GERM CELLS OF COELENTERATES

VI. GENERAL CONSIDERATIONS, DISCUSSION, CONCLUSIONS

GEORGE T. HARGITT

Zoological Laboratory, Syracuse University

THIRTY FIGURES (THREE PLATES)

CONTENTS

I. INTRODUCTION

The study of the germ cells of the Coelenterates was undertaken with the primary aim of securing a series of observations upon the behavior of these cells in a variety of types within the phylum. The original plan included the study of representative forms of Hydrozoa, Scyphozoa, and Actinozoa. The last class

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has been omitted from consideration because of a failure to secure adequate material, but considerable numbers of the other two classes have been carefully investigated.

The earlier studies (G. T. Hargitt, '09 to '18) presented the data obtained from the investigation of particular species, with some discussion of a general sort in interpreting these observations. It is now necessary to consider the observations in the light of the accumulated knowledge of the various species and to correlate the data obtained from different species. It will also be well to discuss the results of these studies in connection with observations upon the germ cells of other phyla. Certain phases of the problem have been reinvestigated and new data obtained; the results of this new study will be considered in place under the appropriate headings.

II. ORIGIN OF THE GERM CELLS

1. Place of origin

The generalization was made many years ago that the germ cells of Hydrozoa always arose from the ectoderm, while in Scyphozoa it was the entoderm which gave rise to germ cells. So far as my observations go, the latter statement is confirmed, but the former is not correct. The genera and species of Hydrozoa which have been investigated are sufficient in number to show that neither the ectoderm nor the entoderm may be considered as the characteristic place of germ-cell origin; on the contrary, these cells may arise sometimes from one layer and sometimes from the other, even in the same species.

A survey of the available literature of recent years on the germ cells of Hydrozoa gave the following results: All who have worked upon Hydra agree upon the ectodermal origin of the germ cells. Thirteen authors record twenty-three other species of Hydrozoa as producing germ cells in the ectoderm and nine authors record thirty-one species in which the germ cells arise in the entoderm. In many cases from two to four authors have studied the same species, in other cases only a single study has been made of a species. The summary made above includes

every record, which means that a few species have been recorded twice when two authors differ in their results. If these disputed cases were omitted, the ratio would remain practically unchanged. Four investigators, working upon six species, agreed that the germ cells might take their origin either in the ectoderm or in the entoderm; Goette ('07) found five species in which the male germ cells were formed in one layer and the female cells in the other; in fourteen species the two sexes agreed in the place of germ cell origin. Other authors have recorded for single species a different place of origin for the two sexes.

Those recent investigators who have studied the Hydrozoa most carefully and extensively are in agreement upon the lack of definiteness in the place of germ-cell origin. They agree that the portion of the polyp or colony where germ cells arise is not always the same, the layer may differ in the same species and in the two sexes of the same species, and they also agree in dismissing the place of origin in germ cells of Hydrozoa as of no significance. The work of the author is in harmony with this opinion.

2. Time of origin

The investigation of the precise time in ontogeny at which germ cells arise comes within the scope of cytological study, rather than in earlier embryological investigation. This change of attitude has developed largely as a consequence of the interest in the germ-plasm theory of Weismann; it is of much importance to the theory to determine the time at which germ cells are differentiated and especially to discover their relation to the fertilized ovum. The studies of Weismann ('83) upon the origin of sex cells of Hydromedusae furnished him with the chief material upon which to formulate his theory. The actual observations of Weismann, did not, in fact, warrant the enunciation of this theory, as has been clearly pointed out by Goette ('07), C. W. Hargitt ('11), the author and others. It is only necessary to refer to the preceding section to note the extent to which Weismann's claim of the ectodermal origin of the germ cells in all Hydrozoa is incorrect; indeed Weismann's own published papers

demonstrate that he often found the germ cells to be first recognizable in the entoderm. The suggestion of an ectodermal origin was proposed on theoretical grounds. But while the ectodermal origin of the germ cells is proved not to be characteristic of Hydrozoa, this does not necessarily discount the germ-plasm theory. If it could be shown that the germ cells arise very early in ontogeny and remain distinct and unchanged to the time of sexual maturity and the formation of the gonads, it would be a matter of no importance where these cells were located in the interim, provided they remained passive and took no part in the functioning of the body.

In certain phyla considerable success has attended the investigation of germ-cell origin; an early differentiation has been noted and these cells have been followed to their position in the gonads. Some of these cases will be discussed later. In Hydrozoa, on the contrary, there has been an almost universal failure to observe the differentiation at any time before sexual maturity was reached. Weismann's studies were made on mature hydroids and medusae, and only as a theoretical suggestion was an early differentiation urged. Harm ('02), in young hydranths of Clava squamata, just developing from planulae, found certain cells which he believed to be primordial germ cells. These cells, figured and described by Harm, are ectodermal cells similar in form, size, and position to the interstitial cells; somewhat later they form elongated, spindle-shaped cells lying directly against the supporting membrane, and possessing a slightly more deeply staining cytoplasm. They were not traced beyond this stage. Wulfert ('02) traced the development of Gonothyraea loveni to the formation of the polyp. While the planula is still within the gonophore, interstitial cells are produced in the ectoderm and entoderm, and these were followed through their differentiation into ganglion cells and nematocysts. After the planula has begun to transform into the polyp, Wulfert finds, for the first time, what he believes to be germ cells. These occur in both ectoderm and entoderm and, according to his figures, are like those cells of an earlier period which became ganglion cells. Furthermore, the cells called germ cells differ from other interstitial cells of all stages only in their staining reaction.

Harm does not describe the formation of ganglion or nettling cells in Clava, but his germ cells follow the same course as the ganglion cells of Wulfert in Gonothyraea, and Wulfert never refers to the spindle-shaped cells as germ cells. It seems clear, that both these investigators are dealing with interstitial cells. Wulfert's results would suggest that what Harm called germ cells were in reality differentiating ganglion cells. Wulfert states that his primordial germ cells arise from interstitial cells, but the evidence he presents in favor of considering these as germ cells is not convincing. The determining characteristic, to him, is the more deeply staining cytoplasm, and this, I believe, cannot be considered a sufficient criterion, as I have pointed out in another place (G. T. Hargitt, '16).

Stschelkanowzew ('06) describes germ cells as present in late cleavage stages of Cunina proboscidea. While the embryo is a solid mass of cells and the ectoderm and entoderm are being separated as layers of a single cell in thickness, he finds one or two cells between the ectoderm and entoderm layers, but neither in description nor in figures does he specify the characteristics of these cells. Their size, form, color reaction, and the size of nucleus seem to be the same for the ectoderm cells, the position alone is different. In this instance, also, we have to do either with the formation of interstitial cells or with the completion of the formation of the cells of the central solid mass. Precisely the same process will presently be described for the formation of embryos of Tubularia.

There is no question of the early formation of interstitial cells; these have been found, described, and their differentiation followed in Hydrozoa by various authors. For example, Schneider, ('90) noted the characteristic and early appearance of interstitial cells in Hydra, and their later transformation into ganglion cells, nematocysts, and germ cells. Morganstern ('01) traced the development of Cordylophora through the larval period, and identified the ganglion and nettling cells produced from interstitial cells, but did not find any evidence of germ cells in larvae or young polyps. The germ cells arose from ectodermal interstitial cells at the time of sexual maturity. Schneider and Mor-

ganstern find, what is probably more or less universal in hydroids,
that some of the interstitial cells remain undifferentiated for a
long time. But such undifferentiated cells are not germ cells,
since they form nettling cells throughout the life of the polyp
and probably act as replacing cells for any of the epithelial cells
destroyed.

In order to test further this question of the presence of germ
cells in embryos, I have made a careful, extensive, and entirely
new study of the cleavage stages and planulae of Campanularia
flexuosa and Gonothyraea loveni; also a similar study of cleavage,
embryo, and young polyp (actinula) of Tubularia crocea.   In this
investigation I have followed the formation of the germ layers,
the differentiation of interstitial cells, and especially have searched
for primordial germ cells.

In Campanularia and Gonothyraea cleavage results in the for-
mation of a solid morula composed of yolk-laden cells whose
boundaries are made out with great difficulty, if at all.   The
outer cells of the morula arrange themselves into an indefinite
ectodermal layer, and later the cells of the solid central mass pull
apart to form an enteron, but during this time none of the cells
take on a columnar form and no interstitial cells are present.
Figure 1 shows the appearance of the embryo after the formation
of the enteric cavity; the cells are not sharply outlined, and
the nuclei, surrounded by masses of cytoplasm, are irregularly
scattered through both the outer and inner layers.   This rather
indefinite condition is replaced in young planulae by the con-
dition shown in figure 2.   The ectoderm cells are now columnar
and a few interstitial cells are present, the cells of the entoderm
are assuming a columnar form, and deep in this layer are groups
of interstitial cells.   The boundary between the primitive germ
layers is not a definitely formed supporting lamella, but only the
cell outlines of the ectoderm.   One is immediately struck by the
appearance of some of the interstitial cells of the entoderm,
and there is little doubt that some of these are similar to the
primordial germ cells of Wulfert.   However, some of these are
spindle-shaped or stellate in form and their nuclei do not differ
from the nuclei of the epithelial cells of the entoderm.   As the

planula develops, these interstitial cells divide to produce such groups as the one shown in figure 3; at the same time the entoderm cells assume a definite epithelial form. During the progress of the development of the planula the entodermal interstitial cells decrease in number, nematocysts are formed from some of them, and others become elongated, as shown in figure 4. Some of these spindle-shaped cells extend, or move, toward the free surface of the entoderm and have the form and appearance of gland cells. None of the entodermal interstitial cells remain in the form shown in figure 2, and none of them, in the older planulae, display. the characteristics of germ cells. During these changes in the entoderm there are very few ectodermal interstitial cells produced (none were present in the region of the planula from which the figure was made), and through the entire history of the planula there are no germ cells in the ectoderm.

Thus, by the time the planula has been perfected, there are no cells in the ectoderm or entoderm which have even the remotest resemblance to germ cells. The almost complete absence of interstitial cells from both germ layers of the completed planulae, and the formation of nematocysts from most of these, renders it certain that primordial germ cells are not present at this stage. Consequently, the cells which resemble those interpreted as germ cells by Wulfert and Harm are not such, but differentiate into specialized cells of the body.

There are some differences in the formation of the morula in Gonothyraea loveni, but once the solid mass of cells is produced the development is so similar to that of Campanularia it has not been thought necessary to describe and figure this form. But it may be said that at no stage could I find even a single cell in Gonothyraea which showed the characteristics of a germ cell. I am forced to believe, therefore, that Wulfert described as germ cells merely interstitial cells which were undergoing differentiation into ganglion cells, gland cells, or some other specialized cell element. Certainly, if primordial germ cells were characteristically present, one should be able to find them, but this study of similar stages of the same species on which Wulfert worked gave no evidence of their presence.

