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CONTENTS

STUDIES ON CHROMOSOMES

III. THE SEXUAL DIFFERENCES OF THE CHROMOSOME-GROUPS IN HEMIPTERA, WITH SOME CONSIDERATIONS ON THE DETERMINATION AND INHERITANCE OF SEX

BY

EDMUND B. WILSON

WITH SIX FIGURES

Since the time of Henking's able paper on the spermatogenesis of Pyrrochoris ('91), it has been known that in certain Hemiptera, and in some other insects, a dimorphism exists in the nuclear constitution of the spermatozoa, one-half of them containing the so-called "accessory" or "heterotropic" chromosome, while in the other half this chromosome is lacking. The meaning of this fact has hitherto remained undetermined. McClung in 1902 developed an hypothesis of sex-production based on the conjecture that the heterotropic chromosome is a sex-determinant, and more specifically that spermatozoa containing this chromosome produce males, for the very obvious, yet fallacious, reason that it is present in the male. This hypothesis was based simply on the fact that the spermatozoa are of two numerically equal classes, like the sexes of the adults; and it was apparently overthrown by subsequent observation. The hypothesis implied that the cells of the female must contain one chromosome less than those of the male; and although McClung did not specifically place his assumption in this form, he considered it extremely improbable that the accessory chromosome, or "any such element," is present in the egg. Sutton ('02) believed that he had found a confirmation of this in the grasshopper Brachystola, where he showed that the number in the male (spermatogonia) is twenty-three, and stated that in the female (oögonia and follicle-

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cells) the number is twenty-two, supporting this statement by a single figure (*op. cit.*, Fig. 11). Sutton was, however, able to examine only a very few of the female groups, and the object is an unfavorable one as compared with the Hemiptera, owing to the less compact form of the chromosomes. McClung's hypothesis seemed to be rendered completely untenable by the later observations of Montgomery on Anasa ('04), and of Gross on Syromastes ('04), both these authors describing and clearly figuring the same number of chromosomes (twenty-two) in the male and the female cells. Gross and Wallace ('05) were thus independently led to the conclusion that only one of the two classes of spermatozoa was functional, namely, that in which the heterotropic chromosome is present. Those of the other class were assumed to degenerate after the fashion of polar bodies.

I am now able to bring forward decisive proof that the apparently adverse evidence brought forward by Montgomery and Gross was based on errors of observation, and that the sexes in Hemiptera of this type do in fact show a constant difference in the number of chromosomes. As far as these animals are concerned, however, McClung's conjecture as to the mode of fertilization proves to have been the reverse of the truth; for it is the female, not the male, that possesses the additional chromosome, as I have determined beyond all doubt in four genera, namely, Anasa, Alydus, Harmostes and Protenor. The facts leave no doubt that both forms of spermatozoa are functional; that all of the eggs possess the same number of chromosomes; that all contain the homologue, or maternal mate, of the accessory or heterotropic chromosome of the male; and that fertilization by spermatozoa that possess this chromosome produces females, while males are produced upon fertilization by spermatozoa that do not possess it.

A second type of dimorphism of the nuclei of the spermatozoa was made known in the first of these studies. In this type all of the spermatozoa contain the same number of chromosomes, but half of them contain a large "idiochromosome" and the other half a corresponding small one. I was led in that paper to suggest the possibility that the idiochromosomes might play a definite

rôle in sex-production, but could at that time produce no evidence in support of the suggestion. I have now the evidence to show that this suggestion was in accordance with the facts; for in at least four genera, Lygæus, Euschistus, Cœnus and Podisus, both sexes show the same number of chromosomes, but the small idiochromosome is present only in the male. Somewhat earlier, and independently, Stevens ('05) determined a precisely similar fact in the case of a beetle, Tenebrio, which indicates that the phenomenon is of wide occurrence in the insects. These results confirm the correctness of my conclusion that the heterotropic or "accessory" chromosome has become unpaired in the male sex through the disappearance in that sex of its mate, and give a complete explanation of the fact that in forms possessing the heterotropic chromosome the male number is odd and one less than the female number. I believe that these facts may give the basis for a general theory of sex-production.

