A. INTRODUCTION.

1. *Purpose and History of the Work.*

THE purpose of the following work from its inception has been to make as careful a study as possible of the cleavage of the ovum, the formation of the germinal layers and definitive organs, and the axial relations of the ovum to the larval and adult axes. At the time when this work was begun, several years ago, scarcely any attempts had been made to trace the history of individual blastomeres through the entire development to the formation of definitive organs. The early stages of cleavage had received a great deal of attention, but the later stages had been largely neglected; and although the origin and homology of the germ layers was perhaps the most frequently discussed subject in embryology, yet the relation of these layers to the individual blastomeres of the cleaving ovum had been determined in comparatively few cases. Since that

time a number of very valuable papers have appeared on this subject of "cell lineage," as Wilson ('92) has aptly termed it. The results of such work are no longer as novel as they were four or five years ago, and yet the general interest in the subject has greatly increased, and that, too, in spite of the fact that there is a growing school of biologists who believe that individual blastomeres have no necessary relation to future organs. The subject of germ layers is no longer so important as it was once considered; in fact, the theory of the homology of the germinal layers has met with so many difficulties of late that it is now generally maintained only in a greatly modified form. However, the fundamental idea which was prominent in germ-layer discussions is of vital interest to-day. In the whole history of the germ-layer theories I see an attempt to trace homologies back to their earliest beginnings. This problem is as important to-day as it ever was, and whether one find these earliest homologies in layers or regions or blastomeres or the unsegmented ovum itself, the quest is essentially the same.

Within this question of the earliest homologies is included another of great present interest, *viz.*, the significance of cleavage. Is it an orderly sifting of materials, a "mosaic work," or, as Driesch ('93) has maintained in the case of the echinids, a mere quantitative division of homogeneous material? Can the cells of cleaving eggs be compared with each other as the organs of adult animals can? Can one properly speak of the homology of blastomeres? Are the chief axes and regions of the egg or embryo homologous in different animals? And finally, are the causes of the various forms of cleavage to be found primarily in the constitution of the egg itself, in other words, in the internal conditions, or rather in the external conditions, such as pressure, surface tension, gravity, etc.? I know that in these days, when "all the world shakes eggs," it may be hazardous to risk an opinion on these questions which is not based on experimental work. And yet, while fully recognizing the value of experimental embryology, we ought not to forget that "Nature is continually performing some very remarkable experiments in her own way," and I believe we need to know

more about these normal processes before we can properly understand abnormal ones. In order to know the significance of cleavage, it is necessary not only to find out how much the egg may be fragmented or the blastomeres transposed without irreparably destroying development, but also and much more, it is necessary to know every step in the normal formation of the embryo. It is less important to know what remedial processes Nature may have for healing broken eggs, than to understand her usual methods of developing unbroken ones. Whether and how much this "secondary," or regenerative development may differ from the "primary," or normal, is still an open question. If there be a difference, as Roux ('93) maintains, the phenomena of regenerative or secondary development are much more complicated and difficult of explanation than the process of primary or normal development, since in these cases we have to explain the phenomena of normal development plus those of regeneration. In any case the phenomena of normal development are the ones to be explained, whatever method may be used ; and before any explanation can be given it is necessary to know the usual development as thoroughly as possible.

It is because of the perennial interest in these questions of the earliest homologies, and of the significance and causes of the various forms of cleavage, and also with the hope that I may be able either directly or indirectly to add something, however little, to the solution of some of these problems, that I now bring forward this long-delayed contribution on the Embryology of Crepidula.

Crepidula is a genus of prosobranchiate gasteropods, whose development has never heretofore been studied so far as I can learn, — a genus, moreover, which is in many respects a very interesting one, apart from its embryology ; besides, it is so abundant all along our Atlantic coast from Labrador to Florida, and its eggs are so easily obtained, so numerous, and so exceedingly favorable for embryological research, that it seems remarkable that no one has hitherto attempted to study its development.

This work was begun in the summer of 1890, while I was occupying the Johns Hopkins University table at the Marine Laboratory of the United States Fish Commission at Wood's Holl, Mass. During the succeeding winter I continued the work in Professor Brooks' laboratory at Baltimore, and in the summer of 1891 I again occupied the Johns Hopkins table at Wood's Holl, and continued to work on the same subject. Since that time my work has suffered long and repeated interruptions owing to the pressure of other duties.

I had hoped to be able to present in one paper both the earlier and the later stages in the development, but the work has grown so much, both in extent and difficulties, that it has seemed best to publish the results of investigations on the early stages first, and to supplement these by another paper on the later stages as soon as possible. Since the study of the later stages is less general in its bearing and more specifically applicable to the Mollusca, such a division of the subject will not be an illogical nor an unwelcome one. Two preliminary papers have been published on this subject, — one on the general embryology of Crepidula and Urosalpinx (Conklin, '91), the other on the cleavage in Crepidula ('92).

During the first year of the work my attention was directed exclusively to the development of Crepidula fornicata, and a large number of drawings of the various stages in the embryology of this species were made; for this reason it forms the chief subject of this paper, although in some respects C. plana is a more favorable object for study. It was not until the summer of 1892 when, through the courtesy of Professor Whitman, I was enjoying the privileges of the Marine Biological Laboratory at Wood's Holl, that I obtained material for the study of the embryology of C. plana and C. convexa. I have, however, made a careful comparison of the development of these three species, and in most respects have found the cleavage and formation of the germ layers and larval organs very similar in all of them.