A similar solid morula is produced by the cleavage of the egg of Tubularia crocea, but the cell outlines are sharply defined and the formation of the germ layers is easily followed. During the separation of an outer ectoderm from the superficial cells of the morula, divisions take place (fig. 5) in such a fashion as to result in the production of interstitial cells. But interstitial cells are also formed by divisions from the deeper cells which make up the entoderm (fig. 6). In this early stage the interstitial cells may, but not always do, show a more deeply staining cytoplasm. The ectodermal layer becomes more distinct, its cells become more columnar, and the interstitials increase in numbers to produce the appearance represented in figure 7. The groups of inter- stitial cells are formed by divisions of the earlier cells, but others are also formed from the cells of the outermost layers. By the time a cavity is present in the center of the embryo the ectoderm has become separated by a supporting layer (fig. 8). At this stage the interstitial cells are numerous, so closely packed as to render their outlines indistinct, and for the most part there is no dif- ference in the staining reaction of the ectodermal and interstitial cells. From this embryonic condition the young polyp or actinula develops. In the development of the polyp the interstitial cells shown in figure 8 are easily followed through their differentiation into nematocysts and other specialized cells. At no time can cells be found which resemble germ cells. This conclusion is in harmony with the earlier results of the author ('09) on this species, for it was then found that germ cells first became dif- ferentiated in the medusoid buds of the hydroid from the ecto- dermal cells.

Other hydroids, as Clava, Hybocodon, Eudendrium, were examined in cleavage and larval stages, but the material was not sufficient in amount to permit a determination of the details noted for Tubularia, Campanularia, and Gonothyraea. No evidences of germ cells were seen in these stages of the forms mentioned. A study of Clava and Eudendrium was made by C. W. Hargitt ('04 b, '06), and the formation of germ layers and interstitial cells was determined. In neither did a differentiation of germ cells occur in the planulae or earlier stages. Other inves-

tigators who have studied the development of Hydrozoa and Scyphozoa record the formation of germ layers and interstitial cells and the differentiation of the latter into ganglion cells and nematocysts, but have not observed the presence of germ cells in these earlier stages.

We may conclude that Wulfert and Harm made no mistake in their observations, but the interpretation of certain cells as germ cells is not justified by their own evidence, nor is it confirmed by this new study. The cells described as germ cells are interstitial cells which were in the process of differentiation into specialized cells of the body. In all the forms carefully studied it is clear that germ cells do not occur in larvae or young polyps, and in the absence of any evidence of their presence in similar stages of other forms, there is ample reason for concluding that an early differentiation of germ cells does not occur.

The germ cells of Hydra have been investigated by a considerable number of investigators, and practically all of these agree upon the origin from interstitial cells of the ectoderm at the breeding season. Brauer ('91) has observed the formation of the interstitial cells before the ectoderm and entoderm are fully separated, and has followed the differentiation of these into ganglion cells and nematocysts, confirming the earlier results of Schneider ('90). Downing ('05, '09) is the only one who has suggested a different conclusion for the germ cells of Hydra. He observes the same origin from interstitial cells, but, in the developing ovary, finds some interstitials to be larger than others; these he believes to be primordial germ cells which have been segregated in early ontogeny to form a 'self propagating' germinal tissue. He has not observed these cells in the embryo, indeed he seems to have studied only the polyps which are producing reproductive organs, and therefore his conclusions are largely hypothetical. The presence of larger interstitial cells in the developing ovary and their identification as germ cells is confirmed by Tannreuther ('08), who also finds similar cells forming spermatogonia. But in every case the formation of ovaries and spermaries is initiated by a rapid growth of interstitial cells and later a multiplication of these cells. Tannreuther thus accounts for the presence of larger

interstitial cells, but shows they are not a germinal tissue. He finds no such cells before or after the formation of the reproductive organs and can trace their growth from ordinary interstitial cells. Later, Tannreuther ('09) followed the behavior of the interstitial cells and could find all gradations between small and large interstitials, as well as trace the transformation of an ordinary interstitial through spermatogonia into spermatozoa.

Wager ('09) finds no evidence of a germinal tissue in Hydra, nor of any difference between interstitial cells. Furthermore, in the very groups of interstitial cells which grow to form oogonia, "one usually finds nematocysts developing in large numbers. In the course of development of the ovarian area these nematocysts either migrate out or are resorbed. Frequently they are found within the egg itself." This is a very striking demonstration of the equipotency of the interstitial cells and effectually refutes the belief of a distinct germinal tissue composed of certain interstitial cells. The characters used by Downing to differentiate germ cells from other interstitials are found to be applicable to most interstitial cells; there is great variety in size and appearance, and Wager finds all gradations between these variations in interstitial cells. He strongly confirms the work of the earlier authors and agrees perfectly with Tannreuther in the absence of a distinct germinal tissue in Hydra. The work of these two authors did not include a complete study of the histogenesis, but the investigations of Schneider ('90) and Brauer ('91) completely fill this gap. Hegner ('14), in discussing the germ cells of Hydra, says he "is inclined to accept Downing's position in the matter." But Downing's position is untenable, for his conclusions are refuted by the work of other investigators. The strongest evidence of the occurrence of a distinct germinal tissue presented by Downing, viz., the presence of larger interstitial cells of a distinct sort, is shown by both Wager and Tannreuther to be merely an incident in the formation of reproductive organs.

From the facts presented in the above discussion there is but one conclusion which may fairly be drawn, viz., in Hydra and other Hydrozoa there is no clear evidence that germ cells are ever differentiated in larvae, young polyps, or any early stage in ontogeny.

In addition to the line of evidence just presented, we have direct observations upon the entire germ-cell cycle of some hydroids from their earliest differentiation. Some of the thirty or more species studied by Goette ('07) give very clear evidence of the method of origin of germ cells. In Podocoryne germ cells arise from both ectoderm and entoderm; in Corydendrium parasiticum the egg cells are formed from ordinary entoderm cells by division, a basal egg cell and a distal epithelial cell resulting, and only this method of formation is applicable in this species. A similar division takes place in Clava, and Goette says, "no doubt exists, that the egg cells of Clava multicornis proceed only from transformed half entoderm cells." He observed epithelial cells dividing, one half forming the egg cells of Sertularia argentea, Gonothyraea loveni, Obelia longissima, and the sperm cells of Eudendrium. In Obelia geniculata the eggs develop only in the medusae by the transformation of entire entoderm cells. Smallwood ('09) traced the egg cells of Hydractinia echinata back to single entoderm cells which underwent no division, but transformed directly into oocytes. Campanularia flexuosa produces its eggs by a similar transformation of entire entodermal epithelial cells or from the basal half of a divided entoderm cell, the distal end of which persists as an epithelial cell (G. T. Hargitt, '13). The author ('16) also observed egg cells typically arising from half entoderm cells in Clava leptostyla, though occasionally from ectodermal interstitial cells.

Such observations upon a number of species by different investigators leave no doubt of the entire normality of the described transformation of tissue cells into germ cells. In such cases there can have been no differentiation and segregation of germ cells in the early ontogeny, for they came from functional tissue cells, a portion of which continued as a tissue cell. Such a cell is a specialized cell and not a latent germ cell. In certain Hydrozoa, therefore, the origin of germ cells has been precisely determined and an early differentiation shown to be impossible; in the absence of positive evidence to the contrary, it would probably be fair to believe that none of the Hydrozoa show a differentiation of germ cells till sexual maturity approaches.

3. Conclusions

In the last twenty years the reports of investigations upon the origin of germ cells of Hydrozoa show more species in which such cells proceed from the entoderm than from the ectoderm. Numerous cases are recorded in which the place of origin differs in the sexes of a single species and where the same individual may produce germ cells from different layers. Furthermore, the germ cells come from different sorts of cells. All of this points to the conclusion that the place of origin is variable and not a matter of any significance. A few cases are reported of the origin of germ cells in the embryos or larvae of Hydrozoa, but new investigation of these gives no confirmation of this. Interstitial cells are differentiated in early ontogeny and undergo early specialization into ganglion, nettling, and other cells, but those not so specialized are alike in all respects and at most persist as somewhat inactive cells. During all the life of the polyps these produce nettling cells, form replacing cells, and, in some species, at sexual maturity produce germ cells.

In none of the Hydrozoa has the differentiation of germ cells been demonstrated in early ontogeny. On the other hand, observations of several species have demonstrated that germ cells may arise from body cells directly, either by the transformation of an entire cell or from the transformation of one half of such a body cell. Obviously in such cases an early differentiation of germ cells is out of the question, and it is believed to be typical of Hydrozoa to form their germ cells only at the time of sexual maturity.

III. THE GERM-PLASM THEORY

1. General statement and discussion of the theory

This theory has been much discussed and many weighty objections have been raised against it; at the same time it has been strongly defended and important evidence brought forward to uphold it. Probably the lines of defense, as well as of opposition, are so well known as not to require further review. There-

fore, the present discussion will be limited to a consideration of the theory in relation to observed facts in the Hydrozoa. In order to have clearly in mind the essential features of the theory and its method of application to the Hydrozoa attention is directed to the statements of the author of the theory.

In every ontogeny, a part of the specific germ-plasm contained in the parent egg cell is not used up in the construction of the body of the offspring, but is reserved unchanged for the formation of the germ cells of the following generation (Weismann, '91, vol. 1, p. 170).

This splitting up of the substance of the ovum into a somatic half, which directs the development of the individual, and a propagative half, which reaches the germ cells and there remains inactive, and later gives rise to the succeeding generation, constitutes the theory of the continuity of the germ plasm, which I first stated in the year 1885 (Weismann, '04, vol. 1, p. 411).

. . . . In hydroids the germ cells do not appear in the 'person' which is developed from the ovum at all, and only arise in a much later generation, which is produced from the first by continued budding.

. . . . In all the last mentioned cases the germ cells are not present in the first person arising by embryogeny as special cells, but are only formed in much later cell generations from the offspring of certain cells of which this first person was composed. These ancestors of the germ-cells cannot be recognized as such: they are somatic cells—that is to say, they, like the numerous other somatic cells, take part in the construction of the body, and may be histologically differentiated in different degrees (Weismann, '93, p. 185).

Invisible, or at any rate unrecognizable, masses of unalterable germ-plasm must have been contained in the body cells in all cases in which such a transformation has apparently occurred (Weismann, '93, p. 19).

In the hydroids, then, Weismann notes the germ cells as unrecognizable till the period of maturity; their origin at that time is from body cells which are morphologically differentiated and physiologically specialized to perform certain functions of the animal. This is a statement of fact which is confirmed by the work of the authors referred to in section II of this paper. These facts do not fall into line sufficiently with the theory as stated in the first two quotations, and Weismann thereupon assumes the presence of invisible and unalterable determinants which lie latent in the body cells till activated in some way not specified. This point of view is one to which the greatest objection has been raised. Lloyd Morgan ('91), in a very searching analysis

and criticism of this position, points out its weakness and considers the recourse to invisible units as a hindrance and not an assistance to an understanding of the facts. In any effort to test the theory by observed results in hydroids one is met by the distinct statement that when germ cells arise from body cells the latter contain invisible and unrecognizable materials. If the germ plasm be really invisible and unrecognizable, the theory need not be discussed, since it cannot be proved or disproved. In the following pages evidence bearing upon the theory is presented from various lines of investigation, but the point of view is taken that there must be recognizable differences of some sort, or else an unbroken line must be traceable from germ cell to germ cell in the life cycle.