I. DESCRIPTIVE

A. *General Character of the Chromosome-groups*

In two preceding papers (Wilson, '05, 1; 05, 3,) (where due acknowledgment is made to previous observers in this field) I have described in some detail the general nature of the chromosomes in these insects. For such an investigation as the present one, the Hemiptera present peculiar advantages, owing above all to the short and regular form of the chromosomes, and the relative lack of crowding in the equatorial plate. I have employed almost exclusively Flemming's strong fluid as a fixative, staining the sections with iron-hæmatoxylin and extracting until the cytoplasm is nearly or quite colorless. The best preparations thus obtained leave nothing to be desired in point of brilliancy and clearness, and show the chromosomes with a distinctness that is hardly exaggerated by the black and white figures here reproduced. The very large number of sections now at my disposal (including all those of Paulmier and a still greater number of new preparations of my own) has enabled me in the case of nearly every species to examine numerous division-figures (of which only the

best have been selected for illustration) and to satisfy myself thoroughly of the constancy of the relations as described. Everyone familiar with such objects will, however, realize that in regard to such matters as the arrangement and size-differences of the chromosomes certain apparent variations appear that are due to slight differences in the form and position of the chromosomes, and to the various degrees of foreshortening thus caused. This introduces a slight error, into both the observations and the drawings, that can hardly be avoided. A second source of error lies in the degree of extraction, which produces surprising variations in the apparent size of the chromosomes—I have found, for instance, that by successive extraction the chromosomes may be reduced almost to one-half their original apparent size, and the smaller chromosomes may thus be caused almost to disappear from view. Camera drawings at successive stages of the extraction show, however, that the relative sizes of the chromosomes remain substantially unchanged, and the comparison of the same object after a shorter and a longer extraction has thus, in a number of cases, given a more certain result than could otherwise have been obtained. I have, whenever it was possible, figured different stages of the same species from the same slide, so as to avoid the error due to different degrees of extraction; but this is not always possible, since as a rule longer extraction is required to give a perfectly clear view of the spermatogonial groups than is desirable for the spermatocyte-divisions. For the comparison of the two sexes, different slides must of course be used, and to this is due, I am sure, some of the size-differences between the oögonial and spermatogonial groups that appear in the figures.

Making all due allowance for the sources of error mentioned, it remains perfectly clear that the chromosomes in each species show among themselves constant and characteristic size-differences; and further, that with the special exceptions in the male described beyond, the chromosomes of the unreduced groups (*i. e.*, those of the oögonia and spermatogonia) may be paired off, two by two, to form equal or symmetrical pairs. The pairing of the chromosomes is most evident in the case of especially small chromosomes (such as the *m*-chromosomes of Anasa, Alydus,

Harmostes, etc., or the small pair of ordinary chromosomes of Cœnus and Euschistus, described beyond) or especially large ones such as the largest pair in Alydus, and in some of the species of Euschistus. Those of intermediate size are also obviously paired in some of the forms (*e. g.*, in Protenor, Fig. 1); but in many of the species the several pairs are not sufficiently marked in size to admit of certain recognition. Nevertheless, a comparative study of many species has convinced me of the correctness of the conclusion, first indicated by Montgomery ('01) and afterward more fully worked out by Sutton ('02), that all the chromosomes (again with the special exceptions referred to above) may be thus paired, and that the chromosome-group as a whole includes two parallel series of chromosomes that undoubtedly represent respectively the descendants of those that originally are brought together in the union of the gametes. This is very clearly brought out by making camera drawings of the chromosomes, and arranging them as nearly as practicable in pairs of equal size. This arrangement conspicuously shows the sexual differences, as may be seen by a comparison of Figs. 2, *f* and *h* (Anasa) and 5, *c* and *g* (Lygæus). There is, of course, a large error to be allowed for in the series as thus arranged, and no pretense to complete accuracy in the selection of the members of most of the pairs can be made. Nevertheless, when all due allowance for differences of form, foreshortening and the like is made, the fact that such a double series exists is unmistakable. When it is borne in mind that the spermatid-nuclei in each case contain a single series of chromosomes showing the same size-relations (*cf.* for instance, Figs. 1, *b*, *c*, *d;* 2, *a*, *d*, *e;* 3, *a*, *e*, *f;* 4, *b*, *f*, *d*, *h*), it becomes in a high degree probable that the corresponding pairs of the somatic groups consist each of a paternal and a maternal member, in accordance with Montgomery's original and fundamental assumption ('01). As may be seen by a comparison of the figures, the members of each pair when in their natural position, do not as a rule lie in juxtaposition but may occupy any relative position. Only at the period of synapsis do they actually couple, two by two, to form the bivalents whose members are subsequently separated by the reducing division.

