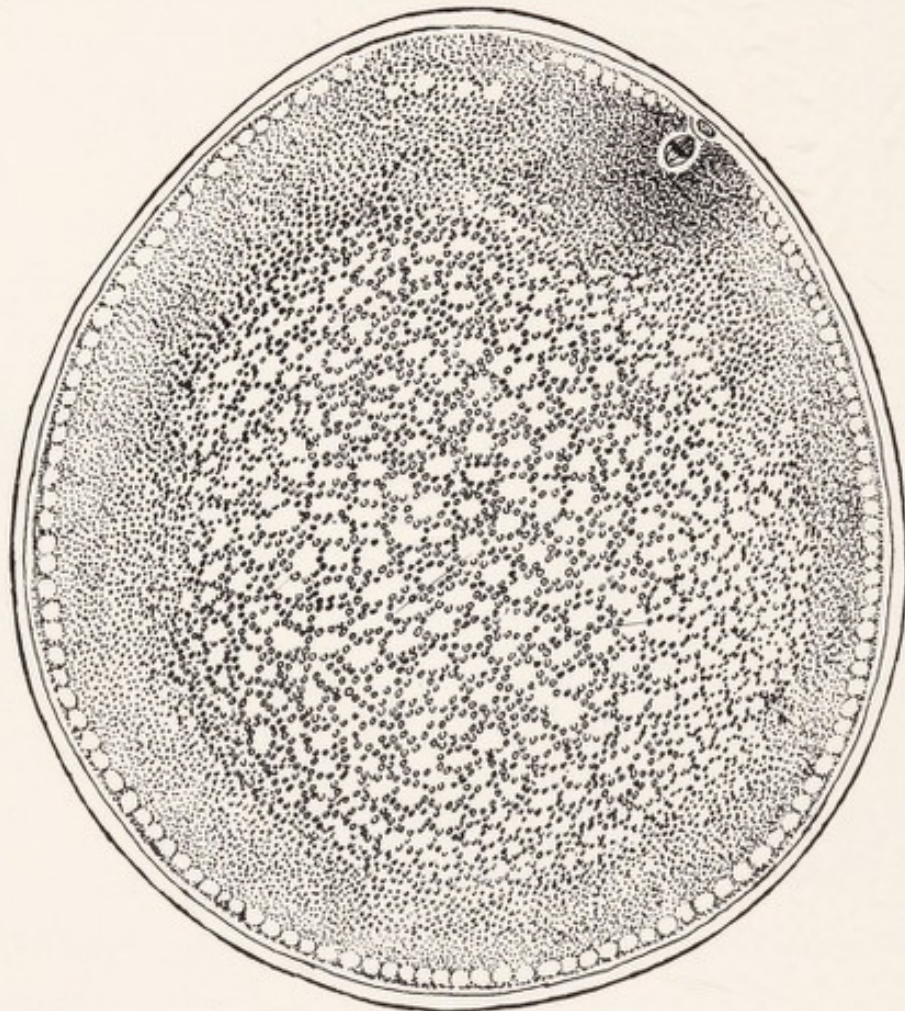
FIG. 16.—Egg of *Petromyzon fluviatilis* (after Herfort).

Its egg has been described by several investigators, among these Calberla, whose beautiful work on fertilization of the lamprey egg is classic. According to him this egg shows regional cytoplasmic differentiation.[1] See also Herfort. (Fig. 16.)

For eggs of selachians (sharks and their allies), His has written as follows:

[1] *Calberla, 1877; Böhm, 1888; Herfort, 1900.*

It thus seems practical to speak instead of kinds of plasma, of zones of the cell-body and we can distinguish: A central condensed zone. . . . A zone of network. . . . A hyaline cortical zone. . . . [1]

In the egg of teleosts (bony fishes) the protoplasm, at first a thin sheath enclosing the yolk, moves at fertilization to one pole to form a disc leaving only a delicate layer to surround the remainder of the yolk mass.[2] Here the relations are as in the eggs of cephalopods.

Living eggs of amphibians (frogs, toads, salamanders) have been long recognized as showing a differentiated surface due to the presence of pigment extending over approximately one-half the egg. In sections amphibian eggs show further differentiation of the superficial cytoplasm.[3]

In the eggs of both reptiles and birds the protoplasm is sharply set off from the large mass of inert yolk. The description given by His for the selachian egg and quoted above applies to the eggs of reptiles and birds.

Recently, Lewis and Hartmann have described the organization of the living egg of the monkey.[4] Other eggs of mammals, as that of the mouse, bat, rabbit, etc., also show some difference between the peripheral and the central cytoplasm.

In addition to these accounts which describe eggs of every group of animals as having ectoplasm, we should note those studies which show that ectoplasmic structure is revealed by experimental treatment. Hammar's description of the ectoplasm on the sea-urchin's egg as composed of radial striations was based on observations on eggs in

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[1] *His, 1897.*

[2] *List, 1887. This process was very beautifully described as early as 1854 by the English physician, Ransom.*

[3] *King, 1901. Earlier: Goette, 1875.*

[4] *Lewis and Hartmann, 1933.*

sea-water made more concentrated by evaporation. In calcium-free sea-water, the ectoplasm of sea-urchins' eggs fairly bristles with delicate filaments. In other eggs also the presence of these filaments can be demonstrated by experimental treatment—especially by placing the eggs in hypertonic sea-water. It is to be emphasized that the experimental means does not cause the formation of these filaments but only makes them more easily visible. Thus, in eggs of *Nereis* and of *Platynereis* the ectoplasmic filaments, seen in the unfertilized eggs as ectoplasmic striations and for a short time after fertilization as strands, can always be more strongly revealed in later stages, when they are seen with greater difficulty, by submersing the eggs in sea-water made hypertonic by the addition of NaCl or KCl. The same observation has been made on eggs of other worms, for example, on those of *Sabellaria* (Fig. 17). The often expressed opinion that the ectoplasmic prolongations thus made visible are artefacts leaves unconsidered the fact that the eggs show normally an ectoplasmic structure, as has been abundantly proved for eggs from those of sponges to those of vertebrates. The descriptions in the literature admit of no doubt on this point.

FIG. 17.—Egg of *Sabellaria alveolata* to show effect of hypertonic sea-water on the ectoplasm (after Fauré-Fremiet).

When we place alongside this fact the observations detailed by students of tissues in culture on the strongly expressed ectoplasm, the generally accepted fact of ectoplasmic structures in Protozoa together with the evidence of intercellular connections between cells in tissues and the knowledge of the mode of union of nerve-cells by processes, we have very good reason for asserting that the filaments and prolongations of the ectoplasm are its characteristic, and perhaps its most important, structure. They have

often been compared to protozoan pseudopodia, especially to those that are extremely tenuous and fine. Years ago in a work that never has received adequate attention, Mrs. Andrews[1] emphasized the capacity of these threads for ceaseless changes which she spoke of as a spinning activity of the protoplasm.

The tremendously impressive fact of the existence of ectoplasmic structure finds here for the first time its proper appreciation. A structure which can rightfully be denominated a *sine qua non* of living matter can be presumed to have significance for the grand problem of biology, the revelation of vital phenomena. In the following pages I aim to establish the thesis that in ectoplasmic behavior we witness the expression of activities that set apart the living thing from the non-living, that mark how life maintains itself ever in harmonious tempo with the ceaseless changes in its surroundings.

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[1] *Andrews, l.c.*

General Properties of the Ectoplasm

$\mathcal{A}$LTHOUGH WE CAN NOT TO-DAY ANSWER THE QUESTION, what is life? we can say what are the manifestations of the state of being alive. Respiration, conduction and contraction are fundamental properties of living matter, exhibited by all living cells. Life never appears without them. Whilst inanimate things as well as organisms after death may display properties like these, only the living thing maintains them in self-regulation through continuous adjustment. Now the question arises: to what extent are these biological indices, these manifestations of life, properties of the ectoplasm? Study of the egg's behavior in the first moments after sperm-contact will throw light on this problem.

The attachment of a single spermatozoon to an egg and the effect produced thereby can best be observed microscopically by adding a drop of sea-water containing very few spermatozoa to sea-water that contains eggs. To eggs of *Arbacia* spermatozoa attach themselves within two seconds after they are added to the sea-water containing the eggs. Under the impact of a spermatozoon the egg-surface first gives way and then rebounds;[1] the egg-membrane moves in and out beneath the actively moving spermatozoon for a second or two. Then suddenly the spermatozoon becomes motionless with its tip buried in a slight indentation of the egg-surface, at which point the ectoplasm develops a cloudy appearance. This turbidity

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[1] *Cf. Derbès, l. c.*

spreads from here so that at twenty seconds after insemination—the mixing of eggs and spermatozoa—the whole ectoplasm is cloudy. Now like a flash, beginning at the point of sperm-attachment, a wave sweeps over the surface of the egg, clearing up the ectoplasm as it passes; careful observation reveals that the ectoplasm now shows a brush-work of threads beneath the membrane. Twenty-five seconds after insemination, a cone of ectoplasm protrudes from the egg and encloses the sperm-head. This is suddenly pulled into the cone; the ectoplasmic threads break at the site of sperm-attachment and release the membrane. Progressively from this point the membrane separates in a wave from the surface of the egg, leaving in its wake collapsing ectoplasmic strands. Thirty seconds after insemination the membrane is separated from the egg by a narrow perivitelline space. During the ensuing twenty-five seconds this space increases in width; the ectoplasmic strands become more sharply defined giving the ectoplasm the appearance of a striated layer. The vitelline membrane becomes equidistant from the egg at all points and the perivitelline space is at its greatest width one hundred twenty seconds after insemination.

This progressive lifting of the membrane, first noted by Derbès,[1] I have studied in several species of eggs in great detail.[2] On the basis of experiments with physico-chemical means which induce membrane-separation, certain investigators have simply assumed the *de novo* origin of the membrane which they called the "fertilization-membrane." However, careful observation shows, as described above, that the membrane is already present on the egg and separates at fertilization by a progressive wave beginning at the point of sperm-entry. It thus is not a "fertilization-membrane,"

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[1] *Derbès, 1847. See also Fol, 1879.*
[2] *Just, 1919a, 1921, 1922e, 1928c and f, 1929a and b.*

but properly the vitelline membrane. I have repeatedly followed the process in the egg of *Arbacia*, though it is not the most favorable form for this study. In the eggs of the genus *Strongylocentrotus*, of *Echinus* and of *Echinarachnius*, for example, the separation of the membrane is more easily followed.

If eggs of *Echinarachnius* be inseminated with thin sperm-suspension, they throw off their membranes that are
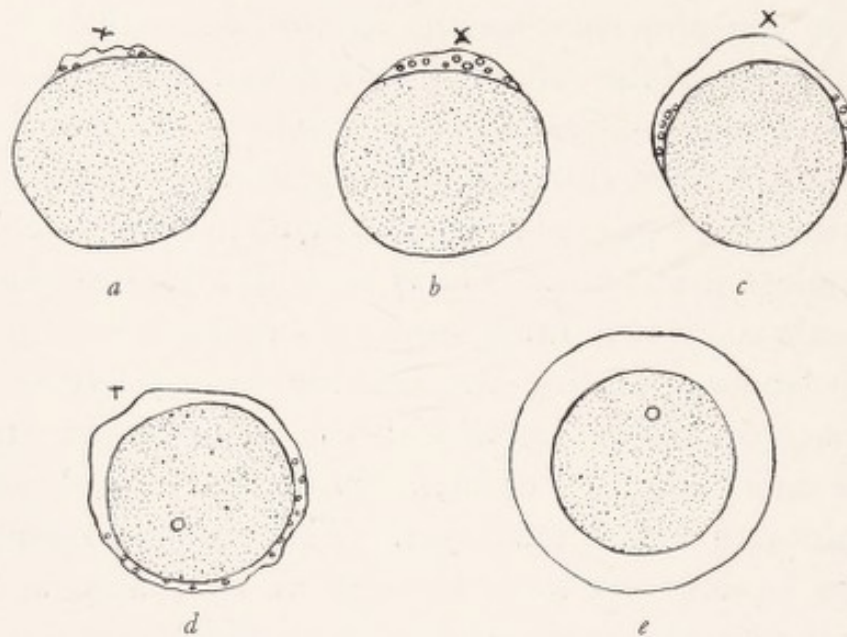
FIG. 18.—Successive stages of membrane-separation in the living egg of *Echinarachnius parma*, site of sperm-entry.

fully lifted and equidistant from the egg-surface in about two minutes. Membrane-separation follows sperm-penetration. After the sperm-head has entered the ectoplasm, a blister appears on the egg-surface at the point of sperm-entry. This blister contains drops that move toward the membrane and go into solution. The process is gradually continued throughout the whole ectoplasm, and thus the membrane is lifted in a wave that sweeps over the surface (Fig. 18). By break-down of material in the ectoplasm, beginning at the site of sperm-entry, the membrane is separated from the surface. After this membrane is fully

off the egg, the ectoplasm again builds a new surface-structure, the hyaline plasma-layer.

Before the actual separation of the vitelline membrane, an ectoplasmic change beginning at the point of sperm-entry sweeps over the egg which immunizes it to other spermatozoa, the pole opposite the site of sperm-entry being the last point affected. The site of sperm-entry becomes a "point of injury" and is "negative" to the entry of spermatozoa arriving at this point, all other points around the egg for a brief moment being "positive." Especially in slowly reacting eggs it can be noted that this "wave of negativity" moves over the egg, at a rate which varies with that at which the sperm-head disappears within the ectoplasm. When only the tip of the sperm-head has entered the ectoplasm, the immediate vicinity of the site of penetration can not engulf another spermatozoon. As more of the sperm-head disappears within the ectoplasm, the "negativity" to sperm-entry progresses still farther around the egg; when the head has disappeared, the egg can engulf sperm only at one point—the pole opposite that at which the spermatozoon entered the egg. The "wave of negativity" thus precedes the actual beginning of membrane-separation. Before the membrane begins to separate at the site of sperm-entry, other spermatozoa can no longer enter at any point on the egg. From the point of sperm-entry a definite gradient of membrane-separation is established. This gradient, therefore, follows that of loss of susceptibility to sperm-penetration.

When a spermatozoon becomes attached not only the penetration of other spermatozoa but also their fixation to the egg depends upon the degree of penetration of the effective spermatozoon, that is, on the rate at which the "wave of negativity" is propagated around the egg. Thus, at the beginning of penetration, other spermatozoa can not become attached to the egg in the immediate neighborhood of the

point of sperm-entry. Those farther removed may become attached, lashing back and forth very vigorously, until with farther penetration of the one successful spermatozoon the "wave of negativity" reaches them and their movement comes to a stand-still.[1]

The ectoplasmic changes in the egg of *Nereis* that take place after sperm-attachment are also worthy of note. Before insemination the ectoplasm of this egg is a broad chambered structure. When eggs and spermatozoa are mixed, sperm-attachment rapidly ensues; then follows the escape of material from the ectoplasm, which rapidly sets in the sea-water as a transparent jelly. The ectoplasmic chambers break down, leaving only strands that cross the space between the inner part of the egg and the membrane. Now comes a period of amoeboid changes in the egg:[2] it shrinks, becomes markedly irregular in outline, its contents become darker and the perivitelline space much reduced. When this period is over, the egg assumes a clearer and more rounded appearance; the perivitelline space widens again. The delicate plasma-membrane beneath the vitelline membrane is now easily visible.

In this egg, thus, the ectoplasmic changes differ from those observed in the eggs of *Arbacia* and of *Echinarachnius*. In all three however the essential response to insemination is the same, namely, an alteration in the ectoplasm. These changes have significance for fertilization and experimental parthenogenesis as we shall see. At present, another aspect of them elicits our interest.

By experiments with dilute sea-water, I was able to correlate the structural changes at the surface of the eggs named above with an alteration in the physical state of the egg. I found, for example, that the egg of *Echinarachnius*

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[1] *Just, 1919.*
[2] *Cf. Torrey, 1907 on amoeboid changes in eggs of Corymorpha.*

is many times more susceptible to dilute sea-water during the period of membrane separation than the fertilized egg before or after this separation, or than the unfertilized egg. The egg is never again so susceptible, although during the cleavage-cycles it exhibits rise and fall in susceptibility which may be correlated with the rhythm of nuclear division. We may consider in detail this susceptibility to hypotonic sea-water first as seen in the egg of *Echinarachnius*.

If to a drop of sea-water containing unfertilized eggs of *Echinarachnius* mounted under low power of the microscope, tap or distilled water be added, one can observe that the eggs take up water, swell, and finally break down in about two minutes. For example: the time of disintegration in tap water for lots of eggs from ten females was as follows:

Number of females	1	2	3	4	5	6	7	8	9	10
Time in seconds to disintegration of the eggs while in tap water	270	243	60	113	150	148	256	247	155	240

The rate of disintegration in eggs from these same females exposed to the action of tap water, five to ten seconds after insemination, was about the same. Eggs from the same females exposed to the action of tap water two minutes after fertilization, i.e., after the membranes are completely off the eggs, withstood the exposure even better than the unfertilized eggs. But with the beginning of membrane separation, twenty to thirty seconds after insemination, the picture is quite otherwise, for then the eggs are highly susceptible. The following table gives the results of such an experiment made on eggs of the same females as were used in the above described experiment.

Once it was well established by experiment with tap water that the period of high susceptibility falls in exactly with the period of membrane separation, attention was

Number of females.	1	2	3	4	5	6	7	8	9	10
Time in seconds to disintegration of eggs exposed to tap water during membrane-separation.	14	17	19	20	9	11	18	16	7	6

directed to the susceptibility of the egg to less dilute sea-water at different stages of the process of membrane separation. In the experiment with the tap water it appeared that when the egg cytolyzed, the break always came in that part of the ectoplasm from which the membrane was lifting at the time of exposure. However, the cytolysis induced by tap water was far too rapid to allow exact observations

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FIG. 19.—Diagrams showing disruption of egg-contents at site of membrane-separation in egg of *Echinarachnius parma*. *a* is a fertilized egg in which membrane-separation has not begun.

of this phenomenon. The experiments with less dilute sea-water proved that the observation made during exposure to tap water was correct: when eggs are exposed to dilute sea-water during the period of membrane-separation they cytolyze by an outflow of cytoplasm at the points from which the membrane is lifting at the moment of exposure (Fig. 19). Thus the susceptibility travels at the same rate as the wave of membrane-separation. As stated above, membrane-separation in the normal process results from the solution of substances in the ectoplasm. Under the microscope one can easily follow droplets of these as they move across the perivitelline space before they completely disappear. Any point on the egg-surface where this dissolution is taking place becomes the point of susceptibility at the instant of exposure to dilute sea-water or tap water.

The most remarkable characteristic of this susceptibility during membrane-separation is its sharp localization. I

have made observations on thousands of eggs and have yet
to see an egg exposed during membrane-separation break in
those regions from which the membrane is not lifting. This
is especially well brought out by exposing eggs during the
early stages of membrane-separation; for then only that
part of the egg from which the membrane is just lifting, at or
near sperm-entry, is susceptible to dilute sea-water.
Similarly, any part of the egg from which the membrane has
fully lifted is resistant, as is strikingly shown by exposing eggs
just at the moment when the membrane is being lifted from
the last point on the egg-surface. Then the break-down is
only at this point; the zones from which the membrane is
already off are resistant. When the membrane is fully off,
the egg leaves the period of susceptibility. We may say,
therefore, that a wave of resistance to dilute sea-water
follows in the wake of the wave of susceptibility. There
is an exceedingly rapid restitution-process in the ectoplasm
following a momentary loss of resistance through the normal
break-down of material in the ectoplasm which pushes off
the vitelline membrane.

If we attempt to come to some conclusion as to the mean-
ing of this susceptibility, we must take into account the
following facts: First, the resistance of the ectoplasm in
those regions from which the membrane has not yet sepa-
rated; second, the very rapid recovery of the ectoplasm
after membrane separation; third, the apparent failure of
the ectoplasm at the site of sperm-entry to show any greater
susceptibility than the remainder of the egg-surface. In
brief, we must keep in mind that this susceptibility is
clearly localized; or, what is more correct, that the break
in the egg which is the expression of decreased resistance
to the hypotonic sea-water is only at the site, and in the
moment, of membrane-separation. Now this does not
mean that only at this point water enters the egg. Rather,
it indicates that the point at which the membrane is sepa-

rating is the point at which the ectoplasm is weakest in that moment. In the zone of membrane-separation it is discontinuous and shows a break. The normal process of membrane-separation being due to a secretory process is bound up with water movement. As the ectoplasmic material goes into solution, it is washed away. In the normal process just enough water is present for this behavior. When the egg is placed in dilute sea-water, the picture is different; the normally occurring recovery in the zone of membrane-separation can not take place, and the ectoplasm breaks down further. Moreover, with the ectoplasm now gone, the endoplasm is without protection and complete cytolysis results. We are thus here dealing with a sensitivity due to actual progressive dissolution of colloids in the ectoplasm of the egg.

The case of the egg of *Arbacia* is very interesting. Here differences in resistance to dilute sea-water shown by the unfertilized and by the fertilized egg at the time of membrane-separation are not so clear-cut. I was, however, able to prove that the period of membrane-separation also in these eggs is a critical one, by studying the swimming larvae reared from eggs treated with dilute sea-water before, during and after membrane-separation.[1] Fertilized *Arbacia* eggs exposed to tap or distilled water before or after membrane-separation and then returned to normal sea-water give rise to normal larvae. But eggs exposed to the tap or distilled water during the period of membrane-separation on return to normal sea-water show marked abnormalities in the late stages of gastrulation. There is here, therefore, definite evidence of a differential susceptibility which appears in clear-cut fashion; dilute sea-water exerts a decidedly deleterious action during the process of membrane-

[1] *Just, 1928c.*

separation and the egg is thereby so profoundly altered that the normal processes of gastrulation are disturbed.

The unfertilized eggs of *Nereis limbata* withstand treatment with tap or distilled water for three or four minutes before disintegration takes place. Beginning twenty-five minutes after fertilization, the eggs are even more resistant than the unfertilized. But during the twenty-five minute period immediately following the mixing of eggs and spermatozoa, the eggs show a very low resistance for they disintegrate within ten to sixty seconds after exposure to tap or distilled water. The surface-changes described above for this egg run over this period of twenty-five minutes. Thus, the period of susceptibility to hypotonicity exactly coincides with the period of break-down of material in the ectoplasm—with release of the jelly-forming material—the amoeboid changes and the darkening of the egg. When the egg rounds up and clears, the period of lowered resistance to dilute sea-water passes off.

These experimental findings on three forms—and I have used eggs of other forms as well—though they show variations, nevertheless point to one conclusion: the period of ectoplasmic changes following fertilization is one of profound physical alteration. The changes that I have discussed here which follow the attachment of the sperm-head to the egg-surface sweep over the egg as a wave of explosions that break down material in the ectoplasm. During this period of visible alteration of the egg-surface occur other measurable physico-chemical changes, as oxygen-consumption and heat-production. These we may consider briefly.

The oxygen-consumption by eggs during various stages of development following insemination has been investigated by several workers.[1] Shearer[2] has reported his find-

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[1] *Warburg, Loeb and Wasteneys, Meyerhof, Shearer.*
[2] *Shearer, 1922.*

ings on oxygen-consumption by the eggs of a sea-urchin during the first minute after their insemination. Says Shearer: "On addition of the sperm to the eggs there is an immediate consumption of oxygen. In the course of the first minute the uptake of oxygen is many times that of the same eggs one minute before the addition of the sperm, and more is usually taken up in the first minute than is taken up in the second and third minutes after the addition of the sperm taken together." Shearer thinks that this "great initial inrush of oxygen into the egg and a corresponding output of CO_2 within the first minute after the addition of the sperm" make it clear "that the spermatozoon sets up an instantaneous oxidation-process in the egg, which is unparalleled in the reactions of the animal-cell for its sudden character."

This period of a great initial inrush of oxygen established by Shearer's findings coincides with the period of surface changes described above during which time also the egg is so susceptible to the action of dilute sea-water. During this period the colloids in the ectoplasm go into solution, material is destroyed. It is this solution at the surface of the egg that is accompanied by the great rise in oxygen-consumption.

Shearer has also investigated heat-production in fertilized eggs of sea-urchins. Rogers and Cole,[1] using methods of higher precision, have reported findings on the heat-production by the eggs of the sea-urchin, *Arbacia*. Their results on the heat-production immediately following insemination are of most interest to us. These workers find that the rate of heat-production at the instant of insemination is ten to twelve times that of the unfertilized egg. Thereafter the rate of heat-production falls constantly for twenty minutes to 65 per cent. of the value at

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[1] *Rogers and Cole, 1925.*

fertilization, remaining constant for the next thirty minutes to drop again by more than 10 per cent., remaining constant as far as the eight-cell stage. Farther than this the observations were not carried.

Rogers and Cole's figure gives the approximate rate of heat-production. In their words, "the greatest period of heat-production occurs immediately upon fertilization."[1] This period falls in with that of the surface changes described above. Say Rogers and Cole: "The fact that the greatest heat-production by the egg comes immediately after fertilization seems to us to make it plausible to say that the entrance of the spermatozoon induces a cortical oxidation-process, and that this process results in the elevation of the fertilization-membrane." On the basis of my observations and experiments, described above, these findings of Rogers and Cole can be more precisely explained. What they have measured is the heat liberated during the disintegration of the ectoplasmic colloids.

In addition to increased oxygen consumption and heat-liberation other cellular processes can be related to these surface changes in eggs.

The wave-like process of break-down in the ectoplasm by which the membrane is separated from the egg of *Echinarachnius* described above, strikingly resembles the transmission of change in various tissues. The nerve fibre may be taken as an example because among animal cells it is the most highly excitable and the most rapidly conducting. As is well known, nerve when stimulated at one point transmits this local effect throughout its course. As the propagated effect, the nerve impulse travels along the stretch of nerve, each point successively, beginning at that where the stimulus was applied, becomes electro-negative to all other regions of the nerve both behind and in advance of the

[1] *Rogers and Cole, l.c.*

point. Thus an electro-negative wave sweeps along the nerve fibre as the impulse traverses it.

During the transmission of the nerve-impulse there are no visible structural changes in the nerve. The propagation of the effect of sperm-attachment over the surface of the egg, however, is clearly visible. Thus, in the egg of *Arbacia* a wave of turbidity followed by one of lightening marks its course. In the egg of *Echinarachnius* it reveals itself: by the behavior of supernumerary spermatozoa that are in contact with an egg and are brought to a standstill progressively, the ones nearest the site of sperm-entry being first quieted; by progressive dissolution of ectoplasmic colloids; and by the break-down of pigment granules in the jelly that encloses the egg.

Also the response of the ectoplasm during the period of membrane-separation to exposure to hypotonic sea-water reminds us of the action-current in a stimulated nerve. The high degree of susceptibility of the ectoplasm in the zone of membrane-separation and the resistance in the zones from which the membrane has not yet separated or from which it is completely lifted again suggest the electro-negative condition in the nerve fibre.[1] It may well be that this is more than a superficial resemblance; and for this reason these ectoplasmic responses would warrant further investigation.

Whilst the egg passes through the period of surface changes under discussion, another property of the ectoplasm rises to sharp visibility, its contractility. Of the eggs described above, that of *Nereis* is the best in which to observe the shrinking and expansion following insemination. But this phenomenon can be followed in other eggs. Metschnikoff years ago called attention to the contractile power

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[1] *Lillie, R. S., 1922.*

of eggs of sponges;[1] Berthold[2] clearly described it for the egg of the sea-urchin as an unmistakable consequence of sperm-contact with egg. A year later O. and R. Hertwig suggested that eggs are endowed with contractility.[3] The earliest description known to me of contraction in the ectoplasm is that given by Ransom in 1854 who very clearly described it as a consequence of insemination, a work which has not received the attention it merits.[4]

That contractility is inherent in the ectoplasm of eggs can be readily demonstrated. Unfertilized *Arbacia*-eggs, for example, may be drawn out into long tenuous strands by putting them among fibres of lens paper. Since in this egg when it is unfertilized the endoplasm is highly fluid, this elasticity is due to the ectoplasm (including the vitelline membrane). The normal contour is readily regained without loss of fertilization-capacity. *Paramoecia* likewise can squeeze themselves through fine tubes and assume most bizarre shapes, returning again to the normal appearance when the pressure is released.[5] Lillie,[6] speaking of the egg of *Chaetopterus*, says: "The protoplasm of this egg is much more fluid than that of any other egg I have tested; in consequence, the egg elongates even with relatively low centrifugal force." And later "The egg is an elastic sphere; it therefore elongates in the direction of centrifugal force."[7] I conclude from these statements that the elasticity of this egg is due to its surface. Mrs. Andrews[8] attributes elasticity of cells to the ectoplasm.

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[1] *Metschnikoff, 1879.*
[2] *Berthold, l.c.*
[3] *Hertwig, O. and R., 1887.*
[4] *Ransom, l.c.*
[5] *Just, 1928c.*
[6] *Lillie, F. R., 1908.*
[7] *Ibid., p. 72.*
[8] *Mrs. Andrews, 1897.*

These manifestations definitely coincide with the break-down of material in the ectoplasm and with the rapid reconstitution whereby the egg-surface builds itself anew. · They are fundamental physiological expressions of vital activity and have their cause in the visible structural changes at the egg-surface which have been observed both on the normal egg and on that exposed to the action of dilute sea-water. Sperm-attachment is for the unfertilized egg the normal and best means of stimulation; as a stimulus the spermatozoon calls forth the same responses in the egg—oxygen-consumption, conduction and contraction—elicited by stimuli acting on other cells. This similarity I hold to be of fundamental significance for cell-physiology for I conceive these modes of response to be properties of the ectoplasm of cells generally. The grounds for this conception follow.

Few investigators to-day support the old theory of the nucleus as the seat of cellular oxidation. The human red blood corpuscle is pre-eminently an oxygen-carrier; it has no nucleus. If there be evidence of increased accumulation of oxygen in the region of the nucleus of a cell, this more likely is caused by the presence of the nuclear membrane rather than by nuclear substance. Positive evidence indicates that oxygen-consumption depends upon cell-structures, that is, upon minute surfaces in the protoplasm; even triturated cells consume oxygen.[1] Then the cell surface constitutes, *par excellence*, an oxygen-uptaking structure. And since this surface is subdivided into processes and its area therefore increased many times, its effectiveness is enhanced. In higher organisms, as the vertebrates, the cells which take up oxygen are rich in surface—as cells of the gills in aquatic and of the lungs in terrestrial animals.[2]

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[1] *Warburg, 1914.*

[2] *The entire inner surface of a man's lungs amounts to about 90 square meters, more than 100 times the area of the skin.*

Red blood corpuscles are discs, measuring seven to eight by two microns; they thus possess a large surface area, the sum of which amounts in an adult man of 78 kilograms, according to one estimate, to 3840 square meters. Hartridge[1] has suggested that the shape of the red blood corpuscle is the best possible one for insuring easy oxygen-intake.

No one can object to the proposition that oxygen to enter a cell must cross the cell-boundary. Even on the theory that in oxygen-consumption only the most deeply located structures of the cell, nucleus or otherwise, are concerned, the oxygen enters the cell by way of the cell-surface. Reasoning by analogy we may say that just as there exist special structures as gills and lungs to obtain oxygen, so in cells generally the modified surface permits easy access of oxygen. These surface-modifications are delicate prolongations. It is also interesting to note that pigment granules, said to be carriers of oxygen, line up at the cell surface.

Oxidation means liberation of heat. The heat is lost by the cell-surface. Here again the cell-surface is admirably constructed for the rapid conduction and radiation of heat. Since heat is produced when colloids take up water and also when they liquefy, it may be that cell-surfaces in breakdown through swelling and liquefaction liberate heat.

Another fundamental property of living substance is conduction. Any living cell when stimulated has the capacity to transfer the effect of this stimulus. The degree of conduction constitutes one of the chief differences that distinguish animals from plants. In animals conduction is the function of the nervous system, which possesses this property, general to all cells, in the highest degree.

Now among other characteristics of nerve cells we note an extreme differentiation of the ectoplasm, as has been stated. Nerve cells show one or more processes, some of

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[1] *Hartridge, 1920. See also Ponder, 1934.*

which attain great length. Even the simplest unipolar nerve-cell, that is, a cell with one prolongation, is a cell in which the ectoplasm is drawn out into an extensive filament. The more richly branched cells and those with the longest prolongations show no cytoplasmic inclusions in the branches, i.e., the fibres; instead, the cytoplasmic inclusions are located in what is called the nerve cell body. Structurally, therefore, the nerve cell is not only rich in surface, but also in a special kind of ectoplasm. It is more than an accident that this structure appears in highly conducting tissues. The minute attenuated threads of nerve fibres are admirably adapted for transmitting impulses.

I have already mentioned the work of Harrison who demonstrated that the growing nerve fibre shows definite surface changes at the growing tip.[1] This tip eventually becomes the means by which one nerve cell establishes contact with another, the junction being known as the *synaptic membrane*. The fibre from one nerve cell does not pierce the cell body of another, but simply comes into delicate contact with it; and so the impulse passes from one nerve fibre to another. Conduction thus is carried on primarily by the fibre.

Conduction in nerve cells, in other words, being a peripheral phenomenon, is not unlike that in an egg cell, since the interior of the egg cell is not involved in the conduction of the fertilization-stimulus. In free living unicellular animals one can not always clearly identify conduction as a surface phenomenon because often conduction is followed so closely by contraction that it is difficult to separate the two processes. Nevertheless, there are forms in which it can be shown that the conduction is purely a surface phenomenon.

It is to be borne in mind that conduction by nerve cells in higher organisms is not restricted to one cell. In the

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[1] *Harrison, l.c.*

propagation of the nerve-impulse, for example, from a sensory ending which receives the stimulus to end-organ (e.g., muscle) which is affected, the impulse in traveling over afferent nerves to central nervous system and from the central nervous system to the efferent nerve, involves at least two and, more frequently, several nerve cells. The transfer of the impulse from nerve cell to nerve cell as well as the connection between the efferent nerve and the end-organ has been clearly demonstrated to be by means of fine fibrils. These, the prolongations of the nerve-fibre, are as clearly ectoplasmic as the fibres themselves. Thus, the integrative action of the nervous system is established by intercellular connections. I conceive all modes of integration in the body of a complex organism, as the vertebrates and man, to be at basis dependent on intercellular—ectoplasmic—connections.

The central nervous system integrates all systems of the body; these are at some distance from it and unlike it; the glands of internal secretion in animals which possess them, exercise even more remote control over one or another system. A more passive integration—and of a lower order, since it binds tissues only—is that by connective tissue. Finally, by means of intercellular connections, like cells, i.e., cells in the same sheet of tissue, are held together. These different modes of integration one can not think of as mutually exclusive; nor can one regard them as categorically distinct and separate from the point of view of function. Only the order or level of integration—of all systems of the organism, of some systems, of organs, of a tissue—is a special, distinct one in each case. So much has been said by authors and in so great detail concerning nerve- and hormone-integrations, that no time here need to be taken to discuss these types of integration. Suffice it to point out that nerve action influences the chemical output of the glands of internal secretion and that the secretions in turn may affect nerve-tissues; to an extent, therefore, they

depend upon each other. Apparently, connective tissue exercises merely a passive restraint upon the subjoined tissues; it isolates and insulates. It is otherwise with the interdigitated cells in a given tissue. Their interdigitations constitute a means for bringing the cells directly into connection.

On the side of theoretical biology intercellular prolongations deserve our interest. Hammar suggested that by them the unity of the multicellular organism may be maintained, seeing in them a basis for some support of Whitman's idea of the inadequacy of the cell theory for development, on which I have commented above; here I wish to point out the following:

A multicellular organism, as a human being, is a unit and acts as such through integration. The cellular connections of the first grade in importance are those of nerve. To secure unified action of the organism is a function of the nerve cells primarily. Alongside with nerve integration, we place that by the hormones; for they come from glands upon which life depends, without which the organism dies; these, except the pancreas, are derivatives of or include derivatives of the nerve tissue or that from which the nerve tissue is derived, namely, the ectoderm; these glands are the pituitary body, the thyroid apparatus and the adrenal body.[1]

This nerve integration, as that of the three important hormones named above, is remarkable because of the fact of the ectodermal origin of its carriers. As we shall see in a later chapter, ectoderm cells arise from those cells in the developing egg which are richest in ectoplasm. Hence the highest form of integration in the complex multicellular

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[1] *The internal secretion of the pancreas is derived from cells which are neither nervous nor ectodermal in origin and therefore the pancreas cells are an exception here.*

organism is by means of ectoplasmic processes formed by originally ectoplasm-rich cells and by chemicals furnished by originally ectoplasm-rich cells.

Now the integration of cell with cell—the simplest mode of integration—is by means of the ectoplasm. Thus it is evident that all forms of integration in the complex organism are in the last analysis ectoplasmic.

Contraction is like conduction a fundamental property of the living cell. The lowest forms of animal and plant life have capacity for contractility either permanently or at some time or other in their life-history. All the cells of a highly organized multicellular organism possess it in some degree. But this endowment may be reduced to such a low point that it is almost extinguished. With evolution animals developed cells which were set apart as contractile tissues. These are the muscle cells in which this fundamental property of living substance is highly emphasized. Muscle cells are also characterized by having great length. The smooth muscle fibre is thicker than either type of the striated muscle. It is also the least responsive and shows the longest reaction-time. Theories of muscle-contraction that have the widest acceptance explain it as a surface-phenomenon. It is certainly true that contractile tissues have relatively large surface area. Their elasticity is undoubtedly a function of the surface— as in the case of egg-cells.

The life-process of a cell is the sum and interaction of its manifold activities. The phenomena which together inhere in the state of being alive reveal themselves as activities of the protoplasm. No protoplasm exists without them. For respiration, conduction and contraction, the foregoing presentation offers proof that they are in particular manifestations of ectoplasmic activity. That nutrition is likewise bound up with the ectoplasm will become evident in the next chapter.

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Water

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*W*ATER, QUANTITATIVELY THE MOST IMPORTANT COM-
pound in the inanimate world, and the most ubiquitous,
holds the same place in the animate. Roughly, living cells
can be said to be made up of two-thirds of water. Water
is a structural part of every living protoplasmic system; life
does not exist without it. Even when in a highly dessicated
state, animals, such as rotifers and tardigrades, are not
completely anhydrous.

Living protoplasm is an aqueous colloidal solution. In
view of the prevailing emphasis nowadays placed upon the
colloid state of protoplasm, it is astonishing how little we
know of the manner in which water forms a part of the
living state. Is protoplasm an emulsoid colloid? To what
extent is it a suspensoid colloid? Is it a structure in which
water encloses other substances or one in which the other
substances enclose water, or is it of both types? With
these questions I refer to the ultra-microscopic structure of
protoplasm—that is, of the ground-substance—for to this,
as already pointed out, the colloid-chemist by his own
definition of colloid-chemistry should address himself if
he wants to study living substance, and not to the micro-
scopically visible inclusions. The solution of many funda-
mental problems in biology is postponed because of our
ignorance of protoplasmic structure. The colloid-chemist
could render great service to medicine and biology by dis-
sipating this ignorance. As I see it, the main significance
attached to the study of matter in that physical state called
the colloidal is that it deals with solutions; and where it

concerns watery solutions it may give not only information concerning the physical properties of particles which lie within a certain range of size but additional knowledge of the chemical action of water in a system of colloid particles. The study of watery solutions is especially valuable inasmuch as protoplasm is such a solution.

Water also is a functional component in the vital reactions which take place in the cell. It plays a leading rôle in the utilization of food materials: when these are broken up into simpler compounds by the process of hydrolytic cleavage water is taken up; when in turn these hydrolyzed cleavage products are synthesized, water is lost. Whether in a cell this cleavage or synthesis will occur, depends upon the presence of water. Thus the movement of water from place to place in a cell hangs together with the direction in which the reactions run. Cellular oxidations demand the presence of water. Water is an end-product of all energy changes. Both conduction and contraction in cells depend upon water. In the removal of secretory products and in the elimination of excretory, water is necessary.

Water thus is both part of the protoplasmic structure and a functional component in vital manifestations. In other words, it is bound up with the two fundamental problems of cell biology, protoplasmic structure and function. The rhythm of water loss and water gain which every cell exhibits during its history, serves by these fluctuations, by its transitory nature, to emphasize the significance of water—to offer us, as it were, a key to the puzzle of the oscillatory changes which characterize life. Although during its development the animal egg progressively loses water —a dehydration imposed upon the rhythmical water-loss and gain—it by no means ever reaches the stage of complete dehydration. Its changing water-content therefore indicates to us a possible approach to the problems, how water

forms part of protoplasmic structure and how it plays a rôle in protoplasmic activities. Since the structure of the whole protoplasmic system is composed so largely of water, and its manifestations as a reacting system depend so greatly upon water, this structure must in some way dictate the distribution of water within the cell and hence determine the entrance and exit of water.

When by chance I saw drops of water leaving an egg-cell, I was alert to seize the opportunity to follow by means of simple observations the fate of water within the egg.[1] These observations will now be detailed and conclusions derived from them. Also suggestions will be made concerning the entrance of substances in solution into cells and concerning the question, why cells take in certain substances and not others. First, some mention should be made of method.

When unfertilized eggs of marine animals are placed in dilute sea-water, they take up water and swell at a rate which depends, for specific eggs, upon the degree of dilution. Thus, for the eggs of the sea-urchin, *Arbacia*, a favorite object for this type of work, the rate of swelling in 40 per cent. sea-water (60 parts tap water plus 40 parts sea-water) has been measured by Lillie[2] and confirmed by others. After thirty minutes in this dilution the eggs break down. If the sea-water is still more dilute, the water-intake is more rapid and the eggs' break-down occurs earlier. If the sea-water is less dilute, the rate at which water enters is slower and break-down comes on later. Thus I found that in 60 per cent. sea-water *Arbacia*-eggs remain intact for twenty-four hours. Unfertilized eggs of *Echinarachnius* are more sensitive, being injured by dilutions which are innocuous to *Arbacia*-eggs.

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[1] *Just, 1926b, 1928b, 1930a and b.*
[2] *Lillie, R. S., 1916.*

According to Lillie, among any lot of unfertilized eggs of
Arbacia which have been placed in 40 per cent. sea-water
are some which disintegrate after a very short time whilst
50 per cent. disintegrate after thirty minutes. From this
observation it is clear that, since more than half of the eggs
are killed by this dilution, the action of the 40 per cent.
sea-water on them is not reversible. Also, it is questionable
that this dilution is innocuous to those eggs that do not
disintegrate during these thirty minutes; they would
scarcely return fully to normal condition when brought
back to normal sea-water. Lillie points out that the eggs
after eighteen minutes exposure continue to swell, i.e., fail
to reach equilibrium with the diluted sea-water; instead
they break down. Thus this dilution gives information
concerning the rate of swelling only in injured eggs about to
die. The very obvious question arises, namely, how much
of the swelling is due to injury. It is thus difficult, if not
impossible, to draw conclusions from these experiments
concerning the behavior of normal eggs. Lucké and
McCutcheon in their many calculations of the swelling of
the *Arbacia* egg[1] have likewise used 40 per cent. sea-water
as well as other solutions including non-electrolytes (sugars).
But inasmuch as to their work the same objections hold as
to that of Lillie—it is almost certain that the sugar solu-
tions used injured the cells further—we can not here con-
sider it, although, for their purpose, the demonstration of
the osmometer-properties of the egg, its moribund condi-
tion is of no consequence.

In all my work I was at great pains to fix very definitely
the lowest limit of hypotonicity that a given marine egg
can withstand with recovery to the perfectly normal condi-
tion. The study of dead protoplasm as it exudes from an
egg membrane because of pressure put upon it either by

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[1] *Lucké and McCutcheon, 1929 and earlier.*

mechanical means or by hypotonicity may be interesting and even fascinating and may allow the drawing of some conclusions concerning the normal processes in the living state. However, in the elucidation of the normal processes in the living state, observations and experiments on living eggs whose conditions are only slightly altered, are, as was said in a previous chapter, of higher value for drawing conclusions than the study of dying or dead eggs. My studies, first made for another purpose, on several species of marine eggs of the rate at which the deleterious effects of hypotonic sea-water set in, led me to establish those hypotonic solutions in which the eggs did not break down though they remained in the solutions for hours; indeed, in these solutions these eggs remained intact as long as, or longer than, normal eggs in normal sea-water. We may for our purpose here at once dismiss those grades of hypotonicity which destroy the eggs.[1]

There are grades of hypotonicity which though allowing unfertilized eggs to survive for hours, do in time destroy them, as does normal sea-water which also finally is destructive. Such solutions one must use with caution because what is essential in these studies is the most satisfactory demonstration that the action of the solution can be fully removed—i.e., that eggs after residence in it on return to normal sea-water are as normal as those which never have been in the solution. Usually one measures the size of the unfertilized eggs in sea-water after they are removed from the hypotonic medium; the return to normal size is taken as the criterion that the egg has recovered completely. This, I find, is not enough. I have pointed out that the great

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[1] *It was deemed necessary clearly to establish in this elaborate fashion that the phenomenon occurred in viable eggs because after the first communication had been given and whenever demonstrations were made, the question, are the eggs alive? was raised.*

advantage of using eggs instead of other cells, especially tissue-cells from a many-celled animal, lies in the fact that one has more and sharper criteria for determining that the cells are alive and normal. One very excellent criterion is the fertilizability of the egg. Eggs that after return from a hypotonic solution regain normal size, may sometimes not be capable any more of fertilization or of development. Or, as has happened in my studies, the hypotonic sea-water induces parthenogenetic development. Fertilization-capacity is then the best expression of normality. If on return to normal sea-water the eggs not only regain normal size but fertilize and develop as normally as eggs that never had been in hypotonic sea-water, we may conclude that they have fully returned to normal condition. In the table appended figures are given to show how far eggs of *Nereis* recover after exposure to various degrees of hypotonicity as measured by the per cent. of cleavage and of swimming larvae.

TABLE I.—PER CENT. OF CLEAVAGE AND OF SWIMMING FORMS OF EGGS FERTILIZED IN SEA-WATER ONE HOUR AFTER HAVING BEEN RETURNED FROM DILUTION OF SEA-WATER, IN WHICH THEY HAD BEEN FOR ONE HOUR*

Per cent. of sea-water	Per cent. of normal cleavage	Per cent. of normal trochophore
60	94	96
55	90	94
50	100	86
45	96	80
40	91	85
33⅓	92	82

* This table represents one experiment of 10 made for each dilution. In most of the 60 experiments the controls showed 100 per cent. cleavage and more than 90 per cent. normal trochophores.

If one bars accidents and uses proper care to guard the eggs against overcrowding and high temperature, they develop as if never having been out of sea-water of normal concentration.

But I went farther in insuring the viability of the eggs. I used dilutions of a degree which allowed the eggs to fertilize and develop whilst in the dilution. Naturally, this development in the more dilute sea-water is far from normal. Nevertheless, if fertilization and development proceed in a solution, that solution is not as destructive as one in which both egg and spermatozoon are killed. The table appended gives figures to show the per cent. of development of eggs fertilized in hypotonic sea-water of various grades in which they develop.

TABLE II.—PER CENT. OF CLEAVAGE AND OF SWIMMING FORMS OF EGGS FERTILIZED IN VARIOUS DILUTIONS OF SEA-WATER, IN WHICH THEY REMAIN DURING DEVELOPMENT*

Per cent. of sea-water	Per cent. of cleavage	Per cent. of swimming forms
60	65	61
55	100	64
50	92	79
45	98	82
40	88	60
33⅓	96	66

* This table represents one experiment of 10 made for each dilution. In most of the 60 experiments the controls showed 100 per cent. cleavage and more than 90 per cent. normal trochophores.

I spent a great deal of time in taking these precautions because I wished very clearly to establish that the eggs in which I observed drops of water were living cells capable of normal processes. Many times indeed I fertilized eggs immediately upon their return to normal sea-water—that is, during the process of their return to normal condition it was possible to initiate development in them; hence the drop-formation did not interfere with fertilization. Thus, these observations were made on viable cells; I can think of no source of error left unguarded.

Now let us follow the process in detail.[1] In the hypotonic

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[1] *Although incidental observations had often been made earlier, the first systematic ones were made in 1925 on which the initial report published the next year was based.*

medium the eggs gradually swell so that finally their bulk greatly exceeds that of the normal egg. With this increase in volume, the yolk-spheres, so striking in the normal egg, seem to disappear, actually they have changed to an unusual degree, losing their refringency and their discrete character; indeed, they may actually merge. These changes in the yolk are worth noting. What happens is this: The indi-

Fig. 20a.—Section of a fixed egg of *Nereis* after 45 minutes' exposure to 40 per cent. sea-water. The egg is swollen, the ectoplasm thickened and opaque, the yolk spheres are in process of fusion. (Figs. 20a to 20e drawn by Mr. L. A. Hansborough from author's preparations).

vidual yolk-spheres increase in size with a liberation of fine drops of oil, thus becoming almost transparent in appearance. If exposure goes farther, the yolk-spheres fuse. Yolk-spheres in fixed normal eggs are homogeneously blackened by iron haematoxylin; the yolk of the swollen egg also stained with iron haematoxylin is seen to be made up of fine threads within a distinct membrane, each yolk-sphere thus resembling a nucleus. The germinal vesicle

(nucleus in the resting stage) is also swollen. The ecto-
plasm is so narrowed that its fibrillae seem absent; in other
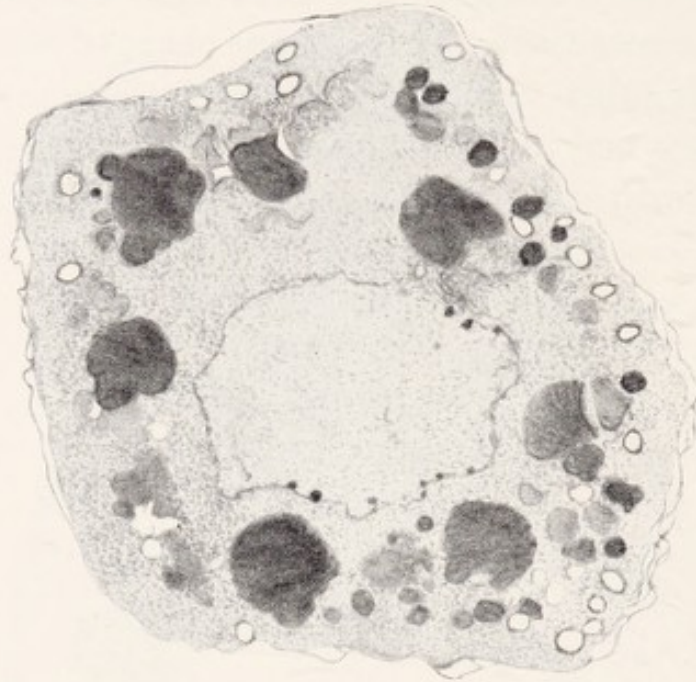
FIG. 20*b*.—Section of egg, in the same stage as that of Fig. 20*a*, after having
been returned to sea-water. The yolk spheres are regaining normal structure;
water in drops moves toward the periphery.
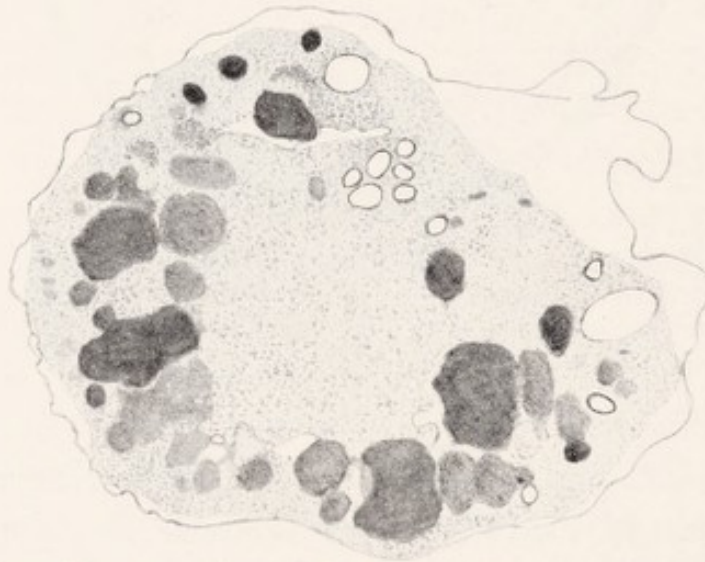
FIG. 20*c*.—Same history as that of Fig. 20*b*, but a later stage of development.

words, the pressure of the egg-contents has reduced the
width of the ectoplasm. On return to normal sea-water,
the egg shrinks and the water-drops then appear. The
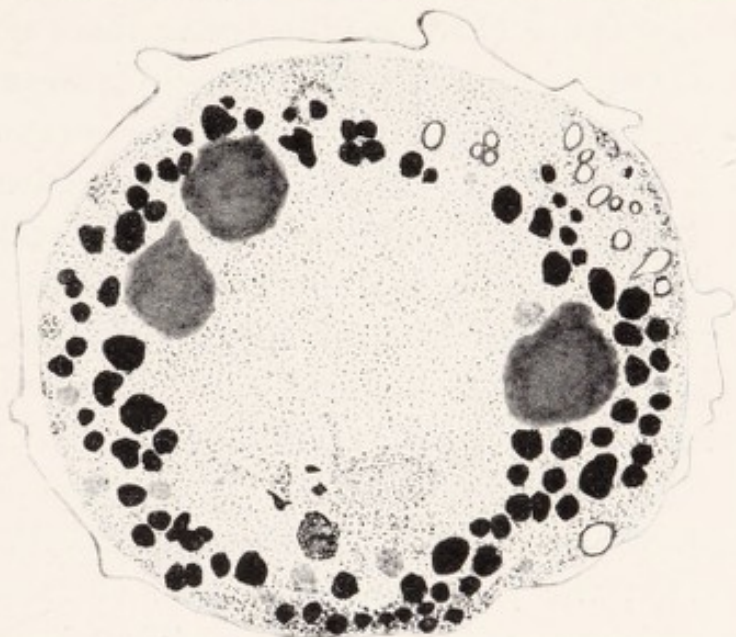
Fig. 20*d*.—Same history as that of Fig. 20*c*, but a still later stage of development.
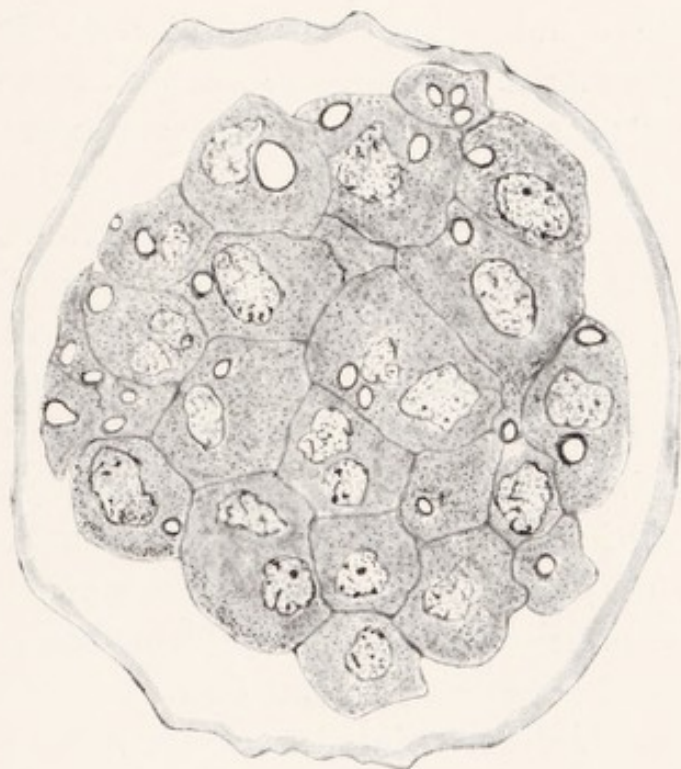
Fig. 20*e*.—Egg of *Platynereis megalops* showing water-drops in cells of a late cleavage-stage on return to sea-water following exposure to 40 per cent, sea-water for one hour.

accompanying figure (Fig. 20) illustrates these changes as seen in fixed eggs. First the drops are at or near the egg-centre, then they move outward. As they cross the ectoplasm they become elongated. Whilst the water-drops form and the egg regains normal shape, the yolk-spheres return to their original condition; they again become distinct spheres in the living egg, appear blackened in the fixed. This change runs parallel with the movement of the fine oil droplets into the yolk.

