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SYMPOSIUM ON EXPERIMENTAL EMBRYOLOGY.

THE APPLICATION OF EXPERIMENT TO THE STUDY OF THE ORGANIZATION AND EARLY DIFFERENTIATION OF THE EGG.

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The central aim of all embryological study is to trace to their origins the principal differentiations of organisms; in addition the aim of experimental embryology is to be able to control these differentiations. In this study experiment and observation can never profitably be separated. More accurate observations will always be needed as a basis for more critical experiments. These two methods of study are, therefore, not antagonistic, but mutually dependent upon each other. Experimental embryology is not a wholly different study from descriptive embryology, but rather a more refined and accurate form of observation, in which emphasis is placed upon physiological processes rather than upon morphological structures. In any large view of the science the results obtained by either of these forms of study cannot be separated easily or profitably.

All sciences as they become more detailed and accurate pass from the descriptive to the experimental stage; and it cannot be otherwise with the various branches of biology. Where the materials with which a science deals are relatively simple, experiments may profitably begin at a much earlier stage than where those materials are very complex. In the case of embryology the materials are so complex that there is still a large opportunity for studies of a descriptive sort, although the experimental method will here play a larger and larger part as the science develops. But experimental studies to be of much value must always be founded upon a knowledge of the normal condition, and the more thorough this knowledge is the better.

Again to be of real value experiments must be of a detailed and individual character. Much of the early work in experimental embryology has been of a general and explorative sort; for example, eggs were treated by the thousands and the end results only in the case of a few of them noted. Now every one who has done such work knows that one of the most usual of all results is the great variation in the types and structures produced. This diversity, probably, depends upon different conditions of the organism at the time of the experiment and also upon varying actions of the experimental conditions upon the organism. Thus it has been shown that eggs are much more susceptible to injury during division stages than during resting periods, and in many cases it can be shown that the precise time and manner of modifying the normal conditions are of the greatest importance in determining the end result. All of the variables should be known for each case, and this requires individual rather than mass experiments.

These general remarks apply with especial force to the study of the organization and early differentiations of the egg. As the result of both observation and experiment we know now that eggs are not unorganized, homogeneous, isotropic, as they were once supposed to be, but that they are morphological and physiological systems, possessing certain differentiations which are correlated with corresponding differentiations of the adult. Although much valuable work has been done in this field, the results so far gained constitute

only a sort of preliminary program of the work which is yet to be done. Some of the chief points to which attention has been and must still be directed are the following:

1. *The Origin and Causes of Polarity and Bilaterality.*

It is well known that the chief axis of the ovarian egg, in many animals, coincides with the chief axis of the fertilized egg and gastrula, and that the latter, either directly or by certain bendings, becomes the chief axis of the adult animal. Is it possible to shift this axis in the egg by shifting the positions of the oöplasmic substances? Lillie (1906) and Morgan (1907) have shown that in the eggs of *Chætopterus*, *Arbacea* and *Cumingia* some of these substances may be shifted into new positions by centrifugal force without shifting this egg axis. Lillie believes that the polarity persists in the 'ground substance' which is not altered by the centrifugal force used. On the other hand, I find that this axis may be shifted in the eggs of *Cynthia* and *Crepidula* if they be centrifuged for a considerable time after maturation and before the first cleavage. Development may be normal after this shifting of the chief axis. In this case there is good reason to believe that polarity consists in the heteropolar arrangement of certain oöplasmic substances, though the results obtained by Lillie and Morgan indicate that not all of these substances are concerned.

Bilaterality appears at different times in the development of different animals; in echinoderms it is first evident in the late gastrula stage; in most annelids and mollusks at the time of the formation of the mesomere, 4d; in some annelids and mollusks at the first cleavage of the egg, in others before cleavage; in the frog and ascidian at the time of fertilization; in cephalopods and insects during the development of the oöcyte in the ovary. Is there a common cause of bilaterality, and if so what is it? According to Roux the path of the entering sperm in the egg determines the plane of the first cleavage in the case of the frog, and this usually coincides with the plane of bilateral symmetry. But it is now known that the first cleavage plane in this animal bears no constant relation to the plane of symmetry and, therefore, the path of the sperm cannot determine

the plane of bilaterality. The fact that this plane frequently coincides with the first cleavage may indicate that although the sperm may enter at any point on the egg there is a line of least resistance along one plane, the plane of future symmetry, and that, therefore, bilaterality may be present in the egg before fertilization. Among ascidians the first plane of cleavage always coincides with the plane of symmetry, and here also there is evidence that this plane is pre-determined in the egg. There is convincing evidence in ascidians that one cause of bilaterality is the bilateral localization of certain egg substances. If this bilateral arrangement of substances is changed, the bilaterality of the embryo is destroyed. The bilateral arrangement of egg substances cannot be changed after the first cleavage is completed, and in general the arrangement of substance cannot be changed after cell walls have been formed. These facts lead to the conclusion that in these animals bilaterality is dependent upon a bilateral arrangement of egg substances.