In order to give a wider basis of comparison I have given new figures of the chromosome-groups of nearly all the species, even in the case of forms already figured in my preceding papers. Since the idiochromosomes or the heterotropic chromosome form the distinctive differential between the nuclei of the two sexes, I shall in the descriptive part of this paper call them the "differential chromosomes."

B.   First Type.   Forms Possessing an "Accessory" or Heterotropic Chromosome

As stated above, I have compared the males and females in respect to the chromosome-groups in four genera, selecting for this purpose the most available cells, which are the dividing oögonia and ovarian follicle-cells in the female, the spermatogonia and investing cells of the testis-cysts in the male. The general result is the same in all, but owing to the conspicuous size-difference of the chromosomes in Protenor, this form gives the most obvious and striking evidence.[1]

a.   Protenor belfragei

Montgomery ('01) first made known the general character of the chromosome-groups in this interesting species, showing that the spermatogonial groups show an odd number, thirteen, that the heterotropic chromosome (Montgomery's "chromosome x") is immediately recognizable by its enormous size—it is fully twice the size of the largest of the other chromosomes—and that it is unpaired (though he considered it a bivalent). My own observation confirms his description in every point, except that I have never seen this chromosome transversely constricted into two halves. The first glance at a good preparation of the spermatogonial metaphase, as seen in polar view, shows this huge chro-

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[1]There can be no doubt of the identification of the follicle-cells; but there is some uncertainty regarding the cells here called oögonia, since they are from the undifferentiated region of the ovary in which the distinction between oögonia and follicle-cells cannot be made out. It is therefore quite possible that some of the groups here described as oögonia may be from very young follicle-cells or nutritive cells; but this does not affect the main result.

mosomed a sa long worm-shaped body obviously without a mate, (Fig. 1, *d–f*). The remaining twelve chromosomes may be grouped in symmetrical pairs (indicated by numbers in Fig. 1,
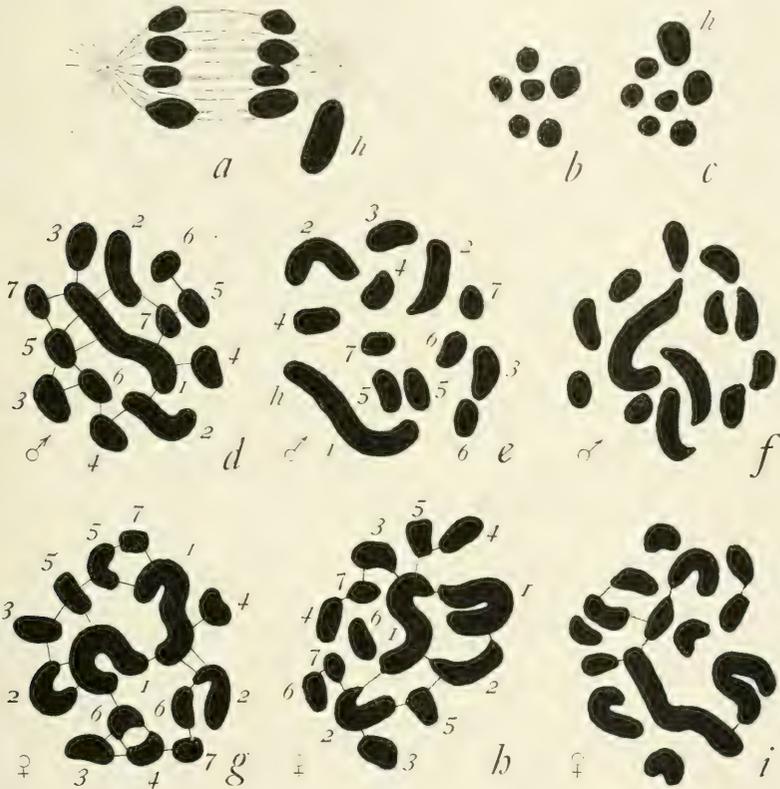
FIGURE 1[1]