Thus the movement of water from the egg is associated with a marked behavior of the yolk-spheres. As the cells take up water, the yolk-spheres lose oil and increase in size, even fusing to make one mass. That is, the yolk-spheres take up water from the water-logged protoplasm. Then as the cells lose water to the surrounding medium, the yolk-spheres lose water to the cell and regain the oil from the cytoplasm. In the case of the *Nereis* egg the yolk-spheres possess a very exaggerated water-holding capacity.

It would be a mistake, however, to conclude from this description that the yolk-spheres are the sole regulating mechanism for the water control in the cell. Water-drops also form in the nucleus, in the clear cytoplasm in cells of stages of late cleavage which contain no visible yolk-spheres, and likewise in the gut-cells of larvae after all yolk has disappeared. Other animal cells than eggs similarly I find show these drops of water. One factor which determines the strong expression of water-drop formation is doubtless the ectoplasm, because eggs and other cells that possess most pronounced differentiated ectoplasm show water-drop formation best.

The rate at which the drops appear I have studied. A comparison of this rate in eggs in various stages is interesting: Drop-formation is more rapid in fertilized than in unfertilized eggs. It varies in fertilized eggs depending

upon the stage in the division-cycle of the egg. The formation of water-drops thus is also a cyclical phenomenon.

From these experimental findings we may draw the following conclusions concerning the behavior of water in these eggs.

First, water leaves the cell as discrete drops. This does not imply that all the water that leaves the cell is in this form. But since, as is shown in these experiments, water leaves the cells in visible drops, a theory concerning the exit of solutions as ions can not apply to these drop-formations. Visible drops of water are of more than even molecular size.

Second, the change in shape of the drops as they cross the barrier of the ectoplasm suggests that they pass through canals whose diameters are less than theirs. Now the ectoplasm shows chambers, the spaces between the radial projections. Since the diameter of the drops is less than that of these ectoplasmic chambers, the latter can not be the canals concerned. The canals therefore through which the drops pass though not sub-microscopic are smaller than these ectoplasmic spaces. The experiments thus strongly indicate that under these conditions the ectoplasm is a sieve with extremely small openings.

Third, the yolk-spheres take up water from the cytoplasm. The yolk in the *Nereis*-egg is a mixture of lipoid and protein.[1] This combination breaks down as water accumulates in the cell and enters the yolk-spheres. Lipoid escapes from the yolk-spheres into the cytoplasm. When water leaves the cell, water also leaves the yolk and the lipoid again enters the yolk. In hydration and dehydration of the yolk the lipoid moves out of and into it. Oil moves out of the yolk as water moves into the yolk-spheres leaving them skeins of protein. As water moves out, oil

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[1] *See Konopacki, 1929.*

moves in and the yolk-spheres assume their original physical appearance. These changes in the yolk-spheres show one very delicate mechanism by which cells hold water.

Fourth, the yolk-spheres are not alone concerned in the water-holding capacity of the cell. The clear, apparently structureless, cytoplasm and the nucleus have the power of holding water and of losing it in the form of drops.

It remains now to be decided how far we can apply these conclusions on the findings on eggs under experimental conditions to normal eggs in normal sea-water. Although these eggs of the experiment were viable and capable of recovery and complete development, they nevertheless were subjected to an experimental treatment.

However, these experiments of mine described above were made with greatest care within limits definitely set; the processes described can not be attributed to extensive or drastic injury. There is thus less danger in making statements concerning them as explanation for normal conditions than in drawing conclusions concerning normal processes from experimental procedures which killed the eggs; the former only exaggerate, the latter extinguish the process that we wish to explain.

If the process of drop-formation of water observed in these experimentally treated eggs is only an exaggeration of a normal process, it should be possible, I thought, to observe it both with other methods of treatment and in the normal untreated egg. As a matter of fact, I have found that the drops form as the result of pressure, of exposure to ultra-violet light and after removing the eggs from normal sea-water at 5°C. to that at room temperature (19°C). During exposure to hypertonic sea-water, the drops are beautifully shown especially by fertilized eggs during every stage of development and by cells in the young worm. In unfertilized eggs in hypertonic sea-water the drops are neither so numerous nor so easily visible.

In the untreated normal egg, drop-formation presumably occurs rapidly and the drops are neither so large nor so numerous as in experimental condition. Actually I was able to observe the appearance of drops in normal unfertilized eggs. They are much smaller and more evanescent than in the experimentally treated eggs. They appear more clearly in the fertilized than in the unfertilized egg and vary in rate of formation during the cleavage cycle. Drop-formation is thus also in the normal egg related to the physiological rhythmical changes coincident with the division-cycle.

Since these findings indicate that the experimental means merely prolong and render more easily visible the more fleeting changes in the normal eggs, we may with more confidence use the experimental results for interpreting normal conditions and processes. The experiments have far-reaching significance for cell-biology in two respects: they permit conclusions concerning an important aspect of the problem of protoplasmic structure and concerning the question of the movement of water into and out of cells.

Although we have some information on the structure of protoplasm, we are far from possessing adequate knowledge of it. What is known of the chemistry of the cell does not suffice to tell us whether protoplasm is an emulsion, a suspension, a foam or a combination of these. As said above, an advance is made the moment that the ground substance is recognized as the cytoplasm *par excellence*. This appears as a menstruum containing extremely minute bodies. Since water makes such a large part of protoplasmic structure, we ought to seek to learn how it forms part of this structure: whether it encloses the particles or the particles enclose it; or, in the words of the physical chemist, whether water is in the external or dispersing or in the internal or dispersed phase. On this

question the experiments showing that eggs of *Nereis* lose water in the form of drops throw some light.

If we think of the water as in the external phase, the effect of increasing the density of the medium—from hypotonic to normal sea-water or from normal sea-water to hypertonic—would be either to cause streams of water to form rather than drops, or to free the water generally and equally everywhere from the eggs, so that the water would not be visible as it moves out of the egg. Since however drops of water form and since water can not appear in water as drops, we must assume that the drops are pressed out of structures. We conclude, therefore, that the water which appears in the form of drops is that which was held by solid structures, i.e., was in the internal phase. The formation of drops in normal viable eggs points to the same conclusion.

As we have seen, the egg on return from hypotonic to normal sea-water regains its normal equilibrium as it shrinks. In this process, inasmuch as drops of water leave the yolk-spheres we conclude that the yolk-spheres possess water-holding capacity and thus exercise function in the distribution of water in the cell under the condition of the experiment. Because it is not easy to discern yolk-free areas of the endoplasm in the unfertilized egg of *Nereis*, one can not so readily determine to what extent water-holding power resides in the cytoplasm. In the various stages of development—cleavage, blastula, gastrula, larva (trochophore) and young worm—as the yolk becomes segregated into certain blastomeres leaving others yolk-free, it becomes easy to learn that yolk-free blastomeres or those in which yolk is not in visible spheres also hold water in the form of drops. Therefore we conclude that the clear homogeneous cytoplasm holds water within its structure which exists in a state of fine subdivision. Moreover, the nucleus reveals water present in drops; these exist apart from chromosomes. Since the water drops can be demon-

strated by several experimental methods we may say that slight experimental modifications of the protoplasm cause movement of water out of the structures which hold it. The conclusion is that water as an integral part of the colloid structure of protoplasm is in the internal phase. This does not mean that no water exists otherwise in living protoplasm. On the contrary, we can only account for the observed initial shrinkage of an egg on return from hypotonic to normal sea-water as due to the invisible escape of water which doubtless represents, in part at least, water held as in the external phase.

Thus we have answered the first question raised: how is water held in protoplasm?

I may point out that these experiments as such have some significance for medicine. The distribution of yolk differs with different eggs, for instance in that of *Nereis* and *Platynereis*, as I have said above. Likewise, during its development the yolk changes in location as has been shown. Undoubtedly some of the difference in water-holding capacity noted among eggs of several species or in the same egg at different stages is to be attributed to this difference with respect to yolk. What is true of yolk may be true of other bodies in the cytoplasm. In diseased conditions as oedema and nephritis in which tissues hold an excessive amount of water, the structure of the cell may be a very important factor in determining this abnormal water content.

I turn now to the consideration of the question of the movement of water into and out of cells. This calls for a statement concerning the nature of the cell-surface.

When this question of the passage of water and dissolved substances into and out of cells is raised, especially in physiological work, by the term, cell-surface, usually is meant a membrane. This membrane is then spoken of as semi-permeable because it is said to take up certain substances and

not others. Much of the work on which the theory of semi-permeability is based is derived from work on artificial inanimate membranes. Many investigators regard the cell membrane as a molecular film; others treat it as such in their physico-chemical and mathematical disquisitions. The chemical nature of the postulated semi-permeable membrane around cells has often been discussed. Depending upon the one or another theory, it is considered protein, lipin, a mosaic of lipin and protein or a more complicated chemical structure. As was pointed out, to some the membrane is a dead, inert thing, a precipitation out of the protoplasm, or merely a surface conditioned by the external medium, etc. For others the membrane is a living part of the cell-structure.

In the above given descriptions of eggs, a vitelline membrane has been spoken of. This is built by the egg as it develops to maturity and is of measurable width. Beneath this vitelline membrane can be seen in many eggs a very delicate structure, appearing in optical section as an extremely thin line, the plasma-membrane, also built by the egg; it is visible and of measurable thickness and not a molecular film. The surface-structure of the cell is, however, not to be thought of as comprising only this plasma-membrane. The deformation of the water drops as they leave the egg takes place in the surface-structure below the plasma-membrane. This cell-structure is the ectoplasm. On these eggs as on so many others it displays amoeboid activity. Changes in a plasma-membrane and certainly those in a molecular film can not account for the rhythmical difference in permeability so often described and postulated,[1] for which, however, the ectoplasm, both in width and in activity, offers a sufficient basis.

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[1] *For examples of rhythmical changes, see Bayliss, 1915.*

In general, while it has been postulated that the differences in permeability noted both in a specific egg during different stages of its development, as before and after fertilization, and in cells of the same type, e.g., red blood cells, but from different animals, are due to the membrane, and that the cytoplasms are the same, the actual and visible changes in the surface-layer of cells have been ignored. Despite the excellent observations made by Mrs. Andrews[1] on the spinning activity of the ectoplasmic surface, which others have abundantly confirmed, this type of rhythmical surface-activity no one has sought to correlate with the postulated rhythmical changes in permeability. Though the measurable ectoplasmic layer of cells differs in width, the size of the layer has not entered into the calculations made on the entrance of water. The cell is a bag of watery solution; and being capable of deformation with return to its normal contour and size, this property of elasticity is resident in the surface-cytoplasm. But the elastic property of the cell-surface has been deliberately ruled out in the mathematical calculations on the entrance of water into cells. On the theory that the difference in rate at which water enters a specific cell in different stages depends upon the permeability of the cell-surface, i.e., the plasma-membrane, we should expect a most exhaustive analysis of the structure and structural changes of that surface—certainly we should scarcely expect that in addition to ignoring these the theory would actually discount the elasticity of the cell-surface.

Vitelline membranes are often chitin or chitin-like substance; it may be that generally they are protein; the plasma-membrane also may be protein, lipin or what else. These considerations lose in interest in view of the fact that

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[1] *Mrs. Andrews, 1896.*

that cell-structure which controls ingress and egress is the ectoplasm which in large part has chemically the same make-up as the remainder of the cytoplasm since endoplasm and ectoplasm are one continuous, though differentiated system.

The question which disturbs the physiologists who say that there must be a membrane in order to keep the cell-contents from flowing out and becoming miscible with the surrounding medium may be answered here. The cell-contents withstand outflow because the cell material is one continuous system. It is not like mercury which is now one mass and then many drops which flow together again; rather, protoplasm is a cohering though extremely thin dilute jelly-like solution, whose biological character is revealed by its strong regional differentiation. Excreted or secreted material, including colloids, gets out of a cell when it is no longer a living part of it. It is broken off and dispelled. Instances in support of this statement are not wanting. In egg cells we have seen that at fertilization the colloids in the surface break down and escape through the vitelline membrane. Material moves out of cells by secretion—that is, the cell breaks off part of itself and washes this part away.

The largest question in the permeability problem relates to the entrance of substances into the cell. If we attempt to answer this question we do it on the basis that the cell-surface is living ectoplasm, continuous with the remainder of the protoplasm, and is made up of a brushwork of filaments.

In part, the question how substances enter cells is bound up with another, namely, why some substances and not others get into cells. For example, in the gut and in the kidney salts or sugars when present in equimolecular concentration do not cross the cell-surface in the same amount. These two cases constitute stock arguments of the vitalists

against a strictly mechanistic point of view concerning the entrance of substances into cells.

The normal living cell carries out certain reactions in which water plays a part; it, therefore, needs either to take in water or to get rid of it. Also in cyclical changes, as those of division, in which the nucleus breaks down and reforms, water moves back and forth between nucleus and cytoplasm as well as between yolk and ground-substance. Whilst the cell-contents may vary from moment to moment with respect to water present, the water within a cell tends to maintain a certain level characteristic of that cell. This level varies with different cells. Thus, the water content of human cells differs: the enamel of the teeth, spermatozoa, and bone cells are poor in water while cells of the liver, kidney, intestine, etc., are rich in water. The level also varies in a given cell with its activity or stage in life-history. Thus, gland cells when actively secreting and when at rest show different levels. An egg-cell will show different levels at different stages of development. Finally, the level varies with changes in the surrounding medium. Every cell tends with respect to water to come into equilibrium with its surroundings. The level at which this equilibrium establishes itself depends upon the cell's specific composition, stage in its life-history and its activity at a given time.

Since the water level within the cell tends to remain constant under the changes brought about by reactions, water moves in or out in order to maintain this level. In a way the movement of water between cell and environment is comparable to the movement of oxygen; it is a diffusion-phenomenon for water moves to the region of most concentrated material, i.e., from the region of more to that of less water.

The air which human beings breathe is composed of 79 per cent. nitrogen, 20.96 per cent. oxygen, less than one per

cent. carbon-dioxide together with the rare gases, helium, argon, krypton, in traces. The permeability of the cells of the lung for these gases is an important property for without the entrance of them into the lungs and their subsequent conveyance to all parts of the body where oxygen is given up to each living cell, life would cease. The air breathed out contains the same 79 per cent. nitrogen, but only 16 per cent. oxygen and an increase of carbon-dioxide amounting to 4.38 per cent. All of us appreciate that the figures reveal how much oxygen the living cells use and need and how much carbon-dioxide they form and get rid of.

The passage not only of water into and out of normal cells in normal condition, but also of substances in solution may be compared to the entrance and exit of these gases. Some pass in and out again in equal, others in diminished, and still others in increased amounts. Without the entrance of food-stuffs and the exit of waste the cells would die. The phenomena of significance are the utilization of incoming materials by the cells and the excretion of effete. What has been said concerning water, therefore, may be said of material in solution as salts, sugars, amino-acids. These enter or leave the cells according to the level at which they are present in the cells. They form part of the protoplasmic structure and also take part in reactions going on within the protoplasmic boundaries. If their concentration in the cell is low, the cell retains them as they come in. In order to learn what substances a cell uses, we should not merely inquire what substances get into a cell, but also what of these remain in it. We may imagine that more of a given substance in solution in the surrounding medium passes into cells than remains in them.[1]

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[1] *The mistake is often made of attempting to learn what can get into cells by too severe treatment of them, injuring them by subjecting them to solutions of too great concentration.*

If the cell-membrane were permeable only to water, none of us could live because the cells in our bodies would receive only water. The physiologist therefore has finally to postulate a membrane whose temporary break-down allows substances other than water to pass.[1] But I think that there is more to the problem than mere passage across an inert boundary. The complexity of the protoplasmic organization and the many reactions going on in the protoplasmic system must determine, in part surely, what substances coming in shall remain. What determines the ingress of substances is the cell-surface. By this is not meant a membrane, semi-permeable or otherwise, a dead or living molecular film, but the whole ectoplasmic structure with its innumerable filamentous prolongations of living active cytoplasm.

Every cell's ectoplasm is built up of such prolongations, as has been abundantly shown in the chapter on the ectoplasm. The fact that this discernible, richly filamentous structure exists, taken alone suffices to render untenable

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[1] *Bayliss, 1922, p. 312: "As a further case of absorption, at all events as it appears to me, the cell-membrane or plasma-membrane may be considered. This is not to be regarded as a fixed permanent structure, but as produced by deposition of cell-constituents which lower surface energy at the interface between protoplasm and surrounding medium. Thus, it changes with cell activity and is in equilibrium with the cell contents as they alter. Thus, there is no difficulty in the membrane becoming permeable in the active state of the cell to substances to which it is impermeable in rest. Moreover, when a fresh protoplasmic surface is produced by mechanical action, a new membrane is naturally deposited on it. This is no doubt why large particles can be taken up in phagocytosis through a membrane which does not permit even sodium chloride in solution to pass. The particles actually break the membrane, which closes again behind them, in the same way as a needle can be passed through a soap film, without bursting it, whereas a gas, such as hydrogen, nearly insoluble in the soap solution, only passes with extreme slowness."*

theories based on a membrane of whatever hypothetical physico-chemical composition. The presence of the filaments means that the exposed cytoplasmic surface is enormously larger than if there were only a smooth membrane, and at the same time that it forms a system of capillary spaces. But this surface is not merely a static structure. The cytoplasmic processes which characterize the ectoplasm are indeed fine pseudopodia and as such display constant activity. By means of them the cell has phagocytic power, ingests fine particles. Phagocytosis is indeed an activity exhibited by all animal cells and not only by Amoebae, white blood cells and fixed tissue phagocytes as those in the vertebrate liver.[1] The moment that we appreciate the normal structure and behavior of the ectoplasm, the problem of the entrance of water and of solutions is placed in a new light. The theories of cell-permeability with their discrepancies and conflicts can with profit be abandoned. Since the ectoplasmic pseudopodia respond actively to the environment they regulate the exchange between cell and external medium.

All these considerations and data indicate that the surface-cytoplasm can not be thought of as inert or apart from the living cell-substance. The ectoplasm is more than a barrier to stem the rising tide within the active cell-substance; it is more than a dam against the outside world. It is a living mobile part of the cell. It reacts upon and with the inner substance and in turn the inner substance reacts upon and with it. It is not only a series of mouths, gateways. The waves of protoplasmic activity rise to heights and shape the surface anew. Without, the environment plays upon the ectoplasm and its delicate filaments as a player upon the strings of a harp, giving them new forms and calling forth new melodies. But these are too nice for the undiscriminating ear of man.

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[1] *Cf. Geddes, 1883.*

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The Fertilization-process

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two processes: nutrition and reproduction. The first is
concerned with the blindly egoistic struggle of the individual
to preserve itself. The second relates to the altruistic
struggle of organisms to perpetuate their kind. Despite
their interdependence the processes of nutrition and repro-
duction have different values for the organism. Without
food or the apparatus for the utilization of food, the organ-
ism dies; without the reproductive apparatus (as sys-
tems, organs, tissues or cells) sexual organisms can still
live. The reproductive (germ) cells are sharply set off
from all other (somatic) cells; they have the special burden
of the perpetuation of the species. The sex-cell is therefore
a thing apart, a tenant housed by mortal somatic cells and
like them mortal while the tenancy lasts. House and ten-
ant have a common origin. Their separation constitutes
the first of the series of those differentiations that mark
the development of an individual animal.

The adult animal is derived from one cell, the egg, which
by the process of cell-division becomes a mass of cohering
cells. They form the germ-layers; these give rise to the
various organs (or systems of organs) which compose the
complex adult individual. In the course of this develop-
ment are set off from all the other cells which make up the
body of the animal certain ones, the primordial germ-cells,
whose function is to produce either eggs or spermatozoa in
bisexual animals or both of these in monosexual or herm-
aphroditic animals. Thus, the germ-cells, first differenti-

ated from the somatic, are in their turn differentiated from each other.

The primordial germ-cells pass through a period of multiplication which ends with the production of many cells called ovogonia (in the female) and spermatogonia (in the male) whose nuclei still possess the somatic number of chromosomes, i.e., the number characteristic for the species. Ovogonia and spermatogonia without increase in their numbers are transformed into primary ovocytes and primary spermatocytes respectively by the pairing of the chromosomes in each nucleus; thus the somatic number of chromosomes is "reduced" to one-half, the gametic or haploid number. Then follows the period of growth which is especially expressed in the egg. The maturation (meiotic) divisions succeed the period of growth and differ somewhat in the male and female sex-cells.

In maturation usually each primary spermatocyte divides into two secondary spermatocytes and each of these again into two cells which are called spermatids. Hence a primary spermatocyte gives rise to four spermatids. By a series of cytoplasmic changes together with nuclear condensation the spermatids become spermatozoa. Only spermatozoa are capable of fertilizing eggs, whilst many eggs, as we shall see soon, can be fertilized before, after or in various stages of their maturation.

The maturation (meiosis) of the egg occurs as follows: at the end of the growth-period two divisions of the primary ovocyte follow each other paralleling those in the primary spermatocyte but the cells so arising are markedly unequal in size. The small cells, the polar bodies, contain nuclei enclosed by a minimum of cytoplasm. Of the three ovotids (or four, if, as sometimes happens, the first polar body divides) from one primary ovocyte there is only one, the mature egg, capable of fertilization; the two (or three) polar bodies are abortive eggs.

This brief account of the history of the germ-cells subsequent to their differentiation from the somatic cells teaches us that they pursue different courses. Without going into details, I call attention further to three facts of general significance for all that follows in my presentation of the problem of fertilization. The first is that there does not exist a single animal whose spermatozoa do not originate from an egg; the second, that no animal spermatozoon ever develops alone; and the third, that fertilization is not indispensable for the development of animal eggs, for, as we shall see in the chapter on parthenogenesis, many eggs develop normally or experimentally without the presence of spermatozoa. These three facts obviously warrant the conclusion that the egg carries the greater burden in fertilization. That the spermatozoon is itself derived from the egg is a fact to be emphasized. Even where it is indispensable for the egg's development, we can not in the light of its origin regard it as the perfect antipode of the egg. Whatever its highly specialized powers and functions, they rest upon derivatives of the egg-substance whence the spermatozoon came. That the spermatozoon never develops without the egg, whilst the egg can develop without the spermatozoon is another way of saying that the egg alone carries the burden of development. In these differences between the gametes we recognize where lie the powers not only of fertilization, but also of the whole course of the future development; the spermatozoon is a cell reduced to the minimum potency through its differentiation, the egg is of all cells known the most potent by virtue of its peculiar differentiation. Structurally, the great difference between spermatozoon and egg relates to the cytoplasm. Out of the egg's cytoplasm the future adult organism is differentiated. I propose in the following pages to prove that fertilization, the initial act in the differentiation of the egg, is likewise a cytoplasmic phenomenon. In order to reach

this definition, I examine (1) the structure of the spermatozoon and of the egg when they normally come together, (2) the changes in both subsequent to union and (3) their behavior at the time of union. The present chapter embraces the discussion of (1) and (2), the succeeding chapter confines itself to (3).

For the majority of multicellular animals, the coming together of the male and female germ-cells or gametes, spermatozoon and egg, guarantees the perpetuation of the species. This coming together of the gametes is fertilization in the widest sense, without which the spermatozoa of all and the eggs of most animals die. In this meaning fertilization marks the beginning of a life though of course both gametes are alive; it is the beginning of a new individual brought into being through the loss of individuality of each of the two cells which are co-partners in the process. If fertilization in all animals which exhibit it were to occur in the same mode and at the same time, our attack on the problem of fertilization would not be so difficult; but we encounter many differences. Always in biology must we reckon with differences and seek to determine to what extent they are "incidentia" or "differentia." The complexity and diversity of animal structure and behavior are either "incidentia" and as such defy reduction to simple terms or, as "differentia," they mask some one common factor to which we can reduce them. That there is one feature common to all fertilization-processes we shall see after we have evaluated the differences in the structure of the gametes as well as those in the time and the mode of their union.

The partners in the fertilization-process are as described above markedly different from each other in their development to the moment of their coming together. In one respect only are they similar: their nuclei contain each one-half the number of chromosomes characteristic of the adult

organisms from which the eggs and spermatozoa come. In this bringing together of the haploid chromosome-garniture from the mother and the haploid from the father, fertilization not only insures the constancy of the species' characteristic chromosome-number, but also, since the chromosomes are concerned in heredity, it maintains equilibrium between paternal and maternal inheritance as far as this is determined by the chromosomes. This similarity of nuclear structure in egg and in spermatozoon has thus significance for the development that results from fertilization, but as a static, non-changing factor it can not be related to that radical and far-reaching change in the egg that we denominate fertilization. We, therefore, turn to the discussion of the other characteristics of the partners and shall find in them many differences.

Animal spermatozoa exhibit great diversity in structure. In general, they may be classified as flagellated and non-flagellated. The larger number of species of animals have spermatozoa of the former class. Typically one such spermatozoon is made up of a head (the nucleus) which may be spherical, bullet- or lance-shaped, to whose anterior end generally is fitted a cytoplasmic cap, the so-called perforatorium, which may be very blunt, as in the starfish-spermatozoon, or more spike-like as in the *Nereis*- or the *Cerebratulus*-spermatozoon. The cytoplasm located behind the head is called the middle-piece; it varies greatly but often contains one or more granules of a lipoid nature. The tail or flagellum, the third cytoplasmic structure, is continuous with the cytoplasmic film enclosing the sperm-head and may be of very great length; it is the locomotor appendage of the spermatozoon.

Non-flagellated spermatozoa may have the form of a truncated cone. Or they are more definitely amoeboid in form, as in crustacean spermatozoa such as the lobster's, crab's, etc. That is, instead of the blunt contracted form

of the *Ascaris*-spermatozoon, those of these crustaceans present more slender projections resembling the finer pseudopodia of an amoeba. These spermatozoa are highly interesting because they are first inverted and explode at fertilization.[1]

Spermatozoa despite these great variations in structure have certain features in common: they possess very little cytoplasm although the volume of this may be greater than the nuclear volume. Compared to the egg, any species of spermatozoon is extremely minute. One fact already mentioned above is to be emphasized again: sperm-cells capable of fertilizing eggs are always fully matured—that is, they are always spermatozoa and never spermatocytes or spermatids.

Whereas most, probably all, species of animal spermatozoa are motile, with few exceptions eggs are incapable of locomotion. Whilst, moreover, animal eggs vary in size from a few microns in diameter to several centimeters (e.g., eggs of cursorial birds), the smallest species of eggs are larger than the largest species of spermatozoa. This greater bulk of the egg is due to its cytoplasmic mass with inclusions, oil, yolk, mitochondria, etc. If these are suspended in the cytoplasm they may amount to about two-thirds of the egg-volume; if they are pendent to the cytoplasm, as in birds' eggs, for example, they make up all but an extremely small fraction of the egg. No eggs, including the so-called transparent ones, are entirely yolk-free. In comparison with spermatozoa, animal eggs as a class at the time of fertilization contain more cytoplasm and are richer in reserve or food materials, yolk and oil.

As was pointed out, eggs in contrast to spermatozoa can be fertilized before, during or after their maturation, depending upon species. We distinguish four classes of

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[1] *See especially Kolzoff, 1910.*

eggs according to the stage in maturation in which they are fertilizable.

Class 1: The egg reaches the stage just prior to the maturation-divisions. This is the so-called germinal vesicle stage characterized by a large nucleus. It there comes to rest and dies unless fertilized. Mere contact of the spermatozoon is sufficient to cause the break-down of the germinal vesicle and the initiation of the maturation divisions. Eggs of various worms, *Polystomum, Gyrodactylus, Ascaris, Sagitta, Nereis, Platynereis, Thalassema, Myzostoma*, and of the clam, *Mactra*, are examples of this class.

Class 2: Here belong eggs which will not fertilize as long as the germinal vesicle is intact. They develop as far as the stage of first maturation and there remain until death unless fertilized. The eggs of the sea-worms, *Thysanosoon, Prostheceraeus, Chaetopterus, Phascolosoma*, and of the clams, *Cumingia* and *Mytilus* as well as eggs of snails and of the ascidians, *Ciona* and *Phallusia*, are examples of this class.

Class 3: Some eggs finish the first maturation before they reach the stage in which they are capable of fertilization. They come to rest with the second maturation spindle formed, one polar body having been extruded. Here belong eggs of many vertebrates; likewise the egg of the worm-like chordate, *Amphioxus*, regarded by some zoologists as the ancestral form of the vertebrates.

Class 4: Eggs of sea-urchins constitute an example of this class in which fertilization is possible only after completion of both maturation divisions. These are called fully matured eggs.

It is here necessary to make a sharp distinction between maturation, referring to the phenomena of polar body formation, and physiological ripeness, referring to the capacity of the egg-cytoplasm for fertilization, which as this classification shows reaches its optimum at various stages in the maturation-process (Fig. 21).

The egg of the starfish is interesting. Whilst other eggs, as we have seen, have a very sharply limited period during which fertilization is possible, the egg of this animal, whose optimum period for fertilization is just after the dissolution of the germinal vesicle, is still capable of fertilization at later stages of maturation and for a time after complete maturation. But inasmuch as the fertilization-process
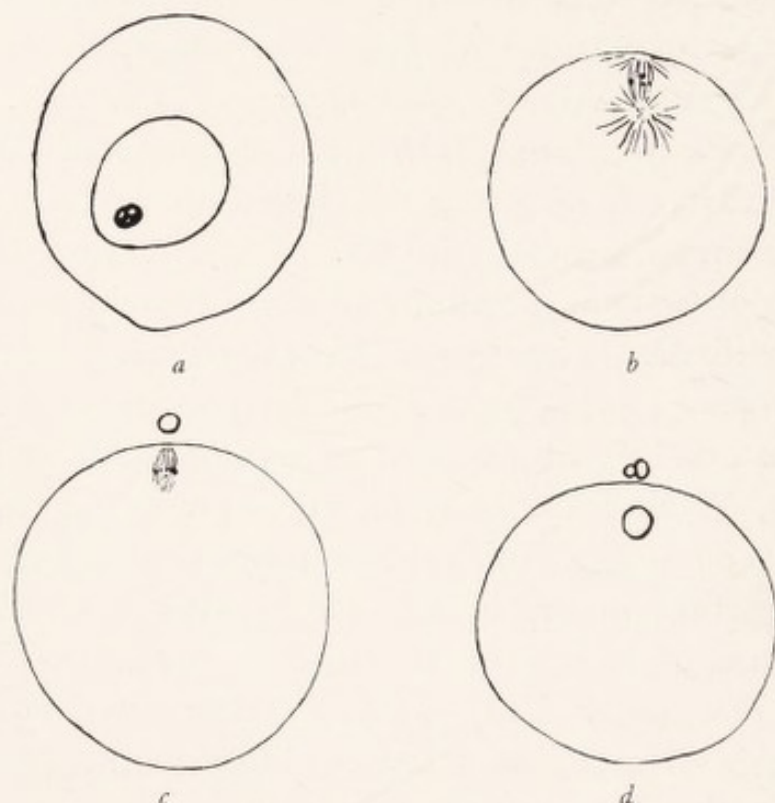
FIG. 21.—Diagrams to illustrate the four fertilization classes (after Wilson).
a, Class I; *b*, Class II; *c*, Class III; *d*, Class IV.

either before or after maturation is below normal, this egg belongs to Class 2.

It is evident that an explanation of fertilization must cover these four classes of eggs. A theory that covers the fertilization of the sea-urchin egg and not that of eggs fertilizable in the germinal vesicle stage and in subsequent stages of maturation would demand a separate explanation for each of the other three classes. This in turn would mean that fertilization differs in different eggs—

that, widespread though the phenomenon is, it would reveal no common factor. This point of view is often maintained for all animal biology, some workers going so far as to say that every animal and every egg is a law unto itself. However, such a point of view does not properly envisage a phenomenon as widespread as fertilization. It is our purpose to look beyond differences, to seek a common factor. This can not be related to a particular stage in maturation, as our classification shows. It thus becomes necessary to describe briefly the process of fertilization as it occurs in a representative of each of the classes in order to learn if any feature is common to eggs of all classes. Said otherwise, having examined the structural make-up of the co-partners in the moment when they come together in the act of fertilization, we address ourselves to an exposition of the events that follow this coming together. At this point one general word concerning the way in which egg and spermatozoon meet may not be amiss.

Eggs of animals, in which the sexes are separate, may be laid before the spermatozoa reach them. In such cases the eggs are shed into the sea—or fresh water—with or without copulation between male and female. In other cases, eggs are reached within the female's body by spermatozoa deposited within her genital tract; here copulation between the sexes is the rule. Eggs in these cases may be deposited at once to undergo development outside the animal's body. Or they may remain within the female where they undergo development completely or in part. The foregoing is true also of normally hermaphroditic animals—i.e., those in which the sexes are united. It should be added that among these, eggs and spermatozoa produced by one individual may unite, so-called self-fertilization; sometimes this is brought about by the presence of some structure that prevents the access of spermatozoa from another individual to the eggs. In many hermaphroditic animals self-

fertilization can not occur because eggs and spermatozoa are not ready for fertilization at the same time. The failure of self-fertilization among hermaphroditic animals is more often assumed than proved.[1] None of these modes by which egg and spermatozoon are brought together can be correlated with the stage in maturation at which the egg-cytoplasm is "ripe" for fertilization. Nor can we say that fertilization and development within the organism are peculiar to higher animals. Among sponges, for example, the lowest form of animals that produce eggs and spermatozoa, the eggs pass through early development in the parent organism. That tapeworms, members of the third lowest group of multicellular animals, are characterized by their strong male copulatory organs, is a fact which discredits a popular notion that copulatory organs are found only among the highest animals. Thus, the mode by which the union of egg and spermatozoon is accomplished has no special significance for the events that follow this union.

In the exposition of these events, which now is given for each class, we shall begin with contact of egg and spermatozoon and end with the first cleavage of the egg. These are common points. Between lie those events whose differences call for evaluation. I request the reader to note them in order the better to appreciate the discussion of their significance. Named in order, these events are: the initial changes at the surface of the egg; the arising of two star-like formations, the asters, associated with sperm- or egg-nucleus (with or without a discrete body, the centriole, at the centre of each aster); the coming together of the egg- and sperm-nuclei (sometimes called pronuclei); and the formation of the cleavage-spindle.

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[1] *Cf. Just, 1934b, and earlier workers.*

The statement made in the chapter, Life and Experiment, that we lack many details concerning the happenings in normal biological processes, can be proved when the history of fertilization in the various examples given below is reviewed. Although I have endeavored to choose a representative for each class whose fertilization is best known, we shall see that in no one have all the steps been completely followed. Fertilization is far from being a sterile field of research; there is no single animal egg for which the events, from the moment of contact of egg and spermatozoon to first cleavage, have been so adequately described in closely set stages that we can say that we possess full information of the chain of events as a continuous process. The description of fertilization in four eggs, that now follows, will give us merely the chief outlines of a picture which we shall try to make more complete by adding lines and details from the process in other eggs. Only then shall we draw conclusions and enter upon the discussion of fertilization.

I describe fertilization as it occurs in the egg of *Nereis limbata* found along the Atlantic shores of America; it represents eggs of Class I.[1]

The fertilization of the egg of this easily obtainable marine worm can be controlled since the eggs are discharged freely into the sea where they are immediately mixed with the spermatozoa; one needs merely to collect the mature males and females separately and then to place them together in pairs at timed intervals to obtain fertilized eggs in a series of as closely set stages as one desires. One draw-back exists, namely, that sexually mature animals can not be found throughout the summer months when the

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[1] *This account is based in large part on that given by Lillie, 1911 and 1912.*

animals breed. Like many other forms, *Nereis* exhibits a lunar periodicity in its breeding behavior and is sexually mature only during the period from full to new moon of each lunar cycle from June to September (at Woods Hole, Mass.).[1]

FIG. 22.—Drawings from photographs of *Nereis* eggs in a suspension of Chinese ink in sea-water (after Lillie). *a*, before insemination; *b*, three minutes after insemination.

Although "ripe" eggs of *Nereis limbata* are available only during this particular moon-phase, their abundance and the clock-like precision of their development make them ideal objects for observation and experiments on fertilization. The fertilization-process as seen in the living egg is as follows:

When discharged or removed from the female the egg measures about 100 by 80 microns. It reveals in optical section at the centre a large formation, the germinal vesicle. Around this are greenish spheres, the yolk, among which are larger refringent bodies, the oil drops. Beyond the area of yolk and oil is a rim, the ectoplasm, made up of coarse strands disposed in a somewhat radial fashion which extend to the clearly discerned vitelline membrane. The eggs die in this stage with germinal vesicle and ectoplasm intact, unless fertilized or experimentally treated by means of inducing parthenogenesis.

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[1] *Lillie and Just, 1913; Just, 1914, 1929a.*

Within three minutes after the addition of a drop of active spermatozoa to the eggs, remarkable changes take place in each egg to which a spermatozoon has become attached: a jelly flows out of the ectoplasm; the dull slightly turbid ectoplasm in the unfertilized egg gives way to a shining space crossed by strands. These changes can best be observed under the microscope by adding spermatozoa to eggs in sea-water which contains fine particles of Chinese ink. The two photographs appended show living eggs before and after insemination (Fig. 22).
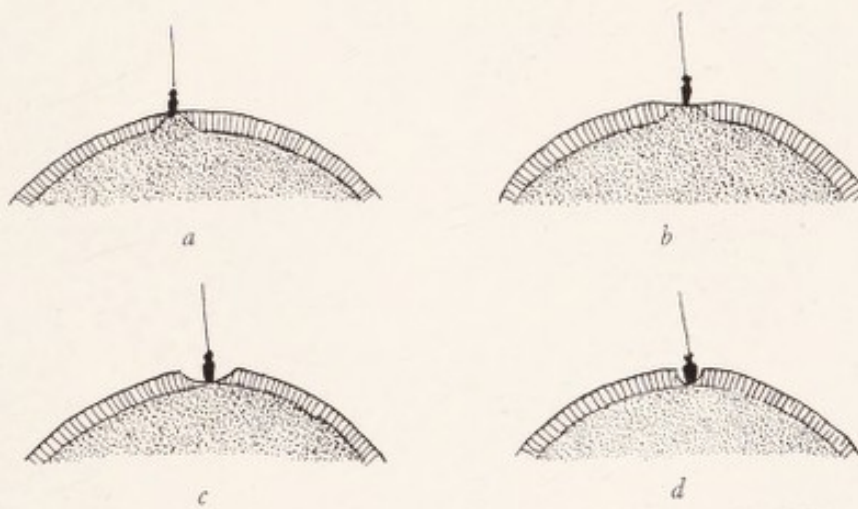
FIG. 23.—The fertilization-cone in the egg of *Nereis* (after Lillie).

After the spermatozoon has become attached to the egg-membrane, the egg beneath the site of attachment forms a nipple-like projection, the cone, which shows itself well developed twelve minutes after the mixing of eggs and sperm, as can be noted in the Figs. 23*a* and *b*. (Compare these figures with those taken from the egg of *Rhynchelmis*, Fig. 24.) Fig. 25 (from a drawing) pictures a living egg in Chinese ink and sea-water fifteen minutes after spermatozoa had been added to it. One marks easily the germinal vesicle (too strongly drawn), the yolk spheres and larger oil drops in the endoplasm, the ectoplasmic strands, the entrance cone and the spermatozoon, plasma membrane, vitelline membrane, the jelly and the surround-

ing ink which extends into the jelly hull where the sper-
matozoon is located.

The entrance-cone below the spermatozoon now gradually
recedes and pulls the membrane along with it so that the
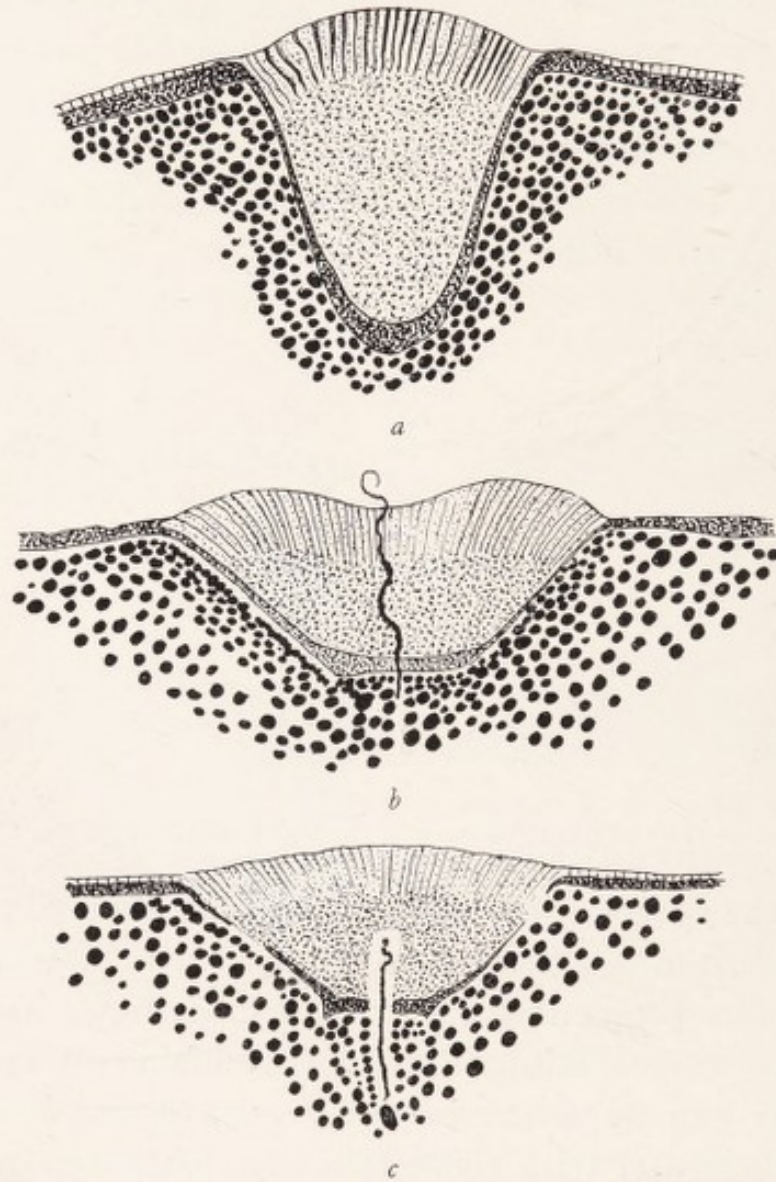
FIG. 24.—The fertilization-cone and sperm-entry in the egg of *Rhynchelmis*
(after Vejdovsky and Mrazek).

spermatozoon lies in a depression. During these minutes
the egg undergoes the irregular changes of form and darken-
ing already described in the chapter on the general prop-
erties of the ectoplasm. When it regains regular form and
clears, twenty-five minutes after insemination, the cone is

no longer visible in the living egg; the spermatozoon, seen with difficulty in the period during which these changes take place, becomes again visible after they pass over. About fifty minutes after insemination, the sperm-head disappears within the egg, leaving the tail and middle-piece outside.
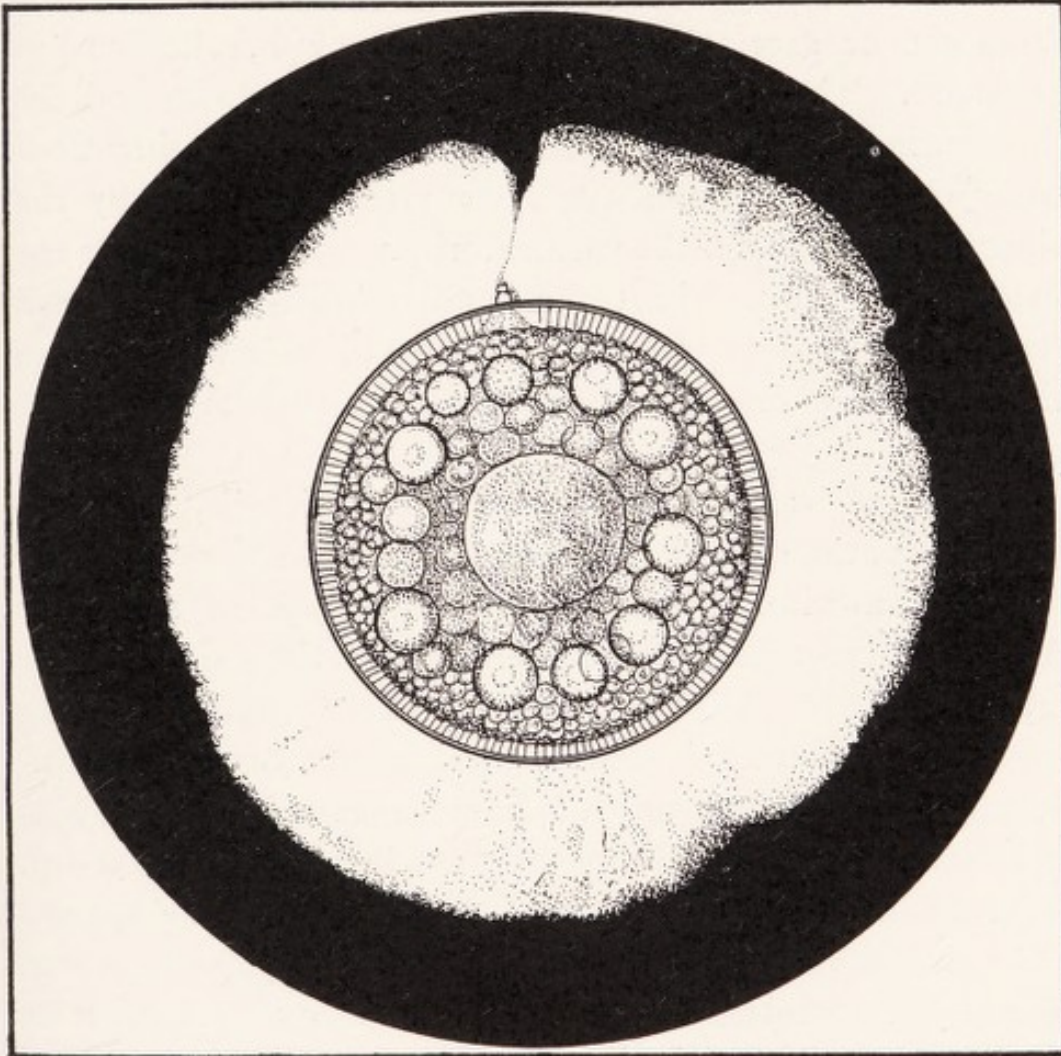
FIG. 25.—Living egg of *Nereis* in a suspension of Chinese ink in sea-water, fifteen minutes after insemination (after Lillie).

About five minutes later follows the extrusion of the first polar body. The second is extruded about fifteen minutes later. The egg cleaves into two unequal blastomeres one hour twenty-five minutes after insemination.

Many details of the fertilization-process in the egg of *Nereis* can be observed only by fixing the eggs with a suit-

able reagent which faithfully preserves the oil and yolk as well as the nuclear structures, cutting them into thin sections (three or four microns thick) and coloring them with a dye which stains both cytoplasmic inclusions and chromatin material.

In sections of eggs fixed after insemination, the following facts can be ascertained: The ectoplasmic breakdown has occurred. The germinal vesicle has broken down and the first maturation spindle has formed. To the fertilization-cone when fully formed the spermatozoon is fixed by the sharp anterior spike, the perforatorium, which traverses the perivitelline space whilst the head, middle-piece and tail remain external to the egg. The fertilization-cone as in the living egg projects beyond the egg-surface and later recedes.

What one learns in addition to these details, verifying the observations on the living egg, concerns the change of the perforatorium of the spermatozoon and of the cone. As the spermatozoon enters deeper into the cone, further granules appear at its tip, whilst it itself gains in staining capacity. The cone is sharply marked off from the remainder of the egg-cytoplasm; it is homogeneous in appearance, free from yolk and actually different in physical make-up.

At about fifty minutes after insemination, the sperm-head disappears within the egg. The cone, as can be recognized by its staining and by its maintenance of form, acts as a solid body which sinks into the egg exerting tension upon the sperm-head which stretches like a ductile strand. Finally the strand breaks at the surface of the egg so that the external portion remains outside. This is the middle-piece; hence, only the sperm-nucleus enters the egg of *Nereis*. Cone and attached sperm-head, acting as one complex, now revolve through an angle of 180 degrees. Soon thereafter a star-shaped formation, the sperm-aster, with a minute granule, the centrosome or centriole, at its centre,

appears in front of the sperm-head (now designated the sperm-nucleus). This minute granule arises from within the sperm-nucleus[1] (Fig. 26). As the sperm-nucleus advances toward the centre of the egg, the aster grows in size and it and the centrosome or centriole then divide; parallel with the growth of the aster the sperm-nucleus
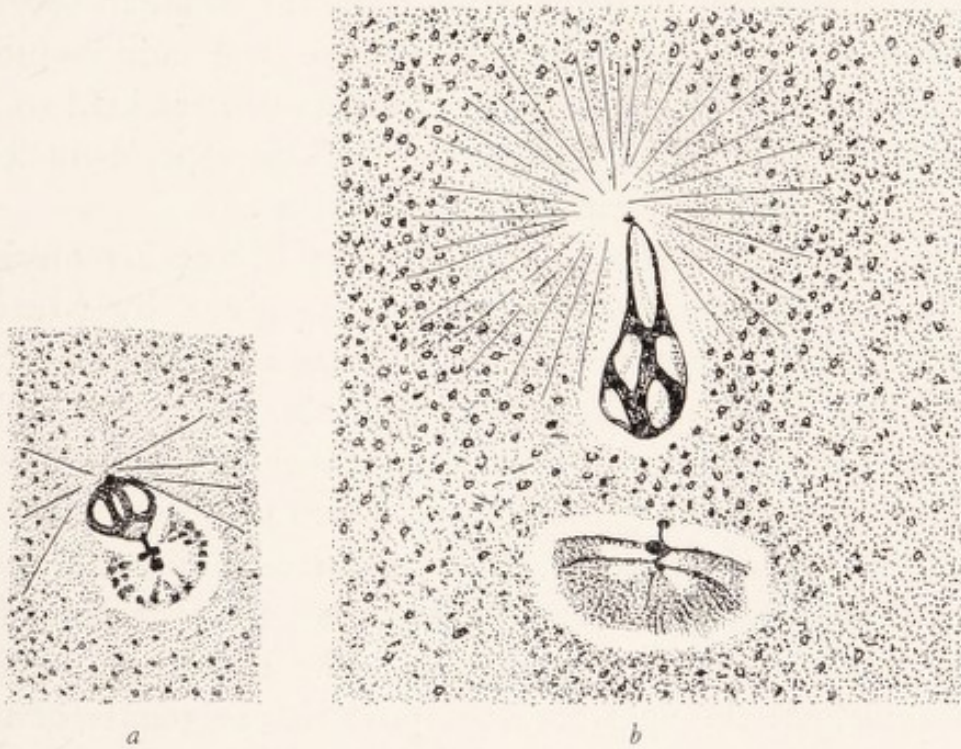
FIG. 26.—Sperm-nucleus within the egg of *Nereis* (after Lillie); *a*, with cone attached and *b*, separated from cone.

enlarges, losing its staining power. In the meantime, the entrance-cone has disappeared.