2. Evidence from Hydrozoa

a. Germ cells. The earlier section of this paper upon the origin of the germ cells is pertinent here, and should be considered in its entirety as a part of the evidence. It may be repeated that the facts show an absence of differentiation of germ cells in early ontogeny; an absence of a definite migration and germ-track; and the formation of germ cells at the time of sexual maturity from different layers and cells of the body. It has been possible to trace the germ cells back to tissue cells and observe the method by which they are produced; Weismann's own observations confirm this perfectly. It is even possible to prove that there cannot be present in the body cells which form germ cells any invisible germ-cell determinants. Goette ('07) and the author ('13, '16) find cases where division of a tissue cell results in the formation of two cells, one of which becomes a germ cell while the other persists as an epithelial body cell. If invisible germ plasm be present in the chromatin, as Weismann distinctly states, how is it possible for one of the two cells to become a germ cell and the other a tissue cell when the chromatin is equally divided and none of it lost? This is crucial evidence, and it gives the facts demanded by Weismann himself to prove his contention incorrect, as Goette and the author have already pointed out.

Without repeating all the evidence presented in sections I and II, the facts may be summarized as follows: there is no definite place of origin of germ cells; there is no definite migration of germ cells and no germ-track; there is no invisible germ plasm in the body cells. Not only is there no continuous germ plasm, so far as can be determined by observation, but the evidence is such as to show the absence of invisible germ plasm. Hegner ('14) is willing to admit the germ cells in Coelenterates do not belong to any germ layer, but he maintains that germ cells are present at all times in a dormant condition. This opinion is based upon the conclusions of Downing, Wulfert, and Harm. The error in the interpretation of these authors has been pointed out and consequently the opinion that germ cells are present in a latent condition at all times is no longer tenable; all the facts are inconsistent with this view.

b. *Budding.* Budding has generally been held to be a process of growth and cell division, often an evagination taking place. But Weismann says, ". . . . I reached the conclusion, that the budding idioplasm, which must be the starting point of the budding process according to my view, could not be divided between both germ layers, but probably was to be found in only certain cells of the ectoderm." At Weismann's suggestion, Lang ('92) undertook to test this hypothesis and studied budding in Hydra and some hydroids. Weismann believes Lang's results ". . . . contain a perfect confirmation of my conjecture that the same [buds] come from the ectoderm and that actually the 'Budding-idioplasm' had its position entirely in the ectoderm cells." These quotations from the preface to Lang's paper show the application made by Weismann of the germ-plasm theory to this form of asexual reproduction. Lang believed his results showed the proliferation of a few ectoderm cells to form a mass from which the ectoderm and entoderm of the bud developed. After the two layers were formed, a cavity was produced in the bud, and this became continuous with the parent enteron. Braem ('94) repeated the work of Lang on the same and other forms, but could not confirm his results; on the contrary, he observed the division of cells in ectoderm, interstitial, and

entoderm, and the participation of all these layers in the for-
mation of the bud by evagination. He says, ". . . . conse-
quently I do not hesitate to proclaim the results of Lang as
erroneous, the conclusions drawn from them as utterly false."

Downing ('05) believed sexual and asexual reproduction in
Hydra to be mutually exclusive, and implied a relation between
budding and germ cells. Montgomery ('06) supposed sexual
reproduction to be the more primitive, and asexual reproduction
to be a secondarily derived process; for him, regeneration and
asexual reproduction were dependent upon the presence of germ
cells. R. Hertwig ('06) found budding and sexual reproduction
proceeding side by side in Hydra and believed buds were produced
by the activity of the cells in all the layers. Mrázek ('07) and
Nussbaum ('07) confirm Hertwig on the simultaneous presence
of buds and sex organs in Hydra. The view of Hadži ('09) was
in partial accord with Weismann and Lang, for he again renewed
the claim of the activity of only a certain layer to form buds in
Hydra. In his opinion the interstitial cells were the active
elements in producing buds, the other layers not participating in
any way. According to this view, the interstitial cells are a
source of all new growth, differentiation, and development in
Hydra, but they do not necessarily form a germinal tissue.
Tannreuther ('09) investigated budding still further, and for two
species of Hydra found, first, an increase in volume, and then a
proliferation of interstitial cells in the budding zone. There was
no migration of interstitial cells into the entoderm as Hadži had
believed, for the layers remained distinct and unbroken through-
out the process. A distinct evagination occurs and cells of all
layers divide mitotically and are active in the budding process.
Furthermore, the division of cells of the ectoderm and entoderm
began about as soon as in the interstitial cells. Tannreuther's
work establishes the fact that budding in Hydra is an evagination
due to cell multiplication and growth, all layers in the budding
zone participating in the process. It seems probable that the
earlier division of the interstitial cells is merely an expression of
a more prompt response on the part of the indifferent cells than
of the specialized ectoderm and entoderm. I believe the fact is

established that budding in Hydra and hydroids is a process of evagination, but the work of Lang, Hadži, and Tannreuther suggests an earlier activity of the interstitial cells. Even if the interstitial cells were entirely responsible for the formation of the bud, proof would not be thereby constituted for the germinal nature of these cells, for they are differentiating into nematocysts throughout the life of Hydra. Also these same cells transform directly into ganglion cells earlier in the life history.

Medusae are sexual individuals and ordinarily reproduce only by eggs and spermatozoa, but there are a considerable number which undergo a process of asexual reproduction and form other generations of medusae by budding. The budded medusae later become mature and form sex cells just as do the parent medusae. The author ('17) has given a detailed account of this secondary budding of medusae and of germ-cell formation in Hybocodon prolifer; the gonads are produced from the ectoderm of the wall of the stomach, while the new medusae come from the tissues of the base of the tentacle at the margin of the bell. In a critical examination of these medusae no evidence was obtained of the migration of germ cells from the old to the budding medusae, but the new buds arose from both layers of cells in the tentacle after these cells had undergone regressive changes and become embryonic. In Hybocodon the asexual budding is not influenced by the formation of sex organs. Müller ('08) is in error in believing the two methods to be mutually exclusive, for C. W. Hargitt ('02), Perkins ('04), and the author ('17) have recorded abundant cases of the simultaneous presence of buds and gonads.

A. Agassiz ('65), Haeckel ('79), C. W. Hargitt ('04), Mayer ('10), and others have described many cases of asexual budding in medusae. Such buds may be formed, a few at a time, or many at a time; a single generation of buds may be produced or many generations; and many regions of the medusae may be concerned in their formation. Haeckel describes the buds on the stomach wall of Sarsia gemmifera (S. siphonophora) (fig. 10), more than twenty being present at one time and several generations being produced; in different species of Cytaeis (fig. 14) enormous numbers of medusae may be budded from the stomach wall at

the same time that gonads are present.   Medusae are formed
from a single tentacle base in Hybocodon prolifer, Amphicodon
amphipleurus Haeckel and others; from the bases of all tentacles
in Sarsia codonophora (fig. 13); from radial canals of Probosci-
dactyla ornata (fig. 9); from the margin of the bell in Niobia
dendrotentaculata (fig. 11); and from the gonads of Eucheilota
paradoxica (fig. 12) and other forms.   These are merely examples
of the variation in the method of budding as recorded for numer-
ous medusae.   In many of these the budding occurs during the
immature period, and only after budding ceases do the gonads
form, but others show no such periodicity and may produce buds
and germ cells simultaneously.

The production of the buds from the gonads has been critically
studied.   Mayer ('10) describes this process for Eucheilota (fig.
12) as involving the activity of the tissues of the gonad and of
the tissues outside the gonad; both ectoderm and entoderm of the
parent take part in the production of the bud by a process of cell
multiplication and evagination.   In Phialidium mccradyi buds are
also produced from the gonads, but only indirectly, since a blasto-
style is first formed and from this the medusae arise by budding.
Sigerfoos ('93), in the formation of the blastostyle and medusae,
discovered no difference from ordinary cases of budding, the
ectoderm and entoderm evaginating to produce the new growth.
The germ cells in the gonad play no part in the process other
than to behave as all other cells of ectoderm and entoderm, which
suggests the probability of the germ cells being merely body cells
capable of acting with other body cells or undergoing a growth
in preparation for sexual reproduction.

Budding in medusae is typically an evagination of the two
body layers, irrespective of the part of the animal which produces
the bud, but a few medusae are known to form their buds only
from the ectoderm.   Mayer describes such a case in Bougain-
villia niobe, the ectoderm of the stomach wall differentiating to
form all the tissues and organs of the bud.   Mayer believes a
possibility exists of the origin of the bud entoderm from parent
entoderm; but could find no evidence of such a connection, nor
of any union of the enteric cavities of bud and parent at any stage

of the process. Chun ('95) describes a similar process in Rathkea octopunctata and Lizzia claparedei. He describes the origin of the bud by the proliferation of a group of ectoderm cells which becomes isolated as a definite mass, though still held in place against the stomach wall, from which all organs of the bud are developed. In these forms the enteric cavities of buds and parents later unite. When sexual maturity is reached germ cells are formed in the stomach wall where the bud was developed earlier, but Chun does not consider the budding as due to a geminal process. Rather, he believes the ectoderm and entoderm of the medusae to be alike in histological and organogenetic structure and potency. Braem ('08) reviews and confirms the work of Chun, but finds germ cells are present in the stomach wall at the same time the bud is forming; he believes the group of cells which start the bud are oocytes, and looks upon the budding process as a short and rapid method of producing a new organism out of cells which are germinal in character. Most budding, he believes, shows no relation between bud and sex cells, and in these cases all layers are essential to the formation of the bud because each tissue has retained only the ability to produce cells of its own kind. Mayer thinks Braem has produced strong evidence that this sort of budding is a germinal process, but does not believe the evidence is conclusive. Child ('15) interprets this case as showing both sex cells and asexual buds come from the functional and more or less specialized cells of the parent medusa.

Nekrassoff ('11) studied Eleutheria dichotoma, which produces buds from the outer wall of the ring-canal. In this form budding parallels sexual development, but does not interrupt it, nor is budding interrupted by sexual development. In a single individual one may find numerous buds, young and old, young and old eggs, cleavage stages and young polyps—all at the same time. The budding takes place in the usual way, involving both ectoderm and entoderm, and while Nekrassoff finds conditions which resemble the observations of Chun and Braem in Rathkea and Lizzia, he can demonstrate the continuity of bud and parent tissues at all times. He does note that the ectoderm and entoderm

cells show a more embryonic appearance after they have begun to form the bud than they did before; especially is this true of the entoderm. Nekrassoff concludes: "on the ground of the observations on the budding of Eleutheria we may conclude that in the Coelenterates already differentiated cells have been given the possibility of a reversible process—the possibility of taking on anew an embryonic character." Regarding the suggestion of the origin of buds from germ cells, he finds in Eleutheria no relation at all between sex cells and buds.