Protenor belfragei.—*a*, Anaphase of second spermatocyte-division; *b, c*, sister groups, from the same spindle, polar view, second spermatocyte-division; *d, e, f*, spermatogonial groups; *g, h*, groups from immature ovaries, probably oögonia; *i*, group from dividing follicle-cell.

d, e), though the members of each pair may occupy any relative position. Of these six pairs, one (2, 2) is always much larger than the others, its members being approximately half the size of the

[1]All the figures are drawn to the same scale. In all, *h* denotes the heterotropic chromosome, *i* the idiochromosomes (large and small in some cases lettered *I* and *i* respectively), *m* the paired microchromosomes, and *s* the smallest pair of ordinary chromosomes.

heterotropic. A second pair (3, 3) may usually be distinguished as the next largest, and a third pair (7, 7) as the smallest, though this is not always obvious. This pair probably correspond to the "*m*-chromosomes" of my preceding paper. The remaining three pairs are of nearly equal size, though sometimes they clearly show a progressively graded series as in Fig. 1, *d, e*. In synapsis the six paired chromosomes become coupled, as usual, to form six corresponding bivalents, while the large chromosome remains as an unpaired univalent. During the whole growth-period of the spermatocytes this chromosome remains in a condensed spheroidal state, forming a very large chromosome-nucleolus. In the prophases of the first division it again elongates and divides longitudinally in this division. Each secondary spermatocyte accordingly receives seven chromosomes. In the second division six of these (the products of the bivalents) again divide equally, while the seventh (the large chromosome) passes undivided to one pole (Fig. 1, *a*). One-half of the spermatid nuclei accordingly receive six chromosomes, the other half seven, the additional one being the large heterotropic chromosome (Fig. 1, *b, c*).

In the female the chromosome-groups of the dividing oögonia and follicle-cells appear with a clearness not inferior to that shown in the spermatogonial groups (Fig. 1, *g–i*). It is at once apparent that in these groups there are two very large chromosomes, equal in size, in place of the single one that appears in the male, while the remaining chromosomes show the same relations as in the male. There are accordingly fourteen chromosomes in all, which may be equally paired off, two by two, and no chromosome is without a mate of corresponding size. Since the largest two are of the same relative size as the single heterotropic chromosome of the male, it is quite clear that one of them must have been derived from a spermatozoön containing this chromosome, while the other is its maternal mate or homologue.

I have not been able to follow by actual observation the phenomena of reduction, maturation and fertilization in the egg; but the data are sufficient to show, with a degree of probability only short of certainty, what must be the history of the chromo-

somes in these processes. Since the oögonia contain fourteen equally paired chromosomes, synapsis in the oöcyte must result in the formation of seven symmetrical bivalents—*i. e.*, seven couples of equal chromosomes—and each egg after maturation contains seven univalent chromosomes, one of which is the maternal representative or mate of the heterotropic chromosome of the male. This group contains one chromosome of each of the original pairs, and is precisely similar to the group present in those spermatozoa that contain the heterotropic chromosome (Fig. 1, *c*). Fertilization by such a spermatozoa doubles this group, giving the condition observed in the female—*i. e.*, fourteen chromosomes equally paired, the largest pair consisting of the heterotropic chromosome and its maternal mate (*1, 1*, Fig. 1, *g, h*). Fertilization by a spermatozoön that lacks the heterotropic chromosome will give the condition observed in the male, namely, thirteen chromosomes, of which twelve are equally paired, while the thirteenth is the large unpaired one which is obviously derived from the egg. There is therefore no escape from the conclusion that both forms of spermatozoa are functional, that females are produced upon fertilization by spermatozoa that contain, and males upon fertilization by spermatozoa that lack, the heterotropic chromosome. Since the two classes of spermatozoa are equal in number, fertilization will in the long run produce males and females in approximately equal numbers.