2. The Potency of Blastomeres and Oöplasmic Substances.

It is well known that many investigators, following the lead of Driesch, have found that individual blastomeres of the early cleavage stages of many animals may give rise to entire larvæ. On the other hand, it is now known that in a large number of other animals isolated blastomeres produce only those parts of a larva which they would normally form. In the one case the blastomeres are said to be totipotent, in the other to be specified. In those forms in which partial development of isolated blastomeres takes place, the oöplasmic substances are either segregated into different blastomeres, or they are very definitely and fixedly localized in the blastomeres. This indicates that in these cases the potency of blastomeres depends upon the completeness with which the different substances are represented in them, or upon the power of localized substances to rearrange themselves into a typical whole.

Are these different oöplasmic substances specified, or is each totipotent? In certain molluscan and ascidian eggs, where these substances are very plainly visible and definitely localized, it has been shown that the development is a strict mosaic work, based upon the

localization of these substances. Here it is possible to speak of organ-forming or histogenetic substances, since each gives rise only to definite organs or tissues. In the absence of a certain substance from an egg, a corresponding part of the embryo is lacking. On the other hand, when substances have been thrown out of their normal positions by centrifugal force, they still develop, in some cases at least, into their characteristic structures. Here, then, we have both negative and positive evidence that these substances are definitely specified, that they are organ-forming. There is no doubt that different eggs differ greatly in the capacity which parts of eggs show for development. This may be due to varying degrees of differentiation or localization of these substances, or to varying powers of regulation. Further experiments must be depended upon to harmonize the conflicting results already obtained.

3. *The Mechanism of Differentiation.*

So far as the process can be directly observed, differentiation consists in the origin, localization and progressive transformation of unlike substances in cells. How these substances arise in the first place is unknown, though there is reason for believing that they are formed through the interaction of nucleus and cytoplasm; further study on this subject is much needed. The localization of these substances in the cell body is accomplished, in large part, through the achromatic portion of the mitotic figure. Lillie has shown that a limited amount of differentiation may take place in the absence of cleavage, and it has been shown repeatedly that there is no necessary relation between planes of cleavage and lines of differentiation. Nevertheless, cleavage is necessary to progressive and orderly differentiation. I have found that cell walls limit and fix the movements and localization of substances, and that the movements of substances within cells take place largely through the instrumentality of the astral systems of the mitotic figure, or of the entering spermatozoon. Differential divisions of the cell body are thus brought about, though there is no evidence that differential divisions of the nucleus ever occur, except in certain maturation divisions of the egg and sperm. Where nuclei become differentiated it is probable,

as Boveri has suggested, that it is through the influence of the surrounding cytoplasm. It is, therefore, probable that while one of the principal functions of the mitotic figure is the equal distribution of the chromosomes to the two poles, another scarcely less important function is the localization and differential distribution of the oöplasmic substances to the daughter cells.

By a series of remarkable observational and experimental researches, Boveri and Wilson have shown that individual chromosomes may possess different hereditary value. There is here open one of the most promising fields in the whole science of biology for the application of experiment to the solution of fundamental problems of cytology and development. In this connection may be mentioned also the fundamental experiments of Loeb, Garbowski, Herbst and others, on the relative influence of the egg and sperm on differentiation and inheritance.

4. *Modifiability of Organization and Differentiation.*

Finally, mention should be made of the need of experimental work to determine to what extent the organization and differentiations of the egg may be permanently modified. Hitherto, such work has been taken up only incidentally, but it is one of the greatest problems of biology, upon the solution of which the artificial production of new types must largely wait.

This brief summary of results and aims of experimental work as applied to the organization and early differentiation of the egg, is, as I indicated at the beginning, in the nature of a preliminary and very incomplete program. Experimental embryology is a new science, and the valuable work so far done is more or less isolated and disconnected. This symposium can serve no more useful purpose than to point out the lines in which work is especially needed, and to stimulate interest in the solution of the fundamental problems of development.