The process of budding in medusae does not, as a rule, involve any difference in principle from budding in Hydra and hydroids, since both germ layers, by cell multiplication and evagination, form the outgrowths which, by later differentiation, become the tissues of the new individual. There are some buds which arise from a small group of cells of a single layer, but.in no case do buds come from a single cell. Budding is not, therefore, a germinal phenomenon, even when the new growth is derived from the tissues of the gonads. Consequently, not only is there no necessity for thinking of the germ plasm as being essential to the formation of buds, but there is no evidence of the presence of germ plasm in these buds. The conclusion of Nekrassoff, that differentiated cells may take on again an embryonic character, seems to explain the facts better than the germ plasm theory. Though quite unaware of this conclusion of Nekrassoff, the author ('17) worked out the budding of Hybocodon medusae and noted the embryonic character of the cells involved in the budding process.

There is considerable variation in the degree to which this 'reversible process' is exhibited by the tissues of medusae, but an unbroken series may be arranged which includes all the known types of budding. At one end of the series we may place the medusae whose tissues do not have such a capacity; these reproduce only from fertilized egg cells. Here are included the majority of medusae. If we accept the conclusions of Braem, we may next place forms, like Lizzia and Rathkea, in which a group of unfertilized oocytes may develop into a new organism. This is a very unusual method and is applicable, so far as known, only to the two forms named. Here the tissues either have no

power to change or the stimulus to such change would be lacking. Following this would come Bougainvillia niobe; the ability to form buds is limited to a definite tissue, the ectoderm. Next are those forms like Hybocodon in which all layers cooperate to form buds, but this capacity for asexual reproduction is limited to a definite locality in the parent. In this category one would place most of the medusae which form buds, and all hydroids and Hydra. Niobia dendrotentaculata represents a type in which the bud is partly new growth and partly the already formed organs of the parent; presumably all the regions of the body in such forms would have the ability to undergo some transformation. This type of budding would really be intermediate between regular budding and fission. A final group would comprise medusae in which a real fission occurs, and such a method of asexual reproduction is recorded by Mayer ('10, vol. 2, p. 280) for Gastroblasta raffaelei Lang. A gradation such as this would correlate the various kinds and degrees of asexual reproduction in Coelenterates with reproduction in protozoa, with regeneration, and with sexual reproduction. It may even mark a possible evolution of reproductive processes in Coelenterates, but would appear to have no meaning according to the germ-plasm theory.

c. *Regeneration.* Weismann ('93) takes the position that regeneration is due to the presence of germ plasm, since the latter is the only substance capable of giving rise to all parts of the body. As applied to plants, this involves the presence of germ plasm in the cambium tissue wherever it is found. There is postulated in plants an accessory germ plasm, concerned with the vegetative development, and a primary germ plasm which is retained unchanged till the germ cells are produced. But vegetatively produced buds may later form reproductive organs and cells; this requires the further assumption that accessory germ plasm also contains primary germ plasm. This same involved and intricate explanation is required to account for regeneration in animals, if we believe that regeneration is due to latent germ cells.

Morgan ('01) discusses a considerable number of theories of regeneration and rejects the germ-plasm theory completely, since he finds so many facts of regeneration utterly contradicting

it. He found, for example, that the regenerating organs in annelids came partly from the old organs and partly from new sources; new muscles came, not from old muscle or even from mesoderm, but from the ectoderm, the pharynx regenerated from entoderm instead of ectoderm as in the original development. Other evidence of the same sort was directly contradictory to the view that regeneration is due to latent germ cells. Morgan ('01, '07) believes regeneration is a growth process. Schultz ('02) thinks regeneration is a primary property of life, limited more or less in consequence of specialization of tissues, but always potentially present. His conclusion is in accord with that of Morgan, and implies development, budding, and regeneration to be exhibitions of the capacity for growth inherent in all protoplasm. Montgomery ('06) and Hegner ('14) reject this view and accept the germ-cell explanation, the latter stating that regeneration in Coelenterates is always due to widely distributed germ cells. C. W. Hargitt ('11) points out serious objections to this explanation in hydroids, and Hegner admits the impossibility of accounting for regeneration of sex organs on this view. But sex organs are readily regenerated in hydroids. Child ('15) has observed that specialized cells of Pennaria may undergo a dedifferentiation and take part in budding, along with the interstitial cells; the same thing occurs during regeneration. Morgan has also found abundant evidence of the formation of masses of indifferent cells by regressive changes, and the production of new structures from such masses in regeneration. Morrill ('18), working upon the regeneration of appendages in salamanders, observed the formation of masses of cells by simplification of old specialized cells, and the differentiation of muscle and cartilage from these cells.

In Hydra and hydroids regeneration may take place at practically any point where a cut is made, and almost as often as new growths are excised. Very minute pieces may also regenerate complete animals, normal in all respects, including reproductive organs. The minimal size is always a group of cells, and yet, according to the theory of regeneration from germ cells, there is no reason why a single cell might not produce a new organism,

for the theory supposes the germ cells to be scattered over the whole body in great numbers. Clearly, there is no evidence that regeneration in Coelenterates, nor in other animals, is a process dependent upon the presence of germ cells. And there is abundant evidence that the specialized cells undergo regressive changes, produce masses of cells or syncytia of embryonic character, and then, by differentiation and specialization form new parts to replace those lost. It is, of course, equally well known that not all tissues can undergo such changes or even regenerate their own kind of tissue to any great extent; but this offers no evidence of a correlation between regeneration and the presence of germ cells. It only shows that specialization may proceed to such a degree that further changes, whether progressive or regressive, are impossible.

So far from regeneration presenting evidence in favor of the germ-plasm theory, practically all the experiments and observations show direct contradictions to this explanation. The germ-plasm theory is not only inadequate to explain regeneration, but it is shown to be incorrect, so far as this process is concerned.

d. Dissociated cells. The tissues of sponges have been broken up by teasing and forcing through fine screens, and the behavior of the isolated cells followed by Wilson ('07). Such cells showed amoeboid activities and fused into masses which later regenerated to form normal sponges. The amoebocytes first began to unite to form syncytia, but collar-cells and other specialized cells also took part in the formation of the masses, first passing through a regressive differentiation. Müller ('11) largely confirmed Wilson, but believes such specialized elements as collar-cells do not assist in the regeneration. Fresh-water sponges also undergo normal degeneration phenomena by a de-differentiation of cells to produce embryonic masses which later produce new organisms. This latter process is quite distinct from gemmation.

Later, Wilson ('11) extended his experiments to hydroids. Here also the isolated cells fused into syncytial masses which secreted perisarc about themselves, then formed ectoderm and entoderm layers, and later regenerated hydranths, complete and

normal, with tentacles, mouth, hypostome, and other structures. In these changes "we apparently have . . . . a plain case of despecialization of tissue elements and their union to form masses of totipotent regenerative tissue." Wilson discusses the question as to whether the tissue cells may not merely retain their specificity and later produce only cells of the same sort. By following the isolated cells with the microscope it was possible to observe the change of the tissue cells from their typical appearance to that of embryonic cells, and their fusion into a mass. The retention of their original specificity seems highly improbable. A histological study of sections of the coalesced cells showed the cells, first, as embryonic in appearance, and, as regeneration proceeds, they undergo changes similar to those seen in normal development and specialization. DeMorgan and Drew ('14), in similar experiments, for the most part confirmed Wilson, but did not obtain hydranths from the regenerating masses. They differ from Wilson in thinking the cells are segregated and rearranged and do not form syncytia by despecialization. They also state their belief that their cell masses are abnormal and pathological, but this does not appear to be the case, as C. W. Hargitt ('15) has pointed out in some detail. This latter author confirmed Wilson's observations in practically every respect, and also noted in detail the behavior of cells immediately after their isolation. The identification of the different cells was easily made, but the characteristic features gradually became less marked and finally disappeared as the cells merged into a common mass. "They have become despecialized into potentially embryonic cells, and probably from this change have acquired their regenerative capacities."

In discussing these experiments, Hegner ('14) claims there are always germ cells present, which would exlain the regeneration from the masses of cells, and therefore a continuity of germ plasm exists in these phyla. He does not attempt to explain the de-differentiation actually observed to occur, though this is a very significant fact and one that cannot be ignored. For, if tissue cells may become embryonic and form other cells and tissues by later differentiation, there is no reason for assuming

the presence of germ cells. The later work on dissociated cells gives clear evidence on this point. DeMorgan and Drew can recognize and follow the isolated ectoderm and entoderm cells and ". . . . in addition such structures as nematocysts, ova and broken down cells, all of which are subsequently absorbed and played no part in the future development." C. W. Hargitt also finds that the presence of germ cells in regenerating masses does not influence the behavior: "Indeed, in those cases in which egg cells were present they took no part whatever in later regenerative activity, either degenerating or being absorbed as yolk material." So far from the regeneration being conditioned upon the presence of germ cells, the latter serve no purpose but to act as food; growth and differentiation are the result of the activity of the tissue cells alone. Since these observations have been confirmed by a number of workers, it is manifestly false to consider regeneration to depend upon germ cells in these plasmodia. There would appear, likewise, to be no ground for assuming any regeneration to be dependent upon germ cells.

The claim of DeMorgan and Drew, of the retention of their distinct structure by the isolated cells, and a later rearrangement to produce the regenerated structures, is not confirmed by any of the other workers. The latter agree in being able to follow the isolated cells through a gradually decreasing sharpness and a final coalescence into a common mass. No doubt occasional cells persist, but the observations clearly show the fusion of the cells into a multinucleate mass. From such a mass a development occurs which parallels the normal development from the egg.

These experiments give such striking and clear-cut results that one is enabled to draw very definite conclusions. Tissue cells have actually been followed through the process of despecialization to an embryonic condition; such embryonic cells behave as any other group of similar cells, and develop a variety of structures which become differentiated and specialized in such a way as to produce a complex, normally organized, and functional individual. The totipotency of the tissue cells of the hydroid is

thereby definitely established, though this is clearly dependent upon the proper stimulus for its exhibition. When we take into consideration, also, the observations upon the origin of germ cells from tissue cells; the observations of Child upon the de-differentiation of cells in a great variety of animals and their later differentiation into a different sort of cell; the observations upon the formation of embryonic masses from which new structures develop in regenerating worms and salamanders; it would seem as though the germ-plasm theory was the very one of all theories least capable of accounting for the facts.

3. Evidence from other phyla

Such phyla as the round worms and arthropods give the strongest evidence of early segregation of germ cells and the best support of the germ plasm theory. This view is not universally accepted, however, and the opposing opinions are worthy of consideration. For instance, Child ('15) states that it is not known whether the primordial germ cells of Ascaris produce only germ cells or the reproductive organs as well. If the latter be the case, "the germ path of early cleavage has not resulted in the segregation of germ plasm from the soma, but merely in the segregation of different organs," since the walls of the reproductive organs are not germ plasm. The same author points to the fact that in no case is a segregation of germ plasm and soma known to take place at the first cleavage, as the theory requires. He believes, even in these phyla, the theory is unproved, and is not in accord with many facts.