The egg-nucleus during the progress of these events has undergone changes. First, as germinal vesicle, it breaks down. The first maturation spindle forms near the centre of the egg and moves to the animal pole where the first polar body and, after the formation of the second maturation spindle, also the second is given off. The chromosomes remaining in the egg constitute the egg-nucleus. The union of egg- and sperm-nucleus follows and thus arises the first

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[1] *Just, 1933b. But cf. Lillie, 1912.*

cleavage or zygote nucleus about which form two asters of unequal size. The larger of these which can be traced continuously is derived from the larger sperm-aster; the smaller, it is believed, though it can not be followed continuously, represents the smaller sperm-aster. Thus in the egg of *Nereis* both cleavage-asters are derived from the sperm-asters, if it be true that the smaller sperm-centrosome and -aster persist. Since the middle-piece remains outside of the egg, the cleavage-centres held to be genetically continuous with those of the sperm-nucleus can not be derived from the middle-piece.

The history of fertilization in the egg of another marine worm, *Chaetopterus pergamentaceus*, as given by Mead,[1] may be taken to represent eggs of Class 2 which reach the fertilizable stage after break-down of the germinal vesicle.

These worms inhabit U-shaped tubes thirty to forty centimeters long, only the ends of which project above the mud. In the laboratory, these bizarre-looking animals, having been removed from their tubes, may be kept for some days in running sea-water. The sexes are readily distinguished: the eggs give the posterior segments of the female a pale yellow color, whilst the similar segments in the male which contain spermatozoa appear milky-white. It is best to use eggs and spermatozoa removed from animals kept each in a separate container of gently flowing sea-water within twenty-four hours after the animals have been collected.

When laid or removed from the animal, the eggs of *Chaetopterus* are in the germinal vesicle stage, but when they come into the sea-water the germinal vesicle breaks down, and the first maturation spindle forms near the centre of the egg. This moves to the animal pole of the egg and in this stage the egg remains until death unless fertilized or

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[1] *Mead, 1898.*

treated by some means to stimulate parthenogenetic devel-
opment. At fifteen minutes after removal from the animal,
every egg shows the first maturation spindle, its chromo-
somes at the metaphase firmly anchored by means of the
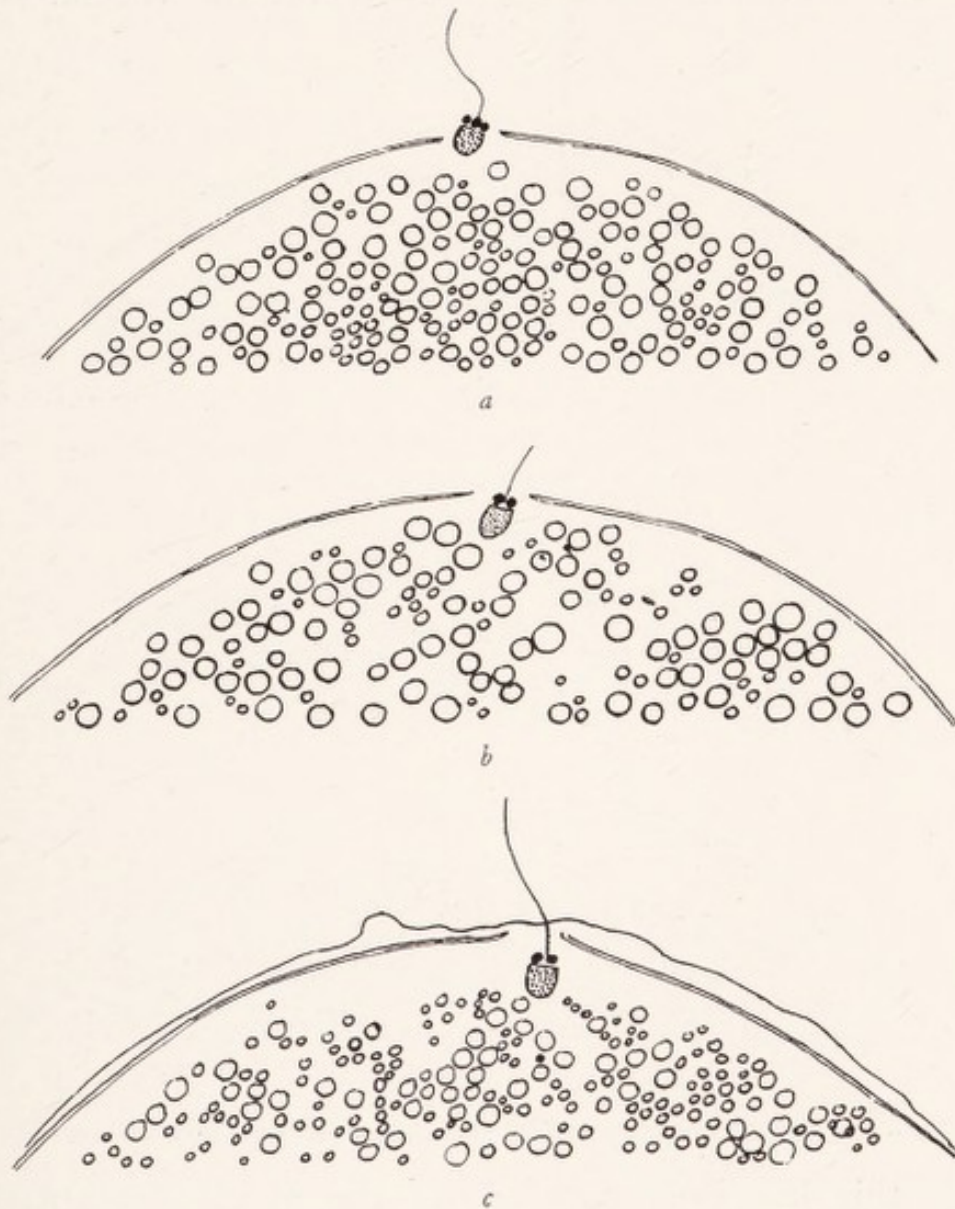
FIG. 27.—For descriptive legend see page 166.

spindle's outer pole to the egg-periphery. At any time after
dissolution of the germinal vesicle the egg is fertilizable.
The egg before rupture of the germinal vesicle while in
the ovary is an irregular pear-shaped body whose upper
two-thirds are covered by a delicate vitelline membrane

under which lies the ectoplasm, showing one or two rows of
granules. As the germinal vesicle breaks down, membrane
and ectoplasm move over the vegetal pole and thus enclose
the whole egg which has assumed an ellipsoid form. Usu-
ally the spermatozoon enters the egg at the vegetal pole
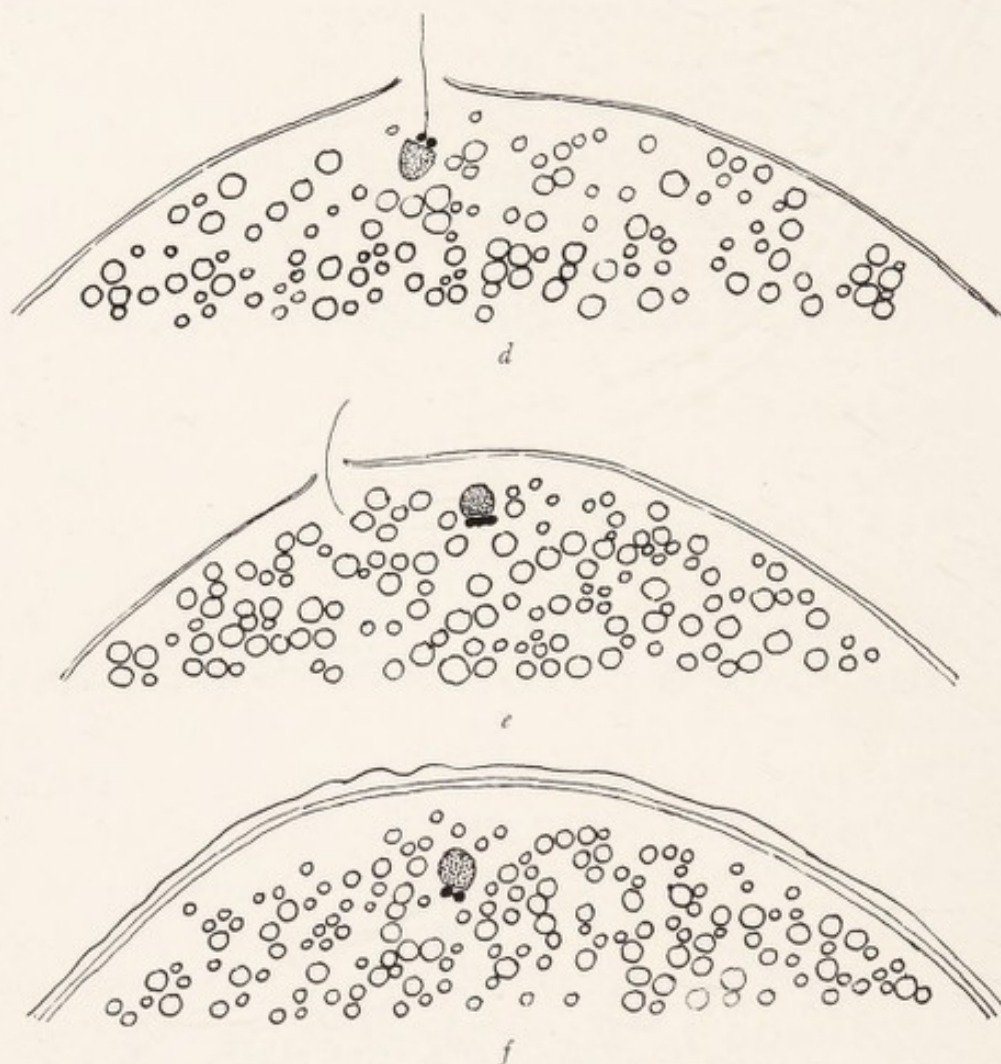
FIG. 27.—Stages of sperm-penetration, egg of *Mytilus* (after Meves).

after this has been covered by the moving ectoplasm.
(Fig. 27 from the fertilization-process in the egg of *Mytilus*
shows ectoplasmic changes after fertilization for this class
of egg.)

Entrance of more than one spermatozoon is rare. Within
the egg, the spermatozoon after having moved some dis-
tance shows an aster with two centrioles; these move apart,

each carrying a separate aster. This configuration, the sperm-amphiaster, together with the sperm-nucleus comes to lie near the centre of the egg. During this change in location the small solidly staining sperm-nucleus becomes converted into a lightly staining larger mass. In other words, the compact mass of chromatin composing the sperm-nucleus is resolved into diffuse faintly staining threads.

The exact origin of the sperm-centrosome (centriole) is not known. According to Mead,[1]

> The behavior of the sperm-centrosomes is in harmony with Boveri's theory of fertilization, but is not necessarily a confirmation of it; for the karyokinetic activities which are revived upon the entrance of the sperm are those leading to the formation of the polar globules. The machinery for these mitotic divisions is already organized, and it is quite as likely that the stimulus which starts it going emanates from the sperm-nucleus as that it emanates from the sperm-centrosomes.

The sperm-amphiaster develops into the cleavage-amphiaster.

The eggs of *Amphioxus*—Class 3—are laid toward evening during the breeding season of this animal. If sexually mature animals are removed from the sea-sand, in which they live, to clean sea-water, they will deposit their eggs.[2] Both Sobotta and Cerfontaine[3] confirm an old observation to the effect that these eggs develop best when shed into sea-water already containing spermatozoa; if they lie in sea-water before spermatozoa are added, they are susceptible to polyspermy and develop abnormally. The eggs form the first polar body while in the body of the female

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[1] *Mead, 1898.*

[2] *Lwoff, 1892, reported that he was able several times during the season at Naples to obtain eggs and spermatozoa shed in the laboratory. On two occasions during 1929 I was able to induce animals to shed.*

[3] *Sobotta, l.c.; Cerfontaine, l.c.*

and when laid are in the stage of second maturation. They possess a strongly marked ectoplasm which remains wholly colorless after treatment with a dye which stains the yolk-spheres. This ectoplasm is made up of spheres lying among a fine net-work composed of radially projecting
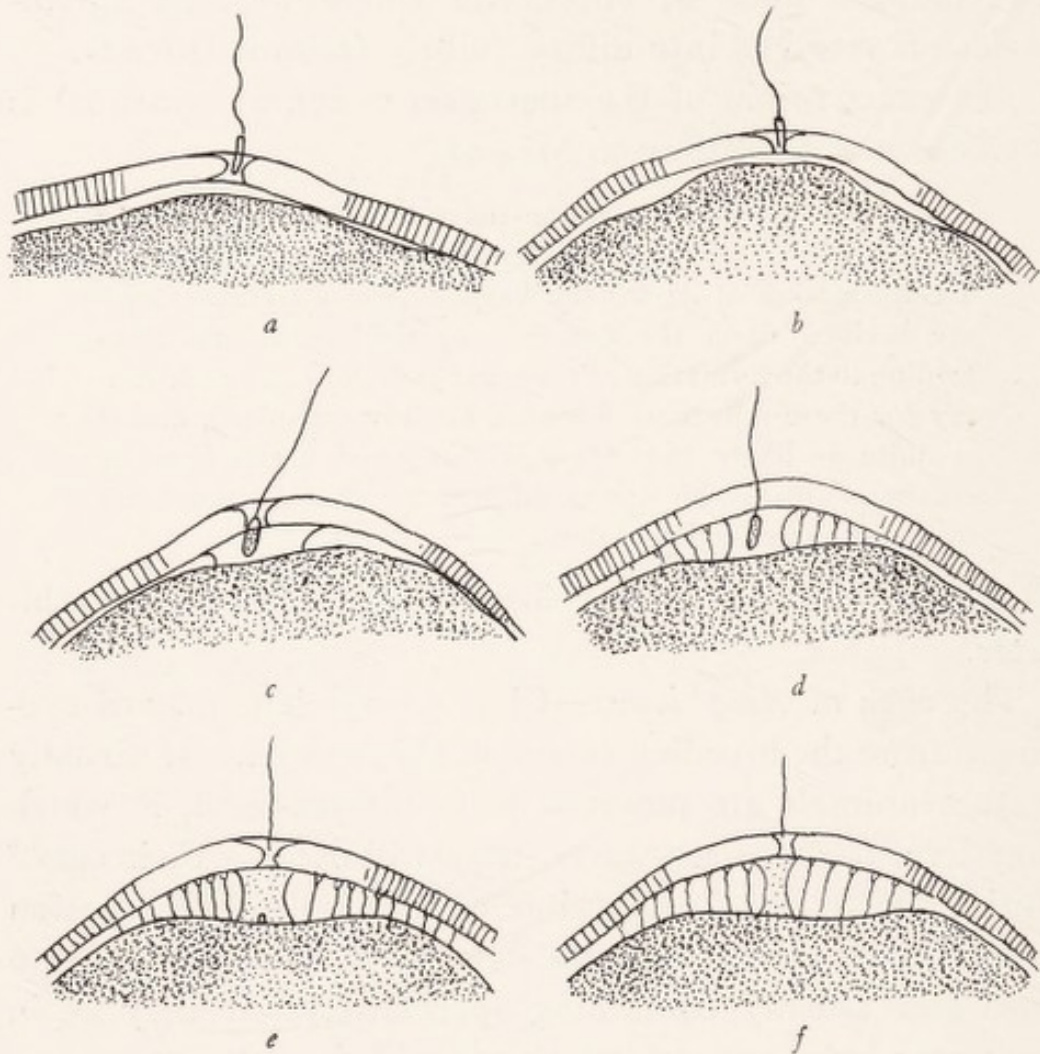
FIG. 28.—For descriptive legend see page 169.

strands of the egg-plasma. (See figure in the chapter, The Ectoplasm, p. 99.)

On entrance of the spermatozoon, which takes place at the vegetal pole, the ectoplasm breaks down; according to Cerfontaine it begins to go into solution at the site of sperm-entry. The ectoplasmic spheres seem to liquefy and become confluent. Both Sobotta and Hatschek affirm that

the membrane separates with extreme rapidity. The latter also says that separation begins at the point of sperm-entry, whilst according to Cerfontaine, actual lifting begins at the animal pole.[1] Appended figures (Fig. 28) of surface-changes are of the egg of *Petromyzon*, a member of this class.

After sperm-entry the bulk of the spermatozoon is carried inward leaving a remnant at the entrance-point which
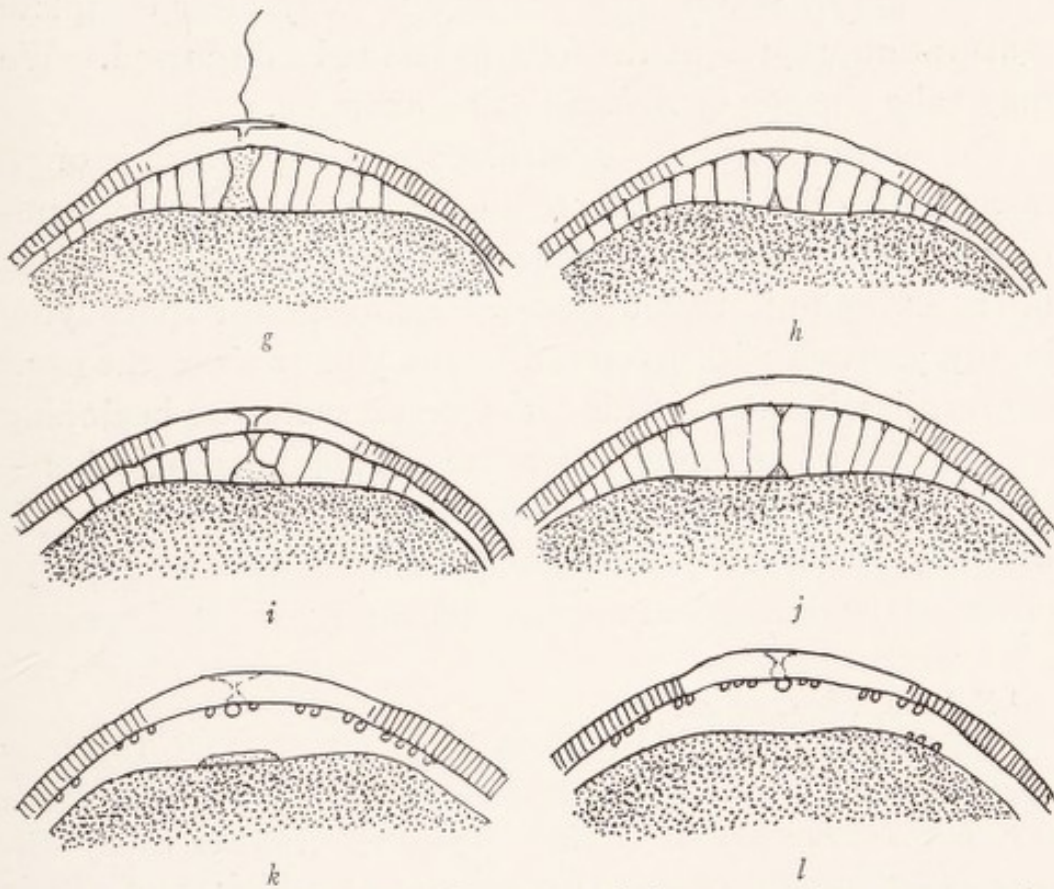
Fig. 28.—Surface changes, egg of *Petromyzon*, during stages of sperm-penetration (after Calberla).

is still visible as late as the four-cell stage. Two equal asters arise from the division of the single aster found in the vicinity of the spermatozoon. Whether or not this aster contains a centrosome is not certain. From an excel-

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[1] *Hatschek has also recorded some interesting observations concerning elasticity of the membrane.*

lent description of fertilization in the egg of *Amphioxus* this statement is taken: "Very close to the head of the spermatozoon one often sees a small intensely stained point, which could very well be the centrosome, but in view of the many yolk-granules in the neighborhood that have the same appearance, I do not dare to decide this."[1]   When the egg- and sperm-nuclei unite, the sperm-centres continue as the cleavage centres.

The eggs of sea-urchins, fertilizable only after complete maturation, represent the fourth class to be discussed.   We may take the egg of *Arbacia* as an example.

The normal,[2] living, unfertilized egg of *Arbacia* is of 76 microns in diameter and bounded by a thin elastic membrane below which is the delicate ectoplasm.   The nucleus in the living unfertilized egg appears as a clear space lying in any position with reference to the polar axis of the egg.[3] On fertilization the vitelline membrane separates beginning at the point of sperm-entry where a nipple-like protrusion— the fertilization-cone—from the egg-surface forms to pull in the sperm-head.   The surface of the egg seen under high power of the microscope appears delicately crenated because

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[1] *Sobotta, 1897, p. 40.*

[2] *Normal, living, unfertilized eggs of Arbacia are spheres of approximately the same size and specific gravity, each enclosed in a jelly hull.   They possess bright red pigment granules evenly distributed and never clumped.   Eggs that do not satisfy these criteria should be rejected for experiment especially if they color the sea-water in which they lie; such discharge indicates the presence of moribund or cytolyzing eggs.*

[3] *This axis is an imaginary line passing through the centre of the egg and the point at which the polar bodies are given off.   In sea-urchins' eggs the polar bodies are always given off at the point opposite a tube in the jelly-hull of the egg.   Because in these eggs the polar bodies are usually lost before the eggs are shed, the location of the tube in the egg's jelly hull is important for determining the egg's polar axis.*

of thread-like projections of granule-free cytoplasm into the space between the vitelline membrane and the egg-plasma. Later by the anastomosis of the free ends of these threads a very thin sheath forms; the threads and their enclosing sheath constitute the hyaline plasma-layer. Fifteen minutes after fertilization (temperature of the sea-water around 21°C.) the hyaline plasma-layer stands out very sharply even under low power of the microscope. During this same period the sperm-head evolves as the sperm-nucleus with attendant cytoplasmic changes.

Although I have found it easy to follow the history of the sperm-head in the egg of *Arbacia*, I prefer at this point to base the following description on the egg of the flat sea-urchin or "sand-dollar," *Echinarachnius*. This I do because the egg of *Echinarachnius* being larger and not so highly colored as that of *Arbacia* lends itself readily to exact observation in the living state. For both, except for minor variations, the process is the same. Because of the significance ascribed to the middle-piece of the spermatozoon in echinids by Boveri's theory of fertilization, it is necessary to take up the history of the middle-piece in some detail.

During the stages of sperm-attachment and penetration, the middle-piece reveals the same structure and position found in the free-swimming spermatozoon. That is, it is closely fitted to the basal end of the sperm-head and shows prominently a bipartite granule. In fixed preparations treated with a dye, haematoxylin, the nucleus of the spermatozoon stains bluish gray; the middle-piece, the outer limits of which are continuous with the nuclear membrane, is also gray. The middle-piece granule is seen as a sharply defined black body, lying—as in the free-swimming spermatozoon—in various positions in the middle-piece. Though in some views it shows up as a continuous horse-shoe shaped body and in others as two rods, it is in reality a body com-

posed of two curved rods joined together by a delicate but clearly revealed bridge.

Once the spermatozoon together with the middle-piece is within the egg—the tail does not enter—it rotates through an angle of 180 degrees. This rotation may, however, take place while the spermatozoon is still in the entrance-cone. At this stage occur the following changes: the sperm-head may reveal a more sharply stained outline, thus giving the appearance of a hollow tube; the outline of the middle-piece is no longer distinguished; and the middle-piece granule is closely stuck to the base of the sperm-head.

After rotation, the sperm-nucleus is directed toward the centre of the egg. The middle-piece granule, hitherto a closely fitting cap over the base of the sperm-head, slips off carrying with it a thread of sperm-substance. Or it may be that the sperm-head swells except in the region at which the middle-piece granule is clamped to its base. Figure 29a shows a sperm-head at this stage. Certainly the sperm-head now shows well defined difference in volume and form; these changes take place with the separation of the middle-piece granule.[1]

The sperm-aster, never found before the stage at which the middle-piece granule draws away from the sperm-head, is now well defined and distinct. Its spherical central portion, the astrosphere, appears in fixed sections as clear, homogeneous substance which does not stain. The astral rays are paths of clear granule-free ground-substance lying between rows of the cytoplasmic constituents—mitochondria, yolk spheres and oil drops. The astral configuration noted in properly fixed eggs bears the closest resemblance to that observed in the living egg.

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[1] *These changes in the sperm-head are not unlike those found in free-swimming spermatozoa fixed after agglutination with specific egg-sea-water that will later be discussed.*

Within the astrosphere the middle-piece granule is brilliantly revealed—a black eccentrically placed body, in sharp contrast to the clear substance immediately around it and the grayish blue cytoplasmic constituents farther beyond. The granule has never been found at the centre of the astrosphere. This is easily established in those stages, like Fig. 29*b*, in which the astrosphere is well developed.

During the ensuing stages of sperm-penetration up to the stage of apposition of the nuclei, the sperm-nucleus increases in size. The greater the distance, and therefore the time, which the sperm-nucleus must travel before reaching the egg-nucleus, the greater is its increase in size. This distance, of course, depends upon the site of sperm entry, which may be at any point on the egg-surface, with reference to the location of the egg-nucleus, which has no fixed position. It is therefore very easy to obtain a large number of stages during penetration.

During these stages I have found a behavior of the middle-piece granule which differs from that described by Meves for the egg of *Parechinus*—a difference which may be due to technique, though I doubt that since the history of the granule in fertilized eggs of *Arbacia* fixed as those of *Echinarachnius* is like that of *Parechinus*. This difference is that the middle-piece granule in the egg of *Echinarachnius* separates from each of its free limbs a more minute granule. Thus each limb of the original bipartite granule in turn becomes bipartite. These two new formations move away from the parent granule, each maintaining connection by means of a delicate thread. Figs. (29*b*), (29*c*) and show this formation.

As the sperm-nucleus increases in size, its basal thread, at the tip of which is the middle-piece granule, becomes longer, an indication of the ductility of the sperm-nucleus during these stages. In form, therefore, it resembles a
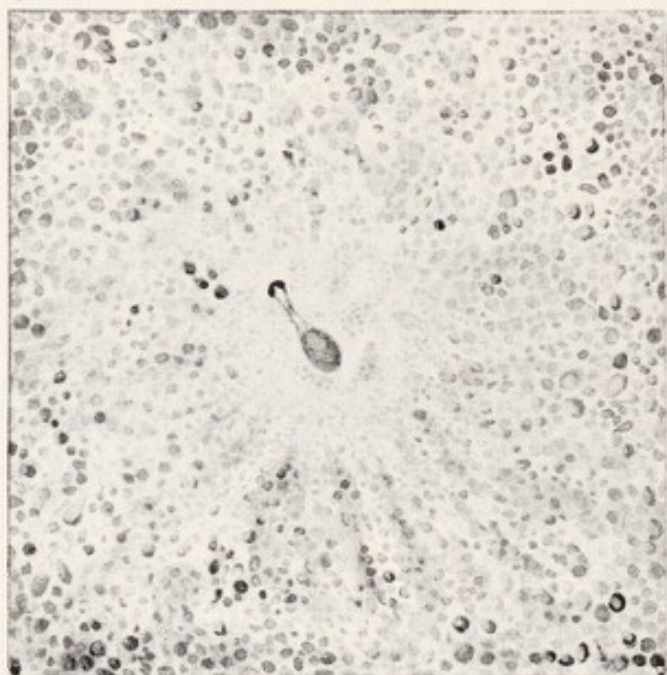
a
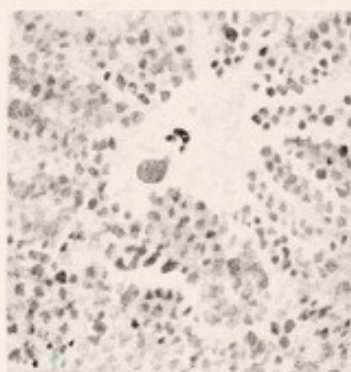
c
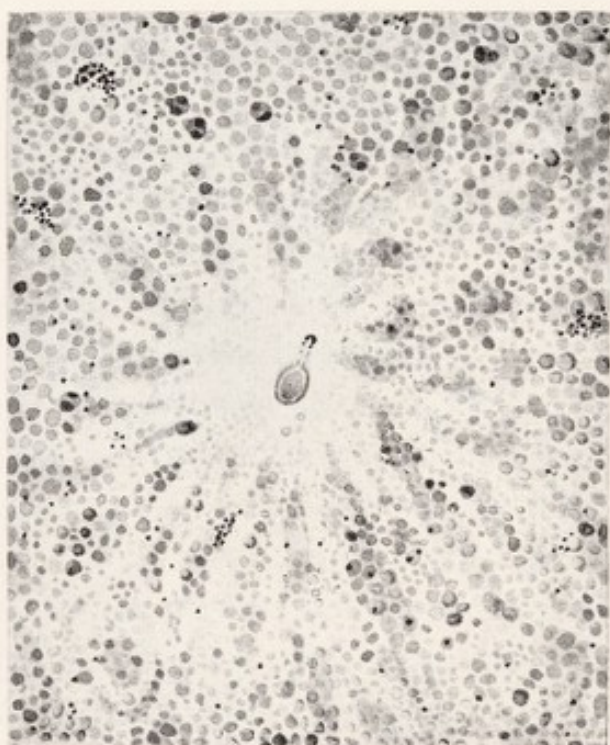
b

d

FIG. 29.—For descriptive legend see page 175.

e
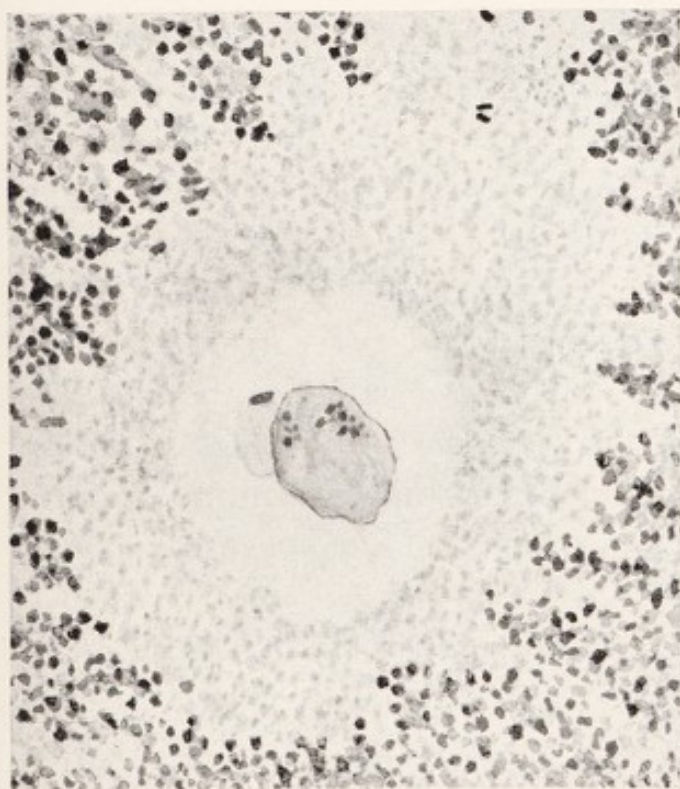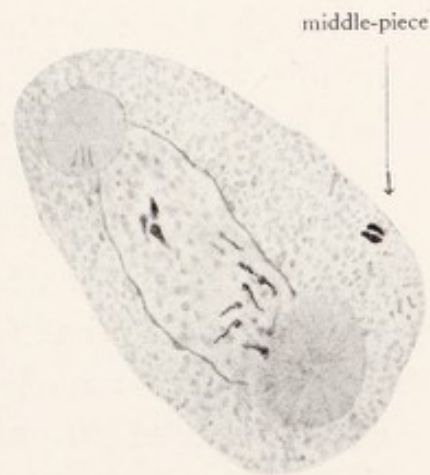
f   *g*

FIG. 29.—History of the middle-piece of the spermatozoon within the egg, *Echinarachnius parma*. (Original.)

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round or oval flask with a long neck. In earlier stages this neck appears solid; in later stages it is really a tube, as Figs. 29*d* and 29*e* show.

Now in this stage the structure of the middle-piece granule and its relationship to the sperm-nucleus are most clearly discerned. The sperm-nucleus and the middle-piece granule form one continuous complex. The tip of the tube arising from the base of the sperm-head spreads out slightly as very delicate threads to connect with the minute granule which arose from the middle-piece body. Thus, the middle-piece granule resembles the top of a parachute, the threads of which come together at the tip of the sperm-tube. At this junction another granule is often found.

With the apposition of the nuclei, the sperm-nucleus loses connection with the middle-piece granule. This is shown in Figs. 29*f* and *g*. There is never a re-establishment; in the succeeding stages in mitosis leading to first cleavage, the granule may lie as a discrete single inert body at any point in the cytoplasm with reference to the spindle. I have never found any evidence of its division or of its taking up a position at either spindle pole. By the time that the nuclei come into apposition, the more minute granules can no longer be traced.

Within the egg the sperm-head, a structure notable for its low water-content, at first maintains its shape and size but as it is carried from the periphery of the egg it slowly approaches spherical form, increasing in volume and its outline losing definition. Observations on eggs fixed during this period reveal what the living egg does not so clearly show: namely, that the transformation of the sperm-head into a vesicular nucleus is the resolution of a greatly condensed mass of chromatin—an exaggerated and rapid evolution of chromosomes from telophase condition to that of a resting nucleus. Though this process is more easily visible before the sperm-nucleus unites with that of the egg, never-

theless it can be discerned after this union. Finally, the sperm-nucleus loses its visible identity through complete fusion with the egg-nucleus. Soon thereafter two asters arise, presumably from the single sperm-aster.

The foregoing accounts of fertilization, embracing eggs of the four classes made with respect to the period in maturation when eggs are in the stage for reception of spermatozoa, reveal two phenomena as common to all animal eggs. First, after attachment of the spermatozoon to the egg-surface, the egg-surface undergoes a change with the result
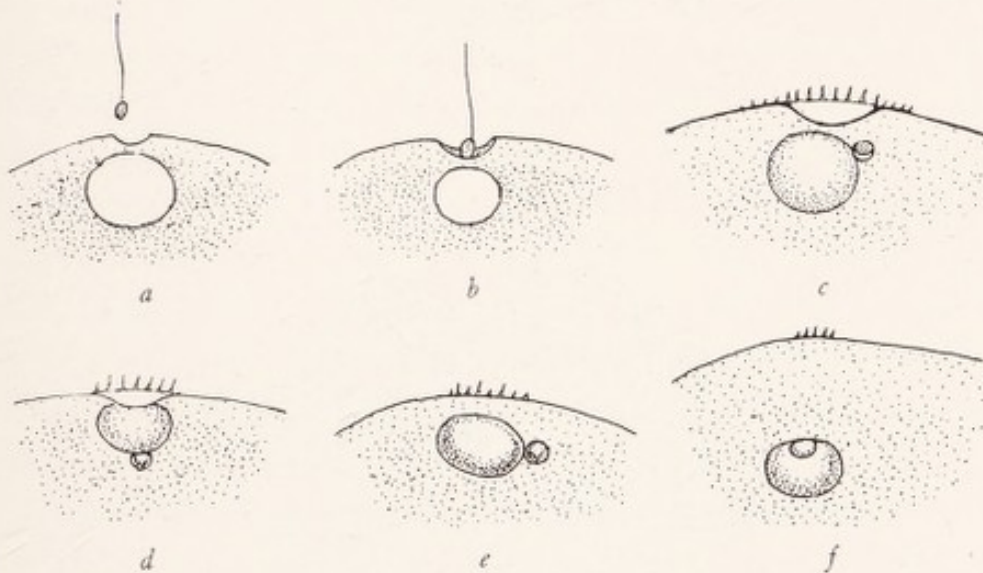
FIG. 30.—Surface changes in the egg of *Mitrocoma* attending sperm-entry (after Metschnikoff).

that the vitelline membrane becomes separated. The surface-changes differ in quality: in eggs of *Nereis* a superficially located jelly is extruded; in the *Chaetopterus*-egg the observable changes are less striking; in the egg of *Amphioxus* discrete bodies flow together and liquefy before the membrane separates widely from the egg; in sea-urchins' eggs the briefly enduring surface-changes are most violent. In order further to elucidate such surface-changes, I include pictures of a Medusa-egg (Fig. 30) described by Metschnikoff.[1] It should also be mentioned that the entrance-

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[1] *Metschnikoff, 1886.*

cones may be exaggerated in unripe echinoderm eggs, i.e., those incapable of fertilization and development (Fig. 31).

a

b
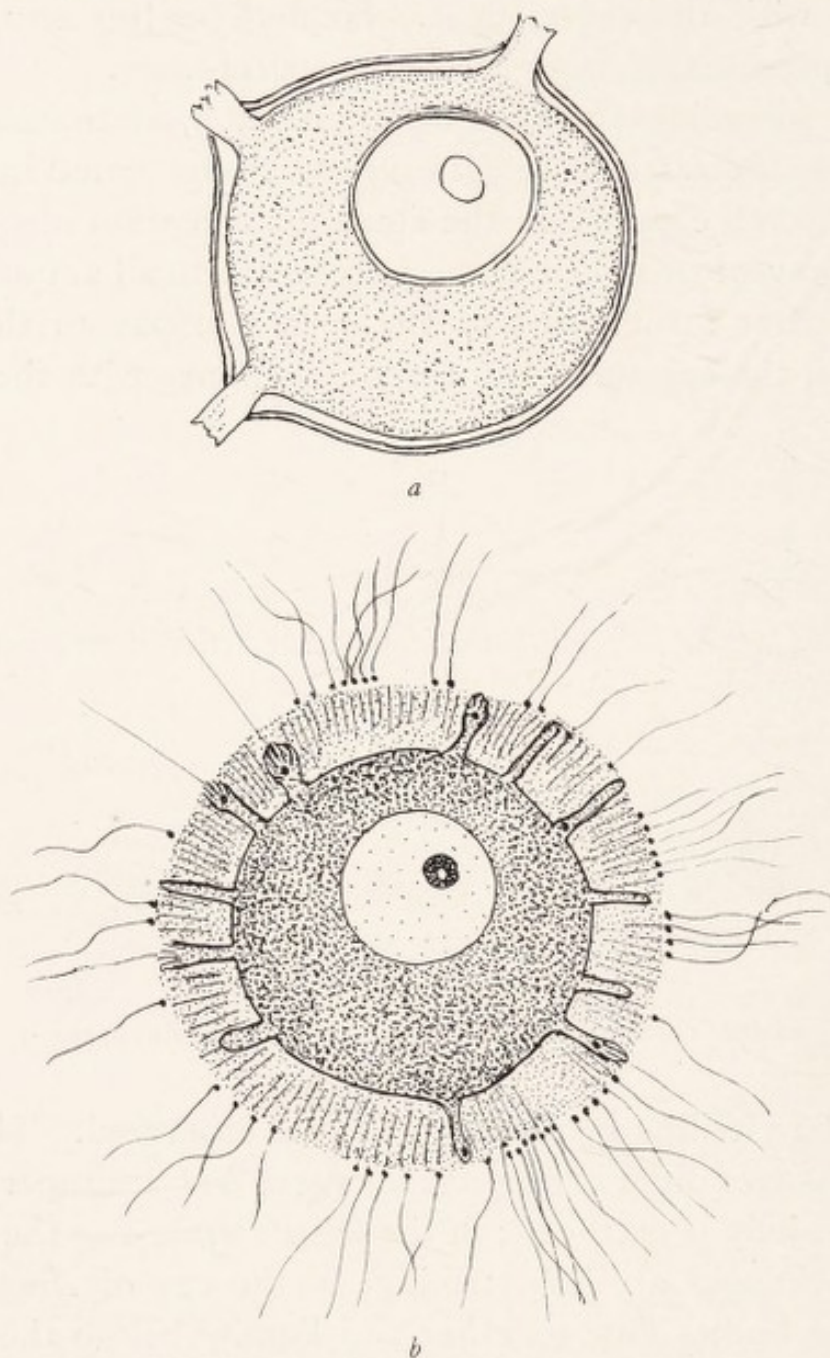
Fig. 31.—Ectoplasmic changes in unripe eggs in response to sperm-entry.   *a*, egg of a starfish (after Schneider); *b*, egg of a holothurian (after Iwanzoff).

The cones may be made more expressed under experimental conditions.[1]   In the second place, the sperm-nucleus having reached the egg-nucleus forms with it the zygote- or

[1] *Fol, 1879; Schneider, 1893; Iwanzoff, 1898; Just, 1929b.*

cleavage-nucleus, about which the first mitotic figure arises. Thus the fertilization-process in these four examples resolves itself into two phases—an external, that concerns the ecto-plasm, and an internal, that concerns the nuclei.

Fertilization means in the strict sense of the word the coming together of egg and spermatozoon, for without it there is no fertilization. Hence obviously, the initial act in the whole chain of events is the contact between the two partners. If we endeavor to state the definite result of these events, we can say that it is the development of the egg. But it would certainly be impractical to define fertilization as some have defined it, namely, that it is complete only when the developed organism has reached that stage in its development when in its germ-cells the chromosomes pair. Since in all animal eggs a mitotic figure is established for the first division-nucleus, it is far more practicable to take this feature, common to all eggs after development has begun, as the end-point of the series of events that begins with the coming together of egg and spermatozoon.

These events we speak of as the fertilization-process; this resolves itself, as said above, into an external and an internal phase. I shall now endeavor to determine which of these must be regarded as the fundamental phase in fertilization, that is, during which takes place the fundamental happening, event, in fertilization. I first discuss the internal phase of the fertilization-process, since it involves more generally known phenomena and since prevailing opinion among biologists places the basic event in this phase. I begin with the question of the union of the egg- and sperm-nuclei.

Were we to conclude from the descriptions of fertilization of eggs of the four classes as given above, we could say that fertilization is the union of the egg- and sperm-nuclei. However, cases exist in which egg- and sperm-nuclei norm-

ally never fuse, although the presence of the sperm-nucleus within the egg is necessary for the egg's development. Indeed, in extreme cases the cleavage-nucleus is distinctly bipartite and remains so during many successive cleavage-stages. In the egg of the water-flea, *Cyclops*, for example, according to Haecker such a bipartite nucleus persists from one generation to the next.[1] Bipartite nuclei have been described for other eggs—it shows clearly in those of the large tailed amphibian, *Cryptobranchus*. The egg of *Pediculopsis* presents an interesting condition: some of the cells resulting from its first division after fertilization contain single nuclei whilst in others each chromosome persists as a separate entity with its own spindle. Between the two extremes of complete fusion at the time when the egg- and sperm-nuclei appose and the persistence of these nuclei as revealed by the bipartite character of the cleavage-nuclei, intermediate grades exist, even in one egg-genus.[2] In eggs of *Rhabditis*, though they must be fertilized in order to develop, the sperm-nucleus remains inert and never unites with that of the egg.[3] Briefly, actual fusion with immediate loss of identity of the egg- and sperm-nuclei is no *sine qua non* of the fertilization-process.

Experimentally, it can be shown that fertilization may take place without the egg-nucleus. The presence of either the egg- or sperm-nucleus alone suffices for the egg's development: the former is concerned in parthenogenesis, the latter in an egg whose nucleus has been actually or virtually removed by experimental means. The fertilization of an egg-fragment without the egg-nucleus is known as merogony.[4] Such fertilizable fragments have been obtained from eggs of sea-urchins, starfishes and

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[1] *Haecker, 1890. See also Heberer for literature.*

[2] *Cf. Boveri's observations on eggs of the genus, Echinus.*

[3] *Krüger, 1913.*

[4] *Delage and others.*

worms. Fertilization of eggs whose nuclei are hindered from taking part in development has been accomplished on eggs of sea-urchins and of amphibia. Experimentally it is thus shown that neither fusion nor even mere apposition of the sperm- with the egg-nucleus is essential for the fertilization-process.

Some observations of my own bear on this point. The first of these shows that in eggs of *Echinarachnius*, fertilized after having been treated with dilute sea-water—or after having lain in sea-water for several hours—the egg-nucleus takes no part in the subsequent cleavages but remains as a "resting nucleus" in every way similar to its condition in the unfertilized egg.[1] In the meantime, the sperm-nucleus divides in the typical manner of the zygote-nucleus. The second observation relates to eggs of the sea-urchin, *Arbacia*, fertilized in various stages of mitosis induced by treatment with hypertonic sea-water.[2] In some of these eggs the egg- and sperm-nuclei divide independently though in different tempo, as one would expect. Here then the stage in the mitotic activity of the egg-nucleus does not inhibit the independent activity of the sperm-nucleus.

The evidence derived from observations on normal fertilization-processes indicates that fertilization can occur without fusion, union, apposition, or even approach of the sperm- and egg-nuclei. The experiments cited show that development ensues when the egg-nucleus is absent or when though present it is rendered inert. We are therefore not justified in retaining the old definition of fertilization as fusion of the egg- and sperm-nuclei.

To speak of the *Rhabditis*-egg, for which sperm-penetration is essential to development, as "nature's bridge between parthenogenesis and fertilization,"[3] because the

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[1] *Just, 1924.*
[2] *Just, 1922b.*
[3] *Brachet, 1917.*

sperm-nucleus once in this egg lies inert whilst the egg-nucleus alone takes part in development, is a pretty statement without much scientific value. If an egg can not develop without fertilization, the criterion of its having been fertilized is its development. If the spermatozoon is essential to the initiation of development, fertilization has been effected, if this egg develops. To speak of this case and of experimental conditions in which the egg-nucleus though present is inhibited from taking part in cleavage as partial fertilization is to ignore the essential problem. If an egg-fragment without a nucleus develops after having been entered by a spermatozoon, it has been fertilized. To retain the old definition of fertilization as the union of egg- and sperm-nucleus is to violate both fact and logic.

But whatever the situation with respect to a union of the egg- and sperm-nuclei, always a division-spindle arises and in this the fertilization-process reaches its culmination. At the poles of this spindle are star-like formations, the asters. These beautiful formations are especially striking in many living eggs and since they are always present in the nuclear division of cleaving fertilized eggs, they have been held to be the cause of fertilization. Thus Boveri postulated a theory of fertilization which, though he himself later abandoned it, is even to-day defended by many writers: namely, that the essential feature in fertilization is the introduction into the egg of two centrosomes by the spermatozoon, about which two asters form which persist as the asters of the egg's division-spindle. Were it not for the fact that many writers still uphold this definition, we could dismiss it at once inasmuch as we have seen in the descriptions of the fertilization-process of the four types of eggs given above that the cleavage-centres can not be always shown to have arisen from sperm-centres. Moreover, whilst in the majority of eggs the cleavage-centres probably are continuations of both sperm-centres, there are eggs in which the centres come one from the egg-nucleus and one from the sperm-nucleus, as in eggs

of many snails. In addition, there are eggs in which the asters of the division-spindle arise entirely around the egg-nucleus, the sperm-nucleus never showing any trace of astral radiations, as in eggs of trematodes. Finally, as we have seen, the division-spindle in eggs of sea-urchins shows asters devoid of centrosomes. What in these Boveri identified as centrosomes have been proved beyond doubt to be bodies located in the middle-piece of the spermatozoon that are cast off into the egg-cytoplasm and have no causal relation to the asters.[1] For other eggs it has also been shown that granules in the middle-piece of the spermatozoon can not be identified as centrosomes.[2] In one egg only, that of *Nereis*, has it been shown that the centrosomes arise out of the sperm-nucleus.

The conclusion is patent: as it is the case with the stage in maturation when eggs reach the fertilizable condition. and with the mode of union of the egg- and sperm-nuclei, so with the origin of the cleavage-centres—all possible variations exist. Hence we find no constancy with respect to the origin of the cleavage-centres and therefore can not define fertilization as the importation into the egg of centrosomes by the spermatozoon.

Failing to discover in any of the events that happen during the internal phase of the fertilization-process the fundamental act in fertilization, we turn to the external phase, to the changes at the egg-surface incident to the attachment of the spermatozoon. All animal eggs in response to sperm-attachment show some visible change in the ectoplasm. In the following chapter I present my hypothesis that underlying these changes at the egg-surface is the fundamental act in the fertilization-process. I denominate this act the fertilization-reaction.

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[1] *See Meves, 1912; Just, 1927a.*
[2] *According to Meves there are not two but five such granules in the sperm-middle-piece of Mytilus.*

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The Fertilization-reaction

*I*T IS A CURIOUS FACT THAT BY FAR THE LARGEST BODY OF data, both of observation and of experiment, on fertilization relates to the end results and final consequences of the coming together of eggs and spermatozoa. The phenomena embracing the union of egg- and sperm-nuclei and the establishment of the first cleavage-figure are end-events of a chain of happenings in a complex, heterogeneous and little understood system and not the end-point of a single one-way reaction taking place in a simple, homogeneous, fully understood system. Many biological processes doubtless may be explained on the assumption of an underlying simple, even mono-molecular, reaction so far as such in chemical experiments with pure reactants be known or postulated. Nevertheless, to ignore the polyphasic nature of the cytoplasm needlessly obscures the problem; comparisons of cytoplasmic processes with simple or even with complex reactions in test-tubes may cause serious retardation in the solution of the problems of biological behavior. Chemistry, in so far as it relates to end-points, offers little help. As in chemistry more information regarding the onset of even the simplest reactions is desired, so here in the problem of fertilization: we stand in need of more exact knowledge as to the initial reaction which leads to the catenary processes culminating in establishing the cleavage-figure with which by rhythmical reduplication the development of the egg ensues. Hence, the study of the happenings immediately ensuing after the mixing of eggs and spermatozoa

assumes great significance for the understanding of the process, fertilization. Moreover, as we shall see, in this study inheres another value, since these happenings are of prospective significance for the whole range of consecutive form-changes by which from the egg the animal emerges, changes which taken together we speak of as the egg's development.

The evidence summarized in the preceding chapter makes it clear that in fertilization it is the egg-cytoplasm that reacts with the spermatozoon. A recital of this evidence is here unnecessary; one fact noted we may recall. This is that whilst no animal sperm-cell is capable of fertilizing an egg except as a spermatozoon—a sperm-cell which has completed both maturations and has become transformed from a spermatid into a spermatozoon by nuclear condensation and remarkable cytoplasmic changes—the egg, depending upon the species, has capacity for fertilization before, during or after maturation. Thus the fertilizability of all animal eggs hangs together with some condition in the cytoplasm of the egg and is independent of its nuclear state, as germinal vesicle, as first or second maturation-nucleus or as a completely matured nucleus. This fact would still stand were the events in the ensuing fertilization-process the same in all eggs; it becomes of paramount significance since we can not reduce these events to a common underlying principle which holds for all eggs.

Some change, then, supervenes in the egg-cytoplasm which transforms it from a condition of non-fertilizability into one of fertilizability. Since we intend to examine the reaction between egg and spermatozoon at the moment of insemination, and since we know that spermatozoa are at this time alike with respect to fertilizing capacity, our task concerns itself with the fertilizable condition of the egg-cytoplasm.

The fertilizable condition in animal eggs arises suddenly. Delage[1] demonstrated that with break-down of the germinal vesicle the egg of a starfish undergoes a change which renders it fertilizable, a finding which I have confirmed in the same and in two other species of the same genus, *Asterias.* Further, not only are these eggs unfertilizable while the germinal vesicle is intact; attachment and entrance of the spermatozoa during this stage actually inhibit completely the break-down of the germinal vesicle. Other eggs which normally are extruded into the sea-water in the stage of intact germinal vesicle are fertilizable only after its disruption, an event which occupies a few minutes.

The fertilizable condition can not, however, in all eggs be correlated with break-down of the germinal vesicle, since, as we have seen, eggs like that of *Nereis*, for example, and of many other animals, are fertilizable only in the germinal vesicle stage. The egg of *Nereis* offers another example of the sudden onset of fertilizability. This is shown by the following: Eggs of this worm are normally shed when the animals are in the so-called heteronereis phase, i.e., when during a period of full-moon they swim actively at the surface of the sea, at which time all the eggs are in the optimum fertilizable condition. Now I have reared in the laboratory to the heteronereis phase many of these worms which had been collected while immature (i.e., in the nereid phase). On five successive days before full-moon I have inseminated eggs removed from these worms without obtaining development despite the fact that under the microscope the eggs resembled fertilizable ova.[2] On the day of full-moon, eggs taken from the same animal, from which others had been removed during previous days and inseminated without

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[1] *Delage, 1901a.*

[2] *If a fully ripe female (one in the swimming stage) be punctured all of her eggs are usually extruded. After the same degree of puncture of an immature female only few eggs exude from the site of injury.*

success, fertilized normally and gave normal development. Comparable results were obtained with eggs of *Platynereis;* never did I observe copulation of the animals and the consequent laying of fertilized eggs before full-moon.

Often during the early days of its breeding season, the sea-urchin, *Arbacia,* contains eggs with polar bodies attached, a condition which indicates that maturation was only then ending; usually, normally shed eggs show no polar bodies. Such eggs with polar bodies either are not fertilizable at all or only in small numbers. During one season I followed the development of the ovaries of this sea-urchin daily beginning in April through to the first of July. Only after the eggs had passed the maturation-stages which occupy a brief period, are they fertilizable.

The fertilizable condition hangs upon or comes with a change which occupies a mere point of time in the egg's history: before it the egg is unfertilizable and after, fertilizable. This is doubtless true also for eggs like *Otomesostoma, Dinophilus, Saccocirrus.* The spermatozoa enter these eggs when they are very young ovocytes, i.e., before the eggs' growth-stage. Only when these eggs are fully grown and matured, which state they attain some time after the precocious sperm-entry, can these eggs be said to be fertilized. Presumably here also something happens in the egg so that it passes from the condition of unfertilizability to that of fertilizability.

The fertilizable condition may endure for hours or even for three days as in eggs of sea-urchins, depending upon the species and for any given species upon the temperature; it persists longer at lower than at higher temperatures. In eggs of the flat sea-urchin, *Echinarachnius,* its duration is very short compared with that of *Arbacia* at the same temperature.[1] Eggs of other animals, fertilized outside

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[1] *By frequently changing the sea-water of the same temperature in which the eggs lie, the duration of the fertilizable condition becomes shorter.*

of the female's body, as those of echinoderms other than sea-urchins, of worms, of molluscs, of ascidians and of vertebrates may retain their fertilization-capacity for hours.