b. Anasa tristis

A comparison of the nuclei of the two sexes in this species gives a precisely concordant result, though the size-differences do not allow of so exact an identification of the differential chromosomes. In the preceding study I showed that the number of chromosomes in the male (spermatogonia) is twenty-one, not twenty-two as stated by previous observers. Study of the spermatogonial metaphase groups shows that twenty of the chromosomes may be equally paired, two by two, while the remaining one is, of course, without a mate (Fig. 2, *e, f*). The unpaired heterotropic chromosome is one of three largest chromosomes,

but which particular one cannot be determined by simple inspec-
tion, since the three are of nearly equal size. In synapsis two
of these large chromosomes unite to form the largest of the ten
bivalents (*1*, Fig. 2, *a*) that appear in the first spermatocyte
division. The third, which retains its compact form as a chro-
mosome-nucleus during the growth-period, remains as the univ-
alent heterotropic chromosome (*h*, Fig. 2, *a*). The first spermat-
ocyte division accordingly shows eleven chromosomes, ten of
which are bivalent, and one (heterotropic) is univalent. The
distribution of these chromosomes in the maturation-division takes
the usual course, the heterotropic chromosome dividing equally
with the ten bivalents in the first mitosis while its products pass
undivided to one pole of the spindle in the second (Fig. 2, *b*).
Half the spermatozoa accordingly receive ten chromosomes, one
of which (*1*, Fig. 2, *c*) is larger than the others, and half an exactly
similar group plus the large heterotropic chromosome, or eleven .
in all (Fig. 2, *d*).

The oögonial groups show invariably twenty-two chromosomes,
which may be arranged in eleven equal pairs (Fig. 2, *g*, *h*). In
place of the three large chromosomes of the spermatogonial
groups appear four similar chromosomes, forming two equal
pairs. Two of these four are obviously the large chromosome,
common to all the spermatozoa, and its maternal mate, while
the other two must be the heterotropic chromosome (derived in
fertilization from the spermatozoön) with its maternal mate.
It is, therefore, clear that all of the matured eggs must contain
eleven chromosomes, that females are produced upon fertilization
by those spermatozoa that contain a similar group—*i. e.*, by those
containing the heterotropic—males upon fertilization by spermat-
ozoa that lack the heterotropic.

The ovarian follicle-cells often show chromosome-groups
identical with those of the oögonia (Fig. 2, *j*). Not infrequently,
however, the number of chromosomes is much greater, and the
same is true of the nuclei of the investing cells of the ovary, of
the oviduct and of the fat-body. In the male similar multiple
groups are not uncommon in the interstitial and investing cells
of the testis. Only in a single case have I succeeded in gaining
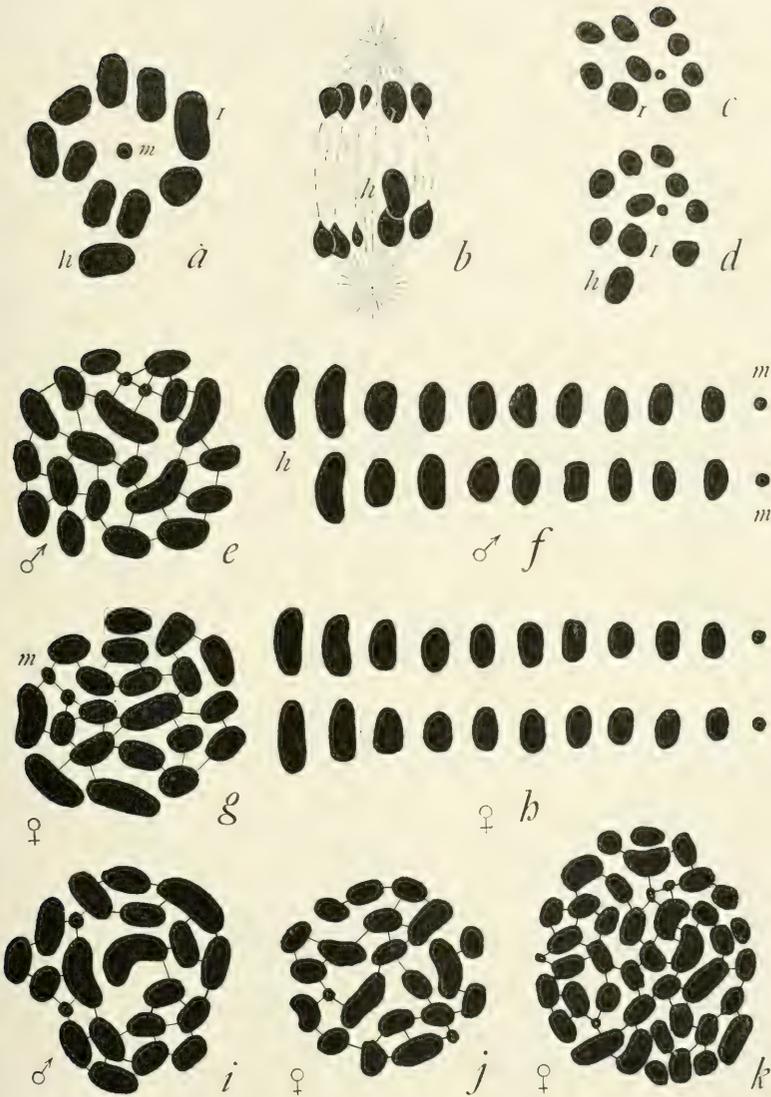
FIGURE 2