In many animals the germ cells are produced periodically at the breeding season, and at no other period is it possible to recognize germ cells, or even reproductive organs. In these cases the germ cells obviously arise from the tissue cells; it does not answer to claim an invisible germ plasm in the tissue cells, since this is not capable of investigation and evades the question. Other animals are produced asexually and at a later period develop reproductive organs; the germ cells to all appearances, in such cases, come from the more or less differentiated cells of the region involved in the formation of these organs.

In the vertebrates the germ cells appear, as a rule, only after most of the other organs are laid down, and in most cases an early segregation of germ cells has not been proved. A review of the work on vertebrates is given by Hegner ('14) and Kingery ('17), and only a few cases will be mentioned here. Von Winiwarter and Sainmont ('08), from studies upon the cat, describe the degeneration of all the germ cells produced during embryonic development; the definitive eggs arise from the undifferentiated germinal epithelium after birth. Bachman ('14) in Teleosts and Witschi ('14) in Rana temporaria find no evidence of the origin of germ cells from the peritoneum, while v. Berenberg-Gossler ('14) believes "that one may no longer speak of a germ track in the Sauropsida," and Gatenby ('16), in Rana temporaria, observes the majority of germ cells arising from the peritoneum. Kingery ('17), working upon the white mouse, gets results comparable to those of von Winiwarter and Sainmont in the cat; viz., all germ cells formed during the foetal period degenerate and have nothing to do with the development of the definitive ova. The latter arise from the germinal epithelium after birth and all transitional stages between this germinal epithelium and graafian follicles were observed and the development followed.

In the vertebrates and some other phyla the evidence seems to be as clearly opposed to a continuity of the germ plasm as it is in the coelenterates. There is, especially in mammals, an increasing amount of evidence that the germ cells arise from more or less differentiated tissue cells at a time approaching the period of sexual maturity.

4. Evidence from tissue cultures

While most of the experiments dealing with explanted tissues have to do with growth, movements, and general behavior of the cells, there is some evidence of a de-differentiation of the tissues into a more embryonic condition. There is very little evidence that such cells re-differentiate into cells of a new kind, but this return to an embryonic condition resembles somewhat the despecialization of isolated cells of hydroids and sponges.

In cultures of skeletal muscle of chick embryos, Lewis ('17) observed the growth of the cut ends of the muscle into embryonic tissue without striations. Streeter ('17) observed a de-differentiation of cartilage cells in the normal development of the ear in human embryos, the cartilage of the membranous labyrinth undergoing a despecialization and a return to the condition of embryonic connective tissue. From experiments with muscle, kidney, eye, thyreoid, and other organs, Champy ('14) observes a characteristic behavior of the cells of the edge of the culture where they receive abundant air and food. These cells form such an indifferent mass as to resemble cells of a young blastoderm; and this is true for all tissues, irrespective of their source or the culture medium. Such a de-differentiation takes place from explanted adult tissues as well as from embryonic tissues.

Danchakoff ('18) mashes adult spleen and grafts it upon the allantois of embryos. The spleen tissue forms a syncytium of embryonic character, and the cells forming the mass contain endothelial cells of blood-vessels as well as reticular tissue of the spleen. The syncytial mass develops and forms cells of a different sort than those which composed it. Danchakoff interprets this, not as a de-differentiation, but as an expression of an inherent capacity of the original cells to undergo a further differentiation. Her point of view is as follows (p. 161):

The changes undergone by the living matter during development are not always specific. They may lead to a specialization of tissue without differentiating them specifically. The difference between these two processes consists in that specialization does not imply a limitation of potencies in the cell, while specific differentiation is a process, by which the constitution of a cell is changed irrevocably and its potencies to development are narrowed. The distinction between the two processes would make it unnecessary to introduce a new concept of dedifferentiation in order to understand certain phenomena.

I am not convinced that this view is simpler or more nearly interprets the phenomena observed than the view of regressive changes in the tissues and a later differentiation of these. Nor does this opinion take into consideration the fact that de-differentiation has actually been observed to take place; that is,

specialized cells do actually become embryonic. But for the present discussion the important point is the observation of the varied potencies of the tissues of a differentiated adult organ like the spleen.

This brief account of some of the experimental investigations upon cells and tissues of adult and embryonic animals is enough to show the degree to which such tissues may change their structure and function. It clearly demonstrates that body cells are not so limited in behavior and so predetermined in potency as to render a change impossible. The difference between body cells and germ cells is proved by such investigations not to be so great as is usually held.

5. Evidence from cancer cells

The studies which have been made upon cancers throw some light upon the potencies of tissue cells. As is well known, it is possible to transplant cancers from one animal to another through many generations. Most of the cancers which have been experimentally studied are tissue growths, not germ-cell growths, and the ability of these cells to continue their growth and proliferation for long periods of time is an indication of the ability of tisssue cells to live and grow indefinitely. It is, of course, perfectly clear that these cells do not produce other cells of a widely different character, but they are more nearly like embryonic cells, physiologically if not morphologically, than the cells from which they originally came. This would probably involve a sort of despecialization of the tissue cells with the resumption of an embryonic potency. The germ-plasm theory postulates a difference between the germ cells and the body cells of such a sort that the former are conceived to have the ability to live and develop indefinitely, while body cells have a limited life. The behavior of the cells in cancerous growths may do no more than show the ability of highly differentiated tissue cells, under unknown or poorly known conditions, to regain this power of repeated and indefinite growth; but this tends to break down the distinction between germ cells and tissue cells in this particular.

Loeb ('15), who has given much attention to the study of cancer cells, discusses the matter from that point of view. He concludes that the observations of fourteen years upon cancerous growths have established certain facts which are contrary to the view of the radical difference between germ cells and body cells. In those cases where it has been possible to detect and study the earliest indications of cancer in mice, he has been able to trace the transformation of the normal tissue cells into the abnormally proliferating tumor tissue, and is thus able to demonstrate the origin of the tumor from the tissue. He believes that germ cells and somatic cells are not so different, and possess no such differences in potency as is often claimed.

6. Summary and conclusions

The germ cells of Hydrozoa are differentiated, at a time just preceding sexual maturity, from different regions of the animal or colony, there being no one region or layer which characterizes the place of origin in this group. These germ cells probably arise in all cases from tissue cells; in some species such an origin is demonstrated, since an entire cell or half a divided body cell produces a single egg or sperm cell.

Budding in Hydra and hydroids involves a multiplication and growth of the cells and an evagination of all the body layers in the budding zone. The claim that latent germ cells are responsible for budding is not sustained by observations. Some medusae reproduce asexually by budding, and as a rule such buds are produced in a manner similar to that of hydroids, viz., by an evagination of both ectoderm and entoderm. In a few cases asexual buds of medusae arise from the ectoderm alone, but in no case does such a development come from a single cell. Buds may also come from the reproductive organs of medusae, but all investigators of this manner of budding agree upon the activity of ectoderm and entoderm cells of that region; such a process is not a development from germ cells. The different types of budding in Hydrozoa suggest an evolution of reproductive processes which may still be in progress. The phenomena of budding give evidence of a considerable degree of

plasticity in the cells of the body, a regressive change to an embryonic condition preceding the formation of the bud.

The germ-plasm theory invokes the aid of latent germ cells to account for regeneration, but there is no evidence of this in Hydrozoa. So many cases are recorded, in many groups of animals including vertebrates, of the de-differentiation of tissue cells and the formation of the regenerated structures from an indifferent or embryonic mass of cells, that it may be doubted whether regeneration is ever related to germ cells. When coelenterate tissues are ground up and the cells isolated, the latter coalesce to form masses capable of regenerating complete and normal individuals, but in all such masses the cells have become despecialized before the regenerative processes begin. The observations upon dissociated cells of hydroids show that germ cells, if present, degenerate and play no part in the ensuing regeneration, while the body cells, under the same stimulus, lose their specificity, become totipotent, and produce the variously specialized cells and differentiated structures of the normal individual.

Many animals of different phyla are known whose gonads are present at the breeding season and entirely unrecognizable at other times, in such cases the germ cells arise from the body cells of the appropriate region. Recent work upon mammals gives strong evidence of the degeneration of all germ cells formed during embryogenesis, the definitive germ cells only differentiating after birth from the germinal epithelium of the gonad.

Explanted tissues, grown in culture media outside the body, may undergo a de-differentiation and form cells more or less embryonic in character. Cancerous growths, originating from tissue cells, display a capacity for long-continued and apparently indefinite growth and division. Such facts are indicative of a less definite distinction between germ cells and body cells than has usually been maintained, and the possession of a considerable capacity in specialized cells to undergo a further differentiation, even in a new direction.

The investigations discussed in this section furnish a great body of facts utterly inconsistent with the theory of the con-

tinuity of the germ plasm. This seems to apply to many phyla, even to vertebrates, but is especially marked in the coelenterates. There are so many facts which contradict this theory that it may confidently be held not to apply in the coelenterates, at any rate.

IV. GROWTH OF EGG CELLS

1. Cytoplasmic growth

The growth of egg cells proceeds by several methods in animals; nourishment is obtained either without assistance from other cells, or else follicle cells, nurse cells, or other accessory structures assist in securing or preparing the nourishment for the egg. In none of the coelenterates is a follicle present nor are there nurse cells such as occur in insects. But there are two distinct methods of growth; one in which the food is obtained directly from the enteric cavity or from the adjoining cells, and the second in which neighboring cells are actually absorbed or engulfed. Often both methods may be employed. The cells which are absorbed have sometimes been called nurse cells, but they do not function in the way nurse cells do in other groups, since they are consumed instead of preparing food. Hydra, Tubularia, Pennaria, and Hybocodon are examples of those eggs which absorb neighboring cells for food, and Campanularia, Clava, Hydractinia, and Aurelia are examples of those which obtain food from the enteric cavity.

A different origin has been claimed for nurse cells and egg cells in those animals whose eggs are so nourished, the germ cells representing real reproductive cells while the nurse cells are held to be tissue cells. The Hydrozoa show no such distinction, for all the oogonia of any ovary are alike in origin and capable of becoming ova; the determination of which shall grow and which serve as food is largely a matter of chance. Even after growth has started, the surrounding cells are like them until degeneration phenomena become apparent in the cells undergoing absorption. One explanation for the initiation of growth is the presence of certain bodies in the cytoplasm. Schaxel ('10 a, '11 a) describes the growth of oocytes of Pelagia,

Aequorea, Forskalia, and Agalma as beginning only when chromatin passes from the nucleus into the cytoplasm. Jörgensen ('10) found similar bodies in the cytoplasm of Sycon sponges at the beginning of growth, Downing ('09) in oogonia of Hydra, and the author ('13 to '18) has noted an apparent correlation between the presence of such cytoplasmic granules and the initiation of the growth processes in the eggs of other Hydrozoa. None of these authors have expressed any thought of these cytoplasmic inclusions acting as indicators of germ-cell or tissue-cell origin, but Hegner ('14), who has collected data from many sources, explains them as germ-cell determinants.