With one exception species of animal eggs undergo no change in maturation whilst the fertilizable condition persists but remain unless fertilized until death in that stage in which fertilization normally occurs. Eggs of the genus, *Asterias*, (starfish) make the exception, as pointed out. When normally laid by the females, these eggs are in the stage of the breaking-down germinal vesicle, their normal stage for fertilization. The steadily accumulating evidence to show that in normal conditions for breeding the males and females of starfishes closely congregate prior to the shedding of spermatozoa and eggs also indicates that the optimum stage for fertilization is that found in the eggs when shed. But if shed eggs or those removed from an ovary with their germinal vesicles intact are brought into normal sea-water without spermatozoa, they may undergo complete maturation. Such eggs fertilized during stages of first maturation develop normally; thereafter, fertilization-capacity steadily falls off. After complete maturation only low percentages both of fertilization and of subsequent development are obtained and are highly abnormal.

The Abbé Spallanzani made interesting observations on the duration of the fertilizability of frog's eggs after deposition. Sobotta points out that fertilization of the eggs of *Amphioxus* succeeds best when the eggs exude from the female into sea-water containing spermatozoa; whereas the addition of spermatozoa to eggs already in sea-water tends to result in abnormal fertilization—an observation indicating the short duration of fertilizability in this egg. Fertilization of the eggs of a fish, the wall-eyed pike, drops from 40 per cent. for the eggs that have lain in water for two minutes to zero per cent. for eggs that have been in

water for ten minutes.[1] Where copulation takes place between male and female before egg-laying and insemination outside of the female's body, the fertilizable condition may pass off rapidly. Thus, in eggs of the minnow, *Fundulus heteroclitus*, a bony fish, the fertilizable condition is at its height as the eggs extrude during the time, when as the male clasps the female, spermatozoa are shed. If one removes the female during the act of copulation, then gently presses her in order to obtain eggs and places these in sea-water, one finds that with residence in sea-water the eggs' capacity for fertilization diminishes. The normally shed and inseminated eggs show one hundred per cent. fertilization. The most interesting case illustrative of a brief duration of fertilizability is that of the eggs of *Platynereis*, fertilized within the female's body.

The male and female of this marine worm go through a most peculiar type of copulation.[2] The sexually mature animals swim at the surface of the sea at night during the period from full to new moon of the breeding season (summer months) and are easily captured. In the laboratory, by bringing a male and a female together in a vessel of sea-water, one can observe more closely the normal behavior displayed at the surface of the open sea. The rapidly swimming male entwines the less active and larger female and thrusts his tail into her jaws. She thus takes up the spermatozoa which pass from buccal cavity to pharynx and through lesions in the wall of the pharynx into the coelom where they become attached to the eggs. The eggs are immediately laid. In one set of observations on 87 females, I found, with the aid of fellow workers who recorded the time, that the whole process, from the moment that the male entwined the female to the beginning of egg-laying,

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[1] *Reighard, 1893.*
[2] *Just, 1914.*

consumed only about five seconds. And yet every egg laid showed an attached spermatozoon and only one. These observations were made soon after capture of the worms: even so, the delicate males are apt to suffer somewhat in handling. In nature, therefore, this period is presumably not longer than five seconds and may very well be shorter.

Only a very brief fraction of this period of five seconds can be concerned with insemination *per se*. Most of the time is consumed by the act of copulation, by the movement of the spermatozoa to reach the eggs, and by the wavelike muscular contraction of the female by which the eggs are laid. That the fertilizable condition is here of extremely short duration is further proved by the fact that eggs can not be removed from the virgin female to a volume of seawater greater than that of the mass of eggs and the sea-water in turn removed quickly enough to insure fertilization. In other words, since it can be shown that sea-water does not impair the fertilization-capacity of the spermatozoa, it must impair very quickly that of the eggs. But the instant that the spermatozoon becomes affixed to the egg, the sea-water is harmless.[1]

The cytoplasm of eggs, then, at one or another stage of maturation becomes suddenly fertilizable and remains so for a longer or shorter period of time. Eggs die unless fertilized, without exhibiting any change in nuclear state. With fertilization, they pass beyond the fertilizable condition. I know of only one report,[2] based on insufficient evidence, which claims that fertilized eggs can be re-fertilized. In my experience, eggs having been fertilized lose capacity for fertilization for neither they nor their fragments can be fertilized again.[3]

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[1] *Just, 1915b, 1915c.*
[2] *Morgan, 1895.*
[3] *Just, 1923a.*

Fertilizability being resident in the cytoplasm, we may ask whether or not it has definite location. As we have seen, in the egg of *Platynereis*, for example, loss of fertilizability may occur very quickly. Since an egg is either fertilizable or not, it is easy to assume that for eggs generally the loss, like the onset, of fertilizability is the event of a moment. Now the rapidity of the loss strongly indicates the superficial location of that upon which fertilizability depends.

Two cases show that with the onset of fertilizability the ectoplasm of the egg exhibits visible alteration in structure. At Naples I often noted that eggs of sea-urchins after complete maturation but before they are fertilizable show very minute projections from their surfaces. Fertilizable eggs, in contrast, present a smooth contour; the ectoplasm is homogeneous in appearance. The egg of *Chaetopterus* undergoes a series of remarkable changes, its ectoplasm flowing from the animal pole to cover the hitherto exposed vegetal pole, before it becomes fertilizable by spermatozoa entering at the vegetal pole. With the flowing movements in the ectoplasm, fertilization may take place at other points.

No one has to my knowledge made a systematic study of a great number of species of eggs with respect to the occurrence of structural changes in the ectoplasm which might be correlated with fertilizability. Until this has been done, we can not reject the possiblity that such changes generally intervene. Also, these changes may be of such extreme delicacy that they are overlooked. Finally, the change to the fertilizable condition may not always express itself as visibly structural.

The rapidity with which fertilizability arises and disappears points to the conclusion that it is a condition of the ectoplasm. Then, also, we may assume that fertilization is concerned with the ectoplasm. But we need not rest

content with mere assumption; evidence at hand amounts to proof that the chief event in fertilization is a reaction between the egg's ectoplasm and the spermatozoon. This evidence will now be set forth.

The proposition that the main event in fertilization is a reaction between egg-ectoplasm and spermatozoon is supported by the following: (1) The ectoplasm is necessary for fertilization. (2) The onset and loss of fertilizability is correlated with the appearance and disappearance of an ectoplasmic substance. (3) Specificity in fertilization depends upon the integrity of the ectoplasmic layer. (4) Polyspermy obtains when the ectoplasm is slow in reacting.

I first cite an observation of my own.[1]

If uninseminated eggs of the flat sea-urchin, *Echinarachnius*, stand in shallow dishes of sea-water, they undergo a change due to the increasing salinity of the sea-water caused by evaporation, a change which manifests itself by an alteration of the eggs' surface. This same change is induced by placing the eggs in sea-water made hypertonic by the addition of sodium chloride (6 parts of $2\frac{1}{2}$ *M* NaCl plus 50 parts sea-water). In either case the surface-layer of the eggs on return to normal sea-water is seen to be of a thickened and translucent jelly-like nature. Many of these eggs, having thus been exposed to hypertonic sea-water, under pressure, as by forceful ejection from a pipette, form each a protrusion. These vary in size. By other methods eggs may be induced to form protrusions; often they show them after having lain in normal sea-water for some time. Eggs of other sea-urchins, *Arbacia, Strongylocentrotus, Echinus, Echinocardium*, treated with hypertonic sea-water likewise form these protrusions when they are brought into normal sea-water. The explanation of the formations is

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[1] *Just, 1923a.*

this: they represent extruded endoplasm escaping through the ruptured surface-layer of the egg, and occur when this layer has been so altered that it loses the elasticity so characteristic of it on the normal unfertilized egg. The residence in concentrated sea-water brings about abstraction of water from the eggs as revealed by their shrinkage and the closer apposition of their cytoplasmic inclusions. The surface-layer also loses water; its greater visibility is owing to its altered structure: on the normal unfertilized egg it appears homogeneous, on the treated egg it appears as a system of fine cytoplasmic prolongations attached to the vitelline membrane. When these treated eggs are brought suddenly into normal sea-water, this altered ectoplasmic surface ruptures; and the endoplasm flows out as a cohering bud. This outflow is checked as the eggs come into equilibrium with their normal medium.

Every egg with a protrusion, then, is made up of two components, one possessed of the altered surface-layer, the other, an endoplasmic bud, devoid of it. Following insemination, that portion of every egg enclosed by ectoplasm separates a membrane, goes through cleavage and develops into a swimming larva. The endoplasmic bud undergoes no change; it can not be fertilized. As an egg with a bud of endoplasm develops, the bud persists as a mass of undifferentiated intact material which finally disintegrates.

The size of protruded endoplasm is without significance; it may be extremely minute, of the size of a polar body, or it may contain most of the egg-substance. In the latter case only the minute component with ectoplasm separates the membrane and cleaves, the cleavage resembling the so-called disocidal cleavage which is normal for the eggs of many animals; but such eggs never form the typical inversion, invagination, of cells by which the egg becomes a cuplike swimming form, composed of two cell-layers. It is interesting to note that this invagination (a form of gas-

trulation) when it occurs—in eggs with buds equal to or smaller than the egg-component having ectoplasm—takes place at the pole where the bud is formed. This fact might mean that the endoplasm is always extruded from the area in the uninseminated egg which is destined to be the site of invagination. But it is also probable that the endoplasm extrudes from any region of the egg, which means that the site of invagination becomes pre-determined by extrusion of the endoplasm. Whilst sea-urchins' eggs lend themselves beautifully to this mode of endoplasmic extrusion, other eggs do not because of the nature of their surfaces and the changes taking place in them after experimental treatment. In these the vitelline membranes must be punctured before the endoplasm can protrude. Such injury leads at once to break-down of the egg-cytoplasm, as happens when one cuts into the tough membrane enclosing the egg of *Nereis*.

In the egg of the starfish one can demonstrate that the egg-surface plays in fertilization a rôle similar to that of the surface of sea-urchins' eggs. Observations reported by Whitaker[1] make it clear that in fertilization of the starfish egg, the effect of the spermatozoon is conducted only by the egg-surface.

The conclusion reached from these observations, that the ectoplasm is necessary for fertilization, though it is definitely proved for only a few eggs, may nevertheless hold for eggs generally, since in them all some kind of surface-change follows sperm-attachment. We should not, to be sure, make a virtue of the necessity imposed upon the spermatozoon that to effect fertilization it must first make contact with the egg-surface; we must know that this particular surface is something peculiar because of special endowments which set it apart from the endoplasm.

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[1] *Whitaker, 1931.*

Mere location does not make the ectoplasm and its behavior peculiar, nor the fact that in all cells cytoplasm becomes converted into ectoplasm. If the ectoplasm plays this rôle in fertilization, for egg-cells this conversion of cytoplasm into ectoplasm must take place in such wise that new and stimuli-receiving substance comes to the surface of unfertilized eggs as they pass from the unfertilizable to the fertilizable condition. We possess some evidence for the appearance of such a substance in the surface-layer of the egg with the moment that it becomes fertilizable.

According to the fertilizin-theory of Lillie, eggs (of sea-urchins and of *Nereis*) in the fertilizable condition contain a substance, fertilizin, located at their surfaces which is necessary for fertilization. Thus, the statement, no fertilization without ectoplasm can be amended to read in these cases, no fertilization without ectoplasm-located fertilizin. Whilst the presence of fertilizin has not been demonstrated for all eggs, its occurrence is not limited to those of the forms studied by Lillie. I have detected its presence in eggs of other sea-urchins and of two other worms (*Platynereis megalops* and *P. dumerilii*). It has been found in the egg of *Ciona*, an ascidian. I have elsewhere reviewed the work of fertilizin;[1] to this review together with Lillie's original papers the reader is referred.[2] Here I give only a resumé with special reference to the ectoplasm-located fertilizin.

Fertilizin is held to be a mid-body in the reaction between egg and spermatozoon. Without it, in the eggs named, fertilization does not take place. No cells other than eggs possess it and these only when fertilizable. Neither in immature nor in fertilized eggs can its presence be detected. Eggs treated with means that induce the surface-changes identical to those induced by spermatozoa likewise do not

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[1] *Just, 1930c.*
[2] *Lillie, 1913, 1919.*

contain it. Repeated changing of the sea-water in which eggs lie can wash them free from it so that fertilization is no longer possible. Also, by bathing the eggs in sea-water containing the animals' body fluid, the fertilizin present in them can be bound: fertilization is inhibited.

That washing the eggs removes the fertilizin is evidence of its ectoplasmic location. Further, I found that after eggs in sea-water containing body-fluid have been mixed with spermatozoa, the spermatozoa attach themselves to the ectoplasm and even penetrate it but do not fertilize the eggs.[1] If however these eggs are soon thereafter thoroughly washed, they develop. From these facts it can be concluded that the inhibition to fertilization, by the blocking of fertilizin by body-fluid, takes place in the ectoplasm. Moreover, the rapid loss of fertilizing capacity following the ectoplasmic changes which underlie the separation of the vitelline membrane whether induced by spermatozoon or by other means, indicates that the fertilizin is located in the ectoplasm.

The presence of fertilizin can be detected only if some of it escapes from the egg into the surrounding sea-water. The means of detection is simple. Sea-water in which fertilizable eggs have been lying has marked effects on spermatozoa of the same species. If a drop of this sea-water be added to a dense suspension of spermatozoa, they are first stimulated to intense activity and rushing together adhere in masses by means of their heads. This agglutination endures for some seconds—or minutes, if the sea-water is highly charged with the agglutinating substance, fertilizin—and then passes off. This agglutination-reaction is a striking and interesting phenomenon capable of nicely quantitative study. Its value is that of an indicator of the presence of the fertilizin that escaped from the eggs; it may itself have no significance for fertilization although

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[1] *Just, 1922c, 1923b.*

it may suggest that the initial stage in the reaction between egg and spermatozoon is the agglutination of spermatozoon to egg-substance. The agglutination here discussed is never induced by treating spermatozoa of one species with sea-water charged with the sperm-agglutinin from eggs of another. This, hetero-agglutination, offers a different picture qualitatively and quantitatively and is due to a substance in the body-fluid.[1] Thus fertilizin, as indicated by the agglutination-reaction which it induces, is specific.

No question in the whole problem of fertilization has more profound significance than this of specificity. Frequently students of fertilization have asked themselves the question: how is it that in the sea, eggs of one species are fertilized by specific and not by foreign spermatozoa? In other words, how do eggs maintain fertilization-specificity?

In the laboratory fertilization by foreign spermatozoa of most marine eggs almost never occurs as a normal event. If one mixes eggs of a sea-urchin and of a worm and inseminates them with spermatozoa of either sea-urchin or worm, only specific fertilization occurs. Also, a mixture of two kinds of spermatozoa added to eggs belonging to only one species of them induces species-fertilization. It has generally been found that before cross-fertilization takes place among sea-urchins, starfishes, etc., the eggs need either a previous treatment with alkali or heat, or long residence in sea-water, so that they become "stale," or an insemination with an excessive number of spermatozoa. All these methods for inducing cross-fertilization can be shown to injure the egg-surface, and so to decrease the egg's vitality. As long as the integrity of the ectoplasm remains unimpaired, cross-fertilization fails.

Specificity in fertilization closely resembles that in immunity-reactions, where a specific anti-toxin combines with specific toxin. It is far more specific than an enzyme-

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[1] *Just, 1919b.*

reaction; besides, there is no evidence that the sperma-tozoon initiates fertilization by means of an enzyme, though enzyme-reactions certainly follow after fertilization.

As I see it, specificity in fertilization depends in part upon the chemical constitution of the blood or body-fluid of the species which produces the eggs and spermatozoa. Whilst the chemical constitution of the egg of a species differs from that of all other cells comprising the animal's body, nevertheless there is a chemical similarity among all cells of the animal's body including the egg, inasmuch as they are all nourished by the same body-fluid. Species-spermatozoa have the identical chemical structure as eggs to the extent that they are products of the same species' body and blood; at the same time they differ chemically in other respects from eggs. Blood (or body fluid) is different from all cells including eggs and spermatozoa. Now where blood inhibits species-fertilization, this inhibition is owing to its being present in such amount that it makes inert the specific substance in the egg's ectoplasm. A smaller amount blocks foreign spermatozoa and this amount can not be wholly removed except by injury of the ectoplasm. In nature, owing to the breeding habits of animals, eggs and spermatozoa of one species tend to be emitted simul-taneously within a fairly restricted breeding ground. Thus the chances for specific fertilization are enhanced. Where copulation takes place, specific fertilization is still more highly insured. On the other side, laboratory conditions for inducing cross-fertilization could scarcely obtain in the open sea. If nevertheless cross-fertilization should happen in nature, we can relate it to an injured state of the egg's ectoplasm.

As stated above, spermatozoa do not enter such fertilized eggs, or fragments thereof, which are normally mono-spermic, i.e., into which only a single spermatozoon enters. The prevailing opinion concerning the block to poly-sperm-

entry has supported the conclusion reached by Fol in his study of sperm-entry into the eggs of the starfish and of the sea-urchin, that the separation of the vitelline membrane prevents the entrance of supernumerary spermatozoa. The physical and chemical changes which take place in the membrane after separation from the egg-surface are certainly such as would bar sperm-entry. But if the block were purely mechanical, removal of the vitelline membrane should make possible the entrance of extra spermatozoa into the egg. It has been shown for the eggs of other animals, in addition to those of echinoderms, that disruption or removal from fertilized eggs of the vitelline membrane does not render the eggs any more liable to poly-sperm-entry.[1] With membrane-separation the eggs undergo some change and it is this change—not its result, membrane-separation—which constitutes the block to the entrance of additional spermatozoa. Thus this block, which is more subtle than the mechanical obstacle interposed by the presence of a separated membrane, is established before membrane-separation occurs.

A rather exact indication as to the moment when the block intervenes is furnished by my observations on the egg of *Echinarachnius*. Here one can follow the wave in the ectoplasm which begins at the point of sperm-entry and sweeps over the egg. As this wave progresses, it renders the egg immune to the entry of supernumerary attached spermatozoa. The behavior of these latter spermatozoa can be observed to change with the entrance of the "fertilizing" spermatozoon into the egg: as the wave

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[1] *By putting inseminated eggs in the best fertilizable condition through bolting-silk at the moment when they separate their membranes one can most easily and with the least harm deprive them of their membranes. Spermatozoa may be added immediately or at any time thereafter but each egg remains fertilized only by the single spermatozoon of the first insemination.*

moves from the site of entry, the movements of those spermatozoa nearest the site slacken and cease; next those farther away become immobile; finally those situated 180° away come to a standstill. These events take place before membrane-separation. Often, however, the process is much too rapid to allow one easily to follow it. Nevertheless I have under a good apochromatic lens followed it in eggs in best condition taken from hundreds of specimens.

The eggs of *Arbacia*, in my experience, if in best condition are never polyspermic. It is possible to fix eggs one second after spermatozoa have been added to them; examined under the microscope, each egg shows a spermatozoon attached to it. If a thick sperm-suspension be added to the eggs as early as one second after the first insemination, no polyspermy occurs. I have also made the initial insemination with the heaviest sperm-concentration procurable, i.e., "dry" sperm as it exudes from the male, and have obtained only mono-spermic fertilization. Thus, the block to polyspermy is most rapidly interposed.

The eggs of *Platynereis* when laid have each one spermatozoon attached. And yet in the body-cavity of the worm, the eggs, especially those in the anterior segments, are in the presence of supernumerary spermatozoa, as sections of the worms made immediately after copulation reveal. In all my slides of normally laid *Platynereis* eggs I have rarely seen one egg on which two spermatozoa are attached. I have never seen more than one spermatozoon within an egg.

Surely under the conditions of the normal insemination of eggs of *Platynereis* one can not postulate a mechanism so precise that it would distribute the spermatozoa in such wise that each egg would receive only one spermatozoon. Rather, within the narrow closed space of the body cavity packed with eggs—even the head segment contains them—prevails a situation most favorable for the aggregation of supernumerary spermatozoa around each egg. And yet

polyspermy does not occur. Eggs in the anterior segments of the worm, where most spermatozoa are found, are as free from polyspermy as those which first issue from the anal segment.

My preparations of starfish eggs which show more than one spermatozoon entering are of eggs fertilized either before break-down of the germinal vesicle or after second maturation. Never in the stage of first maturation when the eggs are in the stage of optimum fertilizability is there polysperm-entry. If, however, fertilizable eggs be injured, supernumerary spermatozoa enter.

These observations lend themselves to the conclusion that the block to polyspermy is rapidly established with the attachment or entry of one spermatozoon.

Now into all these eggs—of *Echinarachnius*, *Arbacia*, *Platynereis*, *Asterias*—the spermatozoon may enter at any point. They thus differ from some other monospermic eggs, as those of the trout or the ink-fish, which possess a tube, the micropyle, through which the spermatozoon gains entrance; or such eggs, as of *Ciona* and of *Amphioxus*, into which spermatozoa enter always at the vegetal pole. Theoretically, therefore, these eggs under discussion are polyspermic; until they establish the block against supernumerary spermatozoa any part of the eggs' surface is responsive to sperm-attachment—leaving aside the unlikely assumption that one site alone admits the spermatozoon and that this would vary even in eggs of the same female. From this point of view the interposition of the block to polyspermy becomes highly significant for it offers a means by which we can set the termination of the initial reaction between normal egg and normal spermatozoon of any of the species named: it ends with sperm-attachment.[1] The

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[1] *This refers to normal eggs, it must be understood, since spermatozoa may enter unfertilizable eggs—i.e., immature ones. See Iwanzoff and others.*

process to all intents and purposes is instantaneous, and therefore ectoplasmic. And this reaction that brings about a fundamental change in the egg which renders it incapable of reacting to other spermatozoa present and initiates the egg's development is the fertilization-reaction.

The rapidity with which the complete fertilization-reaction terminates, obviously depends upon the speed at which the reaction runs. If the egg's ectoplasm is highly reactive the reaction will proceed at a rapid rate. In another egg the rate normally may be slower and yet sufficiently rapid to inhibit polyspermy under natural conditions where eggs and spermatozoa are fully normal. In these, experimental polyspermy may be induced with greater facility. The reaction will be slowest in normally polyspermic eggs. If the block to polyspermy also in normally polyspermic eggs is taken as the indication that the fertilization-reaction is complete, this end would come in such eggs when finally they can receive no more spermatozoa.

Normally monospermic eggs can be rendered polyspermic by experimental treatment as staling (long residence in sea-water), treatment with alkali, acids or poisons, subjection to low temperature, in short, by methods for inducing injury or weakness. The eggs also tend to be polyspermic when below optimum condition as, for example, those obtained toward the end of the breeding season or from moribund animals. Polyspermy in them thus is a sign of weakness and hence pathological. In all conditions favorable to poly-sperm-entry the ectoplasmic response differs from that in normal fertilization. If, for example, eggs of a sea-urchin after having been kept at low temperature, around 5°C., are inseminated, they show polyspermy and the vitelline membrane separates only very slightly, which indicates abnormal ectoplasmic activity. If treated with an organic acid, e.g., 2 cc. $\frac{1}{10}$ normal butyric acid plus 50 cc. of sea-water, for one minute or more, eggs of sea-

urchins are rendered highly susceptible to poly-sperm-entry whilst their ectoplasm becomes thickened and the membrane only slightly separated. The same result obtains after exposing the eggs to the alkaloids, strychnine, nicotine, etc., as the Hertwigs[1] showed in their now classic experiments. If one repeats their observations one marks the striking responses of the egg-surface to insemination, among others, the longer duration of the many "fertilization-cones." The ectoplasm need not be severely injured in order that polyspermy succeeds. If the ectoplasmic response to insemination be slowed down, polyspermy may ensue. Thus, if one knows that eggs of a given lot are below normal by having learned through trial inseminations on some of them that the surface-changes underlying membrane-separation proceed at an abnormally slow rate, one can by inseminating with a heavier sperm-suspension than that usually employed secure polyspermy.

Some animal eggs, as those of cartilaginous fishes, of some amphibians and of birds, are normally polyspermic. Polyspermy has also been reported in insect eggs. All normally polyspermic eggs are large.[2] But since there exist also some large eggs which are not polyspermic, it is, presumably, not so much the size of the egg but the slow reaction of its ectoplasm which makes polyspermy possible.

The suddenly arising fertilizable condition of animal eggs thus resides in the ectoplasm. The indications are that this is a chemical reaction. Consider the problem of spe-

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[1] *Hertwig, O. and R., 1887.*

[2] *Bonnevie's conclusion (1907) that polyspermy obtains in eggs of Membranipora is incorrect as careful study of her paper reveals. See also my failure to find polyspermy in this egg—Just, 1934. MacBride's statement that polyspermy occurs in eggs of Pedicellina is undoubtedly due to his error in interpreting Hatschek's statement that he often observed numerous motile spermatozoa in the perivitelline space.*

cificity. The phenomenon of specificity generally we relate to chemical constitution and not to physical properties. No valid reason can be proffered for assuming that specificity in fertilization is unlike that met with in other biological processes. Unless such reason is forthcoming, we must assume specific fertilization as chemical. Where, as in eggs of some species, a substance has been isolated upon which fertilization depends, we are warranted again in postulating that the fertilization-reaction is chemical—one between this substance and the spermatozoon. Finally, the changes, so strikingly visible in many eggs, whereby the vitelline membrane is separated, can not, as we have seen, be regarded as the initial reaction in fertilization: membrane-separation, a physical process, though common to all eggs, is the result of the fertilization-reaction and not the reaction itself.

What happens is this: the spermatozoon sets off a first explosion in the narrow area of the egg-surface which it touches, and kindles the spark which leads to a chain of explosions. The fertilization-reaction is thus a trigger-reaction. A large portion of the surface is shattered by the explosive effect with gas-exchange and heat-liberation. One can in many eggs see this break-down of the surface by which the egg loses substance and the membrane is separated. The pressure between egg and membrane, in the perivitelline space, probably is considerable as the ectoplasmic colloids disintegrate and go into solution. Water rushes in and further distends the still ductile membrane which then sets as a stiff structure. The fully separated membrane then becomes brittle; one can more easily remove it immediately after its separation than later when it becomes tough. But these changes in the membrane are due to its separation from the egg, for it no longer forms part of the living system. What therefore are important for the fertilization-reaction are the underlying surface-

changes that result in membrane-separation and neither the consequent physical act of separation nor the changes in the membrane itself.

Nevertheless this separation of the membrane constitutes an easily visible indicator of the underlying surface-changes initiated by the fertilization-reaction. It tells us quickly whether or not fertilization has taken place. Also, it gives us information concerning the quality of the eggs fertilized. Membranes that separate incompletely and are not equidistant from the egg at all points and are slow in rate of separation mean eggs of poor fertilizability that subsequently develop abnormally.

Let me emphasize that here I speak only of fertilized eggs which have favorable conditions for subsequent development. If after fertilization conditions are deleterious, the eggs will not develop normally no matter how perfect the membranes. Moreover, as we shall learn in the next chapter, membrane-separation alone, though most perfect, does not guarantee development. Indeed, even in those cases in which perfect membranes are separated by experimental agents, they are not wholly identical with those called forth by sperm. What here looms large is the value of the quality of the surface-changes for foretelling the future course of the egg's development. Quite apart therefore from their value for the study of the fertilization-reaction, the ectoplasmic changes by which the membrane becomes separated have greatest significance. Development embraces a series of surface-changes which vary as the surface-area increases with the march of development. Those occurring at the very outset are most striking; they mark out the course, direct the way toward the final outcome of fertilization, the formation of the complex organism out of a single cell, the egg.

9

Parthenogenesis

BIOLOGICAL PROCESSES OFTEN REVEAL THEMSELES AS themes with many variations. Fugitive incidental nuances embellish the process of fertilization, as we have seen; but though they run the whole range of variation, they never obscure the motive: fertilization as the union of egg-plasma and spermatozoon.

That the egg- and sperm-nucleus are equipotent in the developmental process, since with either alone the egg's development can successfully proceed, has been pointed out. Thus, whilst the development of the egg of any one of several marine invertebrate animals can be initiated by fertilizing it after its nucleus has been removed, the egg-nucleus of the egg of *Rhabditis aberrans* alone takes part in development, for the sperm-nucleus within the egg remains inert. Neither of these examples is a violation of the statement that for the majority of multicellular animals the life of the new individual begins with the coming together of the two living gametes, egg and spermatozoon. Rather, both demonstrate very clearly this essential fact: fertilization is not the fusion of the egg- and sperm-nuclei but the union of egg-plasma and spermatozoon. Now we turn to the discussion of phenomena which reveal that even this union is not always necessary for the elevation of the life-process in the egg from the level of a single cell to that of an individual of multicellular organization. There are eggs which normally develop without spermatozoa. This type of development, encountered among many animal species, is called parthenogenesis. Of the theme, the initia-

tion of the development in the egg, therefore, fertilization and parthenogenesis are variations.

Investigations in the realm of inanimate nature have in the last decades led to such a wealth of new discoveries, have so shattered the foundations of the old point of view that we are warned against beholding the picture of inanimate nature now offered us as final. Full well we know that every day holds the possibility of new discoveries which must make everything known appear in a new light. Also in the investigation of living nature we may be certain that we stand only on the threshold of the knowledge of its deeper principles and we should avoid too quick and final conclusions.

Even when we know clearly the exact goals which living nature reaches, and when we survey the chief paths which it traverses to reach these goals, always does the fact that there are by-paths—detours to our conditioned human comprehension—take us by surprise. We should not look upon this endless richness of nature's creative capacity as an opportunity for us to confirm a preconceived notion or theory. Rather with wide open mind should we be receptive to this richness in all its manifold expressions.

In calling forth a new individual, nature has not limited itself to one mode; both fertilization and parthenogenesis initiate development of the multicellular animal. When it was found that parthenogenetic development could be initiated also in the laboratory by experimental means, we should have taken this discovery as a sign of the manifold possibilities that inhere in an egg-cell to respond to stimuli. Instead, this discovery was held to mean that the final solution of the problem of the initiation of development in animal eggs had been reached, that the final word concerning one biological phenomenon had been spoken—and even, that man here had mastered nature, that he himself could create life. The discovery of isotopes has taught us

that with respect to even simpler material structure nature does not always limit itself to one mode, that we must amend our natural laws as we learn more about nature. Such discoveries also suggest that more complex natural processes wear many guises. Had the parthenogenesis that occurs in nature received due attention—if in fact it had come within the knowledge of those who so greatly busied themselves with experimental parthenogenesis—the discovery of experimental parthenogenesis never would have had the palm awarded it.

Parthenogenetic development is found naturally occurring especially in the groups of rotifers and arthropods. This natural parthenogenesis may be fixed (obligatory)— i.e., the eggs develop normally only parthenogenetically. Experimentally, a change either in the environment or of the food of the mother may render the eggs capable of receiving the spermatozoon. In such cases the period of fertilizability comes during first maturation. Natural parthenogenesis is also variable (facultative)—these eggs develop normally either parthenogenetically or by fertilization: as, for example, eggs of the honey-bee.

In fixed parthenogenesis, the first polar body is extruded, the second is not; hence the egg develops with the double (somatic) number of chromosomes. The chromosomes of the second polar body may unite with those of the egg-nucleus, thus acting as a substitute for the sperm-nucleus.[1] An egg of the variable type of parthenogenesis extrudes both polar bodies and develops with only a single set of chromosomes. A double set of chromosomes is not, therefore, a prerequisite for the onset of naturally occurring parthenogenetic development; nor does the presence of only a single set of chromosomes imply defective development: the male honey bee (or drone) for instance, partheno-

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[1] *Cf. Artemia, Brauer, 1893.*

genetic in origin, is a normal organism though he lacks the sperm-borne chromosomes of his sisters.

With the aid of chemical solutions, changes in temperature, mechanical shock or radiations, eggs which normally never develop without fertilization can be induced to develop parthenogenetically. Up to the present we have succeeded in inducing this artificial or experimental parthenogenesis in eggs of echinoderms, worms, molluscs, a spider, fishes, and frogs. As we shall later learn, the only period in which experimental parthenogenesis is possible coincides with the stage in which the egg is fertilizable. Since, for the eggs of the animals named, this fertilizable period falls, for some of them, in the germinal vesicle stage, for others during first, for others during second, and for the remainder after complete maturation, we can not regard only one of these stages as the prerequisite for induced parthenogenesis.

Unfortunately, of the eggs of the various species of animals that have been treated with means for inducing parthenogenesis only those of a sea-urchin, of a starfish and of frogs have been reared to sexual maturity. The composition of the nucleus can generally be given only for early stages of development. With respect to the end-result of development our knowledge of induced parthenogenesis falls far short of that of the natural. Of the few cases known the sea-urchins' and the starfish eggs show throughout development a single set of chromosomes. The adult frog derived from a parthenogenetic egg is different; such an individual has either cells with single or such with double sets of chromosomes or cells of both types. Nothing indicates that the normality of these adults varies with chromosome-garniture.[1] The same can be said for the

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[1] *We must not overlook the fact that there are variations in the chromosome number in cells of adults from fertilized eggs.*

larval stage of those eggs whose induced parthenogenetic development has not been followed farther.

After successful treatment with a parthenogenetic means eggs whose optimum period for fertilization follows complete maturation develop parthenogenetically with one set of chromosomes (example: echinids). The egg of the frog after puncture (the method used for inducing parthenogenesis in this egg) made after extrusion of the first polar body, which is the fertilizable stage for this egg, extrudes the second polar body and begins development always with only a single garniture of chromosomes, while in later development this chromosome-number has been found doubled. The situation is different with eggs physiologically ripe for fertilization in either the stage of the intact germinal vesicle or that of first maturation. After successful treatment with a means of experimental parthenogenesis, they develop with or without one or both polar bodies—hence, their blastomeres contain single, double or more garnitures of chromosomes.

It is thus clear that development induced by experimental means does not depend upon the presence of two or more sets of chromosomes. In this respect experimental parthenogenesis resembles the normal. An explanation of either type of development can not, therefore, be based upon a bipartite make-up of the first cleavage-nucleus. Here we note, however, the following difference between the natural and the experimental process: in natural parthenogenesis the egg always has the nuclei of both polar bodies available although it develops with or without the union of the second of these with the definite egg-nucleus. In experimental parthenogenesis the polar-body nuclei are not always available for fusion with the egg-nucleus. This difference is due to the fact that in eggs which possess the capacity for natural parthenogenesis, development, either with or without spermatozoon, begins in the stage of first maturation, i.e., before polar body extrusion—whilst eggs

capable of developmental response to experimental means are distributed among all of the four categories made above with respect to the stage in the maturation process, in which animal eggs are physiologically ripe for fertilization.

Eggs which are fertilizable in the stage of first maturation are more widespread in occurrence among animals than those of any other class. Since both the facultative and the fixed parthenogenetic eggs belong to this large class, we might expect that experimental parthenogenesis is more easily elicited in this class of eggs than in any other. This expectation, however, is not fulfilled. Although the egg of the starfish, fertilizable in the stage of first maturation, and the first in which was discovered the capacity of eggs to respond to experimental treatment with development, is of all animal eggs the most readily responsive to experimental means for parthenogenesis, other eggs of the same class, as we shall learn, may fail wholly to respond to means effective upon the starfish egg or they respond with extremely abnormal development. Also, the greatest number of positive results has been obtained on completely matured eggs, i.e., those of sea-urchins. Finally, eggs of the two remaining classes when properly treated develop normally; frogs' eggs, for example, reach the adult stage. We can not therefore correlate the ease with which experimental treatment elicits parthenogenesis with the nuclear state of the egg. Whether or not the restriction of natural parthenogenesis to eggs fertilizable in the stage of first maturation bears a causal relation to this mitotic phase of the nucleus, has not yet been determined. As to the cause of experimental parthenogenesis, however, there are only two possibilities: it rests either in the egg's cytoplasm or in the means that brings about the parthenogenetic development.

The latter view one often meets, namely, that the means carries into the egg something which initiates its development. The brief review of the response of individual eggs

to experimental means for eliciting development which now follows will give evidence against this view and prove that the egg's response like that in fertilization is cytoplasmic.

Above I called attention to the interesting case of the starfish egg. Like that of many other eggs, its germinal vesicle breaks down when the egg comes from the female into sea-water. But whereas other eggs of this class do not develop farther unless they are fertilized, the unfertilized egg of the starfish completes both maturation-divisions. This fact suggests that this egg is extremely unstable and lies close to being normally parthenogenetic. It is not astonishing, therefore, that the starfish egg is found to be most easily induced to parthenogenetic development by one of several means.

The knowledge that sea-water charged with carbon-dioxide brings about the development of eggs of a starfish, *Asterias glacialis*, we owe to Delage[1] who was able to rear the parthenogenetic larvae through metamorphosis. This method has been successfully employed by others[2] on eggs of this as well as on those of other species of *Asterias*. Development also results if one crowds a large number of eggs in the stage of first maturation in a small volume of sea-water and leaves them there for about an hour—in this case the result is due to carbon-dioxide produced by the eggs. In my judgment the parthenogenetic development obtained by Mathews[3] through shaking eggs of *Asterias forbesii* is likewise to be attributed to carbon-dioxide produced by crowding the eggs; shaking of maturing eggs, in my experience at least, is without avail unless the eggs be crowded.

If a thin suspension of maturing unfertilized eggs of this species is placed in large volumes of sea-water in uncovered

[1] *Delage, 1908, 1910.*
[2] *Buchner, 1911 and others.*
[3] *Mathews, 1901.*

shallow dishes so that evaporation takes place, the eggs develop when transferred to normal sea-water. Similarly, sea-water to which solutions of sodium or potassium chloride have been added induces development. Thus, hypertonic sea-water is a means of calling forth parthenogenesis.

Butyric acid in sea-water, like carbon-dioxide, also induces parthenogenesis in the eggs of *Asterias forbesii*. R. S. Lillie has studied the relation of the duration of the treatment with this acid in sea-water to the degree of response elicited. He obtained only membrane-separation with short exposure; membrane-separation and cleavage with a longer exposure, and full development to the swimming stage by a still farther increased length of exposure. Increased temperature of the sea-water alone or combined with butyric acid, and two short exposures to the acid separated by the residence of the eggs in normal sea-water are also successful. Any one of these methods is superior to the hypertonic sea-water method by giving higher percentage of parthenogenetic development.

In 1876 Richard Greef reported that he had observed the parthenogenetic development of the eggs of a starfish, *Asteracanthion (Asterias)* to the ciliated larval stage (gastrula). The larvae obtained were vigorous and corresponded thoroughly with those developed from fertilized eggs. Since Greef had taken every precaution against the accidental presence of spermatozoa, since further the animals from which the eggs came gave no evidence of being hermaphroditic,[1] and since, as he had learned before, fertilized eggs reach first cleavage in one to two hours after insemination, whilst these parthenogenetic ones cleaved first only at ten to twelve hours after having come into sea-water, he was certain that he had observed a true case of

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[1] *Hermaphroditism among starfish is rare. See Retzius, 1911, Vol. 16 of his collected works; Buchner, 1911.*

parthenogenesis. That he had induced parthenogenesis and had not followed a normally parthenogenetic development seems to me to be beyond question. Thus without knowing it he was the first to induce parthenogenesis in marine eggs. Evidence may be adduced upon which this judgment is based.

In the first line I place Greef's work itself. The observed great difference between the fertilized and the parthenogenetic eggs with respect to the time when they reach first cleavage, points strongly to an experimental induction of development. This was probably brought about by an altered condition of the sea-water. If the eggs were crowded, carbon-dioxide was present in high concentration. At the time of year, the beginning of May, when the observations were made, the water in the vessels containing the eggs most probably rose in temperature, in the absence of any precautions taken against such rise. Or, if the eggs were kept in uncovered vessels, evaporation took place. Any one of these possibilities, all well-known means for inducing parthenogenesis in starfish eggs, would account

Fig. 32.—Ripe egg of *Asteracanthion rubens* after having lain two hours in sea-water (after Schneider).

for Greef's results. Schneider in 1883 pictured an egg of *Asteracanthion* which after having lain two hours in sea-water showed many asters. This is clear evidence to any-one familiar with this egg that this change is induced by the residence of the egg in sea-water (Fig. 32).

Secondly, later work on this egg may be considered. In reading O. Hertwig's report on his observations made four-teen years after Greef's, on the egg of *Asterias glacialis* and

especially on that of *Astropecten*, one is struck more by his failures than his success to obtain parthenogenesis.[1] Were the eggs of these two starfishes normally parthenogenetic, he would have secured a higher per cent. of development; also, the development would have gone farther and would have more closely resembled that of fertilized eggs. Three years earlier, when he attempted to observe parthenogenesis in the eggs of *Asterias* and so to repeat Greef's work, he had either changed or aerated the sea-water, so that the sea-water neither increased in hypertonicity nor in temperature and did not become charged with carbon-dioxide. At that time he failed completely to secure development beyond the establishment of the definite egg-nucleus. In other words, the eggs, as is normal for them, merely completed maturation in sea-water. In the later experiments where he observed parthenogenetic development, he does not mention having protected the eggs against changes in the sea-water. The one egg that reached the blastula-stage, though seemingly normal in outward appearance, lacked the separated membrane so characteristic of the fertilized egg and of that which has been induced to develop by means of treatment with CO_2 or with increased temperature. This observation on the single blastula obtained, strongly indicates in the light of my experience that Hertwig induced parthenogenesis by means of sea-water made hypertonic through evaporation.

The strongest evidence that both Greef and Hertwig induced parthenogenesis experimentally in these eggs lies in the fact that although starfish eggs are extremely responsive to experimental means, no one since has succeeded in demonstrating that they are normally parthenogenetic. My experience with the egg of *Astropecten*

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[1] *Hertwig, O., 1890.*

convinces me that it resembles those of three species of *Asterias* which I have studied, and that its classification as a normally parthenogenetic egg is unwarranted.

Eggs of the marine worm, *Chaetopterus*, like those of the starfish fertilizable in the stage of first maturation, when treated with hypertonic sea-water develop into bizarre and wholly abnormal swimming forms through repeated nuclear divisions in the cytoplasm which fails to divide, a condition known as differentiation without cleavage.[1] Following exposure to hypertonic sea-water, other eggs fertilizable in the stage of first maturation, e.g., those of *Podarke*[2] and of *Amphitrite*,[3] both worms, develop without cytoplasmic cleavage. For the egg of *Chaetopterus* and for that of the small marine clam, *Cumingia*, increased temperature of the sea-water induces the best type of parthenogenetic development—with cytoplasmic as well as nuclear divisions.

The egg of *Chaetopterus* merits attention because Mead used it in his interesting experiments. Previously in his paper on the normal fertilization-process in this egg he had set forth a strong argument against the theory that fertilization is due to the introduction of centrosomes into the egg by the spermatozoon. His work on the effect of salt solutions in initiating development was the logical outcome of his main conclusion in the work on fertilization, a later statement of which reveals his clear conception of the initiation of development as a chemical process. His work on inducing parthenogenesis, therefore, was no mere accidental experiment; rather, at its basis was a well defined working hypothesis. His was the first work based on the assumption that development can be experimentally induced. Why Mead so suddenly dropped this promising line of research is strange. It was justly said by Loeb,

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[1] *Lillie, 1906.*

[2] *Treadwell, 1902.*

[3] *Scott, 1906.*

that Mead's work is too often overlooked and deserves greater appreciation than it has received.

Turning to that class of eggs which are fertilizable in the stage of the intact germinal vesicle, I may cite the results of experiments to induce parthenogenesis in three eggs: those of *Mactra*, of *Thalassema* and of *Nereis*.

Eggs of *Mactra*, a marine clam, exposed to 10 cc. of 2½ *N* KCl plus 90 cc. of sea-water for 45 minutes complete maturation and develop as far as the six-cell stage. Longer exposures, 1½ to 4 hours, and to stronger solutions, give development without formation of polar bodies. KCl-treated eggs may also develop into swimming forms by nuclear divisions only, i.e., they differentiate without cleavage as do the eggs of worms mentioned above that are similarly treated.[1]

By the action of either mineral or organic acids, including carbon-dioxide, in sea-water, one can induce the development of 50 to 60 per cent. of unfertilized eggs of *Thalassema mellita*,[2] a marine worm, to the larval stage; this is scarcely to be distinguished from that of the normal, i.e., developed from fertilized eggs. Because of these positive results obtained with acids, the effects of hypertonic sea-water were not thoroughly investigated. Those reported are not only too incomplete but also too contradictory to permit the drawing of a conclusion. In view of the efficiency of hypertonic sea-water on so many other eggs, however, one may hazard the opinion that it is efficacious on this egg also. One is strengthened in this opinion by recent work on the eggs of another species of *Thalassema*, *T. neptuni*.[3]

In the egg of *Nereis* by shaking or centrifugal force ecto-plasmic break-down, with extrusion of jelly, and matura-

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[1] *Kostanecki, 1911 and earlier.*
[2] *Lefevre, 1901.*
[3] *Hobson, 1928.*

tion are brought about. Prolonged exposure to weak or short exposure to stronger hypertonic sea-water gives the same results. With still longer exposure to hypertonic sea-water, differentiation without cleavage is called forth. By treatment with hypertonic sea-water I have never been able to induce development with cytoplasmic cleavage in this egg. Hypotonic sea-water also gives differentiation without cleavage.[1] I find that the percentage of swimming forms may be increased by the addition of an acid or an alkali to the dilute sea-water. Hypotonic sea-water in my experience is an inferior means of inducing parthenogenesis in this egg. Ultra-violet radiation also induces a qualitatively poor development.[2] If eggs from a female from whose body the sea-water has been removed, are placed on a dry glass plate for a few moments, and are then put in normal sea-water, a small percentage develops.[3] Eggs having been kept at a temperature around 5°C. extrude jelly at once when removed directly to normal sea-water at room temperature (around 20°C.). Of these some will develop farther. The best method for inducing the development to the stage of larval worms, which can scarcely be distinguished from those developed from normal, fertilized eggs, is to expose eggs to sea-water at a temperature of from 30 to 33°C.[4] In this method it is necessary that the eggs come directly from the female into the warm sea-water without having lain in sea-water at room temperature. If eggs are taken from sea-water at room temperature, they need an exposure to a higher temperature, around 40°C., for about a minute to be stimulated to develop.[5] In

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[1] *Just, 1928e.*
[2] *Just, 1933c.*
[3] *Just, 1915a.*
[4] *Ibid.*
[5] *Just, unpublished observations.*

this case the percentage of development is not so high as that obtained by use of the method just mentioned.

Eggs of sea-urchins, fertilizable after complete maturation, early became the favorite objects for experiments on induced parthenogenesis.

Although Morgan made experiments which indicated that by means of treatment with hypertonic sea-water, eggs of *Arbacia* can be induced to develop parthenogenetically, he failed to extend his studies sufficiently and thus to obtain the production of larval forms from unfertilized eggs. Despite this failure his work demonstrated that treatment with hypertonic sea-water can call forth the establishment of the mitotic complex, the sign that development has been initiated. By extending Morgan's work, J. Loeb was able to induce eggs of *Arbacia* to develop parthenogenetically to swimming forms.[1] He deserves the full credit because he appreciated his findings, whilst it appears that of his predecessors, Greef, O. and R. Hertwig, Mead and Morgan, only Mead experimented with the definite purpose of inducing development by chemical means.

Loeb found that, whilst after exposure for two hours to a solution of 50 cc. of sea-water plus 50 cc. $1\frac{9}{8}$ M $MgCl_2$, the eggs of the common sea-urchin, *Arbacia*, found at Woods Hole, Mass., developed into swimming larvae, the use of other salt solutions gave no results. The next year, working on the California coast with eggs of the sea-urchins, *Strongylocentrotus purpuratus* and *S. franciscanus*, he was able to induce development not only with $MgCl_2$, but also with NaCl or KCl, either of these salts being added to sea-water, in the proportions 10 cc. of a 2.5 gram molecular solution to 90 cc. of sea-water. Later, other salts as well as cane sugar and urea were also found to be effective. This is the so-called old or original method of parthenogenesis,

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[1] *See Loeb 1913 and earlier.*

sometimes called the osmotic method for inducing development of the unfertilized sea-urchin egg.

This early work established that sea-water, if made sufficiently hypertonic by the addition of electrolytes or non-electrolytes, is capable of initiating development of sea-urchin eggs. It shows that the effective agent is not specific; the original failure to induce development with the chlorides of sodium and potassium was obviously due to an error which Loeb made in preparing the solutions. It was soon learned that sea-water concentrated by boiling is capable of inducing the development of this egg. I have been able to induce experimental parthenogenesis simply by allowing the eggs to remain uncovered in a glass dish with sea-water; in this way, sufficient evaporation takes place to bring the sea-water to that degree of hypertonicity which is effective for stimulating the eggs to develop.[1]

But there are certain shortcomings to this osmotic method. As we have seen, the eggs of sea-urchins separate their vitelline membranes after insemination. The final distance of the membrane from the egg and the rapidity of this separation are indices of the physiological state of the egg: an egg in best condition separates its membrane at a uniform and rapid rate, with the result that it is equidistant from the egg-surface at all points.[2] After the above mentioned treatment with hypertonic sea-water, sea-urchin eggs do not show a separated membrane. Instead, the vitelline membrane present on the egg before treatment remains closely stuck; the hyaline plasma-layer beneath it swells.[3] Furthermore, the fertilized egg, if it be in optimum condition, cleaves at a regular tempo and the blastomeres adhere to each other. After the treatment with the hypertonic sea-water here discussed the blastomeres

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[1] *Just, 1928a.*
[2] *Just, 1928c.*
[3] *Just, 1919c, 1922b.*

cleave irregularly, both as to tempo and as to size, and they tend to fall apart. In the next place, normally the fertilized egg develops into a form which swims at the surface of the sea-water; subsequent to treatment with this hypertonic sea-water, those eggs which happen to develop as far as the larval stage, never become top-swimming forms. Finally, if sea-urchin eggs are in best physiological condition at the time of fertilization, close to one hundred per cent. of them reach the larval stage; on the other hand, with the osmotic method of initiating development, the percentage of developing eggs lies far below that obtained from fertilized eggs of the same female; if the worker is careless, using eggs which are not in best condition, he may obtain no developing forms.

Because Loeb noted that the vitelline membrane did not separate, and that the larvae failed to swim at the surface of the sea-water, he next endeavored to find a method which would overcome these deficiencies. The result of his investigations was the so-called improved method of artificial parthenogenesis for sea-urchins' eggs. This is the famous fatty acid plus hypertonic sea-water method, sometimes called the lysin-corrective factor method. In this method the fatty acid mostly employed is butyric acid. Loeb first studied the eggs of the California sea-urchin, *Strongylocentrotus purpuratus*. He placed unfertilized eggs in a solution of 50 cc. of sea-water plus 2.8 cc. of $\frac{1}{10}$ normal butyric acid and left them in this solution for one and one-half to two and one-half minutes. On removal of these eggs to normal sea-water, they separated membranes. Eggs removed earlier than the minimum time did not separate membranes nor did those eggs which remained in the acid solution longer than optimum time, because prolonged exposure to the acid is injurious. After the eggs had been in normal sea-water for fifteen to twenty minutes, they were immersed in a solution of 50 cc. of sea-water plus 8 cc. of

2.5 gram molecular NaCl. From this solution, beginning thirty minutes after immersion, they were transferred to normal sea-water at intervals of either two and one-half or five minutes. This double treatment of fatty acid and hypertonic sea-water results in a development that closely approximates the normal, because the eggs not only show separated membranes, but the larvae swim at the surface of the sea-water. It is to be especially emphasized that in this method the order in which the means are used is of no consequence to the results obtained: the butyric acid may be used before or after the hypertonic sea-water.

For the eggs of *Arbacia* at Woods Hole, the method is somewhat different. According to Loeb, 2 cc. of $\frac{1}{10}$ normal butyric acid in 50 cc. of sea-water acting from one and one-half to three minutes must be employed. Even so, Loeb did not succeed in inducing the eggs of *Arbacia* to separate their vitelline membranes; they only showed what he called a fine gelatinous layer which was not easily visible. It remained for Heilbrunn[1] to show that the butyric acid treatment for this egg must be shortened; then the vitelline membranes are separated in much the same form as from fertilized eggs. This I have confirmed for the eggs of *Arbacia*. Also, I have obtained with butyric acid perfect membrane-separation in the egg of another echinid, *Echinarachnius*.[2]

Of this resumé, although it is by no means complete, we can make the following summarized statement which holds for all cases of parthenogenesis experimentally induced in marine eggs.

1. Only when they are in their normal fertilizable period do eggs respond to experimental means.

2. For all eggs, one means only—as heat, cold, acids, hypotonic sea-water, hypertonic sea-water, etc.—is suffi-

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[1] *Heilbrunn, 1915.*
[2] *Just, 1919c, 1920.*

cient. We shall soon learn that eggs of sea-urchins which, with the above discussed osmotic method, develop abnormally, are no exception to this rule.

3. Hypertonic sea-water is most generally effective although the result is not the same qualitatively for each species of egg.

4. No experimental means is limited in its action to eggs of one fertilization-class only.

No. 4 requires comment. If we arrange eggs into four classes, on the basis of their fertilization-moment, we do not find that for each of these classes one special treatment is alone successful. Heat, for example, is as successful on the egg of *Nereis* in the germinal vesicle stage as on that either of the worm, *Chaetopterus*, or of the clam, *Cumingia*, both in the stage of first maturation. On the other hand, eggs of the same fertilization-class do not always respond to the same means. Hypertonic sea-water does not induce cytoplasmic division in the egg of *Nereis*, but does in the egg of *Mactra*, which like that of *Nereis* is fertilizable in the germinal vesicle stage. Acid-solutions in sea-water fail to initiate development in the egg of *Nereis;* they call forth development in the egg of *Thalassema*, fertilizable like that of *Nereis* in the germinal vesicle stage. Moreover, consider the egg of the starfish. It responds best when in that stage, first maturation, which is optimum for its fertilization. Carbon-dioxide, butyric acid or heat alone is sufficient for eliciting complete response: membrane-separation, cleavage and vigorous larvae. Butyric acid alone, however, does not initiate development in eggs of other species in whatever stage of maturation they are fertilizable. Shaking, which suffices for the induction of development of the starfish egg, is without effect on other eggs fertilizable in the same stage, though in the egg of *Nereis* it does cause the break-down in the ectoplasm and the dissolution of the germinal vesicle.