Through the kindness of my friend and former pupil, Mr. Harold Heath of the Leland Stanford University, I have recently received a number of adult specimens and a good col-

lection of eggs and embryos of C. adunca, a species quite common on the Pacific coast. I have made a brief study of the embryology of this form. The peculiar features in its development will be referred to later. During the course of this work I have also studied, more or less carefully, the embryology of several other genera of marine prosobranchs, *viz.*, Urosalpinx cinerea, Fulgur carica, Sycotypus canaliculatus, Illyonassa obsoleta, Tritia trivittata, Neverita duplicata.

If space and opportunity permitted, it would be a pleasure to mention the names of many friends who in one way or another have assisted me, but I cannot fail to speak of two or three persons who have placed me under very great obligations. I am indebted to Professor C. O. Whitman, Director of the Marine Biological Laboratory, for the opportunity of working at that excellent institution, as well as for many stimulating suggestions and friendly criticisms ; to Professor W. K. Brooks, my former instructor, for valuable assistance during the first year of my work ; and particularly am I indebted to my wife, who has finished from my camera sketches many of the drawings which illustrate this paper, and has in many other ways rendered me great assistance.

2. *Methods.*[1]

The ova were fixed in many different fluids, — Kleinenberg's picro-sulphuric, picric acid in sea water, Perenyi's, Flemming's stronger and weaker, Merkel's, Auerbach's, Hermann's, corrosive sublimate, chromo-formic, chromo-acetic and absolute alcohol ; but for surface views of the entire egg none of these methods for a moment compares with the first named, *i.e.*, Kleinenberg's stronger picro-sulphuric. The ova were left in this for a length of time varying from fifteen to thirty minutes, and were then gradually transferred to 70 % alcohol. They were left in this until all traces of picric acid had been washed out, and were finally preserved in 95 % alcohol.

[1] The substance of this section was published in the American Naturalist, vol. XXVII (1893).

As a result of many experiments with almost every one of the common staining fluids, I found that the best method of preparing surface views of the whole egg or embryo was the following: (1) Transfer the object gradually from alcohol to water. (2) Stain from five to ten minutes in a solution of Delafield's (Grenacher's) haematoxylin diluted about six times with distilled water and rendered *slightly* acid by a trace of HCl. (3) De-hydrate and clear in oil of cedar or xylol. (4) Mount in balsam, supporting the cover glass so as to prevent crushing. By occasionally softening the balsam with a drop or two of xylol and slightly moving the cover glass the objects can be rolled into any position desired.

By this method wonderfully beautiful surface preparations were obtained, showing with remarkable clearness not only the nuclei and cell boundaries, but also the karyokinetic figures, and in many cases the archoplasmic spheres and centrosomes. One very considerable advantage of this method is that the preparations are permanent — in fact during the first year or two they become better with age instead of degenerating. Most of the preparations from which the figures were drawn are still in existence, and can be consulted at any time.

I have employed this method with almost as good results in the preparation of surface views of the embryo chick and English sparrow, and also with considerable success on other molluscan eggs and embryos, as well as those of annelids and echinoderms.

The objects for sectioning were fixed in various fluids, some of which showed certain points of structure better than others ; for general purposes, however, excellent results were obtained by fixing in the picro-sulphuric solution, though the chromatic filaments and individual chromosomes were brought out much more clearly by the use of absolute alcohol, and the spindle fibres and centrosomes were more clearly shown by the use of Flemming's or Hermann's fluid. In all cases the objects were imbedded in paraffin, and the best results were obtained by staining on the slide. On the whole I have found a double stain, consisting of Delafield's haematoxylin followed by a solution of erythrosine in aniline water, to give the best re-

sults, though many other stains were useful, particularly the Biondi-Erlich mixture and the iron haematoxylin of Heidenhain.

One other thing ought to be mentioned in this connection. I have in no instance been able to follow any one lot of eggs throughout any considerable part of their development. When removed from the mantle cavity of the mother they do not develop normally for more than two or three days. I tried keeping some of the eggs in small dishes, changing the water twice a day; others were placed in a large jar, in which the water was continually aerated by a stream of air; still others were placed in a jar, the mouth of which was covered by silk netting, and the jar was then inverted in a tank of flowing water; the most successful method, however, was to put the eggs in open bottles, which were then placed in an aquarium through which water was constantly flowing. Yet by none of these methods could the eggs be kept normal for more than a few days. It would seem that the circulation of water within the mantle chamber of the mother is more perfect and gentle than could be obtained by any method which I could devise. It was necessary, therefore, to take eggs from a large number of individuals in order to get a complete series, since all the eggs laid by one individual are in nearly the same stage of development. Fortunately, there are such vast numbers of fertile females during the breeding season as to make this an easy task.

B. THE GENUS CREPIDULA.

1. *Natural History.*

At least three species of the genus Crepidula are found on the Atlantic coast of the United States,[1] *viz.*, C. fornicata Lam., C. plana Say, and C. convexa Say, all of which are quite abundant along the shores of New England. All these species are more or less completely sedentary, and they show the most remarkable individual differences in the shape of their shells due

[1] Other species have been described, *viz.*, C. unguiformis Stimson, C. glauca Say, C. acuta Lea. Concerning the first of these there is no doubt that it is identical with C. plana, and I am convinced after a careful anatomical and embryological examination of the last two that they are only local varieties of C. convexa (cf. Verrill '74) Invertebrate Animals of Vineyard Sound.