After growth has once started, it continues rapidly, and reserve food is stored away for future use. The eggs of some Hydrozoa become filled with large yolk spheres, while in others the yolk is in fine particles so diffused through the cytoplasm as to be scarcely noticeable. There is a great deal of variation in the size attained by these eggs, as the figures and descriptions of the following section will show.

2. Nuclear growth

The detailed changes in the nucleus during growth have been described in the papers dealing with particular species; only certain more general relations are here discussed. As the eggs grow, their nuclei also increase, but not in the same ratio. Hertwig's suggestion of a constant ratio between nuclear and cytoplasmic volume is no more supported by the growing eggs of these coelenterates than it has been by other cells investigated by many workers. Jörgensen ('13) has made the claim of a definite relation between the relative size of the nucleus and the mode of nourishment of the egg, basing his claim upon observations of egg cells of a number of different animals. According to this author, eggs nourished by nurse cells or follicle cells, or by the absorption of adjoining ova and oocytes, have very small nuclei; eggs without special nourishing apparatus, but which absorb their food directly, possess relatively large nuclei. In the latter case, he believes, the nucleus of the egg is responsible for its

growth; in the former, the nuclei of the accessory cells govern the growth of the egg, and the nucleus of the egg is inactive till it enters upon the prophase of maturation mitoses.

A brief survey is sufficient to demonstrate a great variation in the relative size of nuclei in coelenterate eggs, and I have undertaken to test Jörgensen's suggestion. Figures 16 to 30 are the outlines of a number of eggs with their nuclei, accurately drawn to the same scale, all representing eggs at the end of the growth period before the prophase of maturation mitoses. Figure 15 is a similar representation of a starfish egg of the same stage, introduced for the sake of comparison. In the accompanying table these eggs are arranged in order, the one with the relatively largest nucleus heading the list. Since the nuclei are not always perfect spheres, and the eggs depart even more from a true spherical form, the figures given in the table for the diameters are averages of the greatest and least diameter of both structures. The measurements, in millimeters, were made from projected images; if each average is multiplied by 1000 and divided by 137 (the magnification of the projected images) the results will give the average diameters in microns. From these measurements was obtained the ratio $\dfrac{\text{diameter of egg}}{\text{diameter of nucleus}}$ indicated in the third column. The figures of this column, squared, give the ratio $\dfrac{\text{surface area of egg}}{\text{surface area of nucleus}}$, and the same figures, cubed, furnish the ratio $\dfrac{\text{volume of egg}}{\text{volume of nucleus}}$ (this computation is given in the last column of the table). The actual volumes are not important, the relative volumes being the thing desired. Some inaccuracies result from the computations based upon formulae for surface and volume of true spheres, but it is believed these are not great enough seriously to disturb the order given in the table. These figures also represent measurements and ratios for particular eggs, and are not of the nature of constants; there is variation in size of eggs of the same species, but this, again, is not of such magnitude as to modify the table greatly.

Table of measurements and computations of relative sizes of various coelenterate eggs, and their nuclei. Diameters, in millimeters, are made from projected images of the eggs and nuclei; these multiplied by 1000 and divided by 137 will give the diameters in microns. The diameters represent the average diameter of the egg and nucleus, since often these are not perfectly spherical

FIGURE	FORMS EXAMINED	AVERAGE DIAMETER NUCLEUS	AVERAGE DIAMETER EGG	DIAMETER EGG / DIAMETER NUCLEUS	VOLUME EGG / VOLUME NUCLEUS
15	Starfish..............	6.0	12.0	2.0	8.0
16	Nausithoë punctata....	8.0	20.0	2.5	15.625
17	Hydractinia echinata...	9.0	23.0	2.555	16.581
18	Pelagia noctiluca.......	11.0	30.0	2.7272	20.153
19	Obelia sp?............	6.0	18.0	3.00	27.0
20	Aglantha digitalis......	5.0	15.0	3.0	27.0
21	Campanularia flexuosa.	7.0	22.0	3.1428	30.957
22	Gonothyraea loveni....	4.0	14.0	3.5	42.875
23	Aurelia flavidula.......	5.0	18.0	3.6	52.656
24	Clava leptostyla........	4.3	18.0	4.186	72.930
25	Corymorpha pendula...	6.0	39.5	6.583	284.848
26	Hydra sp?............	7.0	47.0	6.714	302.6469
27	Eudendrium ramosum..	4.0	31.5	7.875	488.058
28	Pennaria tiarella.......	3.3	32.5	9.848	955.088
29	Hybocodon prolifer.....	4.0	58.5	14.625	3122.794
30	Tubularia crocea.......	3.0	54.5	18.166	5994.8435

Very obviously the table is divided into two parts, 16 to 24 represent eggs with relatively large nuclei, and 25 to 30 have distinctly smaller nuclei. Within each group there is a rather marked gradation, but between the groups a noticeable gap. The relation between the volumes of nuclei and cytoplasm may be expressed in another way. In the first lot the egg volume exceeds the nuclear volume by from 15 to 73 times, but the eggs of the second group are from 284 to nearly 6000 times the volumes of their nuclei. Each egg of the first lot obtains its nourishment from the enteric cavity, from which it is separated by a single layer of cells; the eggs of the second lot (except 27) absorb the surrounding oocytes and ova and appear to depend upon these almost entirely for their food supply. Eudendrium (27), in size of nucleus, belongs to the second series, but does not absorb oocytes; however, its gonophores are adapted to serve as nourishing organs, and the cells of these are later absorbed, so it may properly be placed in the second series instead of the first.

These fifteen coelenterate eggs support the claim of Jörgensen, or at any rate are consistent with his suggestion of the relation between the mode of nourishment of the egg and the size of the nucleus. Perhaps this agreement is incidental, for there are some objections to Jörgensen's views. His suggestion implies a passivity of the nucleus in eggs whose nourishment comes from absorbed ova. I believe, in these as in the others, there is an exchange of material between nucleus and cytoplasm of growing eggs, for there is evidence of the passage of chromatin into the cytoplasm of these eggs during growth. Nor does it seem probable that the nuclei of accessory cells could have anything to do in directing the growth processes, for in coelenterates these cells are absorbed and their nuclei may undergo a degeneration before absorption. All the facts sustain the belief that the nuclei of growing eggs are responsible for the direction of the functional activities of these cells. To this extent, at any rate, Jörgensen is probably incorrect in his interpretation. I think it quite probable that some relation may exist between the method of nourishment and the relative size of the nucleus, and the figures of the table may be an expression of this relation.

3. Cytoplasmic inclusions

In the cytoplasm of growing coelenterate eggs certain bodies occur as characteristic structures. These inclusions, described by the author ('13 to '18) as of nuclear origin, appear to be correlated with the growth processes, either furnishing the stimulus to growth or in some way determining the course and extent of growth. Similar bodies are present in germ cells of other animals at corresponding periods, but there is disagreement regarding their origin and function. Without doubt, some of the difference of opinion is due to the presence of cytoplasmic inclusions of different sorts, both as to origin and as to function. This is clearly established by the work of Cowdry ('16) and other recent writers.

In Campanularia the cytoplasmic bodies in the egg are formed from the dissolving nucleolus and passed through the nuclear wall into the cytoplasm, where they participate in the formation

of yolk. While growth begins before such bodies occur, the period of rapid growth is coincident with the passage of nuclear matter into the cytoplasm. The nucleolus is partly chromatic, and the bodies in the cytoplasm derived from the nucleolus also contain chromatin. Clava shows essentially the same phenomena, but the chromatin which passes into the cytoplasm appears earlier and comes from the nuclear reticulum, the nucleolus being a true plasmosome. After the chromatin enters the cytoplasm of Clava, growth begins. Growth begins in Aglantha shortly before nuclear substances enter the egg, or at least before definite cytoplasmic bodies can be recognized. In this egg it is not possible to determine the fate of the chromatin particles, except for their rapid solution within the cytoplasm, nor whether they have any close relation to cell metabolism. In the egg plasm of Hybocodon, chromatin granules appear before the growth of the oocyte begins; this migration of chromatin is abundant during early growth, but soon ceases, and the particles dissolve within the cytoplasm. Eudendrium shows similar inclusions in oocytes as growth begins, and they continue to form abundantly during practically the whole of the growth period. They are apparently of chromatic nature.

The interpretation of these cytoplasmic inclusions involves, chiefly, the consideration of their origin. Do such bodies arise, in the place where they first appear, out of materials of the cytoplasm, or do they represent nuclear substances in the cytoplasm? If the latter be the case, are the bodies composed of chromatin or of achromatic material? Bodies of cytoplasmic origin have commonly been called mitochondria, those believed to be chromatic in nature are sometimes referred to as chromidia. Tests seem to have demonstrated the reality and difference of these two classes of inclusions, for Cowdry ('16) believes, "we have ample evidence that the chromidial substance (Nissl substance) is a nucleoprotein containing iron . . . ., formed at least in part through the activity of the nucleus, and the mitochondria is a phospholipin albumin complex."

The granulations in the egg cells of the described coelenterates are certainly not mitochondria, though typical mitochondria

have been found in such cells, and no doubt are present in these. Their size, position, time and place of appearance, staining reactions, all seem to distinguish them as extruded nuclear material. They are present in young oocytes at the beginning of growth, and sometimes in later growth stages. They appear, in all cases, first, in the region of the nucleus, usually directly against the nuclear membrane; their appearance is often correlated with signs of activity within the nucleus and indications of currents in the cytoplasm; they stain like chromatin. Within the cytoplasm it is practically universal for them to lie within vacuoles, while other granules are commonly not so situated. In this latter respect they seem to produce a vacuolation or liquefaction of the surrounding cytoplasm in the same manner as Lillie ('02) described for chromatin particles which are free in the cytoplasm.

Jörgensen ('10) found a relation between egg growth and the presence of chromatin particles in sponge eggs; Schaxel, an emission of chromatin into the cytoplasm of coelenterates ('10 a, '11 a), Ascidia ('10 b), and echinoderms ('11 b); and the activation of the cytoplasm upon the entrance of the chromatin. Schaxel ('11 c) finds the mitochondria (chondriosomes) present in practically all cells at all times, while the extra nuclear chromatin (chromidia) occurs only at certain times, performs certain functions and disappears. He also recounts differences in appearance and staining reactions of the two sorts of bodies. Tsukaguchi ('14), using Altmann's technique upon Aurelia eggs, believes Schaxel to be in error, and considers all cytoplasmic granules as mitochondria. But the behavior of the bodies he investigated, especially their disappearance in later growth, is not like the usual behavior of the mitochondria.