Thus, as far as we have seen, a single inducing means is effective in eliciting complete development in all eggs named except in the sea-urchins'. The two exposures to the solution of butyric acid in sea-water used on the eggs of the starfish are not to be regarded as a double treatment since development is initiated by one exposure if it be sufficiently prolonged; in the double or intermittent exposure the effects are additive. Treatment with two different means, such as butyric acid and hypertonic sea-water, is unnecessary for all eggs named above, except for the sea-urchin's egg which does not develop normally with the original osmotic method, as we have seen.

This point is of fundamental significance since a theory of experimentally induced parthenogenesis has been based on the exceptional case of the sea-urchin's egg. Upon the experience that the double treatment brings about good development in the sea-urchin's egg a theory of general application was built. And this despite the fact that for all other eggs the double treatment is not necessary. But, as I was able to prove, also the sea-urchin's egg can be made to develop normally by a single parthenogenetic means.[1]

Using a strong solution made up of 20, 22 or 24 parts of $2\frac{1}{2}$ M NaCl (or KCl) plus 80, 78 or 76 parts respectively of sea-water on the eggs of *Arbacia* instead of the solution employed by Loeb (8 parts $2\frac{1}{2}$ M NaCl plus 50 parts of sea-water), I was able to induce the development of a high per cent. of *Arbacia* eggs. The eggs separate beautiful membranes while in any one of these solutions, but need to remain in it for some time thereafter to give farther development.[2] On transfer to normal sea-water after the opti-

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[1] *Just, 1922a.*

[2] *It should be noted that R. S. Lillie found that to induce complete development of the starfish egg—i.e., to the larval stage—the acid must act for a longer time than that sufficient to cause membrane-*

mum exposure, they cleave regularly, later becoming larvae which swim at the surface of the sea-water. This method is far simpler than the butyric acid plus hypertonic sea-water method; and in my experience it is also superior. Batallion and Batallion and Tchou working with several species of sea-urchins have confirmed my findings.[1]

Thus, the method of double treatment is unnecessary for sea-urchins' eggs. They, like all other animal eggs so far studied, respond to treatment with a single agent as the author of the method of the double treatment himself has shown in his original studies on three species of sea-urchins.

The fact that treatment with a single means initiates development of unfertilized eggs renders less difficult the approach toward an understanding of naturally occurring parthenogenesis.

Every organism, unicellular or multicellular, lives in and depends upon a world of its own. Whilst some have all the oceans, all the earth, as home, or freely roam all the sky, most dwell in a more circumscribed sphere; yet all sense quickly changes in the environment that hems them in. As with individuals, so with the cells in multicellular organisms—to changes in alkalinity, acidity, salinity and temperature they are acutely sensitive. Within only narrow ranges of alkalinity, acid-, salt-content and temperature can the cells of the human body, for example, exist.[2] Vary any one of these factors beyond a certain limit and human life becomes impossible, as we all know. The grand wonder of the human body is the maintenance of these factors as constant.

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separation. This finding is true for all the marine eggs named. The inducing of development in the frog's egg by puncture seems to be an exception.

[1] *Batallion, 1926; Batallion and Tchou, 1926. See also these workers (1933) on Bombyx eggs.*

[2] *See Barcroft, 1932; and earlier, Cl. Bernard.*

Now although other organisms and their cells do not so narrowly depend upon environmental changes, as do man and other warm-blooded animals, nevertheless they quickly respond to changes in their peculiar world, the environment to which they are keyed and with which they are in accord. For practical purpose, we abstract organisms and cells from their surroundings—we could scarcely do otherwise in most cases for we can not always study whole organisms or entire cells at once but only their parts, each in turn. Still we need ever to remember that we indulge in abstracting solely for the purpose of comprehension and that an organism or cell has a dependent relation to its environment. Life can not exist apart from its external world.

Nowhere, I think, in all biology does this most strong relationship, unity, really, of organism or cell and environment more forcibly reveal itself than in the problem of the parthenogenetic development of the animal egg. The problem, how the egg can begin development through the effect of some change in its environment, is to us still mysterious. The findings of experimental parthenogenesis would help to dispel the mystery if they could serve to indicate how environmental changes effect the initiation of development in naturally parthenogenetic eggs.

A plausible explanation is suggested by the fact that the most commonly used means for inducing parthenogenesis are simple changes in the medium, as changes in salinity, acidity, and temperature. The normally parthenogenetic egg probably differs from that which requires fertilization in being more labile and more responsive; as such it would react readily to one or another of these most commonly occurring changes in the environment. That drying, radiations, and puncture also induce development may be significant. Certainly, they are environmental changes by which experimental parthenogenesis can be induced. Since we know these, we should seek to determine whether they

or others start development in the naturally parthenogenetic egg. Until more data are available, we can not go beyond such general statements as to the probable cause of naturally occurring parthenogenesis.

As to experimentally induced parthenogenesis, however, the experimental findings suffice for an attempt at formulating a theory. And indeed, many theories have been proffered. With respect to one of these which became the most widely known, one notes a curious, even an anomalous situation. Although the data on induced parthenogenesis indubitably show that one means alone suffices to call forth development in the eggs studied, including those of sea-urchins, this theory, whose founder is Loeb, runs counter to these findings, including most of its author's, in explaining the phenomenon as due to the action of two distinct means because of the one fact that with a certain method, eggs of sea-urchins develop in more nearly normal manner after treatment with two means.

Since the interpretation of the induction of parthenogenesis in sea-urchins' eggs by the double method came finally to be the most widely accepted theory of fertilization embracing all animal eggs, we must examine it here. In this interpretation it is contended that butyric acid or any other agent which calls forth membrane "formation" tends to destroy, i.e., superficially cytolyze, the egg; and that such an agent acts as do haemolytic agents, those that destroy red blood cells. The second agent, it is held, corrects the destructive action of the first. This interpretation of certain experimental findings on eggs of sea-urchins became the famous "superficial-cytolysis-corrective-factor" theory for experimental parthenogenesis and for fertilization. The question now arises: Do the experimental findings on which it is based, actually permit this interpretation?

Eggs in sea-water, no matter from what species of animal they are, eventually die if they are not fertilized, that is,

they go to pieces, cytolyze. Hence, for the unfertilized egg sea-water is normally cytolytic. The length of time which eggs can remain in sea-water before they begin to cytolyze depends largely upon the temperature of the sea-water and the ratio of the volume of eggs to the volume of sea-water; also it varies with eggs of different species.[1] Now the fertilization-capacity of eggs in sea-water diminishes with time; here also the factors, temperature and volume of eggs come into play. The rate at which the fertilization-capacity drops also varies with eggs of different species. But it holds generally that the fertilization-capacity disappears before cytolysis begins—obviously, it must have disappeared at the latest at the moment when cytolysis begins, for a dead egg can not be fertilized. As a matter of fact, the capacity of an egg in sea-water to respond to an experimental means falls off more rapidly than its fertilization-capacity. This is strikingly brought out by the above mentioned effect of warm sea-water on the egg of *Nereis*. Its response to treatment with warm sea-water is not so good if it has been in normal sea-water before treatment, whilst its fertilizability is thereby not lessened. So we consider the cytolytic action of the sea-water as the antipode of the initiation of development by spermatozoon or by experimental means.

By the addition of any one of a number of substances to sea-water the rate at which eggs cytolyze can be increased. Among these is butyric acid. But the action of butyric acid in accelerating cytolysis is by no means invariable. On the contrary, the effects which this acid produces depend upon its concentration in sea-water and, in the case of each concentration which is effective for calling forth membrane-separation, upon the duration of time that the eggs

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[1] *In making comparisons of the rate of cytolysis of eggs of different species one must be careful that the experimental conditions are uniform in the compared cases.*

are exposed to it. If we designate as optimum the shortest exposures which are effective in calling forth complete membrane-separation, we find, as my own experiments have so clearly established, that sub-optimum exposures instead of accelerating, actually retard the cytolysis which in time normally takes place in sea-water.[1] Eggs in sea-water which show fully separated membranes in consequence of optimum exposure to butyric acid cytolyze more rapidly than unexposed eggs. Over-exposed eggs cytolyze at a still more rapid rate. In his experiments Loeb never used this reagent properly. That is, he exposed the eggs of both the California and the Woods Hole sea-urchins beyond the optimum time for membrane-separation. If he had learned the best exposure, he probably would not have so greatly emphasized in the butyric acid-hypertonic sea-water method for the sea-urchin egg the cytolytic action of the acid. Basing his theory on over-exposure to butyric acid, he created the term, "superficial cytolysis."[2]

The superficial cytolysis-corrective-factor theory explains the action of the two means for inducing parthenogenesis as follows: the fatty acid treatment causes "superficial cytolysis" and the hypertonic sea-water treatment following "saves" the egg from this impending death. As I stated above, the order in which the two agents are used is of no consequence: the exposure of the eggs to butyric acid may precede or follow that to hypertonic sea-water. That is, the corrective factor (hypertonic sea-water), according to the theory, if used first acts to correct where nothing is to be corrected and the superficial cytolysis factor (the fatty acid) used at the second place, tends to kill the more than corrected egg. Thus the sequence in the treatment so strongly demanded by the superficial-cytolysis-correc-

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[1] *Just, 1920.*
[2] *See Just, 1919c, 1920, 1922a, 1922b, 1930c.*

tive-factor theory not only is not supported by fact but is contradicted by it.

We may dismiss the superficial cytolysis-corrective factor theory of parthenogenesis for the following reasons: All eggs including those of sea-urchins need treatment only with a single means. Further, for sea-urchins' eggs, the cytolytic effect that the theory stresses, results from over-exposure to the acid. Finally, the fact that the order of treatment in the double method can be reversed, makes the theory even for the single egg of the sea-urchin untenable.

These obvious failures of the theory however did not prevent the exaggeration of its significance to an unusual degree. The discovery that eggs could be induced to develop without the spermatozoon gave rise to extravagant claims by experimental embryologists themselves and aroused fantastic notions among laymen. Many hailed it as a demonstration of the creation of life. As if the unfertilized egg were not alive! According to one report, to create life is easy since the means for inaugurating the life-process are at once simple and close at hand: one needs only two chemicals, vinegar and salt, found in every kitchen, to start the egg on its long and complicated journey of development, the end of which is the adult form. The occurrence of natural parthenogenesis in the meantime was given scant attention; indeed, in some quarters it was entirely overlooked. The one fact alone, that nature dispenses with spermatozoa for the development of certain eggs—those of rotifers, aphids, bees and others—this recognized fact of natural parthenogenesis that we have known for years, should have saved us from making excessive claims concerning the significance of experimentally induced parthenogensis.

As soon as the superficial cytolysis-corrective factor theory for experimental parthenogenesis had been elaborated, the attempt was made to use it as an explanation for

the phenomenon of fertilization. The problem seemed to be especially easy, since the double treatment—and, therefore, the factors in the explanation—in these experiments on inducing parthenogenesis, appears to have parallels in the two phases in fertilization, the external and the internal phase. I shortly review these here.

All eggs respond to insemination, as was pointed out, by some kind of surface or ectoplasmic changes which produce in some cases very striking results, as the separation of the vitelline membrane in sea-urchins' eggs or the extrusion of the superficially located material in eggs of the genus *Nereis* which sets as a jelly in the sea-water. Underlying these changes is always a disintegration of the superficial cytoplasm upon which follows normally a rapid reconstitution of the surface. This is the so-called first or external phase of the fertilization-process.

Once within the egg, the nucleus of the spermatozoon moves toward the egg-nucleus. Both division-centres arise near one or the other of the germ-nuclei or one centre near the egg- and the other near the sperm-nucleus. The mitotic spindle is established and first cleavage is initiated. These phenomena constitute the so-called second or internal phase of the fertilization-process.

With respect to these two phases, it is true that experimental parthenogenesis in the sea-urchin egg initiated by butyric acid and hypertonic sea-water resembles the fertilization-process inasmuch as the acid calls forth membrane-separation and the hypertonic sea-water initiates the formation of an amphiastral mitotic figure. Therefore, the superficial cytolysis corrective factor theory though it can not be upheld as explanation of experimental parthenogenesis, may, one might think, nevertheless explain fertilization. Attempting such an explanation, Loeb says that the "fertilization by the spermatozoon perhaps depended not upon a single chemical agent, but upon a combination

of two or more[1] which were only *fortuitously combined* in the spermatozoon" (Italics are mine). According to him it is a fact which he has proved that "the membrane formation by the spermatozoon is caused also by a cytolytic agent—a lysin."

I have already pointed out that for the sea-urchin's egg Loeb used butyric acid either too long or in too great concentration; such over-exposures very greatly increase the rate at which this egg cytolyzes in sea-water.[2] The optimum exposure to butyric acid, which for the egg of *Arbacia* Loeb never knew, does not cause this hastening of cytolysis. Moreover, neither Loeb nor any one else has proved the presence of a lysin or of a "fortuitously combined combination" of chemical agents in the spermatozoon. Loeb's so-called proof of a lysin in the spermatozoon is fantastic and wholly specious.

In calling forth membrane-separation the effect of butyric acid is, to be sure, somewhat similar to that of the spermatozoon. But there is never membrane-separation in the butyric acid solution; only after the acid is washed away by bringing the eggs into a large volume of sea-water does membrane-separation take place. There is thus an essential difference between the immediate action of the spermatozoon in calling forth membrane-separation and the delayed effect of butyric acid. This point raises a further question. Since according to the author of the superficial cytolysis-corrective factor theory of fertilization, butyric acid is only an example of a large class of cytolytic (and haemolytic) agents, we must ask if the action of butyric acid is, as we should expect and as the author of the theory claims, characteristic of the class.

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[1] *The "or more" I think is added as a margin of safety since the theory demands only two.*

[2] *Just, 1920.*

Among the cytolytic (and haemolytic) agents named by Loeb are $1\frac{1}{2}$ *M* NaCl and distilled water. Now I find that either of these induces membrane-separation in *Arbacia*-eggs while the eggs are in the solution.[1] In a pure $2\frac{1}{2}$ *M* NaCl solution the membranes separate with extreme rapidity,[2] so that to follow the process one must use a less concentrated solution. The separation of the membrane in hypertonic sea-water is due to the rapid shrinkage of the egg-plasma. In distilled water, on the other hand, the eggs distend as water enters but the elastic vitelline membranes distend more rapidly than the plasma so that in five seconds the membranes are off the eggs, at which time the plasma shows no sign of disruption. With prolonged exposure the egg-plasma swells and reaches the membrane; cytolysis follows. The action of butyric acid in causing membrane-separation resembles neither that of hypertonic nor that of hypotonic sea-water. In my experience all of the cytolytic agents named by Loeb fall into two classes: those that do not call forth membrane-separation directly but only after they are washed away by transferring the eggs to normal sea-water; and those that do act directly, by producing either shrinkage of the egg-plasma or extension of the elastic membrane. If the spermatozoon's first duty to the egg be to inject a lysin, which of the three kinds of cytolysis—that of butyric acid, of hypertonic sea-water or of hypotonic sea-water—does this behavior of the spermatozoon resemble?

An agent which induces membrane-separation in the sea-urchin's egg can not act as a fatty acid, a concentrated neutral salt solution and distilled water at one and the same time. If we consider all the cytolytic agents which have been employed, we fail to discover in their mode of action

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[1] *Just, 1922a, 1928c.*
[2] *Just, unpublished observations.*

any one common factor; they act either as butyric acid, as strong hypertonic sea-water or as distilled water. That is, the cytolytic agents lack a specific factor, whereas the fertilization-reaction reveals a high degree of specificity, borne equally by the components, egg and spermatozoon. If one adheres to the lysin-corrective factor theory of fertilization, one must assume the addition of a specific factor in the spermatozoon imposed upon the nonspecific "fortuitously combined combinations" of factors, resembling butyric acid, hypertonic sea-water and distilled water. From this point of view specificity would reside only in the spermatozoon, the egg playing no part. We are told that "the lysins of foreign animals can get into cells by mere diffusion, while the lysins of the same species can not get into the egg by diffusion. Only through the motile power of the living spermatozoon which acts as a carrier can the fertilizing lysin of the animal's own species get into the egg."[1] Here the author of this theory reveals the failure of well-known physico-chemical agents to act as substitutes for that "mysterious complex" called the "living spermatozoon." The high purpose, to transfer the problem of the initiation of development of the animal egg from the realm of morphology to that of physical chemistry[2] is frustrated at a most crucial point. It is, therefore, needless to discuss the problem of specificity further in this connection.

The order of the two phases of the fertilization-process is constant in a normal egg; always the external (ectoplasmic) changes come first and the internal (mitotic) come second. Never does a normal egg exhibit the phenomena of the internal phase before the ectoplasmic changes characteristic of its fertilization-process have taken place. As I have already pointed out, in the experimental partheno-

[1] *Loeb, 1913.*
[2] *Loeb, ibid.*

genetic development of the sea-urchin's egg by means of butyric acid and hypertonic sea-water, the butyric acid is equally efficient in action preceding or following the treatment with hypertonic sea-water. This notable difference between "chemical fertilization" and nature's process the theory discounts. Though Loeb was the first to know that the order of treatment could be reversed, he made no attempt to explain this difference from the natural process.

Sharply visible ectoplasmic changes are always present in fertilization but not in all cases of experimental parthenogenesis. As we have seen above, the eggs of sea-urchins develop with Loeb's original hypertonic sea-water method, although without membrane-separation. The double treatment is not essential.[1]

Because of these considerations we must flatly dismiss the superficial cytolysis-corrective factor theory as an attempt to explain fertilization as we have dismissed it as an explanation of experimental parthenogenesis; it fails not only for eggs generally but also for sea-urchins' eggs specifically.

But the fact remains that parthenogenesis can be experimentally induced. What is experimental parthenogenesis?

If terms mean anything, we should expect that in experimental parthenogenesis, as in natural, perfect and complete development should result. Yet, as we have seen, generally experimental parthenogenetic development does not extend beyond the larval stage. This is undoubtedly often due to our failures to overcome the difficulty of rearing animals to the adult stage from eggs under laboratory conditions.[2] Some difficulty also inheres in the methods employed: means of experimental parthenogenesis often tend to do

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[1] *It is curious that Loeb insisted that the superficial cytolysis factor is the essential one; he thereby denied his own discovery and deprived himself of all ground in his whole fight concerning priority.*

[2] *I have been able to carry Platynereis megalops through three generations under laboratory conditions.*

too much—though they initiate development, they also impair it.

Even if we in time overcome these difficulties, there still remains doubt that the action of the means of induced parthenogenesis is identical to that of the spermatozoon. For while normal, fertilized eggs do, if properly handled, give at least 95 per cent. perfect larvae, it is difficult if not impossible to obtain this percentage in experimental parthenogenesis. The spermatozoon is more effective than a means of experimental parthenogenesis; frequently one finds that a certain lot of eggs, a sample of which will give more than 95 per cent. fertilization and perfect development, will not respond to a means of parthenogenesis that is usually effective on eggs of this species.

On more general grounds it is still to be doubted that an experimental means duplicates the action of the normal stimuli in the intact organism, though experimental imitation is often possible. Despite these considerations however, it must be clearly emphasized that complete development can be induced experimentally.

Even if we do not yet succeed in establishing the particular way in which either spermatozoa or experimental means act upon the egg so that it develops, we certainly can agree that either spermatozoon or experimental means in initiating the development of the egg produce the same result—a succession or rhythm of mitoses and cleavages which finally lead to embryo-formation. As we have already seen, the experimental means are not specific. Most probably, the nature of the reaction between experimental means and egg differs also from that between spermatozoon and egg, since there is evidence to indicate that fertilization is a chemical union of an egg-substance with the spermatozoon, whereas we can assume that the initial action of the experimental means is physical. But the end-result, however reached, is the same. The conclusion

is therefore this: the egg-cell like many another living cell—nerve or muscle, for example—possesses independent irritability. It has full capacity for development. Neither spermatozoa nor experimental means furnish the egg with one or more substances without which the initiation of development would be impossible.

Here lay at the same time the possibilities and the failure of the work on experimental parthenogenesis. Every single investigator who erred in "proving" an external agent (or agents) to be the cause of development neglected an opportunity to extend our knowledge concerning that fundamental manifestation of living matter, its independent irritability. Some theories of fertilization derived from work on experimental parthenogenesis postulated as the cause of development a change in the egg, e.g., membrane separation, that was however merely one consequence of the ectoplasmic changes, or they related the cause of development to some concomitant of mitosis. Others, as had the Loeb theory, merely offered a substitute for the sperm-borne centrosomes. Instead of these two structural entities which Boveri had for some time postulated to be indispensable for fertilization because he thought them to be either lacking in the egg or present in too enfeebled state,[1] the new school of biology substituted all kinds of means, like lysins, mysticisms no less mystical because appearing to belong to the realm of physical chemistry, having the living condition of the spermatozoon always conveniently at hand as a refuge finally to be sought.[2]

I do not wish by seeming to dwell over long on this point to take unfair advantage of the patent shortcomings of the work on experimental parthenogenesis. And yet I must hold it up as an example of what such a large section of the

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[1] *See also Vedjovsky, 1888-92.*
[2] *Loeb, 1913.*

school of quantitative biology has done for us by over-reaching facts and drawing unwarranted conclusions as to the physical chemistry of vital phenomena. One method used in this work is the "substitution of well-known physico-chemical agents" for living components. But this substitution is only that of a well-known physico-chemical agent for another one. One need not be in complete ignorance of this agent, i.e., the living cell, unless one takes pride in a disdain for the knowledge accumulated by descriptive studies. Not hindered by this knowledge one can easily make great discoveries and, through fecund ignorance, perpetuate error. And if, in addition, with a paucity of mathematics, physics and chemistry one elaborates mathematico-physico-chemical theories, then one by no means wins what quantitative biology so much desires: namely, security of biology, sitting at the right hand of physics. Even those who have an adequate knowledge of physics and chemistry and do appreciate the biological phenomena which they aim to explain in terms of physics and chemistry, should, (when at that point beyond which their data do not warrant conclusions) honestly say: "However much we desire to establish life as a mechanism, here our present knowledge comes to its limits." It is sad irony that a theory of vaunting mechanistic conceptions had, as its basis, work the true value of which lay in establishing the fact that the egg as a living cell is self-acting, self-regulating and self-realizing—an independently irritable system. For the spermatozoon, the theory's mechanistic conception is more vitalistic than even the ardent vitalist could desire: for it says that the living spermatozoon does what it does only because it is alive.[1]

The history of biological research furnishes us with other examples which illustrate the short-comings of such physico-

[1] *Loeb, l.c.*

chemical approach in setting up a singly studied property of living matter as synonymous with the whole complex of life-processes. Witness the theories of oxidation, permeability, electrical conductivity, viscosity and the like. These all have failed; they leave untouched the cardinal problem of biology, the structure of a living thing; they do not relate themselves to the organization which distinguishes a living thing from a non-living. Or take the almost universal fashion in which Hill's work on nerve-conduction was accepted. By "proving" that a nerve conducts without heat-loss this work must logically lead to the conclusion that the nerve-fibre is not living since it gives no evidence of metabolism. Strictly orthodox morphologists have likewise often presented theories of life-processes on the basis of demonstrations that only emphasize anew the capacity of the living thing though debased to respond according to its specific and intrinsic irritability. The now perfect collapse of the organizator theory is a case in point: all that remains of it is a name for the well-known power of protoplasm to respond in the same characteristic, structural and physiological manner to diverse stimuli.[1]

True, terms, as inherent irritability and response to stimuli, are more general than any one denominating a physico-chemical attribute named above, or than the term, organizator, even. But the present state of our knowledge of protoplasmic response does not yet permit the use of more specific terms with reference to the behavior peculiar to matter organized in the living state. The reproach that one retards progress by use of too general terms carries no serious onus so long as we have not progressed far enough to warrant the employment of precise physico-chemical formulation.

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[1] *As an example, cf. Just, 1936c: here an egg responds with the same structural change to most diverse experimental means.*

Experimental parthenogenesis, a problem far from being solved, invites investigations both for its own sake and for the implications it possesses for the biology of cells generally. In the interest of this research it is necessary to define the problem clearly in order that not all kinds of observations are considered as belonging to it: Experimental parthenogenesis must be defined as the development of an egg that has been initiated and carried to at least the normal larval stage by an agent other than the living spermatozoon.

In contrast to this clear definition there exists a great deal of confusion in the literature on this subject. For instance, an agent which produces almost no change in an unfertilized egg, as revealed by the egg's response with perfect development when inseminated, is often called a parthenogenetic agent. Or the so-called parthenogenetic agent induces membrane-separation only, stimulating the egg to the limited extent that makes fertilization now impossible. In other cases the changes induced may be injurious, yet the egg does not lose capacity to respond to fertilization, though its development is abnormal. Or the pseudo-parthenogenetic agent induces cytolysis which begins sooner or later, depending upon the agent's toxicity. Whenever the changes induced, either because of the nature of the agents themselves or the method with which they are used, do not lead to development, they should not be called parthenogenetic. And certainly, death changes, i.e., cytolysis, should be considered to be beyond the limits of the term, the initiation of development.

The establishment of the mitotic figure constitutes, as we have seen, a definite and reliable index for the completion of the fertilization-process. Normally, development then proceeds with rhythmical nuclear break-down and reformation; and in the majority of eggs this division of the nucleus synchronizes with cleavage of the cytoplasm.

In some eggs, e.g., of insects, the first nuclear divisions ensue without cytoplasmic cleavages, whilst in others, e.g., those of selachians and of birds, later mitoses proceed without the splitting up of the cytoplasm. In certain eggs experimentally treated, some kind of development, abnormal however, can go as far as a swimming form without cytoplasmic cleavage. Hence, although development never completes itself without the earlier or later breaking up of the cytoplasmic mass of the egg into cells, we can not categorically define the process of development as related to the sundering of the cytoplasm. Therefore the end of the fertilization-process we define by the appearance of the mitotic figure rather than by the first cleavage of the cytoplasm.[1]

Unfortunately, we have neglected to make nice studies on the differences in the development of two sets of eggs from the same animal, one set fertilized and the other treated with some means of inducing parthenogenesis—such differences, for example, that would reveal themselves in the size of cells and in the time and the place of their appearance in an egg whose cell-lineage is known, that is, one in which the size, order of appearance and location of the cells with reference to each other are definite and invariable. Some differences, as rate of cleavage, we know; others, indicated for the earlier stages of development, are doubtless due to the fact that the experimental treatment never quite duplicates the action of the spermatozoon. But even if we assume that fertilized and parthenogenetically developing eggs, both in the induced and

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[1] *I say mitotic figure because the occurrence of amitotic nuclear division in developing animal eggs has been doubted. If we include the possibility of development with amitotsis in our definition, we substitute in the thesis nuclear division for the term, establishment, or appearance, of the mitotic figure.*

the naturally occurring process, differ in their course of development, we know certainly that by either fertilization or parthenogenesis, if development is complete, an adult animal emerges through a succession of nuclear and cytoplasmic divisions. Therefore, despite any differences that may occur during the developmental process, the beginning stage is always that of the establishment of the cleavage figure with consequent nuclear and cytoplasmic division, the end-stage always being the adult form. The question thus arises as to the nature of the calling forth of the nuclear configuration through whose subsequent rhythmical behavior together with cytoplasmic cleavage the end-stage is attained. The initial action of spermatozoon and of experimental means being different, and differences in action obtaining between one means and another, the question at issue is: Do these differences persist so that the calling forth of the process of nuclear and cytoplasmic divisions springs from various causes, or do these means, whatever they are, elicit the same reaction which sets up the division-process? I suggest that they set up the same reaction.[1] Let us briefly review the experimental findings.

In sea-water sufficiently hypertonic to induce development, eggs of any species which respond to such treatment rapidly and directly lose water. The minimum hypertonicity capable of calling forth development in sea-urchins' eggs does not bring about a sharply defined shrinkage of the eggs from their vitelline membranes; only in stronger hypertonic solutions does this egg shrink from its membrane. Other eggs shrink in hypertonic solutions to such an extent that the vitelline membranes no longer adhere to the cytoplasmic surface. On return to normal sea-water, the eggs take up water but they do not regain the equilibrium with

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[1] *See Just, 1927a.*

the sea-water which they possessed in their untreated condition, instead they establish it at a new and different level.

In hypotonic sea-water eggs take up water and increase in volume. If the degree of dilution is effective, the rapid intake of water induces break-down of the eggs' surface-cytoplasm and rapid distension of their vitelline membranes. Removed to normal sea-water they quickly diminish in volume but do not regain their previous state because of the complete break-down at the surface, i.e., complete ectoplasmic changes. They come again into equilibrium with the surrounding sea-water, but this, owing to the altered surface-structure, is at a new level.

After having been properly exposed to effective acids in optimum concentration in sea-water, eggs on return to normal sea-water separate their vitelline membranes. That is, the acid treatment induces a change which brings about the subsequent break-down in the surface-cytoplasm. With the processes of restitution by which a new surface-layer forms, the eggs come into a new equilibrium with the sea-water.

Warm sea-water when effective causes immediate break-down in the surface-layer. Puncture of the frog's eggs presumably leads also to ectoplasmic break-down. Radiation, as of ultra-violet, brings about ectoplasmic break-down. In these cases as in the others given above, the surface-structure is altered and thus the equilibrium between egg and sea-water is different from that which existed before the treatment.

Also after fertilization an egg having undergone complete ectoplasmic changes does not return to the state which it had before fertilization with respect to its equilibrium with the sea-water. It shows instead a marked difference. With its altered surface-layer, the fertilized egg establishes a new equilibrium with the sea-water. As we shall see in the chapter that follows, fertilized eggs exhibit in their

reaction to environmental influences rhythmical changes of resistance and susceptibility that are both different from those shown by the unfertilized egg.

The new egg sea-water equilibrium is established by the structural changes in the ectoplasm; these changes do not reverse themselves and the egg does not return to the physiological state it had previous to treatment or to fertilization. Also with respect to water-movements these same changes sharply set off the developing egg from the untreated and unfertilized. Since the egg loses and gains water concomitantly with each cleavage-cycle, one can not say that with break-down of substance in the ectoplasm it becomes permanently dehydrated. Rather, a momentary water-loss as a consequence of the stimulus of inducing means or of the spermatozoon, brings about a change to a new level with respect to equilibrium with the surrounding medium, and on this new level the ensuing rhythmical process of water-entrance and -exit which accompanies the developmental process takes place.

The rhythmical movement of water, during the cleavage-cycle, into and out of the egg undoubtedly means a movement of water from place to place within the egg; and this in turn means local and temporary hydrations and dehydrations. These redistributions of water even in minute intracellular dimensions are favorable for reactions.[1] One visible structure of the cell which in addition to the ectoplasm exhibits rhythmical changes during the cleavage-cycle is the nucleus; its break-down and reformation is indeed the criterion used for defining the cleavage-cycle. As we have seen in the chapter on the fertilization-process, the establishment of the mitotic complex constitutes the index of the completion of the initial stage of development. And I suggest that in all modes of initiating development,

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[1] *Just, 1937b.*

that of experimentally induced and naturally occurring parthenogenesis as well as that of fertilization, the establishment of the first mitotic figure is brought about by a dehydration of some constituent of the ground-substance, or as the result of a reaction between nuclear and cytoplasmic ground-substance brought about by dehydration. The mitotic figure then is either expressly a dehydration-formation or the result of a reaction rendered possible by dehydration. In the resting egg this material in the ground-substance is spatially diffused; with the process of parthenogenesis or of fertilization it becomes aggregated— in parthenogenesis and in some cases of fertilization around the egg-nucleus, in most cases of fertilization around the sperm-nucleus. What we see as asters and as spindle may be either this material itself or the sign or product of its activity.

This hypothesis—and more can not be proffered in the present state of our knowledge—is wholly consistent with the established facts as far as one can reduce them to order. According to it the most important factor in all modes of initiating development is a dehydration-process affecting directly or indirectly the ground-substance.[1] In all cases of the initiation of normal development, dehydration begins in the ectoplasm.

Ectoplasmic changes alone, as I have shown above, are not experimental parthenogenesis. They do not of themselves determine that development will follow; but they are a reliable indicator for the quality of the development, if this ensues. That there are cases of ectoplasmic changes without subsequent development on the one side and that the sea-urchins' egg after treatment with weak hyper-

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[1] *Delage in 1901b promulgated a dehydration-theory of fertilization; but this differs from the one here proffered in ascribing dehydration to the influence of the sperm only.*

tonic sea-water develops without complete break-down of ectoplasmic substance on the other, warns us against attributing the cause of experimental parthenogenesis directly to physical changes at the egg-surface, i.e., to membrane-separation.

Far from being an exception to be explained away, the case of sea-urchins' eggs in responding to treatment with the weak hypertonic sea-water without membrane-separation and with poor quality of development reveals clearly what other eggs more sensitive in their ectoplasmic response to experimental means do not: the ectoplasmic changes are significant for the development in prospect. Again, as in fertilization, we see that the quality of development depends upon the quality of these initial ectoplasmic changes.

They are violent eruptions which precede the evanescent spinning that will accompany cleavages yet to come and that will build gossamer-like tendrils to bind cell to cell. Spermatozoon or parthenogenetic means, Nature's or experimenter's, set the life of the egg in quicker motion; the mitotic spindle comes and goes. The web of life gives a pattern which we come to know as larval sea-urchin, worm or clam. But almost with the moment that the egg's vital activity moves at a quicker tempo we know through the ectoplasmic behavior the quality of the events to come.

Cell-division

$\mathcal{W}$ITH THE PROCESS OF CELL-DIVISION, THE FERTILIZED or parthenogenetic egg, a single cell, develops into an adult organism which may comprise millions of cells. The first division or cleavage separates the egg (in most cases) into two portions, blastomeres, which remain attached. Successive cleavages give rise to many blastomeres, the egg becoming a mass of cohering cells. The surface of the frog's egg, for example, in late cleavage so closely resembles that of a golf-ball that one gains the impression that subdivision of its surface by cleavage-planes is the egg's chief characteristic. If one examines a smaller egg, e.g., of a sea-urchin, under the microscope, one notes again the resemblance of its corrugated surface to a golf-ball; looking closer, one observes within each blastomere a nucleus. It is a very simple matter to convince oneself by continuous observation on a living egg throughout its cleavage period that the "golf-ball" has arisen by successive divisions of both nucleus and cytoplasm.

The cells which comprise an adult organism are not all alike in structure. To the naked eye a strand of nerve is readily distinguishable from a strand of muscle of equal length and thickness; and a piece of kidney can not be mistaken for liver. As *en masse*, so singly, under the microscope, the cells of nerve, muscle, kidney, and liver are easily recognized. Since these cells have different functions, development means something more than mere multiplication of cells; it implies the cells' differentiation. This arises in the process of cell-multiplication at an earlier or

later stage, depending upon the species of egg. As we shall see, this old problem of differentiation is still the major one of the study of development.

With successive cell-divisions during cleavage, the blastomeres become progressively smaller. If this process were to continue indefinitely, the size of the cells would approximate zero and cell-division would come to a standstill. But this never happens, since in later stages the cells transform food into protoplasm. In many cases the egg soon after hatching as a larva takes in food; or the embryo, as that of the chick, utilizes the yolk which is present as reserve food material within the egg.

Generally, growth is increase in size. A cell may grow as such by the intake and transformation of food. In the initial stages of the development of an egg, cell-multiplication procedes without any increase of volume. The total mass of the egg of a sea-urchin, for example, at the end of its cleavage-period is approximately the same as at the beginning. In the larval stage, cell-multiplication follows upon the intake and transformation of food by the cells; thus here through the combined process of increase in volume by the single cells and increase in their number, growth of the embryo is attained.

In the adult organism derived from the egg, cell-division operates daily in the restitution process. Cells of the human skin are constantly being replaced. Each day the blood-forming tissues in bones must throw into the bloodstream millions of red blood corpuscles to compensate for the disintegration of older ones. Where there is no more capacity for cell-division, any injury becomes tragic; thus the capacity for regeneration in the human central nerve system is meagre and the possibilities of repair after surgery slight because its cells have lost the power of division. Organs which possess reparative capacity have retained the capacity for cell-division.

Cells in the adult body frequently show a sudden and exaggerated burst of division-activity which results in abnormal growth. Thus tumors are built up by riotous cell-proliferation. The malignant tumor, cancer, in this respect is not different from the benign and to this extent any work on cell-division may be of significance for the cancer problem; this, however, does not mean that every work on cell-division in various forms of animals from *Amoeba* to vertebrate has direct bearing on the problem of cancer, since here malignancy points to a cause lying beyond the facts which can be established for tumors in general, both malignant and benign.

The germ-cells, eggs and spermatozoa, display the most intense activity of cell-division, especially during their periods of multiplication. A great deal of our information concerning cell-division is derived from the study of these cells particularly in their period of maturation.

It is not only among multicellular organisms as egg or as adult that cell-division is of consequence; among unicellular animals and plants it is often the sole mode of reproduction. These single-cell forms live for minutes, hours or days as such and then by dividing into two parts bring into being "new" individuals. Or one cell may divide into several parts each of which becomes a "new" individual.

In the non-living world, substances may divide, and so multiply and also differentiate and grow. But these phenomena are quite unlike those exhibited by living things. Cell-division constitutes a fundamental process which is never observed outside the world of living things. And yet, it still lacks an explanation to which biologists agree. It is, moreover, most often incorrectly defined. The reader should understand that in the following pages my main purpose is to derive a definition. Only after having done this shall I offer an explanation of this phenomenon, the division of the cell.

Before I begin the discussion I should like again to point out how necessary it is that we appreciate differences normally appearing in biological processes before we attempt to evaluate these processes. With respect to the process of cell-division, we recognize two kinds. Based upon the behavior of the nucleus in cell-division as a criterion, two categories of cell-division have been set up: that with direct (amitotic) and that with indirect (mitotic) division of the nucleus.

In direct or amitotic nuclear division, the nucleus by constriction separates into two parts. It first elongates, taking the form of a dumb-bell or hour-glass, and then breaks into two equal or unequal portions. Each of these with cytoplasm constitutes a new cell, if the cytoplasm also divides. But division of the cytoplasm synchronously with that of the nucleus is not invariable. The occurrence and significance of amitosis have been much debated. Some few biologists hold that amitosis is more widespread among animals, even in the development of eggs, than is generally believed and that it may be of equal significance with mitosis. The majority opinion is that amitosis is of restricted occurrence and when found among multicellular organisms is of little significance generally, except as a sign of decadence or of too highly specialized cells.[1] For the Protista we meet the view that what is often called amitosis is a disguised mitosis. Nevertheless, it is generally admitted that among both multicellular and unicellular organisms amitosis does occur. It must, therefore, be embraced by any theory that attempts an explanation of cell-division, especially if the explanation relates division of the cytoplasm to that of the nucleus.

As has been pointed out already, mitosis or indirect nuclear division involves a series of orderly maneuvers of

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[1] *Wilson, 1925.*

the chromosomes on a set of "fibres," the mitotic spindle, beginning with the break-down of the resting nucleus and ending with the formation of two resting nuclei. If from the poles of the spindle where the "fibres" more or less converge, other "fibres," called astral fibres, extend radially into the cytoplasm, the spindle is said to be of the astral type; if these radiations are absent, the spindle is called anastral. In the former type at the centre of the aster may be found either a single granule, the centriole or centrosome, or many finer granules; or, the astral centre may be of such fine structure that it appears to be optically empty. Mitotic division of the nucleus may normally ensue without concurrent division of the cytoplasm; experimentally it is easily possible to suppress or arrest cytoplasmic division while nuclear divisions continue. Since this is true, the failure of cytoplasmic division after amitotic nuclear division that is sometimes observed, does not deserve the emphasis which some writers place upon it. The mitotic complex may arise wholly or in part within or outside of the nucleus.

What should we demand of any theory of cell-division? An explanation of cell-division and not of nuclear phenomena. The theory should cover amitosis and mitosis.[1] It should apply with equal force to all types of mitosis among both Protista and multicellular organisms; to every variation of the mitotic configuration, i.e., to spindles with and without asters, with and without centrosomes. Since cytoplasmic division does not always synchronize with either direct or indirect division of the nucleus, a theory of cell-division based exclusively or too strongly on nuclear division embraces only cells exhibiting synchrony in division of cytoplasm and nucleus; not only does such a theory fail

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[1] *Delage, 1895, p. 758: "Ce qui est essentiel dans la division indirecte, c'est la division directe et cette dernière seule est à expliquer."*

to account for cells lacking this synchrony, but it implies that they are abnormal. The synchrony of nuclear and cytoplasmic division is an interesting phenomenon but not an essential characteristic of cell-division; instead of being over-emphasized in theoretical considerations, it needs itself to be explained.

A theory devised to explain division of the cell-body should also be consonant with the observable phenomena in the cell both whilst living and when properly fixed. Physico-chemical speculations however ingenious when inconsistent with the observed phenomena lend little to the solution of the problem. Indeed, we shall notice that in the attempt to explain cell-division the ignorance of the actual process and the exaggerated application of physicochemical knowledge have together erected serious obstacles to a proper understanding and that, as the research on experimental parthenogenesis, so also that on cell-division can be upheld as an example of the danger which inheres in this kind of physico-chemical study. Again it should be emphasized that the first task in the approach to the problem is to learn as thoroughly as possible the normal process which is to be explained.

Unfortunately, explanations of the mechanism of cell-division have frequently been based on abnormal cell-behavior induced by experimental agents. The chief error in this type of work does not lie in the use of a strong experimental means that brings about abnormality; it lies in the fact that the conclusions overlook this result of the treatment. Further, it is clear that experiments, in which the cells were weak and abnormal at the outset, can not serve as a basis for the explanation of the normal process.

For a theory of cell-division, as for any problem in biology, an appreciation of the normal biology of the cells under their natural conditions is imperative. For example, important as the work on vertebrate cells in tissue-culture

is for that on cell-division, we must always reckon with the fact that these cells are not in their natural medium and also that they have escaped the integration under which they live while in the intact organism. Similarly, it is dangerous to interpret the mechanism of cell-division from the study of living cells after these have been dissected out of the organism. Those eggs normally shed and inseminated in the sea and free-living protozoa offer the best opportunities for observations on cell-division in the living cell under conditions approximating the normal. Since here we deal with metazoa, I concentrate the discussion on them, restricting myself largely to animal eggs. This I do, not only because this book concerns itself with animal eggs, but also because the most prominent theories on cell-division are based on the study of eggs, especially those of the sea-urchins. Nevertheless, we finally encompass cell-division in all animal forms.

Obtainable in large number, easy to handle, of convenient size and comparatively simple in structure, eggs of various species of sea-urchins have been most popular cells for the study of cell-division. The cytoplasm of some is transparent or nearly so and in them one clearly discerns in the successive stages of nuclear division the behavior of the various constituents of the mitotic complex, including the chromosomes once they appear on the spindle; in others because some cytoplasmic granules are brightly colored, one marks by their shift the ebb and flood of the cytoplasmic tides. The study of the pigmented type supplements that of the transparent. Also, the transparent eggs one can color by means of inert dyes that do no harm to the living cytoplasm, and can in the pigmented ones by innocuous centrifugal force mass the colored granules so that the nuclear phenomena more sharply stand out. But the cells must be normal; when fertilized they should be of the same specific gravity and almost perfect spheres, all showing

subsequently at each instant in the division-process the same change in contour and exhibiting a clock-like precision in the cleavage-rhythm.

Another advantage inheres in the use of sea-urchins' eggs for the investigation of cell-division. Because of the fact that these eggs are fertilized after complete maturation, the process of division is most easily observed, being that of a single division-cycle and not imposed upon the events of one or both maturation divisions as in eggs of the other three fertilization-types. Moreover, the cleavage is of the most simple type inasmuch as the first three cleavages form almost equal and close to spherical cells. Since the first cleavage cycle encompasses the union of the egg- and sperm-nuclei while all succeeding cleavages are wholly mono-nu-clear—a fact which we must ever bear in mind—it would appear necessary to appreciate the sequence of events in the second or the third cleavage in order properly to evalu-ate those in the first in which part of the events belongs to the fertilization-process. One must be fully cognizant of what events belong to fertilization and what to cell-division. To obtain a clear picture of the end of the one and the beginning of the other process is not easy since so much of the work on cell-division considers the first division only.

The account now given is based upon the egg of *Arbacia* because of its widespread use by investigators, and not upon the more favorable but more sensitive egg of *Echi-narachnius*, equally familiar to me but not so extensively employed by others. My experience with these eggs as well as with those of four different species of sea-urchins at Naples convinces me that, despite certain structural dif-ferences and some variations in their response to experi-mental treatment—due to habitat, as, for example, temperature and salinity of the sea-water—what I here set forth applies generally to echinid eggs. In order to make comparisons with the work of others who followed the

events of the first cleavage-cycle only, my account will be limited similarly. This record of the happenings in the living egg is supplemented by that obtained through study of the fixed egg.

Let us begin the account of the first cleavage-mitosis in the egg of *Arbacia* with the stage when the two germ-nuclei have disappeared by complete fusion to form a single resting nucleus, a clear vesicle almost spherical in shape lying in the polar axis just above the equator of the egg. At this moment two centrospheres with asters are present at opposite poles of the nucleus; the egg is spherical, oil-drops and yolk-spheres, lying outside of the spindle-area, are evenly dispersed, the pigment granules are trapped at the egg-surface and the hyaline plasma-layer is clearly defined.

In the process of cleavage we shall follow especially these changes: the growth of the centrospheres and asters, the varying shape of the egg, the shifting of the cytoplasmic inclusions and the activity of the hyaline plasma-layer as the nucleus goes through its mitotic cycle. These four changes are easily visible and readily followed in the living egg. In accordance with the principle hitherto followed, I shall endeavor to derive a conclusion as to the meaning of the process by relating the events that can be observed.

The nucleus seen in the living egg does not retain its spherical form; increasing in size it becomes ellipsoid and ruptures at the poles of its long axis where the centrospheres and asters are located. A row of hyaline droplets, the chromosomes, extends across the equator of the transparent ellipsoid nuclear region; soon these form two parallel rows which slowly separate as the ellipsoid nuclear region by a constriction at the equator becomes dumb-bell in shape. As each group of droplets approaches the inner border of the centrosphere (the centrospheres are now hemispheres presenting their plane-surfaces toward the dumb-bell shaped clear region) they become spherical. Suddenly

they are in the centrospheres where they fuse to form five or six droplets; these by farther fusion become one hyaline mass, the resting nucleus.

Concomitantly with this behavior of the nucleus and the movement of the hyaline droplets, the centrospheres steadily increase in size and the astral rays become more extensive until the moment that the hyaline droplets enter the centrospheres. Thereupon the centrospheres diminish in size and the astral rays lose definition and shorten. During their period of growth the centrospheres are spherical; their change to hemispheres marks the beginning of decrease in size.

In the meantime the egg exhibits changes in shape. While the hyaline droplets are moving toward the poles of the transparent dumb-bell shaped area, the egg maintains its original spherical form. With the entrance of the hyaline droplets into the centrospheres, when centrospheres and asters have begun to wane, the egg quickly elongates in the long axis of the transparent region. About one minute later the egg divides by a furrow forming at right angles to the egg's long axis. Thus the egg at first a sphere becomes an ellipsoid which divides and then becomes two spheres.

The site of the future cleavage furrow is marked as early as the stage in which the transparent ellipsoid area is converted into a dumb-bell shape. Already moved by the fertilization-reaction, the various cytoplasmic inclusions, especially the yolk, are further distributed by the growth of the sperm-aster; and later, by the formation and extension of the amphiastral system. The conversion of the ellipsoid area to the dumb-bell shape is caused by the movement of yolk-spheres in the equatorial plane of the egg from the periphery toward the centre. The shifting of the oil-drops can not be easily followed in the living egg. The pigment granules, after fertilization definitely fixed to the outer rim of cytoplasm, at the time of cleavage extend

across the egg in two parallel rows, one on each side of the cleavage-furrow.

Concurrently with these nuclear and cytoplasmic events, the clear peripheral cytoplasm, the hyaline plasma-layer, exhibits changes. During the progress of the sperm-head toward the egg-centre, the hyaline plasma-layer is everywhere of equal width; but with the beginning of the cleavage-process it appears delicately crenated, irregular in contour. The egg at this time being somewhat darker because of closer approximation of the yolk-spheres gives the picture of cytoplasmic contraction, a condition which passes leaving the egg clearer and the hyaline plasma-layer of smoother contour. Immediately prior to the egg's elongation, the hyaline plasma-layer is thinner over the poles of the long axis of the transparent dumb-bell shaped area and seems slightly thicker at the equator, the site of the future cleavage-furrow.[1] With cleavage this thickening apparently increases; actually, the filaments of the hyaline plasma-layer are stretched, because the plasma-membrane to which they are attached does not move inward with the cleavage furrow; they are therefore longer here than elsewhere.

We can confirm and extend these observations on the living egg by study of properly fixed normal eggs. The accompanying figures are from sections of fixed *Arbacia* eggs.

Fig. 33*a* is of an egg with intact nucleus, centrospheres and asters. Fig. 33*b* shows an egg with ruptured nucleus and emerging chromosomes (seen in the living egg as hyaline droplets); here the centrospheres are larger and the astral rays more extended. As the heavily stained chromosomes move apart, the centrospheres and asters increase in size (Figs. 33*c*, 33*d*) finally to reach the maximum

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[1] *Cf. Ziegler, 1904.*

a

b
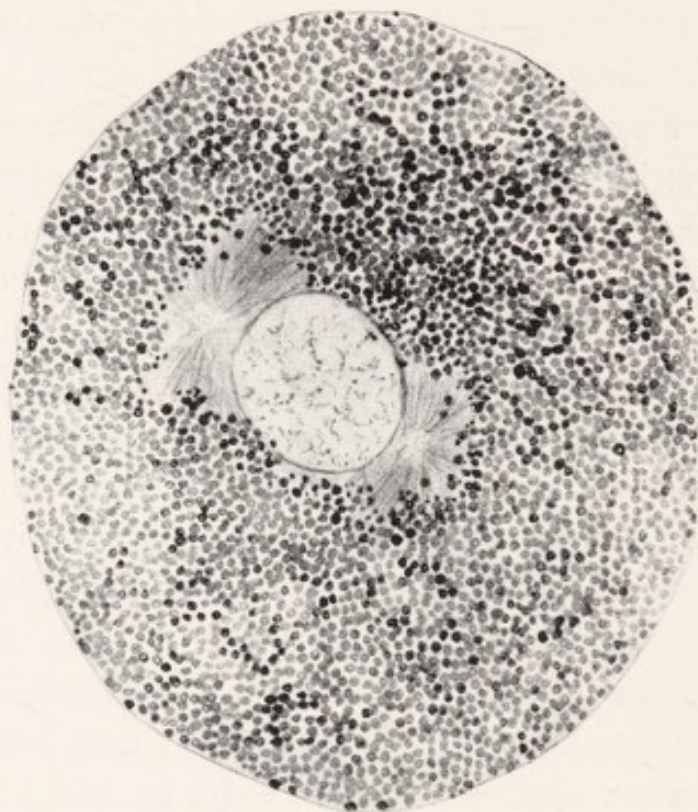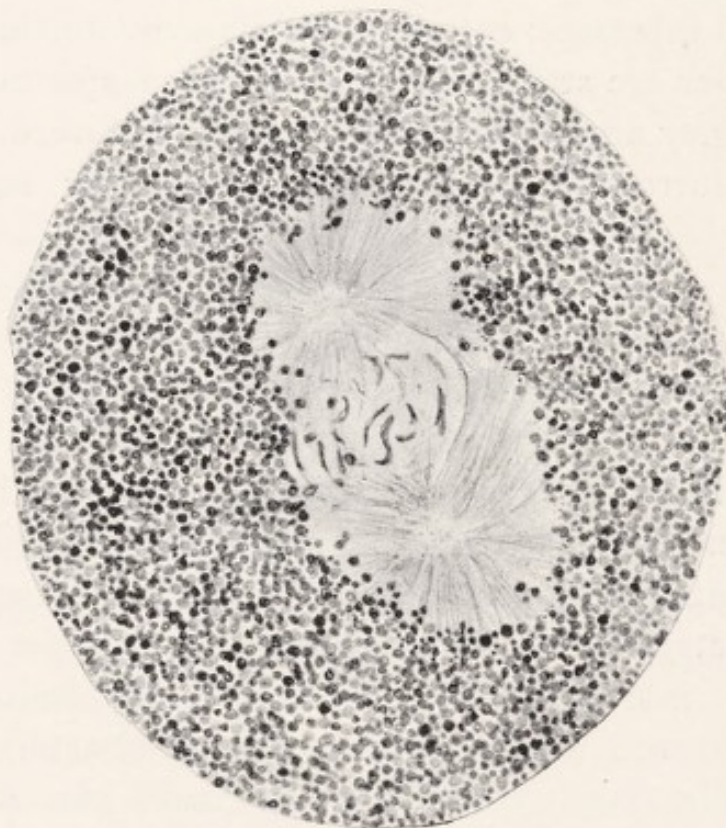
FIG. 33.—For decriptive legend see page 263.

c
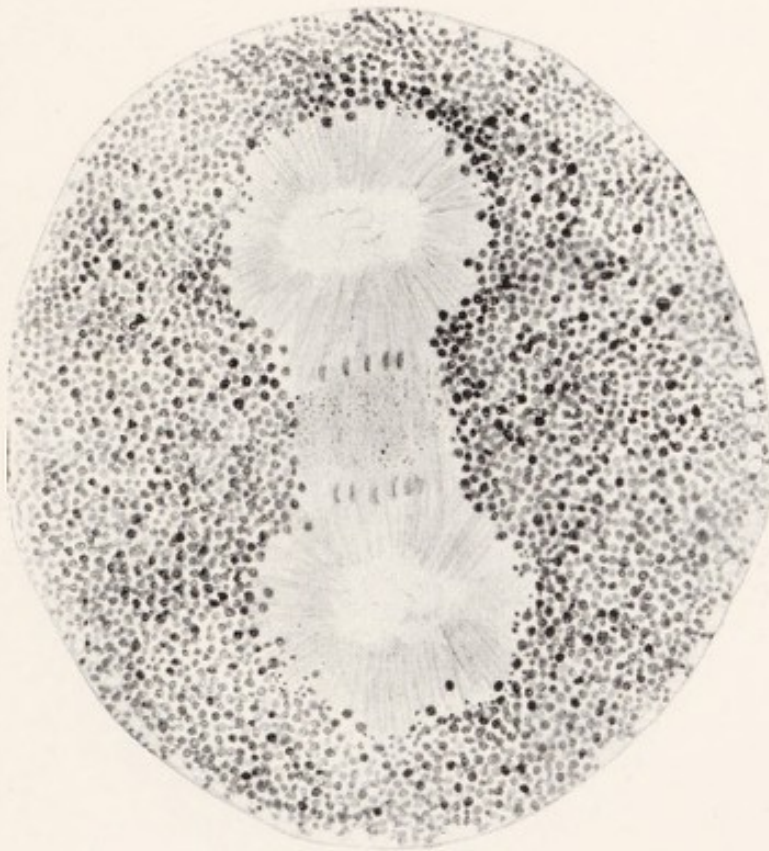
d

FIG. 33.—For decriptive legend see page 263.

e

f
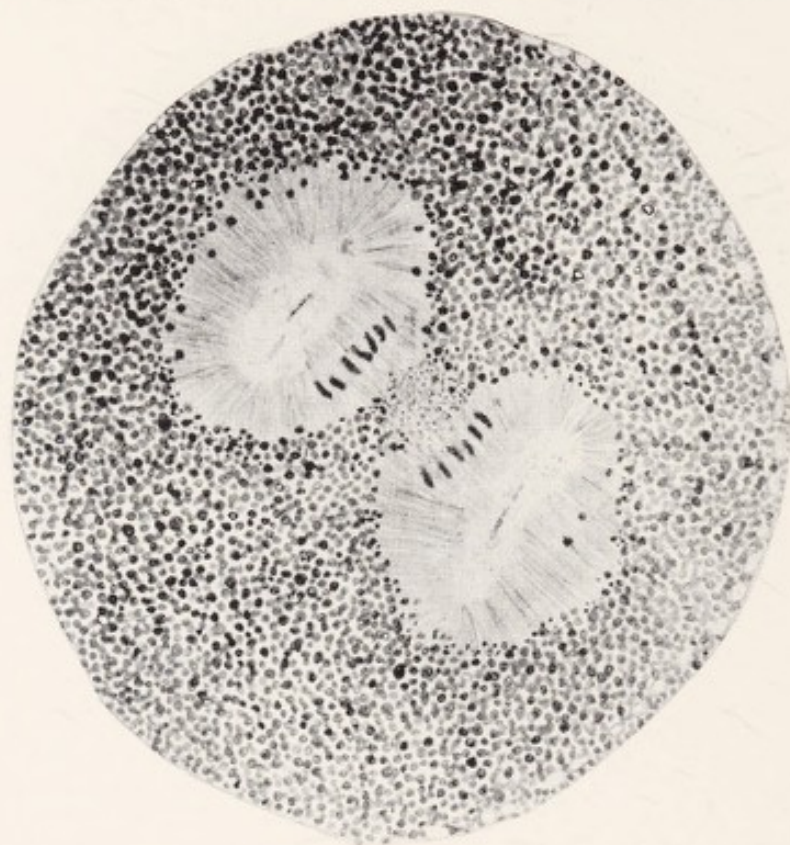
Fig. 33.—For decriptive legend see page 263.

g

h

Fig. 33.—For decriptive legend see page 263.

i

j

FIG. 33.—For decriptive legend see page 263.
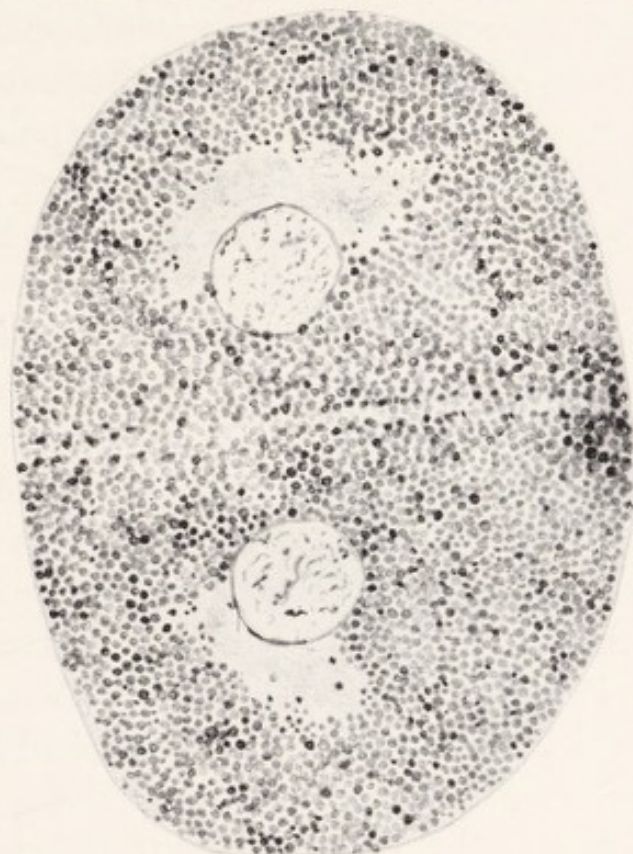
k

F<small>IG</small>. 33.—Stages in the first cleavage-cycle of the egg of *Arbacia*.   (Drawn by Mrs. L. G. Hugueley from author's preparations).

(Fig. 33*e*).   As the variously shaped chromosomes are converted into lightly stained spheres (Fig. 33*f*), they are at the inner border of the now no longer spherical centrospheres; the wide-flung astral rays are losing definition.   When the chromosomes within the centrospheres begin to fuse, centrospheres and asters are on the wane (Fig. 33*g*).   A moment later the egg elongates (Fig. 33*h*); cleavage quickly follows (Fig. 33*i*, *j*, *k*) to give rise to two spheres.

For the sake of simplicity and in order that the reader may not be confused, I omit at this place a description of the evanescent changes in the hyaline plasma-layer. Indeed, the figures that I have given here do not show the layer.   A structure concerning which biologists are at odds, because of lack of knowledge, ought be set off from

the discussion involving better known structures. The changes in the hyaline plasma-layer are therefore taken up beyond, after its structure has been elucidated.

The foregoing description of the changes in nucleus, centrospheres and astral radiations agrees with the accounts given by others for the eggs of several species of sea-urchins. According to all of these the centrospheres and astral rays steadily increase in size until the stage of the telophase whereupon they begin to decrease; later, cleavage occurs. That is, cleavage does not take place when centrospheres and astral rays are at their maximum size. In spite of this mass of well established observations, theories of the division of the cell-body have been fabricated upon the false idea of a coincidence of cell-division with the maximum size of the aster.[1] I do not see any necessity to discuss such theories.

As stated above, this chapter, whilst it deals with cell-division primarily in eggs, has to do with the process in its widest aspects. Thus we are here concerned with all forms of division of the protoplasmic mass. It is to the phenomenon as one widely occurring in all living cells that I wish now to direct attention. I raise the question: Do the phenomena observed in the division-process of sea-urchins' eggs possess general significance for the problem of cell-division? In order to answer this question we must establish those features in the division of the sea-urchins' eggs which have their parallel in the division both of eggs of other species and of other cells, and must eliminate whatever is peculiar to this egg only.

There are other eggs that like the sea-urchins' show that form of mitotic division of the nucleus in which centrospheres and asters are present. But whilst the centrospheres of the first cleavage-figure of eggs of sea-urchins

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[1] *Chambers, 1924; Gray, 1931.*

lack centrosomes, as we have seen in the chapter on the fertilization-process, other eggs, indeed most animal eggs, possess them. The reason for this difference is here beside the point. The difference exists and its existence warns us to be wary in drawing too final and dogmatic conclusions
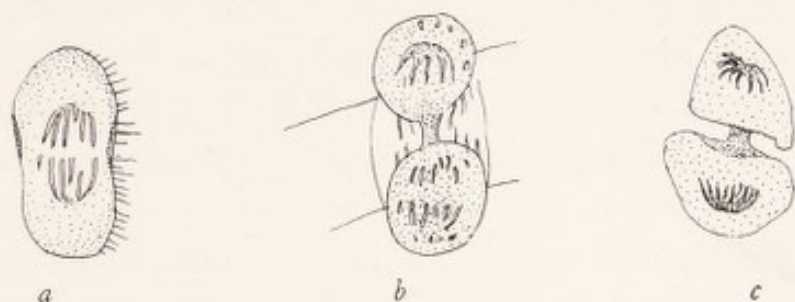
FIG. 34.—Cell-division, living cells of *Triton* (after Peremeschko). Note changes in the ectoplasm.

with respect to these components. Moreover, were we to compare the first cleavage-stage in other eggs with that in sea-urchins', we should find no strict correlation of the appearance of the cleavage-furrow with either the phase of mitosis or the size of the centrosphere-aster complex. When the egg of *Ascaris*, for example, divides, the chromosomes in early telophase are still strongly stained; no such pronounced centrospheres as in sea-urchins' eggs are present. Briefly said, furrowing of the cytoplasmic mass of eggs does not occur always at precisely the same stage of mitosis in all species of them. Nor does it occur uniformly in other animal cells.
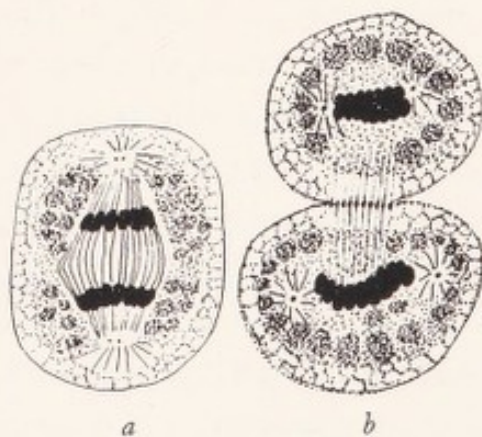
FIG. 35.—Stages in cell-division, spermatogenesis of *Pyrrhocoris apertus* (after Gross).

(Note for example Fig. 34 and Fig. 35.) This is thus my first point: We must be sure by exact observation as to what happens in sea-urchins' eggs; but with this knowledge we are not justified in drawing conclusions concerning division

in cells generally, if we can base these conclusions only on the nuclear behavior and state of sea-urchins' eggs.

Besides, as I stated above, many cells lack asters, many others lack well-defined centrospheres though they possess asters. Certain eggs, such as those of *Ascaris*, of *Ciona*, of *Phallusia*, of *Amphioxus*, etc., have no asters at the spindle-ends in the maturation-divisions. Yet they divide. Many plant-cells go through mitotic division; their mitotic figures are without asters. Asters and centrospheres are not essential for mitotic divisions.

Also we should not forget that in many eggs repeated mitotic divisions ensue without cleavage of the cytoplasm. Take eggs of insects, for example: many nuclear divisions ensue before the cytoplasm divides. Nuclear division without cleavage of the cytoplasm is not limited to eggs. It obtains among unicellular animals and among multicellular, including man, and among plants, in such protoplasmic systems that contain many nuclei, the syncytia. That nucleus and cytoplasm may divide a-synchronously constitutes the strongest evidence against the proposition that in mitosis lies the cause for the sundering of the cytoplasmic mass.

Finally, an explanation of cell-division founded upon the mitotic complex as a whole or upon any component of it, falls to the ground for it can not encompass division of cells whose nuclei divide amitotically. Whatever the biological significance of amitosis, be it a primitive or a degenerate process, undubitable cases of amitosis are on record. Whatever the conditions responsible for its occurrence, we must reckon with this as fact. Even if the mitotic complex were always uniform and cell-division with mitosis were identical in all cells, we still could not explain by reference to mitosis the division of the cell-mass where amitosis prevails.

It becomes obvious in the light of what has been said that nuclear and cytoplasmic division are separate phenomena.[1] Common usage has been loose in giving the term, cell-division, the meaning of the division of the nucleus. The statements made above, however, emphasize that we must define cell-division more clearly and more exactly in order to avoid the building up of theories on improper grounds. Cell-division is to be defined as the division of the cell-body. In what follows I shall adhere strictly to this clear definition.

Finding it impossible to relate division of the cytoplasmic mass to the nucleus, we turn to the cytoplasm itself.[2] Here we may recall three visible events, accompanying the division-cycle in eggs: changes in shape of the egg, movement of the cytoplasmic inclusions, and changes in the behavior of the hyaline plasma-layer. The second and third are not peculiar to eggs and will be discussed in detail. Change in shape not being invariable is not given great emphasis apart from its being a factor in surface-tension-theories of cell-division taken up beyond. I speak first of the movement of the cytoplasmic inclusions.

Movements in the cytoplasm constitute a widely occurring phenomenon, some cells exhibiting them to an extreme degree. The simplest type is Brownian movement, so extensively studied by physicists in inanimate systems. This can be beautifully demonstrated in many cells especially by means of the darkfield in which the suspended particles appear as bright points which shimmer on the black background. Quite different from this phenomenon, which is not peculiar to living cells, are the cyclical movements in cells—often described for plant cells—a streaming

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[1] *Watase, 1891.*
[2] *Cf. Delage, 1895, p. 759.*

that always follows a definite path or along preformed channels; these movements represent specialized conditions. Many cells, especially egg-cells, exhibit streaming which is rhythmical but follows no preformed channels. This streaming can be observed in egg-cells particularly if they contain easily visible formed bodies or if having been bathed in a solution containing a non-toxic dye they took up particles whose movements can be easily followed.

The best of the earliest descriptions of this type of movement in cytoplasm is that by Goette.[1] Since his time many workers, among them von Erlanger,[2] have studied the phenomenon. In living eggs one can of course follow these changes from minute to minute. So-called resting eggs—i.e., those that remain in a given stage, as for instance mature but unfertilized eggs—do not show this type of movement. If such mature resting eggs be fertilized, then the cytoplasm begins to stream. During each cleavage-cycle there is a period of little movement and a period of intense movement; these periods appear to parallel the stages in the cycle of nuclear changes.

With the growth of the asters these movements become most marked. They begin over the spindle poles and move toward the spindle equator. As the opposing streams meet, they have only one possible direction: they can not move outward because of the resisting surface and thus move towards the centre of the egg. In this way the currents foreshadow the future cleavage plane; before the actual division of the cell-body the currents have brought about that disposition of inclusions found when the plane forms.[3]

Before fertilization the inclusions maintain constant positions without reference to their specific gravity. If in such eggs they are segregated by means of centrifugal force,

[1] *Goette, 1875.*
[2] *Von Erlanger, 1897.*
[3] *Cf. Goette, 1875, loc. cit.*

they return in time to their original positions. The oil is the last to return, I find, while the heavier yolk-spheres return first and after them the heaviest bodies, the pigment granules. After normal fertilization the inclusions shift positions and come to a new arrangement which again has no relation to their specific gravity. Thus, in the egg of *Arbacia* after fertilization the heaviest inclusions, pigment granules, remain close to the surface; the oil, shifting back and forth fairly even in distribution except as influenced by the mitotic spindle, forms clusters which mass and break up again;[1] the yolk is evenly distributed outside the spindle area. The behavior of the pigment granules is due to the fact that they are trapped at the surface; they move only as the cleavage furrows are formed. In some eggs, as those of *Nereis*, the yolk and oil come to be massed and are definitely distributed always to certain cells. These facts lead us to assume that the flow in the cytoplasm is not everywhere the same and that there are currents which are circumscribed and limited to definite areas of the cell. If there were only one current of equal velocity in a medium everywhere the same, then we should expect a distribution of particles according to specific gravity, as is for instance the case in the blood-stream in a blood-vessel.

The fact that cytoplasmic streaming in eggs is most expressed directly following the ectoplasmic changes, which result from the fertilization-reaction, gives rise to the suggestion that the ectoplasm by its activity sets up a condition which increases the currents in the cytoplasm. That increased fluidity of the cytoplasm might be brought about by the escape of substances from the nucleus is a less likely assumption for the cause of the increase in streaming. It is true that streaming is not easily discerned in an egg like that of *Nereis*, fertilizable before maturation, until after break-

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[1] *Just, 1927a.*

down of the germinal vesicle; but before this break-down come the intense ectoplasmic changes incident to fertilization. In an egg like that of *Chaetopterus* after the germinal vesicle breaks down the spindle goes to the metaphase and comes to lie at the periphery before fertilization; although this translocation of the spindle indicates streaming in the cytoplasm, this soon diminishes; most intense streaming is resumed after fertilization. The cytoplasm of the unfertilized sea-urchin egg when ripe for fertilization is as fluid as any that I know, the germinal vesicle having broken down some time before in the ovary, but cytoplasmic streaming of measurable degree is demonstrable only after fertilization. Moreover, this cytoplasmic streaming is rhythmical: during cleavage the tides ebb and flow. And always ectoplasmic activity heralds their flood. It may very well be that in all cells ectoplasmic behavior as a response to changes in the environment initiates cytoplasmic streaming.

In eggs so far used in the study of the currents in cytoplasm the two streaming movements from poles to equator by opposing each other seem to bring about cell-division. Theories concerning their cause have not been wanting, the chief of which is that the currents are due to surface-tension.

The theory that the division of the cell-body is caused by changes in surface-tension is upheld by many investigators. Some maintain that the cell divides because of increased surface-tension over the spindle-poles, whereas just as many workers are certain that the increase is at the equator. In many quarters what is considered the strongest proof that the division of the cell is due to changes in surface-tension comes not from observations and experiments on the cells themselves but from study of oil-drops suspended in water or solutions. It is a curious fact that here too the theorists are not agreed: some contend that an oil-drop divides because of increased surface-tension at the

equator, whilst others insist that the increase is over the poles. Despite this lack of accord concerning the efficacy of the infallible principle of surface-tension in the division of oil-drops, to say nothing of the cells themselves, the surface-tension theory persists.

More than once I have protested against the widespread misuse of surface-tension in biological explanations and theories.[1] Drops of oil in water or solution constitute a liquid-liquid system; a suspension of eggs does not. Whilst every oil-drop is a homogeneous liquid, each egg, far from being homogeneous, is itself a suspension of oil-drops, yolk-spheres, etc.—of materials of different chemical structure and physical make-up in cytoplasm, the living continuum, itself heterogeneous. The surface of an oil-drop is a film of molecular dimension chemically identical with the interior of the drop; the measurable surface of an egg is thousands of times the width of the film of an oil drop and is a differentiated structure built by the egg during its development as a germ cell. The fact that one can induce streaming in oil-drops which closely resembles cytoplasmic streaming does not warrant the conclusion that since an oil-drop divides because of changes in surface-tension, division of the egg or other cells is likewise due to changes in surface-tension. Models used in biology to prove the cause of vital phenomena may be interesting but after all they are literally only models—imitations of the real thing and of little value for the analysis of the conditions in living substance.

Equally weak is the support for the surface-tension theory derived from experiments which demonstrate that during the cleavage-cycle, eggs of sea-urchins reveal a rhythm of

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[1] *Herčik (1934) entered a strong protest against the indiscriminate adducing of surface-tension for explaining biological processes. In his opinion the significance of surface-tension is often over-estimated.*

resistance and susceptibility to certain experimental agents. As I pointed out in the chapter, General Properties of the Ectoplasm, unfertilized eggs in hypotonic sea-water disintegrate more slowly than eggs which are exposed to the same degree of hypotonicity when after insemination break-down in the ectoplasm takes place. This period of ectoplasmic changes having passed over, the eggs are more resistant than unfertilized ones. According to many observers this resistance persists until just before or at first cleavage when the eggs become susceptible. It is held by some that this susceptibility is due to increased surface-tension over the spindle-poles.

Now sea-urchins' eggs, as we have seen, are first spheres, then ellipsoids and finally, by cleavage, two spheres. Does the supposed change in surface-tension take place while the egg is still a sphere, when it becomes an ellipsoid or as it forms two spheres? Or does the theory disregard entirely that stage during which the egg elongates in the direction of the spindle-axis? The theory demands the most exact fixing of the moment in the cleavage-cycle when the egg reveals its maximum susceptibility in order to relate this to the act of cleavage. Observations made too far apart on a lot of eggs developing at the same tempo could easily miss the moment of maximum susceptibility. If, on the other hand, closely set observations be made on poor eggs developing at varying rates and therefore not reaching first cleavage at the same instant, the moment of maximum susceptibility would be erroneously fixed as coming in the stage or stages of the intact eggs present whilst the disintegrated ones would be in other stages.[1]

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[1] *Degree of hypotonicity should be great enough to give sharp results; transfer of eggs should be uniform as to number of eggs, amount of dilute sea-water and amount of normal sea-water into which eggs are transferred.*

In the chapter, General Properties of the Ectoplasm, was mentioned the method of study of the susceptibility of eggs by putting them in solutions of dilute sea-water. In the experiments of R. S. Lillie[1] 40 per cent. sea-water and 60 per cent. tap-water gave a solution that destroyed a percentage of the eggs at once, others later. In my experiments[2] on the rhythmical susceptibility of eggs of sea-urchins to hypotonic sea-water during a cleavage cycle, I modified this method. Instead of exposing the eggs to a solution made up of 40 parts sea-water and 60 parts tap-water, I used much more dilute solutions, mostly one made up of one part sea-water and 90 parts tap-water. On eggs of *Echinarachnius* I have also used 10 cc. of tap-water to one drop of a sea-water suspension of eggs, an even greater dilution. The value of this method lies in the sharp results that it furnishes.

I do not wish to imply that I discount the worth of the 40 per cent. sea-water dilution as a means of revealing the susceptible periods. On the contrary; for I appreciate the fact that because it destroys eggs exposed to it during such periods, whilst it does not immediately arrest development of eggs exposed during their resistant periods, it is an excellent experimental means. I have used this dilution as well as others successfully; but I have found that its use should be checked by that of the greater dilutions mentioned above, for the reason that eggs exposed to lesser during terminal stages of resistance prior to the onset of the susceptible period might develop into this period. In such cases the eggs do not break down in the stage which they had reached at the moment of exposure. The dilutions therefore should be so great that they halt development abruptly; then the time in seconds from the moment

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[1] *Lillie, R. S., 1916.*
[2] *Just, 1922e; 1928d.*

of exposure to disintegration can be attributed wholly to the dilution's destructive action and not in part to a possible anesthetic action.

The use of extremely dilute solutions was not my only modification of method. I exposed the eggs during a cleavage-cycle at the short intervals of one and two minutes. Thus, I was able, using always eggs developing at the same tempo, to define with sharpest exactness the briefly enduring periods of susceptibility which otherwise would have escaped observation.

I think that there can be little doubt that my experiments did more than confirm earlier ones which proved the existence of periods of resistance and susceptibility during the cleavage-cycle of the sea-urchin egg; they demonstrate exactly the onset and duration of the period of susceptibility. The significance of my findings for a theory of cell-division needs no elaboration. If we wish to base a theory of the cause of cleavage upon the occurrence of rhythmical resistance and susceptibility parallel to the rhythm of cell-division, we first of all need accurately to relate the susceptible period to a definite stage in the process of cell-division. One finely spun physicochemical theory of cell-division falls to the ground because its author failed to time precisely and to relate definitely the onset and duration of that period of susceptibility which precedes the appearance of the cleavage-furrow.[1]

Perhaps the simplest way to begin the discussion of my findings is to present the data of a typical experiment, on the egg of *Arbacia punctulata*.[2] Accordingly, these data in the form of a table (Table III) are herewith given. The reader will note: First, there are two periods of susceptibility

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[1] *Lillie, R. S., 1916.*

[2] *The egg of Echinarachnius which is less resistant than that of Arbacia reveals much more sharply the periods of susceptibility.*

during the cleavage-cycle of the egg. It is the second, the greater, which comes prior to the appearance of the cleavage-furrow, with which we are primarily concerned. Secondly, during both periods the egg is spherical. The change in form from spherical to ellipsoid occurs once only during a cleavage-cycle; it marks the end of the period of greater susceptibility. These two findings possess especial significance for an interpretation.

TABLE III.—THE RATE AT WHICH EGGS OF *Arbacia* DISINTEGRATE IN DISTILLED WATER AT INTERVALS AFTER INSEMINATION*

No.	Time, in minutes, after insemination	Time, in seconds, to complete disintegration	Comment
1	3	240	
2	6	180	
3	10	60	
4	15	60	
5	17	60	
6	18.5	65	
7	21	80	
8	23.5	75	Streak indicated
9	26	70	
10	27.5	60	
11	29	60	
12	31	60	
13	33	25	
14	35	25	Streak
15	37	30	
16	39	60	"Dumb-bell stage"
17	41	100	
18	43	60	
19	45	60	
20	47	20 ⎱	Eggs burst over spindle pole
21	48	20 ⎰	
22	49	60	Eggs elongate
23	51	60	Cleavage furrow indicated
24	53	120	Cleavage

* Eggs of *Arbacia* are inseminated, and at intervals after insemination 5 drops of them are mounted under the microscope in 15 cc. of distilled water. The first column gives the number; the second, the time after insemination; the third, the time to cytolysis; and the fourth, the stage of the egg at the time of exposure.

As regards the first named finding, that there are two periods of susceptibility in a cleavage-cycle, I should like to point out that an interpretation based on the occurrence of the second which overlooks the first is difficult to defend. If susceptibility as such is the cause for cleavage, then the egg should cleave after each such period. This is not the case. Further, it must be remembered that in many eggs the most pronounced period of susceptibility occurs within a minute after fertilization—that is, depending upon the species of egg, anywhere from $\frac{1}{48}$ or $\frac{1}{120}$ of the total time to first cleavage, at a time long before the onset of the first cleavage-cycle which, as stated above, begins only with the stage of apposition of the egg- and sperm-nuclei. Also if we attempt to correlate the susceptibility with nuclear phenomena, we find ourselves at a loss. During this period of susceptibility immediately following fertilization, neither the sperm-nucleus nor the egg-nucleus is in active mitotic condition. In that period of susceptibility which occurs first in a cleavage-cycle, the apposed nuclei are not in mitosis. Thus only in that susceptible period immediately prior to cleavage is there present an active mitosis. Then susceptibility does not depend upon or run with a definite stage in mitosis. It follows from this that all the learned disquisitions relating the origin of the period of susceptibility to the nuclear state and more specifically to the behavior of the asters have no basis in fact.

Whilst it is true that the periods of susceptibility can not be correlated with mitotic phenomena, they can be with ectoplasmic state or behavior. At the first period of susceptibility that appears during a cleavage-cycle, the ectoplasm manifests peculiar behavior as it does during the period of susceptibility immediately prior to the act of cleavage. The greatest observed susceptibility is that occurring immediately after fertilization; in this stage the

egg exhibits the strongest ectoplasmic changes ever shown in its development. The periods of susceptibility thus can be related to structural and behavior-changes in the ectoplasm. No other constant change being associated with the period of susceptibility than the visible and easily demonstrable ectoplasmic state, we can assume that ectoplasmic state is wholly or in part responsible for the temporary lowered resistance of the egg to dilute sea-water.

Not only did I fix the period of maximum susceptibility of sea-urchins' eggs during their cleavage-cycle to hypotonic sea-water; I was able also to localize precisely the point on the eggs at which they break down during this period. Properly to appreciate this latter finding, we need fully to understand the structure of the hyaline plasma-layer. This, part of the ectoplasm, though often the subject of discourse by many writers, has never been properly understood. I give therefore a detailed description of the origin and structure of the hyaline plasma-layer in sea-urchins' eggs, thus supplementing the description given in the chapter on the ectoplasm.

Selenka[1] years ago described the surface of various echinoderm eggs as composed of a sheath of clear cytoplasm. Later Hammar[2] and others also described this external layer on fertilized sea-urchins' eggs. It is variously known as the ectoplasmic layer, hyaline plasma-layer, Hammar's layer, etc. It should not be, as it often is, confused with the vitelline membrane.[3]

I have found that in several species of sea-urchins this hyaline plasma-layer arises in the same way and has much the same structure as described for the fertilized egg of *Arbacia*. Into the perivitelline space which arises with separation of the vitelline membrane fine filaments of the cyto-

[1] *Selenka, 1878, 1883.*

[2] *Hammar, 1896, 1897.*

[3] *Just, 1930g, 1933c.*

plasm project.[1] By anastomosis of their free ends a very fine covering membrane forms.[2] Thus the hyaline plasma-layer is clear granule-free cytoplasm in the form of finely spun threads covered by a thin membrane. Other eggs, as shown in the chapter, The Ectoplasm, possess these ectoplasmic filaments. They are present on every marine egg that I know. Unfertilized sea-urchins' eggs treated with strong hypertonic sea-water also show that the surface-cytoplasm is made up of radial strands. Eggs of *Nereis* and of *Platynereis* show especially well after fertilization immediately below the vitelline membrane a delicate plasma-membrane, which is the covering film of cytoplasmic prolongations. It is these prolongations, of greater length and diameter than those of sea-urchins' eggs, which give the surface of the eggs of these worms their striated appearance. These prolongations constitute the outer region of the ectoplasm. They and their covering film compose the hyaline plasma-layer of the egg.

Others before me have described on the fertilized egg in later stages this layer as made up of filaments. Meves,[3] for example, has pointed out that some time after fertilization the layer is composed of threads and a thin covering membrane. This structure of the hyaline plasma-layer can be demonstrated by placing fertilized eggs in calcium-free sea-water; the covering membrane becomes destroyed leaving the filaments free.

During the whole cleavage-period, sea-urchins' eggs show on the surface of each blastomere these fine cytoplasmic projections covered by a delicate membrane which together make a continuous film enclosing the blastomeres. The filaments are most easily seen between adjacent blastomeres as they are separating at cleavage. Since there is no

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[1] *Cf. also Berthold, 1886, and Théel, 1892.*
[2] *See also Mrs. Andrews.*
[3] *Meves, 1912.*

great decrease in width of the hyaline plasma-layer as cleavage progresses and since by treatment with calcium-free sea-water the presence of the filaments can be demonstrated on eggs throughout cleavage, we reach the conclusion that the egg builds new hyaline plasma-layers constantly. One can not say exactly where in the cytoplasm the hyaline plasma-layer begins, for each filament, the essential structure of the layer, is a granule-free prolongation of the cytoplasm. The activity—amoeboid, spinning, and contractile—of the hyaline plasma-layer, so well described by Mrs. Andrews as early as 1897, is due to the behavior of these continuations of the egg-plasma. The filaments of the hyaline plasma-layer are a living part of the living protoplasmic system (see also Fig. 36).[1]

As a living and integral part of the egg, these filaments, we should expect, react as the cytoplasm of which they are continuations. Because of their tenuous and granule-free structure they are physiologically different from the remainder of the egg. However, any very striking difference in their physical behavior needs to be fully established. It was claimed by Goldschmidt and Popoff[2] first and later by Gray[3] that hypertonic sea-water increases the volume of the hyaline plasma-layer and decreases that of the egg and thus reveals a marked difference between the osmotic properties of the surface-cytoplasm and the remainder of the egg. If this interpretation were correct, I would call this difference in osmotic properties—what Gray calls a fortunate coincidence—a most important discovery. We should use every available fact concerning the behavior of colloids in living protoplasm. Is it true that the hyaline plasma-layer increases in volume whilst the remainder of

[1] *Ectoplasmic protrusions on living dividing cells have been early described by many workers.*

[2] *Goldschmidt and Popoff, 1908.*

[3] *Gray, 1931.*

the egg decreases because "the former is freely permeable to electrolytes whereas the latter is not"?[1]   The answer is simple: the existence of such a difference has actually not been proved.

The correct interpretation of the changes in sea-urchins' eggs induced by means of sea-water made hypertonic by the addition of either electrolytes or non-electrolytes is as follows: placed in hypertonic sea-water, the whole egg, including its ectoplasmic filaments, loses water and shrinks.   The
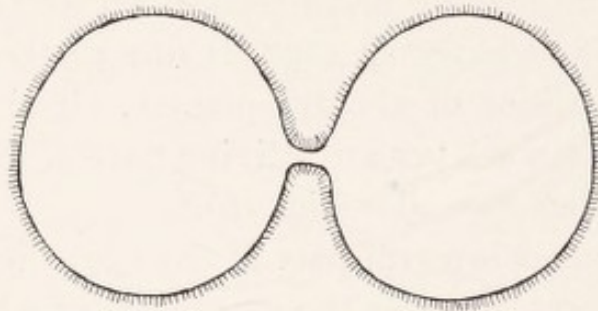
Fig. 36.—Cleavage of an egg of *Echinus microtuberculatus* in calcium-free sea-water (after Herbst).   The covering membrane of the ectoplasm is destroyed, leaving the filaments free.

filaments decrease in diameter and appear as lines thereby becoming more easily visible.   In every egg known to me, the ectoplasmic filaments appear in the same way as an effect of hypertonicity.[2]

We can not escape these facts: that after fertilization and with membrane separation the surface of the egg is studded with filaments; and that also unfertilized eggs as they shrink in hypertonic sea-water show these filaments.   It is patent, therefore, that the filaments are not new formations called forth by the action of electrolytes.   The action of electrolytes does not demonstrate a difference in permeability between endoplasm and ectoplasm.

Gray also errs when he says that the hyaline plasma-layer dissolves in calcium-free sea-water.   Rather, eggs in this

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[1] *Gray, l.c.*

[2] *Cf. Fauré-Fremiet.*

medium show the filaments very beautifully as the figure taken from Herbst so clearly shows[1] (Fig. 36). Herbst himself thought that he had altered the structure of the hyaline plasma-layer, changing its homogeneous structure to a collection of radial threads whereas actually by the treatment with calcium-free sea-water, he only removed the covering membrane of the threads. This change in the eggs' environment prevents the formation of a new membrane which takes place when the eggs are returned to normal sea-water. The so-called removal of the hyaline plasma-layer by microdissection is also a removal only of the covering membrane.

Goldschmidt and Popoff advanced the idea that the ectoplasmic filaments on the sea-urchins' egg are due to astral radiations from the nucleus. But there are no astral radiations in an unfertilized egg momentarily subjected to the action of strong hypertonic sea-water; nor are any present in an egg fifty seconds after insemination. In both cases, filaments can be observed. Fertilized sea-urchins' eggs shrink slightly when the astral rays are first most extensive so that the eggs' surface appears somewhat crenated because the filaments stand out strongly. It is this crenation that these authors observed.

Gray and Goldschmidt and Popoff have derived theories of cell-division from their observations on the behavior of the hyaline plasma-layer. Since these observations are incorrect, the interpretation given them is unwarranted. This is thus another example of the danger of applying physico-chemical notions to a biological process in the absence of knowledge of the underlying structural condition.

During a cleavage-cycle of sea-urchins' eggs the ectoplasm undergoes changes. Until the stage[2] when the

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[1] *Herbst, 1899.*
[2] *See Ziegler, 1904. Compare Ziegler, 1898, on egg of Beroë.*

chromosomes are at the anaphase and the egg is still spherical, the hyaline plasma-layer is everywhere of equal width and the filaments in it are all the same length. In later anaphase or early telophase the ectoplasm shows a rapid change, moving from over the spindle poles toward the equator, the site of the future cleavage-plane, in an amoeboid or wave-like fashion. The ectoplasm then begins to move inward toward the egg-centre, in the direction of the course of the cytoplasmic currents, and the egg elongates. As the ectoplasm continues to move inward, the covering film of the hyaline plasma-layer resists. Thus the moving ectoplasm exerts a pull on its filaments in the region of the oncoming cleavage plane so that they are longer here than elsewhere. With the beginning of the moving of the wave of amoeboid changes from the area over the spindle poles to the equator, the filaments in the former region appear to lose connections with their covering membrane, whilst those in the latter seem more firmly bound to it.

By the very simple method of treatment with extremely dilute sea-water, I was able to ascertain that while the egg is still spherical and when the ectoplasm exhibits the wave-like movement from the circumpolar areas to the equator, it has definitely localized weaknesses in this, the most susceptible, period of its cleavage-cycle. At this time the egg breaks down in about ten seconds after immersion in the dilute sea-water by an outflow from the areas over the spindle-poles. Though eggs of some species of sea-urchins show this localized disruption more clearly than others, it can be observed in all; none ever show an exudation elsewhere than from the circumpolar areas. With elongation the egg is again resistant.

As we have seen, the eggs' period of greatest susceptibility to hypotonic sea-water occurs during the process of membrane-separation. At this time the ectoplasmic filaments appear more sharply because of break-down of material

at the surface of the egg. Though this susceptibility is not of immediate concern here, since it does not relate to the cleavage-cycle, we adduce it since it shows susceptibility as due to ectoplasmic change. In the first period of susceptibility which occurs during the cleavage-cycle and which falls in with the stage of union of the egg- and sperm-nuclei, when there is a normal shrinkage of the egg-contents and the ectoplasmic filaments are most easily visible, the egg does not disrupt at definite points. In some other eggs, those of *Crepidula*,[1] for example, it seems that the weakness that appears at this time or soon after, persists. In the period prior to cleavage, there are localized points of weakness due to ectoplasmic activity; they are limited to the egg-poles. In all periods, susceptibility is due to changes in the ectoplasm and where these are local, break-down of the egg is likewise localized.

In none of these three periods of susceptibility can the break-down of the egg be ascribed to changes in surface-tension. It is not possible to regard the complex structure of the ectoplasm with its palisade arrangements of filaments and their covering membrane as a simple surface-film. The width of this covering membrane alone surpasses by far that of a molecular film. Besides, this membrane is passive in cell-division; ectoplasm and filaments play the active rôle, changing from moment to moment first in one region and then in another. These changes in structure and behavior parallel the periods of susceptibility.

The ectoplasm of marine eggs is a highly mobile, constantly changing structure. In eggs as in other cells, protozoan, nerve, muscle, etc., it is no mere inert sheath. Instead, it reflects in its spinning, in its suceptibility, the irritability of the living system in response to environmental changes. In addition to the significance that its general

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[1] *Conklin, 1912b.*

properties have for cell-life, the ectoplasm by its behavior as here described in the cleavage-cycle, plays a definite rôle in the process by which the cytoplasmic mass becomes two cells.

When a cell divides, the plane of cleavage arises either from within the cell-mass and extends outward, or from the periphery and extends inward. The former mode, cleavage by formation of the so-called cell-plate, is frequently met with in plant cells. Division of animal cells takes place by a furrow extending from the periphery inward. Cleavage by formation of a cell-plate in animal cells is only seldom found and is a modified process occurring together with furrowing. The problem of cell-division for animal cells is that of the origin of the furrow which separates the dividing cells. The phenomena observed during the process of furrowing in the living sea-urchin eggs by which the single cell becomes two we summarize as follows:

Currents in the cytoplasm move toward the site of the future cleavage-plane; meeting there the opposing streams they move inward. The ectoplasm, in whose behavior lies the cause of streaming, then actively shifts by amoeboid movement from the circumpolar areas toward the equator. With this the egg quickly elongates. The ectoplasm moves then inward along the plane pre-delineated by the cytoplasmic currents. Cleavage in a sea-urchins' egg is therefore accomplished by active movement of the ectoplasm.

Neither cytoplasmic movements nor changes in ectoplasmic behavior are confined to animal eggs. Cytoplasmic streaming has been described in plant cells, especially by Ewart. Movement in the cytoplasm of Protozoa is well known. Changes in shape and in activity of the ectoplasm are similarly familiar from descriptions made by the earlier workers on cells. Recently, investigators using the method of tissue-culture have, particularly by means of the cinematograph, recorded the evanescent changes at the cell-

surface which accompany cell-division. They are also well defined in other living cells when freshly dissected out of the organism. We need not, therefore, confine our explanation of cell-division to eggs of sea-urchins. We dismiss as cause for cell-division division of the nucleus, by mitosis with or without asters, and by amitosis, whether occurring simultaneously with cytoplasmic division or not, since no constant relation between nuclear division and that of the cell-body can be established. Since not all cells are spherical and not all spherical ones elongate during the cycle, we dismiss also elongation of the cell as a factor. Movement in the cytoplasm depends upon ectoplasmic activity; it is thus not a primary factor. There remains the ectoplasm found in all cells.

Cells do not extend indefinitely into space. They possess surfaces. Cell-division means that one cell becomes two by the rise of a new partition. Does this arise within the cytoplasm or does it come about through the extension of the ectoplasm inward? Until we know more concerning the origin of cleavage by cell-plates, we may only hazard conjectures as to the formation of cell-walls within the cytoplasm as found in plants. For animal cells, generally, we must seek the cause of cell-division in ectoplasmic activity.

Cleavage and Differentiation

THE ESSENTIAL PROBLEM OF ANIMAL DEVELOPMENT LIES in the question: How does the egg, a single cell, become an adult organism? If one take the transparent eggs of a common marine fish like the mackerel which float like bubbles on the surface of the sea, one can easily follow their development under low power of the microscope. Before fertilization a thin film of cytoplasm beneath the membrane encloses a core of yolk and oil. With fertilization this cytoplasm flows to one egg-pole to collect there as a disc.[1] This disc is next crossed by one furrow and then by another at right angles to it; so by this cleavage two and later four cells arise. Cleavage progresses until many cells form, whilst some of the yolk beneath the cytoplasmic disc is transformed into cytoplasm. Soon one discerns an opaque line running the length of the disc. Here there are more cells than elsewhere, hence the opacity; from some of these will come the future embryo. Under the microscope even one who has very little knowledge of the development of eggs can follow the origin and formation of the nerve tube out of which the brain and spinal cord emerge. One looks, as through an open window, at the very mystery of life, wondering at the heartbeat, first uncertain and then in definite, sure rhythm; the bright red blood moving in jerks with each beat of the heart; the first spasmodic muscular twitch; the appearance of the purple-black eye-pigment; the definite fish form; the color of the skin which will give

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[1] Cf. Ransom, 1854.

the markings that make the adult mackerel one of the most beautiful creatures of the sea.

There are other eggs, those of ascidians, which like the mackerel belong to the highest group in the animal kingdom, that hatch as swimming larvae in eight hours after fertilization instead of seventy-two hours, the time the mackerel egg in a warm laboratory requires. From egg to complex animal in eight hours! The problem of the development from a single cell to a complex animal is thus a fascinating one.

In the following exposition we shall see again that exact description of the process which we aim to explain plays a chief and significant rôle. Whilst here as with many other problems in biology we must realize that a description does not explain the process described, nevertheless description is the prerequisite of a successful attack on the problem of development, a problem which doubtless more than any other in biology is complicated by the interplay of simultaneously occurring reactions and by rapidly succeeding events. To determine cause and effect in this manifold process of differentiation is a task whose difficulty is so manifest that we must before all else seek to follow the process as exactly as possible in closely set stages. We may then be able to relate smallest changes in form and in expression to general phenomena of development, that is, to the differentiation which accompanies the cleavage-process. Such a task is undertaken in this chapter.

Following the classification of Karl Ernst von Baer, one can separate the development of fertilized or parthenogenetic animal eggs into four periods: cleavage, formation of the germ-layers, development of the organs and histological differentiation. This classification represents a scheme which holds more nearly true for the higher animals than for the lower and should not be taken as meaning that the development of any egg is sharply divisible into fixed

periods.   Development is a catenary process of overlapping stages and can not be categorically separated by rigid lines. With periods three and four, which have to do with the establishment of the organs and the finer differentiation within them, we have here no concern.   Period two includes the formation of the primary sheets of cells, the ectoderm and the endoderm in the lowest multicellular animals, the sponges and the coelenterates, and the ectoderm, endoderm and mesoderm in all the other multicellular animals. Period two represents the end-result of period one.   It is in this first period, cleavage, that our problem lies.   And this for the following reasons:

Coming first, this differentiation during cleavage stands nearest to the condition in the egg at the moment of fertilization, a moment that forms in time the limit between the condition of the egg as single cell and those conditions of the egg as multiplying cells which we call the differentiation during cleavage.   Hence studying the period of cleavage, we approach the source whence emerge the progressively branched streams of differentiation that end finally in almost quiet pools, the individual cells of the complex adult organism.

But just as the source of a river though single may not be simple but compounded of rivulets, so the fertilized egg though single is not simple, being itself a complex of many contributary streams of differentiation.   For the fertilized egg is already a differentiated system.   Indeed, the whole history of the egg from the moment when it became distinguished as such and separated from body cells is a succession of differentiations.   In form, growth, mode of building up of food reserves, and in nuclear structure, a young egg-cell shows itself different from every and any other cell of the animal's body.   The egg-cell is subjected to the same environmental influences as other, fully specialized cells of the animal's body.   The changes and processes

by which it becomes an embryo thus spring from an initial differentiation, an intrinsic organization of the egg that distinguishes it from all other cells.

Consider the chick at the moment when it emerges from the egg-shell: it has all the organs that it will ever have and it had the precursors of these at the moment that it was fertilized. Nothing of organic material did it gain during the 21 days of incubation. It took in water, oxygen and heat. But not one single milligram of living protoplasm is in the hatched animal but what was within the unincubated egg. Early the embryo formed blood with a red pigment; this pigment contains iron. But not the most minute fraction of this iron was gained during development; all present in its body at hatching was already there before the egg was laid.

True, a mammalian embryo, like that of the human, develops by the material of the blood which comes to it. But in the minute human egg must be present all that from which comes a human embryo for the blood which nourishes it is the same as that which supplies any other cell in the mother's body, no one of which becomes a human embryo. Hence, though the human egg differs from the bird's, at basis the same is true: the egg at beginning of development is a differentiated and a differentiation-capable system.

Following its period of primary differentiation the egg cell grows, reaching a stage prepared for fertilization but unfertilizable. Then it becomes fertilizable—another phase of differentiation. If fertilization ensues, it becomes differentiated again. Thus differentiation flows on as a stream without breaks and if later for purposes of convenience I use words that seem to connote sharply discontinuous phases, the reader should keep in mind that I regard these phases, periods or stages, only as moments in time when the stream alters its course. Although we can not yet assert what in all this history is cause and what effect,

nevertheless we may say in general terms that each suc-
ceeding differentiation is conditioned in part by extrinsic
and in part by intrinsic factors, the latter of which are
conditioned by the preceding differentiation.[1] One great
task in the study of the differentiation occurring during
cleavage is the analysis of these factors, a task especially
difficult because we have no known point from which we
may start, for in this respect the egg also must be regarded
as an unknown system.

In the second place we concentrate attention on the
cleavage-period in our effort to trace the differentiation
of development, because cleavage is a process common to
all animal eggs.   Every species of animal egg passes through
cleavage, but not every animal egg, e.g., of sponges and
of coelenterates, passes through the three other periods
enumerated above. Then the differentiation that takes
place during cleavage we regard as that embracing in
a wider sense those characteristics common to animal
development and most readily reducible to general terms.
As we shall later see, whatever the period of development,
in which differentiation reveals itself to us, the mode of
differentiation is always the same. This being true, study
of the differentiation in the most generally appearing stage
has distinct advantage.

In the third place we have the practical reason that, as
experience has taught, of all the periods of development the
cleavage period lends itself most readily to resolution of
the processes into closely set stages; thus by experiment it
is possible to analyze the factors which set up the conditions
for differentiations in a more normal or natural manner
than, for example, in experiments with transplantations
involving conceptions of "organizators" and the like.

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[1] *Cf. Delage, 1895, pp. 765–766.*

For these three capital reasons, then, I consider it best to limit my discussion of differentiation to the cleavage-period. Since the fertilized uncleaved egg is itself differentiated, and in this respect an unknown system, a distinct advantage accrues in pushing back our inquiry as far as possible to it as a single cell whence originate the many different kinds of cells that make up the embryo. For the great question is: how out of a single cell do these manifold differences arise? I repeat: singleness does not mean simplicity. And yet, inasmuch as the complex organism has its genesis in a single protoplasmic system, the unknown complexity of this system may in some measure become known by means of a resolution of the events by which a single cell organism becomes one of many cells. It follows from this that we should define very exactly the cleavage-period. I turn therefore to a brief resumé of cleavage processes in various animal eggs. Such a resumé will further be valuable, because in the later discussions I shall need to refer to the various cleavage-types; and because by evaluating differences in the mode of cleavage we may arrive at a basis of similarity.

Every developing animal egg normally passes through a period of successive cell-divisions or cleavages. The cleavage-cells or blastomeres always arise by binary fission, that is, two "daughter-cells" arise from the division of one cell. In this fashion is established a cleavage-pattern which differs with different eggs. Upon these differences depends the classification of eggs according to their type of cleavage. Cleavages are classified as total and partial: total, if the whole egg cleaves; partial, if only a part of the egg cleaves—this cleaving portion is always either the whole surface or a definite surface-area.

In total cleavage the blastomeres of first cleavage and of several subsequent divisions may be approximately equal

in size; they may be at first equal in size and early in the succeeding cleavages show marked size-differences or they may from the first cleavage show striking disparity in size. At the end of the cleavage-period the blastomeres are of unequal size in all totally cleaving eggs.

The position of the blastomeres also plays a part in making the cleavage-pattern in totally cleaving eggs. If they stand directly above each other the final pattern is radial; if the four smaller cells, instead of lying directly over the larger ones, lie above the furrows of the first and second cleavage, the pattern is spiral.

The derivation of the blastomeres may be so regular that one can determine exactly in the cleavage stages the time and place of the origin of each. For many eggs it has been shown that the blastomeres in late cleavage can be traced unerringly back to the stage of first division. Thus, for these eggs, a cell-lineage, as it is called, can be traced. In other eggs for several cleavages the blastomeres arise always in the same way and hence show constancy in origin; after this period, they vary both as to time and place of origin. In other words, the cleavage pattern of animal eggs may be constant or not. If their cell-lineage can be traced, the pattern so far as the origin of the blastomeres is concerned, is constant. It is to be noted that the cleavage of eggs of clams, worms, ascidians, showing cell-lineage is closely similar. Hence this type of cleavage is not restricted to any one group of animal eggs. If the lineage of the cells can not be traced, this means that the cleavage-pattern is composed of cells whose positions are not determined by fixed origin.

Partial cleavage is of two forms, discoidal and superficial. In the former, cleavage is confined to a disc at only one pole of the egg, whilst in the latter the entire superficial cytoplasm cleaves. Discoidal cleavage is found in eggs of cuttle-fishes and their allies, in many bony fishes and in

reptiles and birds; superficial cleavage in eggs of some coelenterates, of many insects and of some other arthropods. Superficial cleavage might be said to be the mode among arthropod eggs for with few exceptions in the eggs of the members of this group that embraces the crabs, spiders, insects etc., only the surface-located cytoplasm shows cell-boundaries.

In figures of the discoidal cleavage in the egg of an ink-fish or squid, *Loligo*, as seen in section, one notes at the upper pole of the egg a heavily drawn line which represents the thin sheet of superficial cytoplasm. It is in this sheet that the cleavages ensue. In the unfertilized egg of a fish a thin band of cytoplasm encloses the yolk. After fertilization the superficial cytoplasm moves to the upper pole of the egg to form a disc where cleavage now takes place. In eggs of reptiles and of birds the process is the same: cleavage is limited to a small area of the total egg. As this disc becomes cleaved, the underlying yolk is converted into active cytoplasm which by cleaving adds to the area of the original disc.

Superficial cleavage was first clearly described by Weismann in 1864. Since the appearance of this classic memoir on the development of the insect egg, the clear superficial cytoplasm of the egg, which encloses the egg-yolk and to which during cleavage cell-boundaries are confined, has been spoken of as the blastema. Following entrance of the spermatozoon into the egg through a canal, the micropyle, located at one pole of the ellipsoid egg, the egg- and sperm-nuclei come together and form the cleavage nucleus located below the egg surface. This nucleus divides mitotically several times without cleavage of the egg. Later most of these daughter nuclei become located in the blastema, a few remaining in the central yolk-mass. With the arrival of the nuclei in the blastema, cleavage planes appear in it, the interior of the egg remaining uncleaved.

The foregoing general statement on the cleavage-process in animal eggs, though it makes no pretension to be exhaustive, nevertheless covers the essential points on a process which has been the subject of much admirable and painstaking investigation. In a book like this a comprehensive treatment of cleavage would be out of place, because it demands more space than we can give it. Moreover, a certain advantage obtains here, as with other biological processes, in setting forth a plain and simple statement of salient features that stand as accepted and established facts. Without further discussion we may note the following points concerning the cleavage-process in animal eggs:

1. Cleavage may involve the whole or only a part of the animal egg. Hence, the pattern varies depending upon the area of the egg which undergoes cleavage; the pattern at the termination of the cleavage-period resulting from total cleavage, as in the egg of a snail, is markedly different from that of its near relative, the squid, which undergoes partial (discoidal) cleavage.

2. The size of the blastomeres contributes a distinguishing feature to the cleavage pattern. Where in developing insect eggs the cell-size has been investigated, it was found that the blastomeres in the superficial cytoplasm show equality in size. This is not true for the cleavage-cells in the eggs of the ink-fish and in other eggs exhibiting discoidal cleavage. In total cleavage the blastomeres may be markedly unequal in size. Hence, not only the extent of the egg undergoing cleavage, but also size-differences of the blastomeres in the cleavage-area constitute a factor which determines an egg's cleavage pattern.

3. In all forms of cleavage, except superficial, the initial splitting up of the egg-substance takes place at that pole from which the polar bodies have formed or will form. In total cleavage the first and second cleavage planes cut through this pole at or nearly at right angles to each other.

In discoidal cleavage the cleaving disc is located at this egg pole. In superficial cleavage, the initial nuclear divisions are confined to the region directly below the point whence the polar bodies form, whilst the cleavage itself involves the whole egg-surface.