Beckwith ('14) discusses the origin of the plasma structure of one of the hydroid eggs, and observes basically staining bodies, which she calls 'pseudochromatin-granules,' scattered through the cytoplasm. She also observed a second plasma granulation, "large drop-like masses which appear near the nuclear wall and which are also probably not chromatin;" these also are stained with nuclear dyes. Various stains were tried, and it was common

to find the nucleus and cytoplasmic granules staining alike, but some vital dyes gave a difference in staining reaction. If young eggs were digested in pepsin, the nucleus and the cytoplasmic granules were unaffected. Beckwith clearly points out the lack of precision in selective staining, but believes her evidence shows the non-chromatic character of the protoplasmic granules. "In all cases which seem to indicate the contrary conclusion (some staining and digestive tests and tests for proteid) the results can be interpreted in some other way." This author believes the contrary conclusions of Smallwood, Schaxel, and others are due to faulty technique. Differences in technique may undoubtedly account for difference in appearance, but it would appear rather improbable that these investigators, in addition to others not mentioned, all working independently and by different methods and arriving at similar conclusions, should not have worked out a reasonably satisfactory technique and should have been unable to distinguish between artifacts and real structures. It is permissible for Beckwith to differ in her interpretation of observed facts, but not to attack the methods of those who differ in this interpretation, with no more grounds than she offers. According to Beckwith herself, the evidence implies that these other authors were correct in interpretation; the weight of evidence of her own observations supports their contention of the chromatic character of the protoplasmic bodies under discussion, for she says, "the balance of the evidence . . . . indicates the non-chromatic nature of the granules in question." I do not believe the balance of her evidence outweighs the evidence in the other direction.

Jörgensen ('13) discounts his own earlier work on sponges, all of Schaxel's work, the work of Goldschmidt, Montgomery, and others, so far as they relate to questions like the present one. He believes undue weight has been placed upon staining reactions; it is necessary, in his opinion, to identify nucleic acid in plasm granules in order to show their chromatic origin. Pepsin digestion experiments convinced him of the presence of nucleic acid compounds in the cytoplasm of some eggs, and he admits the occasional migration of chromatin from nuclei, but he thinks this is

of no significance where it occurs. Jörgensen finds chromatin stains and mitochondrial stains and technique to be very uncertain, and neither of these, or any other staining method, is to be depended on, since they do not differentiate bodies of diverse origin and chemical composition.

An even stronger criticism of our staining methods and all microchemical tests is made by van Herwerden ('13). Our technique, she holds, is so primitive as to be useless in the identification of chromatin; evidence from stained, fixed preparations is not valid; action of weak or strong alkalis or acids does not give satisfactory results; digestion by pepsin and trypsin leads to no intelligible information; none of the usual tests are of any great service. This author uses nuclease as an enzyme in digestion experiments to test for chromatin (nucleic acid content) in the basic cytoplasmic granules of echinoderm eggs. Using ripe eggs, very simple experiments demonstrated the basophile granules of the cytoplasm to "consist of a nucleic acid compound." In younger oocytes, where chromidia had been described against the nuclear membrane, the nuclease experiments show the presence of nucleic acid compounds. Van Herwerden is somewhat doubtful as to the origin of these chromatin particles and hesitates to interpret it as a migration of chromatin from the nucleus. However, by observing living oocytes of Sphaerechinus, she could follow a movement of refractile granules to the nuclear membrane where they disappeared, and at the same time granules appeared in the cytoplasm close to the nuclear wall. Van Herwerden concludes that there is a possibility of the diffusion of nucleic acid compounds from the nucleus into the cytoplasm, but no direct proof of this. I suppose, in the very nature of the process, one could not expect to secure absolute proof of this passage, but van Herwerden seems to have obtained evidence which renders such diffusion highly probable. In all experiments with nuclease, the chromatin of the nucleus was affected in the same way (though to a much less degree) as the basophile granules of the cytoplasm. From the experiments and observations of van Herwerden there would appear to be ample warrant for the belief that nuclear material passes from the nucleus into

the cytoplasm of growing eggs; in other words the morphological conclusions appear to be supported by experimental results.

Outside the forms already mentioned, the insects are described as showing a passage of chromatin into the cytoplasm. Wassilieff ('07) finds the nebenkern of the cockroach spermatid has come from chromatin of the nucleus by a diffusion through the membrane. Hegner ('15), in the honey-bee and carpenter-ant, thinks the oocyte nuclei give off chromatin, which appears in the cytoplasm of fixed eggs as granules. In echinoderms Danchakoff ('16) finds basic granules, indications of cytoplasmic movements, and other conditions similar to those described by the author ('13) for Campanularia, but believes these mark the passage of basic material of the cytoplasm into the nucleus, where it becomes differentiated and helps to form chromosomes.

There are abundant records in the literature of the presence of basophile granules in the cytoplasm of eggs and other cells of animals. These have been observed and studied by cytologists, following their usual technique and have been interpreted in accordance with the morphological appearance; relatively few attempts having been made to check these by chemical or physiological tests. It would appear from some of the recent work that staining reactions are much less specific and selective than has been assumed; conclusions drawn from stained material, therefore, would have little significance and would be misleading, since morphological structures of a very diverse chemical composition and varied functions may stain alike. From this point of view, all interpretations based upon staining are of little value until they have been checked by appropriate chemical or physiological tests. I believe there is a large element of truth in these criticisms, and we have probably gone to an extreme in interpretations based upon purely morphological studies. For present purposes we are very fortunate to have had such a test of basophile granules of echinoderm eggs, with an application of these to the chromidial hypothesis of Goldschmidt and Schaxel. This hypothesis is not entirely substantiated by van Herwerden, and some of the 'chromidial apparatus' described for echinoderms is believed to be artificial. But the fundamental principle of the

theory is confirmed, viz., that basic granules in the cytoplasm contain nucleic acid components, which are similar to the nucleic acid compounds within the nucleus.   Moreover, it appears quite probable that this cytoplasmic nucleic acid has come from the nucleus, van Herwerden having followed a nuclear emission in living echinoderm eggs.   From this evidence we are warranted in believing that the passage of chromatic material (nucleic acid compounds) into the cytoplasm is a reality.   According to the tests on echinoderms, it is the basophile granules near the nuclear wall in young oocytes which represent this material; probably the similarly placed granules in the coelenterate eggs are the same substance.

The determination of the functions of these bodies is not so simple, and there is a good deal of difference in interpretation. Hegner believes the chromatin bodies in egg cells are germ-cell determinants; Goldschmidt thinks they represent the chromatin which is responsible for all the vegetative functions of the germ cells; Schaxel looks upon them as regulating some of the cell functions, but not governing all vegetative activities; the author has held the view that they are related to yolk production, and possibly have an enzyme action in stimulating growth and synthesis of reserve food in eggs.   Others view these bodies as of no significance in cell metabolism.   If they play a single definite part in the cell metabolism, further work is necessary for a decision.   My own impression would lead me to discard the view of a total absence of any significance.

V. CHROMOSOMES

The maturation phenomena, characteristic of germ cells, are exhibited by both male and female germ cells of coelenterates. In the egg cells polar bodies are formed by means of mitosis, and a reduced number of chromosomes remain in the egg.   This reduction apparently takes place at the beginning of the growth of the oocyte, and evidence is not lacking of a conjugation of chromosomes.   The coelenterates do not appear to offer material favorable for the determination of the method by which such conjugation is accomplished.   Differences are noticeable in such

details as the form of spindle, distinctness of chromosomes, and the like, but the principles involved are those characteristic of similar phases in germ cells generally. In some instances conditions are found which have been interpreted as synizesis, in other cases such phases were not found. The coelenterates do not, therefore, add anything definite to the evidence concerning the normality of this process.

While the chromosomes appear to show a characteristic behavior, they are lacking in the variety of form and size which obtains in the chromosomes of some animals. In most coelenterates whose chromosomes have been studied, there is a similarity which renders it very difficult even to identify synaptic mates in maturation mitoses. Of the forms studied by the author only Aglantha had chromosomes which offered a reasonable opportunity for a study of details. Oogonial chromosomes did not, however, readily lend themselves to a grouping into homologous pairs. Some doubt was expressed as to whether these chromosomes behaved in quite the fashion believed to be characteristic and typical of maturation mitoses. The evidence is not sufficient to warrant any definite conclusions of a difference in the chromosome behavior of the coelenterates.

The question of the individuality and continuity of the chromosomes has been in mind during the study of the coelenterate germ cells. On one point the evidence is clear. During interkinesis there is no indication of the persistence of the chromosomes, the 'resting nucleus' is typically a single vesicle clearly without division into smaller vesicles. In certain forms chromosomal vesicles are produced after maturation or cleavage mitoses, but it is very common for two or more chromosomes to form a single vesicle. In any event, if the period of interkinesis is long, these vesicles unite into a single one. On the matter of the maintenance of chromosome individuality during interkinesis Wilson ('13) says:

Some of the most careful recent cytological studies in this direction seem to show that such is not the case. Nevertheless these same studies, together with recent experimental evidence, give very strong ground for the conclusion that a definite relation of genetic continuity exists between the individual chromosomes of successive generations of cells.

The evidence obtained from coelenterate eggs would not permit one to dissent from this view. In the absence of contrary evidence in this group, the evidence from other groups would lead me to agree that there is no reason to believe the coelenterates differ in this regard.

Recently Robertson ('16), McClung ('17), and others have expressed a more radical view. Robertson believes the chromosomes are 'individually indentical' in succeeding generations and 'persist as entities' from one cell division to another. McClung is likewise convinced that each chromsome persists as a distinct structure; during interkinesis the chromosome may extend its boundaries and diffuse its substance, but each body retains just as precise a limit (though it is usually unrecognizable) during this period as it does during its stay in the usual form. This is a return to the older view of a distinct morphological individuality which Wilson and others have abandoned. McClung says of the chromosomes, "either they actually persist as discrete units of extremely variable form, or they are entirely lost as individual entities and are reconstituted by some extrinsic agency." It is quite unwarranted to state that extrinsic agencies are all that can explain a reintegration of chromosomes under these conditions. McClung gives us a very valuable critique of chromosome individuality, and, in his chief arguments, makes use of analogies between chromosomes and other organic behavior. The restitution of the normal form in regeneration and the production of a typical adult form by developing eggs are due to internal organization and not to 'some extrinsic agency.' On the same basis, the restitution of chromosome form is scarcely to be ascribed to external agencies, even if there have been a loss of identity in interkinesis. McClung contrasts organization with lack of organization in urging a persistent and continuous individuality, but organization does not involve preformation, as his discussion assumes.

In discussing chromosomal relationships Payne ('16) says: "It seems to me it is time we were realizing that evolution of chromosomes as morphological units, in chromosome numbers, and in chromosome behavior has been as diverse as it has been in

external morphological characters." Nor have we any reason to believe this evolution has ceased. It is quite conceivable that the chromosomes may be tending toward a persistence throughout the entire life of the cell in all its changes, and in some cases may now be distinguishable in interkinesis as well as in mitosis. But the evidence does not warrant a belief in such a continuity as Robertson and McClung postulate for all chromosomes of all organisms. The work of Hance ('17) furnishes him with no evidence of a persistence of individuality during interkinesis, and he can only subscribe to such a view by broadening the present concept. That is, he believes the chromatin particles may persist from generation to generation, but the bodies which they form do not persist. This view could hardly be tested, since we are without means of identifying or following particular chromatin particles at the present state of our technique. If such a belief could be confirmed, we should have a chromatin individuality hypothesis which would be without many of the objections of the present one.