Because hitherto some writers have preferred to assume a simplicity for the uncleaved egg, thus disregarding its previous history as a succession of differentiations, fruitless attempts have been made to interpret the cleavage-patterns of the animal egg, diverse though they are, as the mode for or even the cause of the setting-up of those differences whose sum-total defines the differentiation made manifest during cleavage. It is, however, more correct to see in the cleavage-pattern a display of the differentiations present before cleavage set in. The separation of the egg as egg from other cells of the organism, its position in the ovary, its relation to food-supply, its intake and elaboration of food both in quantity and quality —in short all those processes that long before fertilization and cleavage endowed the eggs with those characteristics to which many authors relate cleavage as total or partial and as variants of each of these—these are the processes which condition the respective cleavage-patterns. Thus, a cleavage-pattern is established by earlier occurring differentiations; it does not express differentiation occurring during cleavage. We therefore consider the cleavage-pattern as only the frame-work within which we try to find the answer to our question. By analysis of the events which occur during cleavage, we may reach an agreement upon a proposition as to the primary and basic factor which underlies all forms of differentiation. For the cleavage these chief events are six.

(1) The egg is split up into cells; (2) the embryonic axis and the plane of bilateral symmetry are revealed; (3) the cytoplasmic inclusions shift positions; (4) nuclear material

increases; (5) water is redistributed; (6) the ectoplasm increases.

Before I begin the discussion of these events, I must deal with the loss by an egg of capacity to produce more than one embryo—i.e., with the change of the egg from a pluripotent to a unipotent system. This loss of pluripotency, often spoken of as a chief problem in development, in my judgment, is only a revelation that embryogenesis is a series of progressive restriction.

Loss of pluripotency is revealed at that stage in cleavage when the blastomeres having been experimentally separated develop into defective embryos. In the eggs of snails, for example, this loss can be demonstrated to have taken place at first cleavage; blastomeres separated at this time develop each to a swimming form, which is an incomplete embryo made up of those structures which in the normal embryo would have arisen from this blastomere. In eggs of echinids, on the other hand, loss of pluripotency occurs later because only after the third cleavage do the blastomeres when separated develop into defective embryos; blastomeres isolated after first and second cleavage develop into perfect though dwarf embryos, one-half and one-fourth, respectively, the size of the normal. Briefly, we find that animal eggs vary with respect to the time after fertilization when they lose capacity for multiple embryo-production.

Cleavage has been classified as determinate and indeterminate. In eggs with determinate cleavage, the history of each cell or blastomere in a late period of the cleavage-process can be traced back to the two cell stage; that is, some eggs as early as the two cell stage reveal an organization which fixes the destiny of the blastomeres derived from the products of the first cleavage. In so-called indeterminate cleavage, although for a time the blastomeres show constant origin, they later arise in undetermined fashion. This classification of the cleavage of eggs as

determinate and indeterminate I omit from the discussion for two reasons. First, since in an indeterminate egg, as that of an echinid, the initial cleavages are determinate, it can not be called strictly indeterminate. Second, since, all monoembryonic eggs finally become unipotent, we can not say that only determinate eggs are unipotent.

The evidence at hand shows clearly that in one respect eggs are the same: I refer to the fact, never sufficiently emphasized, that before fertilization all eggs, amenable to the experiment of merogony, have capacity to produce many embryos.[1] If eggs in the fertilizable stage be broken up into fragments and these be inseminated, each fragment produces an embryo. The developmental capacity of such egg-fragments is as great in the egg of *Chaetopterus*, in which pluripotency is lost early during cleavage, as in that of echinoderm eggs, in which pluripotency persists longer. Some eggs normally give rise to many embryos, as that of the Texas armadillo that always forms four, and those of some insects which give rise each to hundreds. The fertilizable egg thus is a system of multiplex embryonic potency. This pluripotency however, except for normally polyembryonic eggs, is never realized in the normal process of development. Instead, the egg develops as a mono-embryonic system which becomes successively restricted within the domain of monoembryogenesis. It is in this domain that our problem of differentiation during cleavage lies.[2]

The primary event of the cleavage period is the act of cleavage, a rhythmical phenomenon. This sundering involves both nucleus and cytoplasm; for although in some cases the nucleus divides without cytoplasmic division at one time or another during cleavage, normally no egg ever

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[1] *For literature on merogony see Delage, 1898, 1899a and b.*
[2] *See also Just, 1937b.*

develops throughout this period without cytoplasmic division. The furrowing of the cytoplasm, that is the act of cleavage itself, has been thought of as the cause of differentiation. This assumption however, is untenable, as I shall now show.

By means of cleavage the cytoplasmic mass, the egg, is successively sundered into smaller parts, the blastomeres. This sundering, even if regarded as a means for the separation of constituents, does not bring about a new distribution of them by which arise embryonic parts. True, the division into smaller parts will facilitate distribution, but it can not be its cause. It is not the mere division into cells that makes the head, neck, arms of a human being or that distinguishes the liver as an appendage of the gut from which it is derived. Rather, concomitantly with the act of cleavage changes take place by which the embryonic areas subsequently arise.

From yet another consideration we conclude that cleavage as such is not differentiation. Even if we should assume that the uncleaved egg were an undifferentiated system, cleavage-partitions would only set off undifferentiated areas and the differentiation which arises during cleavage would be due to some other cause—as for instance, chemical reactions induced in a previously homogeneous mixture by the act of cleavage in setting up separate reaction-chambers of minute capillary dimensions. But these reactions and not the act of cleavage would then be the cause for differentiation. Or were we to suggest that by the establishment of cell-boundaries brought about by cleavage, the increase of surface renders possible surface-reactions characteristic of capillary spaces, we should still not support the proposition that the mere act of cleavage in sundering the protoplasmic mass is responsible for the progressive differentiation which can be followed throughout the cleavage-process.

Finally, consider the experimental evidence. This, although not embracing all eggs, indicates clearly that fertilized eggs may reach a certain degree of differentiation without cytoplasmic cleavage.

Differentiation without cleavage has been noted by several observers to take place in eggs of annulated worms and of ascidians having been subjected to experimental treatment. The most thorough-going description of this type of development is that given for eggs of *Chaetopterus* having been exposed to hypertonic sea-water.[1] These eggs develop into most abnormal swimming forms made up of either uninucleated or multinucleated undivided cytoplasm due either to complete failure of cytoplasmic cleavage or to the disappearance of cleavages and fusion of the blastomeres.

As development progresses, the cytoplasmic inclusions take up new positions in almost the same way and at the same time as in fertilized normally developing (untreated) eggs. But the ectoplasm behaves in a quite different manner. A portion of it moves to the animal pole, a smaller amount rests at the opposite pole. Later the ectoplasm at the animal pole moves toward the vegetal pole so that the endoplasm is covered, thus simulating the overgrowth of the larger yolk-containing blastomeres by the smaller ones in normal development.

Another characteristic feature in differentiation without cleavage in the egg of *Chaetopterus* is the behavior of the nuclei in uninucleated eggs. In these eggs when the nucleus reaches the size of the germinal vesicle of the normal egg there appears a strong mutual attraction between it and the ectoplasm: the ectoplasm is drawn into the egg to form a mass that lies close to the nucleus or the chromatic part of the nucleus is drawn out to the ectoplasm. Eggs which show ectoplasm drawn into the interior do not

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[1] *Lillie, F. R., 1906.*

develop as far as those in which the chromatic substance of the nucleus is drawn into the ectoplasm.

We conclude therefore that although cleavage and differentiation normally go hand in hand, the relation is not one of cause and effect.

The second event enumerated above as occurring during cleavage which has been held as a cause of differentiation, is the appearance of the embryonic axis and of the plane of bilateral symmetry, the so-called median plane.

For egg-cells the term, polarity, means that with reference to an axis the egg in some way, as arrangement of pigment, location of the nucleus, site of polar body formation, etc., shows structural polar arrangement. Now it is often held that the polar axis of the egg bears some constant relation to the axis or the median plane of the embryo and on this assumption are elaborated theories of differentiation as determined by the egg's polar axis, of a differential axial gradient, and the like.

In order to clear this problem, we must first distinguish between the polar axis in eggs of radially symmetrical animals and that in eggs of the bilaterally symmetrical with respect to the axis of the embryo in the former eggs and to the median plane in the latter. Although this distinction is easy if only we appreciate certain simple geometrical truths, nevertheless we must give it prominence here inasmuch as so many writers have most unfortunately used axis and plane interchangeably in their theories of differentiation.

Polar and embryonic axes coincide in eggs whence radially symmetrical embryos arise. A line drawn through the egg from the site of polar body extrusion to the pole opposite is also the gastrular and the embryonic axis. In the eggs of these organisms—Porifera and Coelenterata—the gastrula forms in such wise that its axis is along traces of the polar axis, no matter by which of the several modes of gastrulation that obtain in these eggs the gastrula arises.

The gastrula may develop from a morula (a solid mass of cells) or a blastula (a mass of cells that contains a cavity); the gastrulation may be that of invagination, delamination or epiboly; finally the gastrula is a two-layered radially symmetrical structure enclosing a cavity whose polar axis, now the embryonic, for the most part traverses empty space, and at whose poles only are cells located which are part of the two-layered covering. Components of an axial gradient, if there be such, can become effective only in the cell-layer.[1]

Eggs of most bilaterally symmetrical animals begin their development as radially symmetrical structures and therefore show a polar axis. But at the moment after fertilization when bilaterality appears in such an egg, we can no longer speak of an axis. In a bilaterally symmetrical organism—egg or adult—there exists no line common to planes as in a radially symmetrical one. Here, accurately speaking, we can use only the term, plane of symmetry.

Certain eggs out of which develop bilaterally symmetrical animals—as, for example, those of *Loligo*, *Hydrophilus*, *Amia*—reveal bilateral organization before fertilization. In these, obviously, we can not speak of a polar axis. For even if we know that at some time in their history the eggs were radially symmetrical, the moment that bilaterality appears in them there can be no axis with respect to which the parts are symmetrically arranged.

But even if we allow the incorrect use of the term, axis, in bilateral embryos, we find no constant relation between this axis and that of the egg. A survey of embryogenesis

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[1] *J. W. Wilson and I were never able to repeat Child's observations on the effect of KCN on eggs of Arbacia which he interpreted as demonstrating an axial gradient. My own experiments (1928c) on this egg, which gave results similar to Child's, strongly indicate that these are due to changes in the ectoplasm. Child's own work, as that on protozoan forms, can be explained in terms of effects on the superficial cytoplasm.*

in eggs whence develop bilaterally symmetrical embryos does not permit the conclusion that "the axis of the egg shows a definite relation to that of the gastrula, of the later embryo, and of the adult body"; and that "this relation, broadly considered, appears to be constant throughout the Bilateralia."[1] In many radially symmetrical eggs that subsequently show bilaterality it is true that a line drawn from the site where the polar bodies lie to the opposite pole is perpendicular to one drawn along the length of the embryo. In others,—e.g., the egg of *Amphioxus*[2]—the intersection of these lines forms an acute angle. In eggs that are bilaterally symmetrical before fertilization, the medium plane of the embryo lies in the egg's plane of bilateral symmetry.

A survey of embryogenesis in the entire animal kingdom permits the conclusion that the embryo, with the possible exception of the mammalian, arises from the egg-surface. In the present state of our knowledge, the mammalian egg does not seem to fall in with our generalization, since its surface-cells form trophoblast. In all other eggs embryonic axis or median plane is normal not to the core of the egg but to its surface where the embryo lies. Axis or plane appears, due to new configurations in the protoplasmic system;[3] the embryo-axis or the plane of bilaterality is an expression and not the cause of the differentiation which unfolds itself as cleavage progresses.

We turn now to another of the events occurring during cleavage which were enumerated above, the shift of the cytoplasmic inclusions, again seeking the cause of differentiation.

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[1] *Wilson, 1924.*

[2] *Cerfontaine.*

[3] *One such configuration is undoubtedly set up by the entrance of the spermatozoon into the egg—see Just, 1912.*

The positions especially of the oil and the yolk in the egg-plasma change after fertilization, as comparisons of the unfertilized with the fertilized eggs of either *Nereis* or *Chaetopterus* reveal. In the egg of *Arbacia* as of any other echinid the shift in oil and yolk is not so well marked. That is, in eggs with determinate cleavage there is a more clear-cut change of the cytoplasmic inclusions to new positions than in indeterminately cleaving eggs. The progressive differentiation from the single cell, the egg, to a complex organism, the embryo, was once thought of as due to the distribution of the visible materials in the cytoplasm: embryo-formation was ascribed to the segregation of these fat- and fat-containing materials which were therefore denominated organ-forming substances. To-day, we can appreciate the naiveté of this theory, wondering how we could have given it such serious consideration.

Eggs differ[1] with respect to content of cytoplasmic inclusions. This is especially true of yolk. Indeed, eggs are sometimes classified on the basis of yolk-content. Once we spoke of yolk-less (alecithal) eggs; now we know that all eggs, even the human, contain yolk. Thus it is better to classify eggs not as yolk-free and yolk-rich, but rather as telolecithal (yolk in one part), centrolecithal (yolk in the center) and homolecithal (yolk evenly distributed). Yolk also differs physico-chemically in different eggs; and I have some evidence which indicates that it differs in a given egg before and after fertilization. What is true of yolk, the food material for the embryonic organism, is true of mitochondria, pigment-granules, etc.: they vary as to amount, distribution and physico-chemical make-up. Whilst all of these cytoplasmic inclusions of which I here speak differ

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[1] *The reader will have noted that I often emphasize the differences in animal eggs. In this my general purpose is to sift from the differences that which is similar and equivalent and thus to order and to define the biological problems.*

from each other, they are, so far as I know, wholly or in part composed of lipin (fat or fat-like substance). Though there is no reason against the postulate that the organ-forming substances are composed of fats, nevertheless, I believe that we are on unsafe ground in attempting to relate the formation of the many various organs to fat-containing compounds.

Sometimes the cytoplasmic inclusions show differential distribution to very definite blastomeres. Consider the egg of a low form of chordate, for example, that of the tunicate, *Cynthia*. One substance (yellow) maintains a definite distribution to given blastomeres. This is an egg with determinate cleavage. From the blastomeres containing the yellow substance always in the normal egg muscle and mesenchyme develop. Apparently, therefore, here is a very clear case of an organ-forming substance definitely localized in the egg before first cleavage. But experimental findings do not permit such a conclusion. To these I shall later turn.

Other eggs with determinate cleavage for the most part do not exhibit prelocalization so clearly as that of *Cynthia*, though their cytoplasmic inclusions shift from their original positions before fertilization to new ones after fertilization and at first cleavage; thus the yolk and oil come to lie in cells which are destined to form the gut. This is not evidence that oil and yolk "determine" gut-formation. Also, there are many other eggs that do not show any special or constant disposition of the cytoplasmic inclusions.

On other grounds the theory that the visible cytoplasmic inclusions are organ-forming substances is untenable.

In the first place, I call attention to the egg of *Strongylocentrotus lividus*, a sea-urchin commonly found in the Mediterranean, which may exhibit a beautiful superficial band of orange pigment below the equator in the hemisphere opposite the animal pole. Boveri used the pigment band

of this egg as a means of orientation in experiments. But the occurrence of this band is inconstant; not every specimen of *Strongylocentrotus* gives eggs containing it. There is no virtue in colored particles as such suspended in the cytoplasm for differentiation, though they may be useful indicators in other respects.

Another cogent argument against the theory that the visible cytoplasmic inclusions are organ-forming substances lies in the fact that cleavage in many eggs may ensue for several cell generations apart from the underlying yolk and oil; this is true of all eggs with discoidal cleavage. In them the cytoplasm is at first a transparent and apparently homogeneous sheath and later a disc below the vitelline membrane. Cleavage is confined to this inclusion-free cytoplasm. When in later stages of development—after the differentiation occurring during cleavage has taken place—the embryo grows at the expense of the yolk-laden region of the egg, the yolk is transformed into clear cytoplasm. In other words, in these as in all animal eggs, yolk and oil play no direct part in differentiation.[1]

Let us recall another fact: many living eggs under the microscope appear to be transparent. Rigorously to adhere to the theory of visible organ-forming substances is to deny organ-formation in transparent eggs. By ascribing a primary rôle to the visible inclusions in the cytoplasm, we have had the tendency to reckon the flood by its burden, taking the traffic for the stream.

Finally, certain experimental results set up an unsurmountable obstacle to acceptance of the theory of organ-forming substances as an explanation for differentiation.

Above I have pointed out that due to the ectoplasmic changes caused by fertilization, eggs exhibit cytoplasmic

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[1] *In trematode eggs exists the strongest separation between egg and yolk, because the egg alone is a product of the ovary; the yolk is produced by accessory glands and spun around the egg.*

currents which move the inclusions. This movement is most striking in eggs with determinate cleavage—especially if the inclusions be colored and hence easy to identify. They come to lie constantly in definite regions of the egg at first cleavage. If these fixed positions of the inclusions be responsible for differentiation, then altering them should modify or hinder development.

By centrifugal force the normal positions of the cytoplasmic inclusions can be altered. The materials, which as spherules and granules are suspended in the cytoplasm normally without reference to their specific gravity, are when the eggs are centrifuged moved to new positions and stratified according to their respective specific gravity. Thus oil, yolk and pigment form layers in the order named with a zone of clear cytoplasm beneath the oil. Here our interest focuses on two results obtained from the centrifuging of eggs.[1]

The first deals with the fact that centrifugal force in altering the position of the inclusions does not alter the direction of the cleavage planes. Hence, the new arrangement of the inclusions induced by centrifugal force, which shifts them to blastomeres in which in normal development they would not come to lie, has no effect on the development of the embryo. It should be emphasized that this effect of centrifugal force is the same in eggs with determinate and indeterminate cleavage. Investigators are agreed that the altering of the position of the cytoplasmic inclusions has no effect on embryo-formation: the eggs develop normally although the inclusions come to lie in

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[1] *Although centrifuging may be a violent method of treating eggs, capable of causing their destruction, it can, if used with discretion, leave them quite uninjured. One can soon learn the least amount of centrifugal force necessary to stratify the cytoplasmic materials; there is here no point in using a greater, as Conklin did on the ascidian egg.*

blastomeres other than those that normally hold them. For the cleavage planes bear any relation to the axis of stratification.

Even more significant is the second result, namely, that fragments of clear inclusion-free cytoplasm taken from centrifuged eggs, whether these eggs cleave determinately or indeterminately, develop when fertilized as whole eggs. Many unfertilized eggs—e.g., those of sea-urchins and those of other species whose germinal vesicles have broken down —can be strongly deformed by pressure, centrifugal force, etc. This is due to the fact that the fluid protoplasm is enclosed by a highly elastic membrane. Such eggs when centrifuged are pulled out into strands, a fact known already many years ago. With a greater degree of centrifuging the strands break up into fragments. Some of these fragments, with or without the egg nucleus, are composed of the clear hyaline cytoplasm only. Such clear fragments devoid of inclusions are capable of fertilization and development; they cleave as whole eggs.[1]

These two experimental findings alone render untenable the theory that considers the visible inclusions as organ-forming substances. The differentiation from the single cell, the egg, to the complex multi-cellular embryo can not be related to the distribution of visible materials suspended in the cytoplasm. That these materials have functions, no one would deny. Most of them, oil and yolk, are food for the embryo or larva; the remainder are doubtless for respiration, secretion, etc. They move with the cytoplasmic ebb and flow but are not themselves the tide of life.

The orderly progressive shifting of the visible multi-colored spherules and granules in a living egg, especially in one with determinate cleavage, is an entrancing spectacle: in defiance to gravity, oil, yolk, mitchondria and

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[1] *Lillie, F. R., 1906.*

pigment granules, each at a different rate, shift to new positions, always the same in every undisturbed egg of a given species and definite at its every cleavage. In the order and amplitude of this shifting we recognize the power of the so little known cytoplasmic currents. Since these in turn are directed by ectoplasmic changes, as I have shown, we may by study of the shifting of the inclusions come to know more about the ectoplasm and its interaction with the inner cytoplasm.

In this inner cytoplasm is situated that fixed and little changing cell-component, the nucleus, to which has been assigned the rôle of maintaining intact the inheritance of the species. What is its rôle in differentiation?

Along with the successive acts of cleavage by which the egg substance is divided up into blastomeres and as the embryo-axis or plane of symmetry becomes recognizable, the original single nucleus of either the fertilized or the parthenogenetic egg undergoes division so that each blastomere receives a nucleus. In discussing the probable rôle of the nucleus as a causal factor in the differentiation during cleavage, I shall now deal with the following points: the quantity of nuclear material finally found at the close of the cleavage-period, the origin of this material, and how the nuclei, with special reference both to chromosome-content and to the genes making up the chromosomes, may be conceived as taking part in determining the development of the egg.

Although with each successive nuclear division during cleavage of the egg the nuclei progressively diminish in size, no individual nucleus in any blastomere at the end of cleavage ever vanishes. On the other hand, the total quantity of nuclear substance, where this has been determined, is greater at the end than at the beginning of the cleavage-period. The quantitative increase in nuclear substance continues through the whole embryonic period

and becomes therefore a definite characteristic not only for cleavage but also for the whole course of development. The body of a newly hatched chick contains nuclear matter far in excess of that in the uncleaved egg which gave rise to the animal. Indeed, as long as the chick lives and is capable of producing germ-cells it elaborates nuclear substance. Moreover, every organism that regenerates lost parts, every one whose tissues exhibit pathological growths, as tumors, builds nuclear substance. Hence, the building up of the chemical constituents of the nucleus represents a basic property of protoplasm as a self-reproducing system. In other words, as protein-synthesis represents that fundamental chemical characteristic of living matter that distinguishes it from non-living, so nuclear synthesis (in part a protein-synthesis also) stands as one of the primary chemical activities of the self-regulation and the self-differentiation exhibited as special attributes of cells capable of reproducing themselves. The course of development is marked by syntheses. By these arise secretions as those of the thyroid, the pancreas, etc., and such compounds as haemoglobin, the iron-containing respiratory pigment of the blood, as found in the embryos of animals which possess these substances. By synthesis also nuclei arise.

The question now is: out of what are the nuclei synthesized? With the progressive increase in amount of nuclei runs during the period of cleavage a decrease in that of the cytoplasm without any change in the amount of the yolk and oil. This fact, holding for all animal eggs, is especially clearly shown in totally cleaving eggs. In the egg of *Nereis*, for example, I have followed very exactly the composition of the blastomeres throughout cleavage and can state that in the blastula-stage the total amount of cytoplasm is less than that in the egg at first cleavage, whereas the oil and yolk do not change in amount. The clearest evidence

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that nuclei arise out of the cytoplasm is furnished by the development of those clear hyaline pieces of protoplasm already referred to, for in the cleavage-process of such fragments also the nuclear matter increases and the cytoplasm decreases. It has been shown that the nuclei in yolk-free blastomeres are larger than those in yolk-rich blastomeres and that the size of the nuclei is proportional to the hyaline content of a blastomere rather than to the total, encompassing the inclusions.[1] These facts prove that in totally cleaving eggs during cleavage the nuclei are synthesized from the cytoplasm. In partially cleaving eggs as well the yolk takes no part in development during cleavage. When, as happens in these eggs, at the end of cleavage new cells are added to the embryonic area from the yolk region, the yolk, as the evidence indicates, is transformed into cytoplasm and does not go directly into the nucleus.

The increased nuclear content at the end of cleavage therefore can only mean that the nuclei are elaborated out of nuclear stuffs or their precursors; it can not mean an automatic activity of the nucleus capable of building nuclei out of nothing. Nuclei are built up from the cytoplasm. So too their constituents, the chromosomes.

In preparation for fertilization both egg and sperm-cell go through a process by which the number of chromosomes characteristic for the species is halved; at fertilization this number is restored, half from the father, half from the mother. This is the strongest evidence in favor of the chromosomes as the means by which the off-spring inherits qualities from both parents. The number of chromosomes of the fertilized egg is the somatic or so-called diploid number characteristic for the species. During development this number remains constant. Every somatic cell of the adult organism no matter how complex contains in its

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[1] *Conklin, 1912.*

nucleus the number of chromosomes equal to that of the fertilized egg. The germ cells of the adult in their terminal stages after the process of reduction again possess half the somatic number. Thus the cycle from germ cell to germ cell is complete.

During the cleavage stages, the chromosomes are halved in quantity at each cell-division. Since, as we know, they never reach the zero-point, they must increase in mass during the period of cleavage. Says Morgan[1]: "During development, especially during early cleavages, the amount of chromatin steadily increases in amount, giving an exponential curve resembling the first half of a curve of a mono-catalytic reaction." What evidence we possess definitely warrants the statement that the chromatin material increases in amount during cleavage.

Suppose we consider the egg of a large fish, a carp or salmon. Such an egg after fertilization has two groups of chromosomes, one from the sperm-nucleus and one from the egg-nucleus, which united give the total number of chromosomes characteristic for this particular species of carp or salmon. Eventually from this single fertilized egg the adult fish develops, which is composed of millions and millions of cells constituting all of the organs of the fish; if it is a female, it produces thousands or even millions of eggs, if it be a male, billions of spermatozoa. Eggs or spermatozoa of an adult fish have chromosomes equal in number, size, form and, presumably, in weight to those found in the egg- or sperm-nucleus of the fertilized egg from which this adult fish was developed.

If the chromosomes grow—and it is inconceivable that they do not—they grow at the expense of something; they can not grow directly out of themselves without obtaining the new materials for themselves. The synthesis of chro-

[1] *Morgan, 1927.*

matin demands the utilization of raw materials out of which chromatin is constituted. Then the question arises: Is chromatin synthesized directly from the elements of which it is composed or from compounds? We know that the synthesis, upon which directly or indirectly all living stuff depends, comes from CO_2 and HOH which in the presence of chlorophyl and sunlight make the simplest sugars. From these by polymerization polysaccharides are derived and, with N, proteins and fats are built up. In other words, the protoplasmic synthesis of organic compounds like proteins is never from elements directly but from compounds in the cell. If such protoplasmic syntheses are from compounds, why not chromatin, itself a conjugated protein? Chromatin grows because more of it is made; and it is made from compounds available in the cell. Even if we assume that it is made out of that material which is already in the nucleus as non-chromatin, we must conclude that this non-chromatin in being part of the nucleus has come from the cytoplasm.

It is clear, first, that chromatin grows; second, that it can not grow in some magical way but most probably grows as other cell-stuff grows—i.e., according to principles governing protoplasmic syntheses as far as we to-day know these, that is, from preexisting and simpler compounds. The fact that during the cleavage stages of most eggs the cytoplasm decreases as the chromatin increases further indicates that chromatin is synthesized directly out of the cytoplasm. Oil and yolk as such play no part in this process since they tend to be segregated and the cells containing them are the least actively dividing and those free of them most productive in the elaboration of new chromatin substance.

Thus we deal with the definite increase of a chemical stuff, nuclein; a measured weight of this must mean a definite weight of precursors that produce it. Briefly, in

dealing here with this conversion of cytoplasm into nuclei we are concerned with the formation of a known chemical stuff. If now we relate differentiation to this chemical process, a process whereby a definite chemical stuff is removed from the cytoplasm so that cytoplasmic reactions are rendered possible, we may have close at hand an explanation of differentiation as a series of chemical changes. In this wise it may be possible to detect certain chemical stuffs as potencies. Surely, the more we can substitute for such terms as "potency" chemical reactants in chemical reactions, the closer shall we come to the solution of the problem of differentiation of development. In relating differentiation in part to the synthesis of nuclear material, we take the first step in this direction.

The progressive differentiation of the egg during cleavage according to this conception is brought about neither by the pouring out of stuffs by the chromosomes into the cytoplasm nor by segregation of embryonic materials as postulated by those who uphold the theory of embryonic segregation, but by a genetic restriction of potencies through the removal of stuff from the cytoplasm to the nuclei.

Consider a fertilized egg, *ABCD*, with determinate cleavage, which at first cleavage forms two blastomeres, *AB* and *CD*; there must be differentiation, since the *AB* and the *CD* blastomeres when separated give rise to partial larvae. This would mean according to the theory of segregation that *AB* is minus *CD*'s material and *CD* is minus *AB*'s. *AB* thus would be a cell in which the prospective *AB*-potencies are present; the same would be true in the *CD* cell for the *CD*-potencies. After the second cleavage, *A*-, *B*-, *C*- and *D*-potencies would be present in blastomeres *A*, *B*, *C* and *D* respectively. Similar segregation would happen at each succeeding cleavage. For eggs with indeterminate cleavage the presence of material—and conversely, the loss

of other material—would take place at that stage in the cleavage-process when the blastomeres on separation lose the potency for development into complete though dwarf larvae. But is an egg-cell (in this case with determinate cleavage) $ABCD = AB + CD$, as we would express it according to the theory of segregation? Or can we say that $AB = AB + (-CD); CD = CD + (-AB)$? Can we say that $ABCD$ is really $AB + (-CD) + CD + (-AB)$ and do we gain thereby?

Restriction is, as I shall show, preferable to segregation for it more nearly expresses the process. Restriction implies a loss, in this case of developmental potencies, without necessarily a rearrangement of materials. Segregation connotes new arrangement or sorting out of materials without any implication of change in them. If as cleavage progresses cytoplasmic materials for embryo-formation are merely shifted to new positions, then reversal of the cleavage process should be possible. Stages of differentiation then would be repetitive. I do not wish to indulge in hair-splitting definitions; nevertheless I must say that I for my part can not see how the materials of an egg by mere acts of separation can account for differentiation. Against the assumption of a mere transposed order of materials stands the fact that as development proceeds the individual blastomeres lose potency.

At first cleavage blastomere AB becomes such because it loses CD-material, and blastomere CD becomes such because it loses AB-material. A separation into AB- and CD-blastomeres means that AB somehow removes all CD-material and CD all AB-material. So does genetic restriction begin and so it continues. This takes place through the absorption of CD-material by the nucleus of the AB-blastomere-to-be and of the AB-material by the nucleus of the CD-blastomere-to-be. The subtrac-

tion of the cytoplasmic materials by the nucleus takes place with each mitotic cycle.

We know, as has been pointed out, that as cleavage progresses the nuclei increase in number and *en masse* in volume. That is, they grow. We know also that in this same period the total volume of cytoplasm decreases and that the growth of the nuclei is at the expense of the cytoplasm. Genetic restriction then depends upon the removal by the nucleus of certain materials from the cytoplasm, leaving others free. The free materials determine the character of the cell—as ectoderm, mesoderm or endoderm and later as any one of the organs arising therefrom.

Something leaves the cytoplasm with each cell-division; then why not the materials of the cytoplasm which are *nonspecific* for the given blastomere? Certainly, the mechanism for the removal of material is present in this growth of the amount of nuclear substance as development progresses. With each cleavage each nucleus fixes all material other than that which makes the blastomere what it is, *AB* or *CD*; *A* or *B*, *C* or *D*, etc., to the end of cleavage. Then the nucleus of cell *AB* is different from that of the *CD* cell since the *AB*-nucleus contains bound *CD*-material and vice versa for the *CD*-nucleus.

Then the potencies for embryo-formation are all present in the uncleaved egg; cleavage serves to remove these and this removal is fast or slow, early or late depending upon the species of egg. The question arises: When does the cytoplasm of the egg gain its potencies for differentiation during cleavage?

Pluripotency becomes demonstrable through merogony, as was pointed out, with the onset of the fertilizable condition inasmuch as now fragments of the egg are when fertilized each capable of development. From this we might reason that the potencies that were stored up in the nuclei

during the previous development of an egg are restored to the cytoplasm at this time, that is, to the cytoplasm of the new egg. However, the demonstration that pluripotency is present gives no evidence of the time when it arose. Merogony may be only an indicator of an earlier established pluripotency. Having no other criterion than fertilizability for the demonstration of pluripotency, we can not exactly define the moment when the egg becomes pluripotent.

An attractive hypothesis is that pluripotency arises with breakdown of the germinal vesicle when there escapes into the egg-cytoplasm residual nuclear stuff greatly in excess of what remains to form the mitotic complex of the first maturation-division. The potencies might be regarded as identical to, or associated with, this extra-nuclear material. According to this suggestion, the rise of pluripotency would be separated from the fertilizable period, for the fertilizability does not depend upon a particular stage in maturation. But since many species of eggs are fertilizable whilst their germinal vesicles are intact, not a single one of these should be pluripotent if we are to relate the rise of pluripotency to substances escaping into the cytoplasm after breakdown of the germinal vesicle. Where, however, fragments taken' from such eggs develop when fertilized, it must be ascertained that the fragments are devoid of stuff having escaped from the germinal vesicle. The lack of data on merogony in such eggs does not warrant a decisive conclusion in these questions.

There remains another possibility. After the germ-cells have become differentiated from somatic cells through the loss to their nuclei of all potencies, with only the potencies for germ-cells left free in their cytoplasm, they become isolated from the soma. This isolation brings about the escape of all potencies that were up to that time bound in the nuclei, into the cytoplasm. Thus the eggs would

become pluripotent. The condition of the spermatozoon is discussed later.

Any theory offered to account for the differentiation that takes place during cleavage should be consistent for both the cleavage-period and the succeeding periods of development during which differentiation occurs, since in essentials, every period of differentiation is alike.

Certain phenomena occurring in the organism as egg, as cleaving mass, as embryo and as adult are based on some differentiation. A theory of differentiation during cleavage should hold for these phenomena. They are: (1) polyembryony and experimental twinning; (2) merogony; (3) the development of diploid fragments; (4) the development of isolated blastomeres; (5) haploid parthenogenesis; (6) experimental and natural polyploidy; (7) asexual reproduction by budding and fragmentation, and alteration of generations; (8) regeneration of lost parts; and (9) the origin of tumors. I shall now endeavor to show that these phenomena are better explained on a theory of differentiation as genetic restriction than on one that postulates segregation.[1]

1. The capacity of some eggs, as those of certain insects, normally to produce many embryos from one egg as well as the possibility of producing twins experimentally from normally mono-embryonic eggs including those of the determinate type, as the eggs of *Nereis*, *Chaetopterus*, *Tubifex*, etc., shows that eggs have more latent potency than that required for producing one animal. A theory postulating a restriction of potencies seems to meet these facts better than one suggesting that materials for the embryo are segregated.

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[1] *Cf. Perez (1912) who lists much the same experimental evidence against the mosaic-theory (an earlier form of the segregation-theory) of development, which he justly considers a modern version of the preformation doctrine.*

2. This same conclusion holds for the data on merogony. It may be regarded as proved that all eggs which can be fragmented before fertilization have the capacity for the production of many embryos. Merogonic development indicates strongly, especially in determinate eggs, that differentiation ensues as a restrictive process—restricting potency for multiple embryo-formation to one. The theory of segregation in these cases would imply telescoping of several embryos.

3. Some fragments of eggs contain the egg-nucleus and hence when fertilized develop with two nuclei constituting a diploid nucleus. In such fragments restriction obtains as in whole eggs, except that the chromosomes remove less cytoplasmic stuff since less is available. From the point of view of embryonic segregation, we would have to assume that since perfect though dwarfed embryos result from the fragments, the segregates in whole eggs are pluralistic. From this it should follow that all eggs when separated into blastomeres should develop into entire organisms or into embryonic regions, each containing parts reduplicated.

4. The development of blastomeres isolated during cleavage into perfect though dwarf embryos stands as strong evidence that restriction and not segregation underlies differentiation. For how from already segregated areas could perfect embryos arise? Or, if it be postulated that the areas are not yet segregated at the stage of isolation, why do the blastomeres in an intact egg not develop each into an embryo? On the basis of our theory of restriction, potencies bound in the cytoplasm in the intact egg become free in the isolated blastomeres because of the new conditions set up by isolation.

5. If we put forward the theory of segregation to account for the differentiation occurring in a parthenogenetic egg containing a nucleus with half the number of chromosomes found in the eggs of this species when they are fertilized,

we encounter the difficulty of explaining how under the domination of this "half" nucleus the cytoplasm is ordered into regions whence the organs arise. The theory of restriction by means of the nucleus removing potencies from the cytoplasm does not encounter this difficulty for according to it the nuclei extruded as polar bodies relieve the cytoplasm of potencies.

6. Similar reasoning applies to eggs developing with polyploid nuclei—i.e., with more chromosomes than those contained in the united egg- and sperm-nuclei. In the egg of *Nereis*, for example, fertilized after treatment with ultra-violet light,[1] the polyploid nucleus, made up of the three nuclei from the suppressed polar bodies plus the egg- and the sperm-nuclei, remove cytoplasmic potencies precisely as these are removed in normal fertilization by polar bodies and egg- and sperm-nuclei. But from the point of view of segregation, conditioned by nuclear material or power escaping into the cytoplasm, development should not take place because of the over-powering influence of the super-abundant nuclear matter.

7. Certain eggs develop into animals which have the capacity for asexual reproduction by the formation of buds or by the process of breaking up into two or more fragments. Both the buds and the fragments develop into complete organisms similar to the form whence they arose. If sexual and asexual phases regularly succeed each other, they constitute a life-history said to reveal alternation of generations.

Asexual reproduction can be explained by assuming that potencies in the egg, present in excess of those for the formation of a single individual, remain free. The reduplication of the animal by bud or fragmentation though it occurs late in the life-history is thus comparable to poly-embryony. In those cases of alternation of generations, where egg- and

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[1] *Just, 1933c.*

sperm-producing areas as motile swimming organisms arise, restriction has taken place by removal of these free potencies.

8. Regeneration of lost parts is encountered in all animals; in some, as the flatworms, it reaches the level of asexual reproduction; in others, capacity for regeneration is limited. In some forms regeneration is said to be due to the presence of formative cells, cells in which it is assumed that differentiation has been arrested; in others regeneration of structures occurs from tissues that normally never give rise to them.[1] In either case the capacity for regeneration may be thought of as a reorganization under the influence of the liberation of potencies, previously bound by the chromosomes, into the cytoplasm induced by the changed condition of the cells as a result of the injury or loss.

9. Abnormal growths or tumors can be explained in the same way. Some change in the environment of the cells stimulates the throwing out by the nuclei of potencies into the cytoplasm where a new type of development is set up. A tumor-cell is one which has escaped the domination exercised by contiguous cells. It becomes physiologically isolated.

In view of all of these facts that support my theory of differentiation as genetic restriction, I state this again: As development progresses, the egg-potencies are restricted through their withdrawal from the cytoplasm by the chromosomes with each successive cell-division. Thus the cytoplasm forms functional areas. At some time in the history of the egg's development the potencies, having been previously taken out and stored by the chromosomes during cleavage and succeeding stages of differentiation, escape into the cytoplasm. The cytoplasm of the fertilized or parthenogenetically developing egg restores them again

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[1] *Reed, 1904.*

to the chromosomes. This theory of differentiation as a genetic restriction is consonant with the nine phenomena enumerated above.

This conception of the rôle of the chromosomes in the process of differentiation stands in contrast to the attempt of genetics to explain differentiation.

The last thirty years have seen as an outgrowth of the well-known Mendelian laws that flourishing branch of biology, genetics, nourished by the knowledge of chromosome behavior, develop almost to the proportions of a separate science—at least it has a very rich vocabulary of its own. Mendel's laws are not causal, but statistical conclusions concerning the regularity with which offspring show the characters of parents. The chromosome theory of Mendelian heredity attempts a causal explanation.

According to the current conception each chromosome is a string of units, the genes, which are the carriers of heredity. The gene theory has been developed by work done on the small fruit-fly, *Drosophila*, raised in the laboratory under fairly constant conditions of temperature, of food, of periodic subjugation to heavy doses of ether, etc. Thus divorced from the rigors of nature these animals imprisoned in large numbers in small glass containers have undergone changes which endure for so many generations that they are without doubt constant. Animals exhibiting these enduring changes are mutants. For each mutation genetics has assigned a place on one or another chromosome; sometimes determiners of the same mutation are located on several chromosomes. Among geneticists the gene-theory is widely accepted. Among biologists, on the other hand, even of those who do not accept the gene theory of the geneticists, the majority agree that the chromosomes are the bearers of heredity.

According to the geneticists the chromosomes in every cell of the most complex organism are identical with those of the fertilized egg. That is, as they express it, the chro-

mosomes maintain their individuality.   The linear arrangement of the genes in each chromosome[1] is the same for the given chromosome whether it is in a fertilized egg, in a kidney-cell, a muscle-cell or any other cell of the adult organism.   Now at each cell-division though the chromosomes are split they must somehow maintain their identity or individuality; this maintenance is the prevailing doctrine among geneticists.

Speaking of the individuality or genetic continuity of the chromosomes, Wilson says[2]:

> In any general account of the history and genetic relations of the chromosomes in the life-cycle, we inevitably find ourselves speaking of them as if their identity were really lost when they disappear from view in the resting or vegetative nucleus.   The vast literature of the subject is everywhere colored by the implication that chromosomes, or something which they bear, have a persistent individuality that is carried over unchanged from generation to generation.   This view has met with some determined opposition; but with the advance of exact studies on the chromosomes escpticism has gradually yielded to the conviction that the chromosomes must, to say the least, be treated *as if they were* persistent individuals that do not wholly lose their identity at any period in the life of the cell but grow, divide and hand on their specific type of organization to their descendants. This does not mean that chromosomes are to be thought of as fixed and unchangeable bodies.   Beyond a doubt they undergo complex processes of growth, structural transformation and reduction, in some cases so great that no more than a small fraction of the substance of the mother-chromosomes at its maximum development is passed on to the daughter-chromosomes.   Whether we can rightly speak of a persistent "individuality" of the chromosomes is a question of terminology.   What the facts do not permit us to doubt is that chromosomes conform to the principle of genetic continuity; that every chromosome which issues from a nucleus has some kind of direct genetic connection

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[1] *Delage, 1895, p. 751, very clearly expressed the idea of a linear arrangement in the chromosomes.*

[2] *Wilson, 1925.*

with a corresponding chromosome that has previously
entered that nucleus.

What here is individuality? If I am able to keep alive
all the descendants which arise from a single slipper ani-
malcule by cell-divisions at the rate of three divisions a day,
in seven days I shall have 2,097,152 organisms, each of
which has 1/2,097,152 part of the original *Paramoecium*.
Only this fraction, then, of individuality is maintained.
And this is a much simpler case than that of individuality
of each member in a chromosome-complex from fertilized
egg to adult of a complex organism. I think that the
question of the persistent individuality of the chromosomes
is more than a question of terminology.

If we turn from the chromosomes to the genes themselves
that compose each chromosome, we find the same difficulty.
It has been suggested that the gene is a molecule. The
two daughter-cells arising from first cleavage would then
possess chromosomes made up of half molecules; in the four-
cell stage there would be quarter molecules; and in each
succeeding division there would be a corresponding reduc-
tion of the original gene-molecules found in each chromo-
some of the fertilized egg. But in chemistry we know of no
fractional molecules. Then, let us start with the adult
organism. On the assumption that each gene in each
chromosome of every cell of the whale, for example, is a
molecule, it is necessary to assume that each gene of the
fertilized egg is a complex of molecules whose number
equals that of the sum total of all the descendants from
this gene which are in each and every cell of the adult. It
would be necessary further to assume that the gene-mole-
cules vary in size in stages of development from egg to
adult. The gene therefore can not be a molecule according
to physico-chemical usage. The proponents of the gene
theory should be the last to postulate any non-physico-
chemical molecule.

Thus the term, individuality of the chromosomes or of their constituent genes, is of restricted meaning. I can not see how a given chromosome or a gene in the fertilized egg should be the same individual in every cell of the body of an adult male including every one of the billions and billions of spermatozoa that such an animal, like the trout, for example, sheds during its life-time. With regard to the "individuality" of the genes or the chromosomes genetics has offered us nothing except a term, which, as we have seen, is not even clear. From this point of view the gene theory certainly can not give an explanation of the development of an egg into an adult organism.

Let me hasten to state that in company with the majority of biologists I consider the weight of evidence sufficient for the assumption that the chromosomes have to do with heredity. As I have pointed out already, I see in the nucleus that component of the cell which tends to maintain the specificity of the cells by its change-resisting character. Then the chromosomes maintain individuality or identity. This maintenance however, as we have seen, can only come through growth at the expense of the cytoplasm. This fact allows us to see in a new light this maintenance: it is brought about by growth, the up-take of material that carries or assumes the characteristics of that which is already present in the chromosome. Further, this maintenance of identity must stand in some relation to the source that furnishes the material, namely, the cytoplasm.

Counter to this fact of the growth of the chromosomes at the expense of the cytoplasm runs the postulate of those geneticists who seek to explain differentiation by the gene-theory of heredity, namely, that the genes are factors which order the developmental processes by giving up substance to the cytoplasm. But even if the upholders of the gene-theory of heredity accept the fact that the chromosomes grow at the expense of

the cytoplasm, their conception of the action of the genes as unalterably fixed entities can not explain differentiation of development. For how could the genes be responsible for differentiation, if they are the same in every cell?[1] Unless the geneticists assume that their genes are omnipotent, we can not understand how the problem of differentiation can be solved by the gene-theory of heredity.[2]

The gene-theory of heredity is an ultra-mechanical rigorously bound concept. This mechanistic rigidity renders it inadmissible as an explanation of the process of development, a process marked by the egg's inherent capacity and by its mobile responses to external influences. On the other hand, the gene-theory, postulating factors in chromosomes, fits in well with the stable and change-resisting character of chromosomes. But in thus accentuating the relative non-mutability of the chromosomes as carriers of heredity, the most stable possession of the organism, the gene-theory sets off the process of heredity from differentiation of development; hence, as we have seen, it offers us no help in our attempt to explain differentiation.

Now the gene-theory as formulated may not be the only way of interpreting the vast amount of reliable data accumulated by the numerous geneticists the world over. These many data, let us say at once, we accept. If, however, we can offer another interpretation of them, can for example substitute for the factorial concept in the gene-theory another concept which is less rigid, more consonant with our knowledge of physiological processes and one

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[1] *Cf. Lillie, F. R., 1927; Conklin, 1924, et. al.*
[2] *Untutored savage man made his god as big as possible because his god could do everything. It remained for the geneticists to make one of molecular size, the gene. Here obviously infinite minuteness means infinite capacity. According to one geneticist, Demerec, the gene has almost magic power. This is physicochemical biology with a vengeance!*

substituting a means of protoplasmic reactions for a non-physical concept of molecules, we should be able to do what so far has been attempted in vain in biology, namely, to envisage differentiation and heredity as merely two expressions of development.

It has been said that differentiation of development and genetics must remain forever separated.[1] And when and wherever geneticists have attempted a union of the two, they have failed.[2] But since heredity is expressed during the process of development and indeed is a kind of differentiation, biology must attempt to find ground common to both. Up to now this common ground has not been located. I here propose a suggestion concerning the rôle of the gene in heredity and in differentiation.

The moment that we postulate that differentiation during cleavage turns upon the taking up of potencies leaving free in the cytoplasm those that give the blastomeres their characters as such, we see that an organ becomes such because in the cytoplasm of its cells are those potencies free which make it a special organ. Every cell in an organism becomes what it is because its cytoplasm has free its particular potencies whilst its nucleus binds all others. These latter would, if left unbound in the cytoplasm, act as obstacles to the display of the special potencies. Thus, the removal of potencies at the same time means removal of obstacles to cytoplasmic reactions.

My fundamental thesis is that all the differences, i.e., differentiation, that appear during development, rest upon cytoplasmic reactions. These are made possible through removal of obstacles by nuclei, hence, by chromosomes and genes. The nuclei by removal of substances release the activity of the cytoplasm in one direction. The genes also

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[1] *Lillie, F. R., 1927, p. 367.*
[2] *Morgan; Goldschmidt.*

act by removing impediments to cytoplasmic reactions. Let us examine this proposition more closely.

I begin with the assumption that with the onset of the fertilizable condition of the egg there are in the cytoplasm all the potencies—chemical reactants—whence arise the future organs. Indeed, as experiments on fragments of eggs obtained during this period show, such cytoplasm has capacity to produce several embryos. The chromosomes in such an egg now begin to remove from the cytoplasm potencies so that others remain free to initiate reactions responsible for differentiation. Thus progressively restriction ensues.

Contrast this condition with that in the history of the spermatozoon. With the two rapidly ensuing maturation-divisions the cytoplasm of the spermatocyte is divided among four spermatids giving rise to mature spermatozoa with less cytoplasm than the mature egg possesses. Egg and spermatozoon thus are markedly different with respect to the amount of their cytoplasm at the moment of fertilization. We may recall a further difference mentioned earlier in this book. Whilst eggs can develop in some cases without the spermatozoon, no spermatozoon has ever been found capable of development without the cytoplasm or at least a fragment of the cytoplasm of an egg. In capacity for development, thus, the egg is superior to the spermatozoon. This superiority is related to the egg's cytoplasm since this can develop either with egg- or sperm-nucleus. Now, according to our conception, the egg-nucleus gives up its potencies to its cytoplasm at some moment before the fertilizable stage. The sperm-nucleus, being likewise capable of taking up potencies again in the development of the egg which it fertilizes, must have lost its own potencies also before it takes up those residing in the egg-cytoplasm. When this loss occurs we do not know. A probable assumption is that as early as the moment when

the sperm-cell as such is differentiated from all other cells and retains in its cytoplasm only the potency for sperm-ness, this conditions the nullification in some way of those potencies borne by the chromosomes. Or they may be lost during the process of the ripening of the sperm-cell, when, as is known, concomitant with morphological changes amino-acids are lost. A less likely assumption is that potencies of the sperm-nucleus are lost and nullified at fertilization.

In any case, both egg- and sperm-chromosomes are pre-pared for removing stuffs from the cytoplasm of the egg. I bring an example: If a spermatozoon from a *Drosophila* possessing pure red-eye fertilizes an egg of an also purely red-eyed female, in the cells which show redness, the egg- and sperm-genes remove from the cytoplasm the hindrance to the reaction leading to redness. The "factor," redness, is resident in the cytoplasm and expresses itself because the genes remove stuff opposing this reaction. If on the other hand the sperm-chromatin is descended from a white-eyed animal and the egg-chromatin from a red-eyed one, the sperm-chromatin, when in those cells which give the color to the eye removes stuff which releases whiteness-reaction, and the egg-chromatin stuff which releases redness-reaction, with the result that the cytoplasmic reaction is now no longer $r + r = R$ as in the first case, but $r + w = R(w)$ where $R(w)$ gives either dominant red-eye color or an interme-diate color between red and white. Thus this conception, whilst consonant with the experimental findings of Men-delian genetics, differs from the theory of the gene for it places the determination of characters in the cytoplasmic reactions. The active "factors" for Mendelian characters do not reside in the genes; rather, the genes by extracting definite materials from the cytoplasm render possible the reaction of the cytoplasm-located hereditary factors. Only so far as they take out substance do the genes determine heredity.

Thus finally every cell in the most complex organism has in its nucleus all the potencies bound except that one which is free in the cytoplasm and which makes the cell specific. The egg-cell, for example, the moment it becomes differentiated from other cells of the body is such because it has in its nucleus all bound potencies except that which makes it an egg-cell. And it is this potency in the cytoplasm which determines the future growth and differentiation of the egg to that moment, when these bound potencies are thrown again into the cytoplasm. The sperm-cell is such because in its nucleus are all the potencies bound except that which determines the sperm-character of the cell. This potency determines the further differentiation of the spermatozoon and most probably is responsible for the nullification of the potencies in the sperm-nucleus.

This conception of mine concerning the action of the gene offers a far better interpretation for several phenomena than the hitherto proffered conceptions. Thus, for example, can we state that sex-differentiation is resident in the cytoplasm. For the majority of animals, the taking out from the cytoplasm of more sexual potencies by the chromosomes results in femaleness. So in those experimental conditions, where more than one set of chromosomes is present, the organism becomes female. The taking out of fewer sex-potencies by the chromosomes gives rise to a male. Thus an egg with half the somatic number of chromosomes, the so-called haploid number, is always, so far as we know, a male. Finally, hermaphroditism means equal abstraction of sex-potencies by the chromosomes, leaving in the cytoplasm both male- and female-factors.

These considerations warrant the assumption that the genes play a part in heredity but are not the factors of heredity in the sense of the geneticist; the genes act through their binding of potencies in such wise as to free the action of the cytoplasm-located factors of heredity—that is,

chemical reactions in the cytoplasm underlie both differentiation and heredity.

In thus relating heredity and the action of the genes to reactions in the cytoplasm, we come to a more physiological conception for heredity than the theory of the gene and one which instead of running counter to the facts of differentiation of development is consonant with them. From fertilized egg to the least active cell in the adult organism, the visible manifestations of life are outside the nucleus. It is the cytoplasm therefore that we consider to be the field of activities be they concerned with heredity or differentiation.

With reactions in the cytoplasm deals the fifth event during cleavage enumerated above with which our discussion of the cause of differentiation is concerned: the redistribution of water during cleavage.

From fertilization through cleavage eggs of marine animals certainly and all others probably show a visible altered distribution of water. This is best observed in experimentally treated eggs, but it can also be seen in normal development, as I stated in the chapter on water. After the initial ectoplasmic dehydration which occurs when eggs are fertilized or subjected to experimental means that initiate development, the egg establishes a new equilibrium with the sea-water. On this level, water, as discrete drops, moves from place to place within the egg or from egg to external medium. The formation of water-drops is a rhythmical phenomenon which accompanies each division-cycle of the cleavage-period.

The addition or removal of water in a reversible reaction determines hydrolysis or synthesis, respectively. Thus, water plays a rôle as a component in chemical reactions; in addition, it is both a solvent for other components and a part of the cytoplasmic structure, the chamber of the reactions. Then the demonstration that water is inter-

mittently present as discrete drops in viable cells having had any one of several kinds of treatment—whose action is not deleterious since one can prove that the cell after treatment returns completely to the untreated state—has far-reaching significance: it becomes an index to the nature and rate of reactions. In both normal egg and blastomeres, the rhythmical appearance of water-drops allows us to correlate their occurrence with the rhythm in a cleavage. Differences in the formation of the water-drops in different blastomeres we may regard as an index to chemical changes underlying the progressive restriction that runs with cleavage.

With each succeeding cleavage the egg is further sub-divided into chambers of capillary dimensions. At the end of the cleavage-period the total surface-area of the blastomeres is much greater than that of the uncleaved egg. Thus, during cleavage the ectoplasm increases in amount. This increase of ectoplasm constitutes the last of the six enumerated changes that occur during cleavage. The cleavage-period may be defined as one in which the cytoplasm diminishes through its conversion on the one side into new nuclear substance and on the other into new cell-partitions. Having examined the rôle of nuclear growth in differentiation, I seek now to evaluate the evidence from which I derive a postulate concerning the rôle of that part of the ground-substance located at the cell-surface, the ectoplasm, in differentiation.