So far as coelenterate chromosomes are concerned, there is nothing to disprove the view that the chromosomes of one generation are descended from the chromosomes of a previous generation. All the evidence obtainable, however, is quite inconsistent with the view of the persistence of chromosomes as distinct entities during interkinesis. A genetic continuity is very probable, a morphological continuity is highly improbable.

VI. SUMMARY AND CONCLUSIONS

In the Scyphozoa and Actinozoa all observations point to the entodermal origin of germ cells. The former widespread belief in the ectodermal origin of germ cells in Hydrozoa cannot be maintained, for literature records show a greater number of species whose germ cells arise in the entoderm than of those in which the ectoderm produces them. The germ cells of Hydrozoa may originate in either or both germ layers; the same individual may even produce germ cells from both ectoderm and entoderm. There is no characteristic place of germ-cell differentiation in this class.

The germ cells of some animals have been observed to form relatively early in ontogeny, but such is not the case in coelenterates. It has been claimed by some writers that in Hydra and a few hydroids germ cells are differentiated in the larvae. This claim has been refuted by later studies upon Hydra, and a new investigation of larval stages of hydroids furnishes no evidence of an early differentiation of germ cells in those forms. Furthermore, there are a number of the Hydrozoa whose germ cells have been observed to arise directly from differentiated body cells. This happens either by the transformation of an entire epithelial cell into a germ cell or by the division of a body cell and the transformation of one of these division products into a germ cell. In the latter case the sister cell persists as a functional tissue cell. In at least ten species of eight different genera the germ cells have been observed to form in this way. This positive evidence, together with the refutation of all contrary claims, points to a single conclusion, viz., in the Hydrozoa (probably also in all coelenterates) germ cells are not differentiated in early ontogeny, but only much later as the time of sexual maturity is at hand.

The theory of the continuity of the germ plasm postulates the formation of a somatic blastomere and a germinal blastomere at the first cleavage of the egg; in no animal is such a result known. As applied to hydroids, the theory originally admitted the origin of germ cells from histologically differentiated somatic cells, but invoked the aid of invisible and unrecognizable germ substance lying latent in such body cells. The production of a germ cell by the transformation of half a tissue cell, and the persistence of the other half as a tissue cell, is sufficient to disprove the claim of the presence of an invisible germ plasm in such tissue cells. This fact, together with the origin of germ cells only as sexual maturity approaches, indicates a lack of continuity of the germ plasm in the coelenterates.

As explained by the germ-plasm theory, budding is always due to the presence of latent germ cells. But budding in Hydra and hydroids involves the activity of all the layers of the budding zone; in Hydra it is possible that interstitial cells first

become active, but there is no evidence that germ cells are present. The budding phenomena of medusae resemble the same processes in hydroids, since, in most cases, both body layers evaginate to form the bud. In a few forms buds are produced from the gonads of the parent medusa, but even here this is not a germinal process, for the buds are formed from all layers of the animal; the germ cells of the gonad may participate in the process, but only by behaving as tissue cells. A few medusae form their buds from the ectoderm alone, and one investigator claims that the bud originates from a group of oocytes, though he admits this is a very unusual method, not applicable to most buds in coelenterates. In no case are buds known to arise from a single cell. While it may be possible, therefore, that budding is occasionally a germinal process in medusae, this is rare; as an alternative explanation, other investigators believe both germ cells and tissue cells are able to undergo regressive changes and become embryonic. The embryonic cells have the ability to form a new organism. The latter explanation would correlate various types of reproduction, both sexual and asexual, in coelenterates; would correlate fission and budding in coelenterates and other groups of animals, and would outline a possible evolution of reproductive processes in coelenterates. The germ-plasm theory, therefore, may be held not to apply to budding in coelenterates, for it is contradictory to most of the facts of this phenomenon.

Regeneration is also held to be dependent upon the presence of latent germ cells. There seems to be no direct evidence in favor of this view, and the great body of facts concerning regeneration in many phyla of animals contradict such an interpretation. Especially do the observations upon regeneration from isolated cells of hydroids disprove the germ-plasm theory. When hydroid tissues are broken up into isolated cells the latter undergo a despecialization and fuse to form syncytia. From these masses complete and normal hydranths are regenerated. When germ cells are present they are absorbed as food, and take no part in the regenerative processes. The behavior of the isolated cells has been followed with the microscope and

sections made of the regenerative plasmodia. All the facts
point to the totipotency of the tissue cells under such stimulus.

In some animals of different phyla reproductive organs are
present only during the breeding season, and at other periods no
germ cells can be recognized. In such cases the germ cells
must be differentiated from the tissues of the region which gives
rise to the reproductive organs. There is no evidence of a con-
tinuity of the germ plasm in these animals. In the vertebrates,
especially in mammals, recent observations point to the degen-
eration of all germ cells which are formed during foetal life;
the definitive germ cells are differentiated from the germinal
epithelium after birth.

Pieces of tissue removed from the body will grow in culture
fluids, under certain conditions. In some cases the new growths
from this explanted tissue are embryonic in character, due to a
despecialization of the old differentiated tissues. Cancers, de-
veloped from tissues, are composed of cells more embryonic in
character than those from which they arose. These cells may
continue to live, grow, and divide indefinitely. Such observa-
tions indicate a less marked difference between body cells and
germ cells, and a greater plasticity and a more varied potency
in differentiated tissue cells, than has commonly been believed.
Such a weakening of the line of demarkation between these
two categories of cells tends also to weaken the germ-plasm
theory.

So far as the coelenterates are concerned, the observations
upon the time and method of germ-cell origin; upon budding of
all types; upon regeneration of the usual sort, and regeneration
from plasmodia formed by coalescence of isolated cells, all point
in one direction, viz., that there is no germ plasm in the sense
of Weismann. Furthermore, the origin of germ cells in some
phyla other than Coelenterata, the despecialization of differ-
entiated cells, and their behavior in tissue cultures and in normal
development, and the continued growth and division of body
cells in cancers, also present evidence contradicting the germ-
plasm theory. There are so many facts, from such different
sources and from so many phyla, which are inconsistent with

the theory, that it may be questioned whether the theory applies at all extensively to animals of any phylum.

As a rule, those coelenterate eggs which secure nourishment from the adjoining enteric cavity have large nuclei; and the ones which absorb oocytes or other cells possess relatively small nuclei. Whether this correlation be incidental, or whether it have a deeper significance, is not known.

Cytoplasmic granules which stain in nuclear dyes are a characteristic feature of coelenterate eggs. Typically, these appear in young oocytes about the time growth begins, and they may also form at other times during growth. From their initial position, close to the nuclear wall; from their staining reactions; from the behavior of other cytoplasmic and nuclear substances; the author has interpreted these granules as chromatin. Observations by other investigators, upon the eggs of other animals, have led them to conclude that chromatin does migrate into the cytoplasm. The criticism that the usual tests for chromatin are not specific is justified in large measure, but digestive experiments have demonstrated the presence of nucleic acid compounds in cytoplasmic granules, similar to those described for coelenterate eggs. Also van Herwerden has observed a migration of nuclear material into the cytoplasm of living oocytes of Echinoderms. Using these experiments to check the other observations, it seems probable that the cytoplasmic bodies described as chromatin do, in fact, represent this substance. There is considerable diversity of opinion as to the functions of these inclusions, and further work is necessary to determine this with certainty.

The chromosomes of most coelenterates do not lend themselves to a study of details of behavior to the degree possible in some animals. This is due, chiefly, to the lack of variety in form and size. It is not possible, therefore, to determine whether the chromosomes reappear in each generation in precisely the same form and size they had in earlier generations. During interkinesis the nucleus is a single vesicle with no subdivisions into smaller vesicles, and the chromatin is in the form of a continuous reticulum.

Since there is no evidence to disprove the view that chromosomes are genetically related, this may be accepted for the coelenterates. But all the evidence from this phylum is opposed to the view of a persistent morphological continuity and an individuality of the chromosomes retained during interkinesis.

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EXPLANATION OF PLATES

All figures of plates 1 and 3 were made by the aid of a camera lucida. These plates have been reduced in reproduction to three-quarters the original size; the magnifications given for each figure are the actual magnifications as reproduced. The figures of plate 2 are copied from the sources indicated.

PLATE 1

EXPLANATION OF FIGURES

1 to 4, Campanularia flexuosa, approximately × 1150; 5 to 8, Tubularia crocea, × 620.

1   Young planula with coelenteron present. Primitive ectoderm and entoderm present, but cells walls are to be detected in only a few places. Both ectoderm and entoderm are filled with yolk spheres.

2   Older planula with walls becoming more plainly marked. There are few interstitial cells in the ectoderm, but a number are present in the entoderm. The cell with the large nucleus may be like Wulfert's germ cell in Gonothyraea. but the nucleus is similar to that of other entoderm cells. Some of the interstitial cells are differentiating into gland cells, muscle cells, and the like. No germ cells are present.

3   Same planula as figure 2, showing only a portion of the entoderm. A group of typical interstitial cells is represented.

4   A still older planula with definitive ectoderm and entoderm. There are fewer entodermal interstitial cells than in earlier stages. The interstitial cells are undergoing differentiation, but no germ cells are present.

5   Section of egg about the end of cleavage. The germ layers have not been separated. The cell in division may be forming an ectodermal and an interstitial cell.

6   Embryo with a definite outer layer of cells and a solid central mass of cells. Two interstitial cells have been produced, one of which was formed by the division of a cell of the central mass.

7   A later embryo with cubical ectodermal cells, and groups of intersitial cells. One of the ectoderm cells is dividing to form an interstitial cell. None of these interstitial cells form germ cells at this time.

8   An embryo with coelenteron, about the period of the formation of tentacles and the production of an actinula. The ectoderm and entoderm cells are distinctly separated by a supporting lamella. Groups of interstitial cells are present and others are forming from the ectoderm. These interstitials form nematocysts and other structures, but not germ cells.
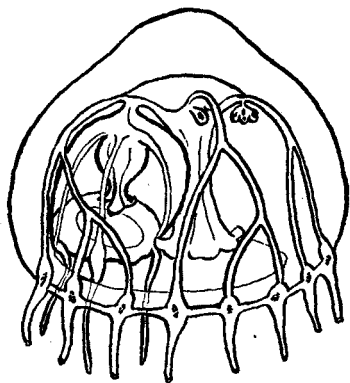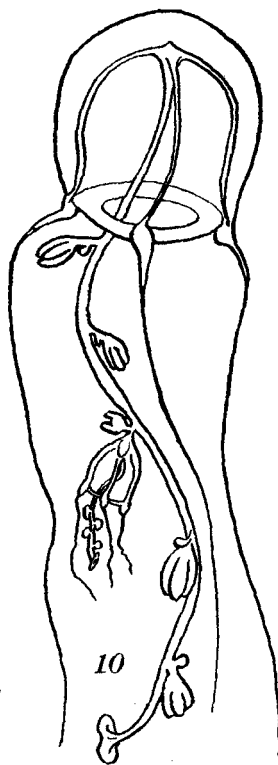
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PLATE 2

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PLATE 3

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