In the first line stands the fact that the ectoplasm increases in amount with the progressive sundering of the egg into blastomeres, regardless of the type of cleavage by which this result is reached. Whilst no animal egg is a perfect sphere and never by cleavage gives rise to blastomeres which are perfect spheres, nevertheless the ratio of total surface to total mass which obtains for a sphere when sub-divided into spheres is closely approximated by the egg

during cleavage; the mass of protoplasm does not increase but the total surface-area does. Increased surface-area is best seen in an egg with total cleavage which develops into a blastula. It is clearly shown by the egg of the starfish for during early cleavage the blastomeres are at first separated from each other. In totally cleaving eggs which give rise to morulae, the increased surface area appears in the covering blastomeres; they are more flattened and the ratio of surface to mass thus is greater. For eggs with discoidal cleavage, it has been established that new cells are added to the embryonic disc from the underlying yolk where nuclei undergo mitotic division without being partitioned off into cells; only when around those nuclei lying next to the disc cell-boundaries form, are new cells added to the growing embryonic disc. Cleavage-planes arise in superficially cleaving eggs only after the nuclei reach the ectoplasm. The difference between totally and partially cleaving eggs lies in the fact that the initial cleavages in the latter are confined to an ectoplasmic region which is utilized in the formation of cell-walls before additional ectoplasm forms; in the former, cleavage and increase in ectoplasm run more synchronously.

Since the ectoplasm is a part of the living system and since during cleavage no living substance is added to the egg from the outside world, the source of new ectoplasm is from the egg-substance itself: ground-substance alone constitutes this source.

But the interpretation of the rôle of the ectoplasm in differentiation does not rest only upon its amount and its source; in some of the events named as occurring during the cleavage-period, the ectoplasm plays a part.

In the first place, consider the fact that a fertilized egg, which possesses pluripotency, develops normally only into one embryo. This means that only if the ectoplasm of the blastomeres is removed from its normal contact with the

other blastomeres—as happens in the experiment of separating the blastomeres—do they develop separately. In the intact cleaving egg the ectoplasm interacts with that of the neighboring blastomeres and thus insures the normal development into one embryo.

Secondly, in normal development the egg cleaves into blastomeres, that is, new cell-partitions arise. When experimentally eggs are induced to differentiate without cleavage, abnormal development results. In such cases of differentiation without cleavage there is always an abnormal accumulation of ectoplasm.

In the third place: the embryonic axis in eggs of radiate animals and the plane of bilateral symmetry of the embryo in eggs which develop into bilateral animals, arise in the surface and not within the bulk of the egg-substance. If there be gradients of development these are ectoplasmic and not axial, polar or otherwise.

Fourthly, the ordering and shifting of cytoplasmic inclusions, in terms of primary causes, depend upon the ectoplasm, since the changes in the ground-substance, responsible for these movements, are themselves dependent upon activity in the superficial cytoplasm, as was shown in the chapter on cell-division.

The importance of the ectoplasm as a causal factor in differentiation is in addition predicated upon its general properties, its rôle in the exchange of water between egg and outside world, its necessity for fertilization, its significance in parthenogenesis, its part in initiating division of the cell. These attributes alone would suffice to establish the ectoplasm as a leading factor in differentiation. But there are other and more general considerations.

The establishment of new surfaces during cleavage alters the physical properties of the protoplasm. With the separation of the egg-substance into blastomeres the original physical state of the uncleaved egg is altered since some

of it goes to make up new nuclei and another portion forms the new cell-boundaries. Thus, the viscosity of the cytoplasm, for example, in the individual blastomere can not be the same as that of the cytoplasm of the uncleaved egg. Furthermore, the differential distribution of the cytoplasmic inclusions during cleavage certainly alters the original physical state of the egg.

With subdivision of the egg into blastomeres, changes in the chemical reactions take place. Each blastomere represents a separate reaction-chamber of capillary dimensions favoring especially those reactions which are confined to surfaces.

Cleavage means a change in the space-relations of the original egg-substance. With the formation of new cell-partitions, especially in totally cleaving eggs which form blastulae, the subdivision into cells means new disposition of materials which at first were in more intimate contact. The orderly arrangement of blastomeres may be looked upon as one of the most characteristic attributes of the cleavage-period. The cell-surfaces are not simple ones but are made up of prolongations of unequal distribution, length, and activity.

With cleavage, then, by virtue of the ectoplasm, the original egg-substance is separated into blastomeres and the blastomeres are integrated by means of intercellular connections, that is, the ectoplasmic prolongations. It is well known that cells in a strip of tissue, like a strand of ciliated epithelium, behave differently when part of the strip and when isolated. The beating of the cilia can be observed in an intact strip to run in order from one cell to the next; whereas when the cells are isolated the cilia beat irregularly. The same phenomenon has been observed in suspensions of spermatozoa.[1] In a normal sperm-suspension

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[1] *Lillie, F. R., 1913.*

each spermatozoon moves by the lashing of its tail. If the spermatozoa are caused to form balls by the addition of some substance which agglutinates them with their heads sticking together, the tails now beat one after the other in succession. It is also well known that cells of a tissue when isolated and grown in tissue-culture manifest form-changes and behavior which never occur when they are part of the tissue.[1] Finally, blastomeres when isolated reveal behavior arising out of their isolation. In brief, therefore, the behavior of blastomeres during cleavage must be in part due to an integration brought about by the intercellular connections arising from the ectoplasm.

The movement of blastomeres is an undoubted factor in development and this movement is a function of the ectoplasm. The rise of cells out of which is formed an embryonic area in a specific region is to be attributed to a movement on the part of the cells which is closely akin to amoeboid movement. True, physical factors may be concerned in the displacement and new alignment of blastomeres, but these are to be regarded as subsidiary. No purely physical theory has as yet accounted for the process of invagination by which a hollow blastula is converted into a gastrula. On the assumption, however, that cells take up a certain position with reference to others through their active movement we may approach the explanation of many processes, as invagination and evagination, epiboly, etc.

Finally, there is clear experimental evidence that during cleavage the ectoplasm reveals structural changes and activities which parallel cleavage-rhythms and which indicate that the ectoplasm plays a rôle in determining the direction of differentiation. Some simple observations of my own may be cited.[2]

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[1] *Harrison, Lewis and Lewis, et al.*
[2] *Just, 1928c.*

If eggs of *Arbacia* be exposed to hypotonic sea-water during the time after fertilization when the vitelline membrane is being separated and are then returned to normal sea-water, they develop throughout the cleavage-period much as untreated fertilized eggs. A sharp difference is noted, however, in the blastula stage, for then, whereas the normal blastula is made up of cylindrical cells enclosing a cavity, the eggs which have had treatment with hypotonic sea-water are made up of cells approaching the cuboidal form; the cilia of their cells are longer than those on the normal blastula.

If the eggs of the same species of sea-urchin are treated three minutes after fertilization, that is, two minutes later than those used in the observation described above, part of the egg protrudes beyond the membrane and these eggs develop as twin embryos. In some cases the twins remain joined, in others they become separated. I have found by exposing eggs from the same females at different intervals of fifteen or thirty seconds after fertilization that the most favorable time for the production of these twins comes immediately following the separation of the membrane from the entire egg. Thus, the same treatment if given in a different stage of the egg's development, yields a different result. This statement holds generally for the different periods during the cleavage-process; to treatment with hypotonic sea-water, eggs in different stages of development respond differently as shown by the difference in the types of embryos resulting.

Now the variety of the results obtained in these observations can be related to ectoplasmic behavior. Beginning with the moment of fertilization the ectoplasm goes through changes in behavior which are easily followed under the microscope. One needs, therefore, only to treat the egg during periods when by observation one notes a difference in the quality of ectoplasmic behavior in order to obtain an alteration in the development.

The evidence that the treatment affects the ectoplasm alone is derived from the following considerations: First, there are no other noticeable or as striking changes elsewhere in the egg. For instance, the time for obtaining blastulae with cuboidal blastomeres and long cilia comes when the egg-surface is breaking down in the process of separating its vitelline membrane. On the other hand, the best time for the production of twins comes when the ectoplasmic surface is being reconstituted, or, one might say, a new surface arises. Now at these moments, separated by a time interval of about two minutes, no profound changes either in the egg- or sperm-nucleus or in the cytoplasm below the ectoplasm can be noted. Secondly, the method employed in these simple observations is that of treating the eggs experimentally for fifteen or at the most thirty seconds. An exposure to hypotonic sea-water of this duration is most certainly to be regarded as affecting the egg-surface only. I conclude, therefore, that the various alterations in the development called forth by treating eggs with hypotonic sea-water at intervals after fertilization are due to an effect on the ectoplasm. As development ensues, the ectoplasm undergoes definite changes correlated with the definite periods in development. If these surface reactions are modified, development is modified. I may refer again to the phenomenon of differentiation without cleavage. Certainly this represents an extreme type of developmental modification. As certainly it indicates that the alteration is due to modification in the behavior of the egg-surface.

Finally I cite an observation on the egg of *Asterias*[1]. 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 blasto-

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[1] *Just, 1931.*

meres 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, by surrounding them with fibres of lens paper, 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. For example: two lots of eggs from the same female were fertilized at an interval of one minute apart from each other. At second cleavage, the four blastomeres were removed from one egg of each fertilized lot. Two blastomeres of the one were next brought together with two blastomeres of the other; no union was established. Observation revealed that the surface-changes in the transposed blastomeres were different. This observation furnished the clue for the failure of union between transposed blastomeres taken from a lot of eggs which had been fertilized at the same time: For the establishment of the interconnection of blastomeres, the ectoplasmic condition must be identical. Therefore, the union of blastomeres can be made certain if by direct observation their surface changes have been found to be in the same stage. Since in any lot of eggs in best condition fertilization takes place in every egg at almost the same instant and the development ensues at much the same rate, at second cleavage the difference in the ectoplasmic behavior between the most slowly and the most rapidly developing egg is never as great as that in eggs fertilized at an interval of one minute. The failure of blastomeres from two eggs of the same fertilized lot to unite therefore is to be attributed to changes in ectoplasmic behavior resulting from a difference in rate of development which is of seconds only. To my knowledge, there exists no observation which so

clearly and so beautifully shows the quickly occurring changes in ectoplasmic activity.

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.

The moment to moment changes in the ectoplasm in its response to the cell's milieu set up conditions in the cytoplasm which are favorable to the mechanism of the synthesis of nuclein, i.e., of the building up of nuclei out of cytoplasm. This synthetic reaction constitutes the removal of a barrier to those cytoplasmic reactions leading to differentiation. Alongside this function of setting up conditions favorable to the releasing-reaction of nuclear syntheses, is the growth of ectoplasm through the transport of ground-substance to the cell-surface. The ectoplasm impresses the cytoplasm, and this ectoplasm-induced cytoplasmic activity brings about the nuclear behavior which underlies genetic restriction. Ectoplasmic behavior is thus the direct and so far the only visible manifestation of the cause of the differentiation of development which takes place during cleavage.

Chromosomes and Ectoplasm

IN A LARGE MEASURE OUR KNOWLEDGE OF THE EFFECTS of experimental means on cells is the result of studies on eggs. But in these studies the primary effect of the experimental means on the protoplasmic system was often misinterpreted. Enthralled by the dramatic manoeuvres of the chromosomes in mitosis investigators assumed for the chromosomes an independence from the remainder of the cell; this point of view easily led to the assumption that external agents directly affect the chromosomes. So also, the origin of mutations has been predicated upon inherent activity of the chromosomes.

The effects on marine eggs of changes in temperature, in salinity or in hydrogen-ion concentration vary, depending upon the eggs employed. For a species of eggs these effects vary before and after insemination. For the fertilized egg again there is a differential effect of the means which runs with the mitotic cycle. But there is here no evidence to indicate that this effect is primarily or only on the nucleus or its chromosomes; what the experiments do show is that the effect is first on the ectoplasm.[1]

These changes in the surrounding medium bring about changes in the nucleus only secondarily. Always the ectoplasmic changes come first. Indeed, within optimum range, the magnitude of the ectoplasmic changes determines that of the nuclear. There is also a relationship between the duration of the exposure of the egg to these changed

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[1] *Just, 1932.*

environmental factors and the quality and character of the ectoplasmic reaction. Again the intensity, that is, the strength of the change in the environment, determines the rate of the ectoplasmic response; and within optimum range, this rate determines the degree of the nuclear response.

Specific examples to support these statements one may find in the literature on fertilization, experimental parthenogenesis, experiments on cell-division and on development. I may mention again the observations on the egg of *Chaetopterus* which after treatment with hypertonic sea-water develops and differentiates into an abnormal swimming form; here clearly ectoplasmic activity conditions nuclear behavior. In sea-urchins' eggs treated with potassium cyanide, the failure of mitosis runs with an exaggerated activity of the ectoplasm.[1] Also, in sea-urchins' eggs, the experimental prolongation of the monaster stage is conditioned by an exaggerated ectoplasmic activity.

Egg cells have been subjected not only to changes in temperature, in salinity and in hydrogen-ion concentration of the surrounding sea-water; they have also been exposed to radium, Roentgen and ultra-violet rays. The first noticeable effect of such exposures is on the ectoplasm.

Some years ago Packard[2] reported results of exposing eggs of *Nereis* to radium. The first effect observed is cytoplasmic. In consequence or at least in sequence to this follow modifications in nuclear behavior. Redfield[3] especially has analyzed the ectoplasmic response displayed by this egg after exposure to various rays. In this same egg I found that ultra-violet rays induce profound local ecto-

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[1] *Just, 1927b.*
[2] *Packard, 1914.*
[3] *Redfield, 1918.*
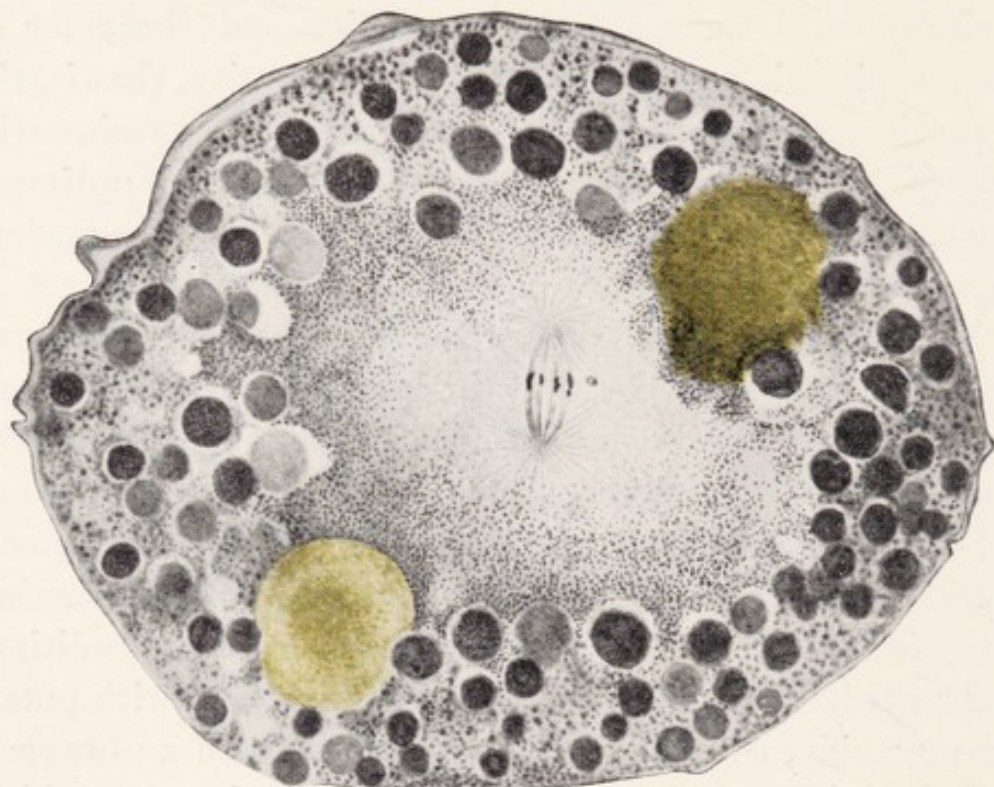
Fig. 37.—Egg of *Nereis* with first maturation spindle held near the centre of the cell as an effect of ultra-violet irradiation.
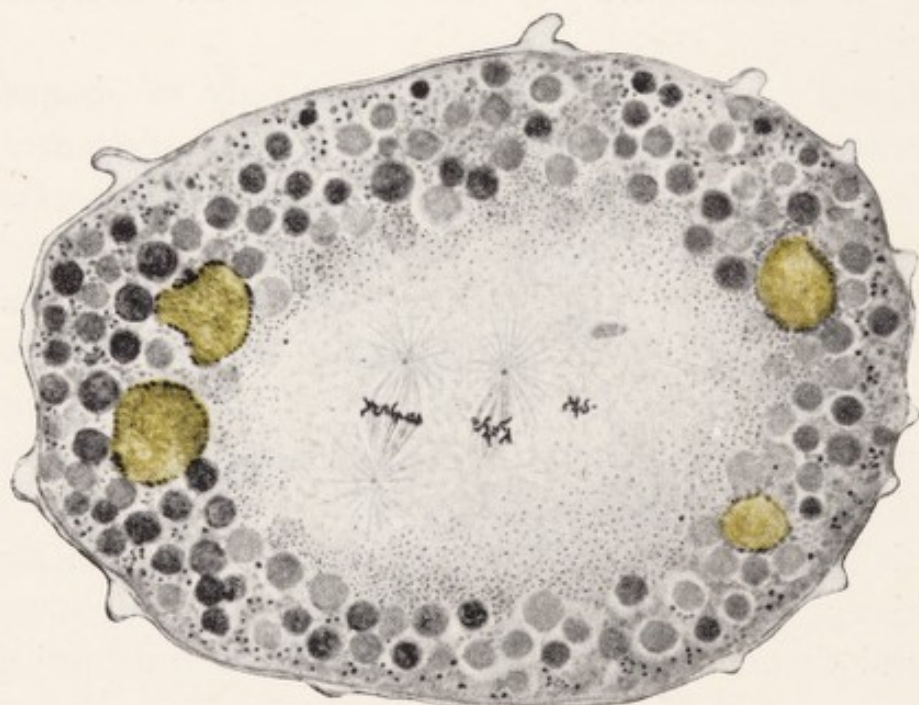
Fig. 38.—Irradiated egg of *Nereis* showing nuclear division without extrusion of polar bodies.

plasmic changes which are followed by abnormal nuclear behavior.[1]

The effect of the rays is limited to the superficial cytoplasm, whose altered condition brings about the remarkable result that the eggs develop with seventy chromosomes instead of the normal number, twenty-eight. The process is as follows: Consequent to the injury of the ectoplasm, the normal cytoplasmic movements are inhibited. As an effect of this inhibition the first maturation-spindle formed

FIG. 39.—Irradiated egg of *Nereis* with four groups of chromosomes resulting from division of the two spindles in Fig. 38.
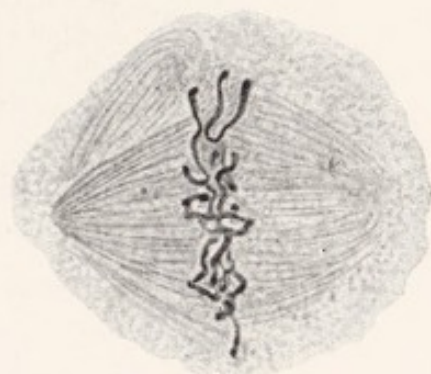
FIG. 40.—First cleavage spindle, irradiated egg of *Nereis*, showing some of the seventy chromosomes.

after break-down of the germinal vesicle fails to move to the surface of the egg. Instead of at the periphery—to which normally the spindle moves and where after separation of the chromosomes their outer group is extruded with the first polar body—the first maturation mitosis takes place at or near the centre of the egg (Fig. 37), two nuclei arise and from each of these a spindle forms, each giving rise to two groups of chromosomes (Fig. 38). In other words, the first and second maturation mitoses take place near the centre of the egg and four egg-nuclei arise (Fig. 39). These four nuclei with the sperm-nucleus, each containing fourteen chromosomes, give rise in some cases to

[1] *Just, 1926a, 1933b and c.*

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a cleavage-spindle with seventy chromosomes (Fig. 40). In other cases multi-polar (two, three or more) spindles
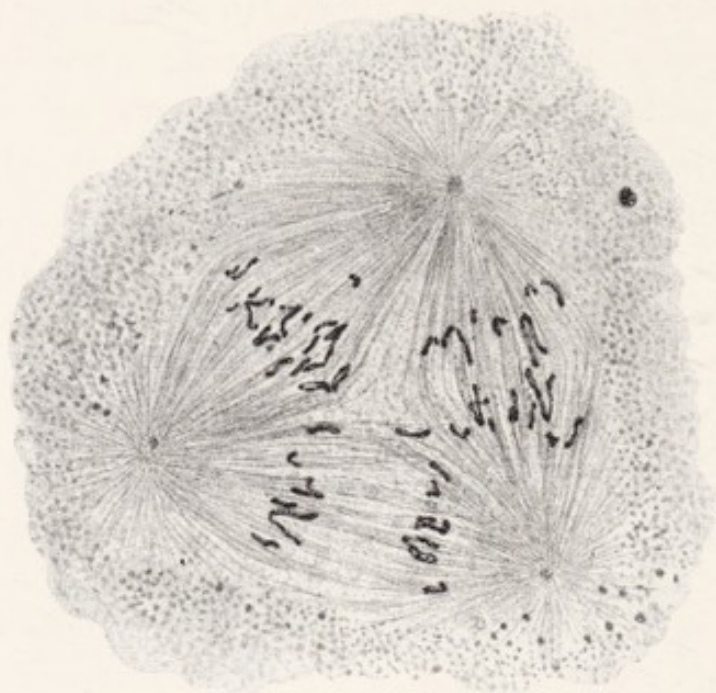
Fig. 41.—A multipolar first cleavage spindle, irradiated egg of *Nereis*.

Fig. 42.—Another type of multipolar first cleavage spindle, irradiated egg of *Nereis*.

arise as the appended figures (Figs. 41 and 42) of the radiated *Nereis* egg show.

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The striking similarity between these figures of abnormal mitoses and those found in cells of human cancer deserves some comment.

Almost fifty years ago Galeotti described and figured abnormal mitoses in human cancerous cells. Later he showed that poisons, acting on skin cells of the salamander, induce pathological mitoses closely resembling those found in cancer. Subsequently various investigators have confirmed Galeotti's findings. Cancer cells of man and of other mammals in tissue culture likewise show abnormal mitoses. If one compares under the microscope my preparations of these abnormal mitoses in eggs of *Nereis* with those of human cancer cells, one notes at once that, except for their greater clearness and sharpness due to more excellent technique, the mitotic figures in the eggs bear the closest resemblance to those in the cancer cell. How far this resemblance has common cause, it is hazardous to assert. And this for the following reasons.

In the first place, eggs of a worm, freshly discharged into the sea, are far removed from the cells of warm blooded animals, which in the intact organism stand in a quite different relation to their environment, conditioned by nervous and humoral integrations. Conclusions for human cells derived from studies on cells of other mammals even are not always safe. Hence we are wary in making final statements on the basis of animal experimentation concerning human disorders, the more so since man varies so much one with the other, and since in one individual the harmonious adjustment of cells, their capacity for self-regulation, so greatly depends upon that so little understood principle of individuality, which in the final analysis goes back to the chemical make-up of the ground-substance of every single cell;[1] from it emanate what we designate individual resistance and susceptibility.

[1] *Just, 1936b.*

In the second place, the fact that abnormal mitoses arise in response to most diverse means needs to be considered. The *Nereis* egg which portrays them as an effect of radiations, responds in like manner to other means.[1] Then the reaction is an expression of independent irritability which reveals specific structure. So with somatic cells which react similarly. Thus, abnormal mitosis does not as such mean that the cell is one of cancer. Moreover, not every human cancer cell displays abnormal mitosis.

None the less is the resemblance striking. Far removed though any cell—egg of worm or cell of an onion root tip—is from a human cell of cancer, it might not be too far fetched to see in the similarity of response a manifestation of a fundamental structure common to all protoplasmic systems. It is as imperative for the problem of cancer as for any problem in biology discussed in this book that we add to our knowledge of this structure.

The fact that the egg of a worm and a human cell show comparable configuration in abnormal condition points to a similarity in a basic pattern of organization in living matter upon which the characters of speciation are imposed. If therefore we could designate in the egg one characteristic deformation as diagnostic of its response to changes in the external medium, we should have at hand a point of departure for study of the cancer cell. Now such diagnostic is, as I shall show, available: All means that elicit the rise of abnormal mitosis do so through alteration of the ectoplasm. A more exact cytology of human cancer cells with reference to ectoplasmic behavior is thus indicated.

The effect of the radiation on the ectoplasm of the egg of *Nereis* is shown in the following ways. First, the normal egg after fertilization extrudes a jelly throughout the whole ectoplasm so that every fertilized egg is enclosed in a hull

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[1] *Just, 1936c.*

of jelly of the same consistency throughout and of equal width. The ectoplasm whence the jelly escaped shows no persistent localized inequality. The changes taking place in it are not confined to any particular region. The egg fertilized after radiation, on the other hand, extrudes jelly of lesser degree of consistency so that the eggs tend to fall out of their jelly hulls. Always is there present in the ectoplasm thereafter an injured region marked by the change in the structure of the ectoplasm as well as by the greater distance of the vitelline membrane at this site. This change in the physical make-up of the jelly is an index of the ectoplasmic injury induced by radiation. In the second place, in the radiated eggs the first cleavage plane passes through the mark of injury revealed after the extrusion of the jelly. Third, a localized area of injury in the ectoplasm persists through the egg's development and can be traced into the larval worm.

This observation on the effect of ultra-violet rays on the *Nereis* egg is supported by my findings on the egg of *Chaetopterus;* in a similar, but perhaps not so striking a manner, ultra-violet rays affect the surface cytoplasm of this egg.[1]

Since in some cases radiations, especially radium and Roentgen rays, are most effective after nuclear breakdown, it is often held that irradiation does not affect the cell while its nucleus is intact. From this it is argued that radiation directly affects the chromosomes. Indeed, some workers consider that the action of Roentgen rays, for example, is limited to the chromosomes alone. Against this position arguments may be adduced.

For many cells it has been learned that the display of normal mitotic activity depends upon some condition in the cytoplasm which often may even be visible. It is well

[1] *Just, 1930d, 1934c.*

known that a certain complex of physical and chemical conditions in the cytoplasm is necessary for the initiation and completion of mitosis. The rhythm of nuclear division is itself parallel with the rhythm of structural and physico-chemical changes in the cytoplasm. Abundant evidence indicates that parallelling the rhythm of mitosis is a rhythm of susceptibility and resistance of the cytoplasm to many and diverse experimental means. In some cases, notably that of the egg of *Nereis*, the experimental means including radiations are as effective on the egg in the condition of the resting nucleus as in stages of mitosis. The susceptibility displayed by a nucleus is to be correlated with changes in the cytoplasm; and since radiations are not alone among means which may be more effective on cells in stages of mitosis than on those with the so-called resting nuclei, we can not denominate Roentgen or radium rays as specific chromosome-affecting means.

What is true is rather this: radiations are far nicer means than, for example, hypotonic and hypertonic solutions, heat and cold—all of which give effects similar to those of radiations. Radiations produce effects which are more rapid, more exactly measurable and more widespread in a given population of cells. This, in my judgment, is the chief value of radiations as experimental means.[1] Failure to appreciate that in experiments with radiations the cytoplasm is affected and that radiations belong to a large class of experimental means has given birth to the fertile error that Roentgen, radium and other rays are causative factors for inducing directly profound changes in chromosomes. The offsprings of this notion, nursed by the gene-theory of heredity, are theories concerning the origin not only of species but also of the whole realm of living things.

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[1] *Just, 1936c.*

The fact that in Roentgen therapy there is a difference in the susceptibility of human cells does not vitiate the argument. Among these cells those most highly endowed with division and growth capacity are most susceptible—a fact which does not prove that the rays are chromosome-specific in their action. Rather, because of such division- and growth-capacity, the cells have cytoplasm whose condition renders them more susceptible to radiation than other cells.

As we have seen, the effect of the feebly penetrating ultra-violet rays is a sharply localized ectoplasmic injury. Hence, it is not the penetrating power of the rays and so their power to reach the more deeply lying chromosomes which is responsible for their effects. Even the more deeply penetrating rays, Roentgen and radium, as Redfield has shown, also affect the ectoplasm; we therefore can not assume an effect of these radiations that is limited to chromosomes only.

Further, materials entering the cell—gases, water, etc.—come into relationship first not with the nucleus but with the ectoplasm. We may here reason from analogy. Take oxygen consumption, for example.

There was a time when biologists assumed the nucleus to be the seat of cellular oxidations. The presence of iron in the nucleus was postulated as part of the oxidation mechanism. On *a priori* grounds one would assume that oxygen entering the cell would combine with cellular constituents lying in the cytoplasm between the cell boundary and the nucleus. Now we know that this is at least nearer the truth: oxygen consumption is a function of cytoplasmic structure. Moreover, the attempt to demonstrate the presence of iron in the nucleus of spermatozoa failed though impeccable methods were used.[1] What is true of oxygen is

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[1] *Cf. Lynch, 1922.*

doubtless true of other substances that enter cells: they react first with the cytoplasm.

Experimental cell-study gives instances of aberrant behavior of the chromosomes in egg cells. All of this work indicates that the experimental means first affects the ectoplasm. According to the theory here advanced this ectoplasmic effect is responsible for secondary effects on the whole egg cell. Among these is the aberrant behavior of the chromosomes.

In the early days of the modern work on genetics, the experimental analysis could not have got very far without the descriptive studies on the behavior of the chromosomes. In the first line of this classic period for cytology stands Boveri's work. According to his analysis of experiments on dispermic fertilization, normal development depends not on the number but on the proper combination of chromosomes. These experiments furnished part of the basis for the theory of the individuality of chromosomes.

To secure dispermic or polyspermic fertilization of an echinid egg, one must first weaken its ectoplasm or employ heavy insemination. Where polyspermy ensues without experimental weakening of the eggs, we must assume that they were weak at the outset. The aberrant development of the blastomeres in his experiments Boveri related to the wrong combinations of chromosomes. But these combinations themselves depend upon the weakened conditions in the cytoplasm which make dispermy possible. In the normal egg the unimpaired ectoplasm protects against disorder of the chromosomes.

Aberrant behavior of paternal chromosomes is revealed in cross-fertilized echinid eggs when in certain crosses the paternal chromosomes, failing to take part in the mitotic process, are eliminated from the spindle. The possibility for cross-fertilization in all these cases is rendered greater by injuring the ectoplasm of the eggs. Here again, there-

fore, the condition of the cytoplasm determines the behavior of the chromosomes. To induce cross-fertilization especially between widely separated species, for most eggs at least, one must impair the integrity of the eggs' ectoplasm. Such impairment means a weakened cytoplasm. As with the aberrant behavior of the chromosomes in experimental polyspermy, so with that in cross-fertilization: it follows injury to the egg's ectoplasm.

A point here must be emphasized. Too frequently biologists speak of the incompatibility of chromosomes to account for the elimination of chromosomes in cross-fertilization. As a matter of fact, chromatin may be eliminated in straight fertilized eggs. I have found in straight fertilized eggs of *Echinarachnius* that the whole egg-nucleus may fail to take part in the cleavage-mitosis.[1] Such eggs are injured.[2] Here there can be no question of the "incompatibility" of chromosomes to foreign cytoplasm. Rather, the chromosomes fail to take part in the ensuing mitoses because of the weakened condition of the cytoplasm previously treated. Work on the effect of temperature and of ether on the sperm-nucleus in the echinid egg are amenable to the same interpretation. The behavior of monaster eggs is also a case in point: the abnormal behavior of chromosomes is clearly due to injury of the superficial cytoplasm brought about by vigorous shaking at a time after insemination when the ectoplasm is very susceptible to experimental treatment. The effect of radiation referred to above on straight fertilized eggs may be recalled. Here again the aberrant behavior of the chromosomes is in consequence of a definite ectoplasmic injury. Finally, Dubois has shown for the egg of *Sciara* that chromosomes are normally elimi-

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[1] *Just, 1924.*
[2] *See also J. Gray on Echinus-egg.*

nated during cleavage and that this elimination is determined by the ectoplasm.[1]

In all the foregoing it is safe to conclude that the injury to the cytoplasm is an ectoplasmic injury. We may therefore proffer the hypothesis that in ectoplasmic behavior lies the cause of the behavior of the chromosomes. Normal chromosome-distribution and -combinations depend upon the integrity of the ectoplasm; their aberrant behavior is the effect of the loss of this integrity. Such behavior may manifest itself in chromosomal elimination, fragmentation, and the like.

To me the conclusion seems inescapable: ectoplasmic behavior determines the cytoplasmic reactions that lie at the basis of nuclear activity in both normal and abnormal mitoses. Where experimentally induced mutations are related to visible changes in the chromosomes, as their fragmentation, translocation, etc., these are not to be regarded as direct effects of the experimental means employed,—e.g., temperature, radiations—but as expressions of the antecedant altered cytoplasmic reactions brought about by changes in the ectoplasm that evolved in response to the action of the means.

If one observes under a microscope, equipped with a dark-field condensor, a suspension of Chinese ink ground up in water, one sees these inanimate particles scintillating back and forth in Brownian movement. One knows that the shining particles are moved and are not themselves motile. It is a far cry from the dance of ink-particles to the orderly movements of chromosomes in a living cell. And yet, it is warrantable to assume that as we look upon the shifting of chromosomes in cells, their constant distribution during cell-division, generation after generation, we behold, not a motility inherent in these bodies themselves

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[1] *Dubois, 1933.*

but a behavior which expresses a force in the cytoplasm enclosing them. In normal mitosis, in experimentally induced mutations, the chromosomes express the underlying behavior of the medium in which they lie. They mark for us the ebb and flood of the cytoplasmic tides. These in turn are under control of ectoplasmic activity.

13

Ectoplasm and Evolution

THE PRINCIPLE OF EVOLUTION IS AS FIRMLY ESTABLISHED as any in biology. The evolution-theory constitutes a fundamental postulate of the science of biology and has proved a guiding principle of uncalculable value for biological research. Among biologists exists the almost unanimous verdict that evolution took place. According to the prevailing opinion, the world of living things was evolved from a unicellular organism.

It should however be emphasized that this first form of life was not that of some now existing protozoon. The word, Protozoa, literally means first animals; but we should bear in mind that among the Protozoa themselves evolution has taken place. We therefore assume that the first form of life was a simpler unicellular structure than the protozoan. From this both the Protozoa as we now know them and multicellular animals came, the former evolving in one direction, the latter in another.

We encounter two questions: How did this first living thing arise? What was (and is) the cause of evolution?

With the first question most biologists will not concern themselves—they deem it unanswerable and thus only provocative of fruitless speculation. And yet such speculation will always be alluring. The drama of the universe in the act now before us is a tremendously moving spectacle, but the prologue to its pageantry is also capable of moving the dullest imagination. One need, therefore, make no apology in voicing a note concerning the origin of the world of living things. The answers to the second question are

innumerable, as is attested by the many and various theories of evolution. Those biologists who, after having weighed the evidence, accept the theory that animals and plants exist as products of evolution and reject the theory of a special creation for every living being that ever was and now is, by no means agree concerning the cause of evolution.

To take up our first question: How did the first living thing arise? To put it otherwise: How out of non-living matter did life arise?

The combination of chemical compounds from the environment to make up the first living thing must obviously have meant a separation from the environment, that is, the combination must have been peculiar, both physically and chemically; otherwise, there never could have come about its separation and the maintenance of its integrity apart from the environment. Now the moment that this peculiar combination of compounds arose, there must have begun reactions or responses of it to the environment—especially to temperature, to gases and to electrolytes. The chief characteristic of this original substance was its peculiar and complex organization, which set it apart from its environment; but at the same time it must have been responsive to environmental changes. Environmental changes must in the first instance have brought about the combination of compounds peculiar to living substance, and in the second place must have conditioned its activity.

This original mass of primitive protoplasm at first perhaps showed no high degree of differentiation, but we can scarcely imagine it as a homogeneous structure throughout; as such it could not have endured for any great length of time. The moment that we assume that a combination of chemical compounds was separated out from the environment as a peculiar system, we must postulate some differentiation in the mass—which differentiation served to keep the combination of compounds intact.

If, however, we assume that this combination of compounds separated from the environment was first purely homogeneous throughout, then there must soon have come a time when factors in the environment played upon this structure and so modified, if they did not determine, its behavior. It would be difficult to imagine a structure made up of the same elements or compounds found in the environment and maintaining its separateness from the environment without some structural difference from it.

The moment that such a peculiar combination of compounds arose, it assumed life; it had response to its environment, both because it arose from it and had to exist in it.[1]

Living substance can not be considered abstracted either from time or from space. The organism can not be separated from its environment; they form together one inter-acting system. Two predominating characteristics exhibited by living organisms are: first, those changes which are time-ordered; and second, those which are environment-conditioned. Thus, the organism—a single cell, for example—changes from moment to moment and the rate of such changes becomes its differentiating characteristic; within the organism these changes tend to run in one direction—the building up of protoplasm from simpler compounds while life lasts. External to it the environment plays a part in yielding up the raw material for these changes and setting the conditions for the reactions in the living matter. In a certain sense we should not speak of the "fit-

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[1] *Although there is the possibility of life below that size that can be made visible by the microscope, we must admit that we know little concerning the organization or structure of such ultramicroscopic organisms. Therefore, we might most profitably begin with a hypothetical organism whose size is within the range of resolution by the microscope. Nevertheless, what is said of such a hypothetical structure might also hold for one of ultramicroscopic size.*

ness of the environment" or the "fitness of the organism"; rather, we should regard organism and environment as mutually adapted.

The play of factors in the environment—of temperature, of gases and of electrolytes—upon the living organism must be first on the cytoplasmic surface. Even if we assume that the primordial living thing was a mass of homogeneous protoplasm structurally the same throughout, there must have early arisen a differentiation between surface and interior.[1] In the constant interchange between environment and organism reactions must have taken place first in the more superficially located cytoplasmic structure; these reactions would condition succeeding ones in the endoplasm. The first step in the evolutionary process, then, was a differentiation of the cytoplasm into ectoplasm and endoplasm. The second step, according to this theory, was a nucleo-cytoplasmic differentiation.

We have thus a picture of the primordial living thing as a mass composed of the prototype of the ground-substance in cells as we know them to-day which limited itself in space by a changed surface, its ectoplasm. In time this primordial thing showed a farther differentiation of a substance which by opposing its more fixed character to the ever-changing mobile character of the ectoplasm tended to maintain the stability of this primitive protoplasm. Thus, nuclear substance arose.

In thus postulating for the nucleus only a secondary origin I reject the theory that the first form of life was a chromatin-granule.[2] However attractive this latter theory may be to those who regard the ultra-filterable virus as living and to those who believe that the gene represents the fundamental living unit,[3] my speculations concerning evolu-

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[1] *Cf. Child 1915 on "Surface-interior patterns."*

[2] *Cf. Minchin, 1915.*

[3] *E.g., Jennings, 1936.*

tion derive their plausibility from their consistency with known facts of cellular phenomena: one of these indicates the elaboration of chromatin out of cytoplasm. More than once have I in the foregoing chapters referred to the origin and growth of chromatin out of the cytoplasm. On the other side, there exist no data to indicate that chromatin builds up cytoplasm.

The same emphasis that I place upon the differentiation of ectoplasm out of the ground-substance in the genesis of the primordial living thing, I place upon it as a cause in the evolution of the animal and plant kingdom.

Protozoa are classified into ascending orders on the basis of their ectoplasmic structure and behavior. Eggs develop into adults by virtue of ectoplasmic changes during differentiation. Among multicellular adult organisms, grades of complexity can be recognized: the more complex the organisms, the richer are their modes of integration as shown by comparative studies on nerve-systems. What makes man's brain the greatest among animals, is not the number of its nerve-cells—indeed, in a given area, the primate brain has fewer cells than that of other mammals—but the richness of their connections. Other forms of intercellular integrations in multicellular organisms are also ectoplasmic.

Let us recall once more the fundamental functions of living protoplasm. These are contraction, conduction, respiration and nutrition. The primordial contraction, let us say that exhibited by the egg or protozoan cell, involves the cell interior to a secondary degree only. The cilia of ciliated Protozoa are ectoplasmic structures. Muscle-contraction in the highest organisms is a phenomenon of the cell-surface.[1]

Also conduction, we found, is an ectoplasmic function. The transfer of the effect of a stimulus is ectoplasmic, as

[1] *Hill et al.*

for instance in the fertilization of the egg. In tissues highly endowed with conductivity, as in those highly endowed with contractility, the predominating characteristic is relatively large surface area. In nerve cells, conduction travels over the nerve fibre (ectoplasmic prolongation), and is transferred by means of ectoplasm from one unit to another. By and large, it is reasonable to assume that both contractility and conductivity are greater in surface-rich than in surface-poor protoplasm.

The subsequent differentiation from a hypothetical first form of life into animal or plant we may suppose came about through the higher development of contraction and of conduction by that form which evolved as animal. A deviation or less emphasis on these brought plants as such into being. It is surely on the side of the nervous tissue that animals and plants differ most.

Cellular oxidation is a function of cytoplasmic structure. Again it is reasonable to assume that oxygen coming into the cell makes first some union with the superficial cytoplasm, as we have seen.

Now of three fundamental life-processes—contraction, conduction and respiration—respiration may be regarded as primary: on it depends all vital activity. Respiration, the same in both animals and plants, may have been largely responsible for the early separation between plant and animal. That is, those primordial individuals possessing most rapid rates of oxygen-consumption tended to oxidize themselves, or as cannibals, their like. Some, because of lesser intake of oxygen, tended to accumulate CO_2 and thus developed photosynthetic power as a means of protection. With this went also the building up from CO_2 and water of carbohydrate polymers, including cellulose.[1] The presence of cellulose determined the disposition of the cytoplasm found in higher plants—that is, a cytoplasm of peripheral

[1] *Cf. also Geddes.*

location enclosing a vacuome. Thus, respiration may have determined the difference between animal- and plant-nutrition.

Animals evolved slowly or rapidly, farther and farther, depending upon the degree to which ectoplasmic behavior, its faculty for contraction and conduction, developed. The greater became these two forms of behavior, the more imperative became the need of exquisite means for respiratory-exchange. Respiration, according to our assumption, played a part in establishing the difference between animal- and plant-nutrition. Animal nutrition advanced showing progressively increased utilization of large surface-areas for the play of reactions—in digestion and for absorption of the digested end-products. With this evolved in increasing complexity a circulating system which gained steadily in structural perfection whilst its correlation with the respiration-system increased; and a more exact integration by nerves and finally by internal secretions arose.

Thus all forms of behavior by which we recognize that a thing is alive express themselves in response to the environment in the activity either of the ectoplasm itself— as in unicellular organisms—or of structures which are rich in ectoplasm. The fineness and nicety of ectoplasmic organization increase progressively in the animal kingdom from the lowest to the highest organisms and thus parallel the course of evolution. This course, from the emergence of life out of non-life to the separation of animals from plants and farther to the unfolding of progressive complexity of animal-form, makes manifest the rôle and importance of the ectoplasm in evolution.[1]

This hypothesis of the evolution of the living world holds also for the more restricted problem of evolution, the origin of species. As I see it, the most valid criterion for

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[1] *Just, 1933a.*

determining that animals of a genus belong to the same species is the capacity of normal fertilization and the production of fertile offspring. As has been abundantly shown, fertilization of an egg by a foreign spermatozoon is only possible if the ectoplasm has been debased. The unimpaired ectoplasm is a barrier to fertilization by non-specific spermatozoa. Then species arose through changes in the structure and behavior of the ectoplasm.

In the differentiation of ectoplasm from the ground-substance we thus must seek the cause of evolution.

14

Conclusion

*T*HE IMPOSING MASS OF EVIDENCE PRESENTED IN THE chapter, The Ectoplasm, can not be gainsaid. In all cells, of unicellular and multicellular animals, the existence of the ectoplasm can be demonstrated. We have seen what is its rôle in the phenomena of conduction, contraction, respiration, the intake and output of water—these all being general properties of all animal cells. More specifically for the animal egg, we have seen that without the ectoplasm, fertilization can not take place, that in both fertilization and parthenogenesis the response of the ectoplasm to the inciting means for development is prognostic for the quality of the future development; that in cell-division the ectoplasm initiates the event by regulating the movements within the cytoplasm and that by redistribution of its structure and relocalization of its activity, it establishes new cell-surface; and that during differentiation the ectoplasm increases in amount and reveals a differential activity. This behavior of the ectoplasm was shown to be one causative factor in differentiation of development, the other being the building up of nuclear material out of the cytoplasm. Thus the reactions underlying both differentiation and heredity were shown to be under the domination of cytoplasmic reactions, resulting from an interplay of both ectoplasm and nucleus with the cytoplasm. On this basis an interpretation of the action of the gene was offered. The behavior of the chromosomes themselves in normal cells and in experimentally induced mutations was shown to be dependent upon ectoplasmic activity. It was finally

suggested that ecto-endoplasmic differentiation is a factor in evolution.

Thus briefly the argument in favor of my theory of the ectoplasm. Let it be clearly understood, however, that the theory in no wise invalidates the conception of the protoplasmic system as the unit of the state of being alive. Rather, the whole argument upon which the theory rests aims at encompassing this conception in a fuller and more complete fashion than hitherto was possible. Because of the up to now existing failure to assign to ectoplasmic behavior a rôle in the integrative action of the living system, it has been necessary to emphasize what this rôle is. I wish very clearly again to state my position: life resides in the whole of the protoplasmic system taken as a unit—the phenomena of life are not to be dissociated from the integration of the system's constituent regions, the integration which is the basic manifestation of the state of being alive.

While life rests in the protoplasmic system as a unit, life is not static, is not structure only, but is the sum-total of activities; protoplasmic organization or integration, therefore, means orderly reactions as surely as it means space-distributions of the protoplasmic components. We may never come to know what life really is, but if we do approach such knowledge, it will be through appreciation of activities which constitute the protoplasmic integration; these we learn by their expression. Life is not confined to nucleus only and certainly not to constituent genes; nor yet does it reside in the ectoplasm alone. The significance of the ectoplasm is that it gives visible expression to these activities.

On the one side every living thing tends to fixity and rigidity, resistant to change. This tendency aids to preserve individual integrity which hands over intact from generation to generation the character of the organism. In the protoplasmic system the nucleus represents this

conserving static *Moment*. On the other side, the living thing is highly mobile, changing with every change in the environment, accommodating itself and thus evincing capacity for self-regulation. In the protoplasmic system the ectoplasm is the region of active momentary changes in response to environmental conditions.[1] Within the protoplasmic system is a stability that is derived from the interplay of the more static and the more mobile factor. This stability thus brought about determines both that organization of matter called living thing and that specificity which sets off one living thing from another.

To the varying states of the protoplasm due to the concomitant and succeeding reactions we need especially to address ourselves. The region of the protoplasmic system in which these changes are most strongly revealed is the ectoplasm. Its most important characteristic is its change in time, its rapid response to outside conditions. No other cell-component exhibits this characteristic in a like degree.

By re-evaluating structure and function of the protoplasmic system, my theory of the ectoplasm has significance not only for the advance of biological investigation; it offers a point of view also for medicine—the highest form of applied biology—whence medical problems can be surveyed.

Medicine, as human biology, a welter of complex relations, even less than the biology of other animals can depend upon the ultimate particles of physical science for the solution of its problems.[2] As protoplasmic systems, human cells are capable of study by comparable cytological technique and experimental methods which used on eggs have furnished the particulars that give basis for my generalization. An insistent demand for medicine today is, therefore, a far-flung attack on human cells as such. We

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[1] *Cf. Montgomery, 1904.*
[2] *See Sauerbruch, 1926, 1937.*

need more and more to supplement that histology of normal and pathological states which although it suffices for diagnosis nevertheless remains the study of tissues—and more often of organs—by most exhaustive study of the minute structure of every single type of human cell both in its normal and in its every available pathological state.

The study in every type of cell of its ground-substance—i.e., of the protoplasm minus the inclusions and the products of metabolism—may win a new understanding of the functions of the liver, the kidney, the gut, the pancreas, etc. Extension of the descriptions of the ectoplasm in these various cells given by the older medical histologists may serve for a closer correlation of structure and function in health and disease. In spite of the view that cancer, for example, is caused by external agents and that purely microscopic investigations here are fruitless, nevertheless it may be a problem intrinsic to the cell. In oedema, forms of nephritis and all other pathological states in which the balance of water is disturbed, careful microscopic investigation aiming at the fullest possible description of the minutiae in the cell is called for. The white blood-cells used every day in the diagnosis of disease have not been sufficiently investigated beyond diagnostic needs; studies made by means of new methods of cell fixation and staining might yield results that would throw additional light on the changes both in number and in quality which white blood-cells show in various human diseases. In these three problems of medicine are strong indications that the ectoplasm is involved.

From consideration of the theory of the ectoplasm as a working principle in the attack of general problems in biology and its application to problems in medicine, let us turn to its philosophical implications.

Biology being limited to protoplasmic organization and not being able to go below it without that life is destroyed,

must establish its philosophy on this basis. Any philosophy of biology must take into account the activity of the superficial cytoplasm.

The living thing is part of the natural world; it grows and lives on the stuff of which it is made and whence it came. Then living thing and outside world constitute one interdependent unity, as evolution teaches, as the development of an animal egg reveals. As the boundary, the living mobile limit of the cell, the ectoplasm, controls the integration between the living cell and all else external to it. The ectoplasm is the means of exchange for incoming and outgoing substances. It is keyed to the outside world as no other part of the cell. It stands guard over the peculiar form of the living substance, is buffer against the attacks of the surroundings and the means of communication with it.

If we trace the development of the brain in higher animals, including man, we find that always it arises from ectoderm cells, those cells that possess most ectoplasm. The whole nervous system arises in animals always on the outer surface of the embryo; only later in development does it become enclosed within the body by other cells. Then the functions of the brain represent highly developed general properties of primitive ectoplasm. The brain can not be fully appreciated unless we bear this in mind. As with brain, so with sense organs.

The origin and development of the brain show that a conception which assumes that either the individual alone or only the outside world is real, has no biological basis. The interdependence between individual and outside world is a postulate which has its sanction not from any abstract philosophical principle, but is true because of the biological basis here set forth. The best system of philosophy, then, is that which recognizes living thing and outside world as one interdependent continuum. Instead of building our

philosophical theories of life on the behavior of electrons, it is safer to erect them on a biological basis. We conceive human behavior in terms of the history, the evolution, of the differentiation of the cytoplasm, as this differentiation appears in the course of development of the living world, attaining its highest degree in the human race and in the human individual.

The activity of the brain means a manifestation of ectoplasmic properties which we may regard as evolved from primitive ectoplasmic relation to outside world. Every mental state may thus be conceived as having behind it this old relation. Perhaps it is because of this that man is able to trace the evolution of the universe. Our minds encompass planetary movements, mark out geological eras, resolve matter into its constituent electrons, because our mentality is the transcendental expression of the age-old integration between ectoplasm and non-living world.

Life is not only a struggle against the surroundings from which life came; it is also a co-operation with them. The Kropotkin theory[1] of mutual aid and co-operation may be a better explanation of the cause of evolution than the prevailing popular conception of Darwin's idea of the struggle for existence. The means of co-operation and adjustment is the ectoplasm. But we can go farther.

Man with his highly complex nervous system constitutes a species apart from the rest of the animal kingdom. Nevertheless he maintains communion both with animate and with inanimate nature. Still closer is his relationship with fellow man. These relationships rest upon a purely biological principle. The foregoing pages have established this thesis. Here, then, is indicated where we may seek the roots of man's ethical behavior.[2]

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[1] *See also Ch. IV of Darwin's "Descent of Man."*
[2] *In a forthcoming essay, I deal with this point at greater length.*

Nature is both continuous and corpuscular. In the former sense, we pass from lower to higher revelations of organization almost insensibly and with scarcely a break. Every form of matter follows upon another. In the latter sense, we recognize breaks in natural states from electron to atom, from atom to molecule, from molecule to compound, and from compounds in association to living matter. But even conceived of as corpuscular, matter, as we know it, is never purely discrete and absolutely independent from the remainder of nature. Whether we study atoms or stars or that form of matter, known as living, always must we reckon with inter-relations. The universe, however much we fragment it, abstract it, ever retains its unity.

The egg cell also is a universe. And if we could but know it we would feel in its minute confines the majesty and beauty which match the vast wonder of the world outside of us. In it march events that give us the story of all life from the first moment when somehow out of chaos came life and living. That first tremendous upheaval that gave this earth its present contour finds its counterpart in the breaking up of the surface of the egg which conditions all its life that is to follow. The sundering of the egg into many parts, to be woven again into a whole is no less wonderful than the breaking up of the primeval unit out of which the sun and the stars, the earth and the moon were made separate and brought together again in the pattern of the heavens and the earth.

The lone watcher of the sky who in some distant high tower suddenly saw a new planet floating before his lens could not have been more enthralled than the first student who saw the spermatozoon preceded by a streaming bubble moving toward the egg-centre. And as every noviate in astronomy must thrill at his first glance into the world of stars, so does the student to-day who first beholds this microcosm, the egg-cell. For the student of Nature there

is always that which moves him, that something which he can not reduce. And out of this intuition he comes to know what otherwise would remain hidden.

We feel the beauty of Nature because we are part of Nature and because we know that however much in our separate domains we abstract from the unity of Nature, this unity remains. Although we may deal with particulars, we return finally to the whole pattern woven out of these. So in our study of the animal egg: though we resolve it into constituent parts the better to understand it, we hold it as an integrated thing, as a unified system: in it life resides and in its moving surface life manifests itself.

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