Anasa tristis.—*a*, Metaphase of first spermatocyte-division, in polar view, showing the nine large bivalents in a ring, the univalent heterotropic chromosome below it, and the *m*-chromosome bivalent in the center; *b*, anaphase of second division; *c*, *d*, sister-groups from the same spindle, polar view, second division (1 the macrochromosome); *e*, spermatogonial group; *f*, the same chromosomes arranged in pairs; *g*, oögonial group from a larva; *h*, the same group arranged in pairs; *i*, spermatogonial group; *j*, group from a dividing follicle-cell; *k*, double group, from a cell toward the periphery of a larval ovary.

a clear and complete view of such a group; but this one case suffices to give, with great probability, the explanation of the increased number of chromosomes. In this case every chromosome of the metaphase group may be clearly seen, and the number is exactly twice the oögonial number, namely, forty-four (Fig. 2, k). Careful study clearly shows that this group contains four microchromosomes and eight macrochromosomes, in each case twice the number of those present in the oögonia. This leaves no doubt that in this case all the chromosomes have divided once without the occurrence of a cytoplasmic division, and makes it probable that the increase in number in the cells in question is always due to a process of this kind. I have not been able to obtain faultless preparations of the dividing cells of other tissues, and can only state that in the ectodermal cells of the larva the number of chromosomes is approximately the same as in the oögonia. The multiple chromosome-groups were only observed in the cells mentioned above, all of which, it may be observed, are degenerating or highly specialized cells.

c.   Alydus pilosulus

Despite the small number of chromosomes (♀ 14, ♂ 13, as in Protenor) this genus is in some respects less favorable for detailed analysis than either of the ones described above, for the size of the heterotropic chromosome does not distinguish it sufficiently from the other chromosomes to allow of its certain identification in the spermatogonia. The main fact appears, however, as clearly as in Protenor or Anasa that the female has one more chromosome than the male.

In polar views of the second spermatocyte-division this species shows the sister spermatid-groups with great beauty, one having six chromosomes and one seven (Fig. 3, e, f). These chromosomes show at least five distinguishable sizes that are constant, namely, (1) a largest; (2) an extremely small one (m-chromosome); (3) a second smallest (the heterotropic); (4) a second largest, and (5) three others intermediate in size between (3) and (4), one of which is frequently a little larger than the other two.

The sister groups are practically exact duplicates save for the heterotropic which varies considerably in appearance as seen from the pole owing to foreshortening (*cf.* the side-views given in my preceding paper). The spermatogonia correspondingly show always thirteen chromosomes (Fig. 3, *a*), of which the largest and the smallest pair are at once distinguishable. Next follow four chromosomes nearly equal in size, two of them often appreciably smaller than the other two. Of the remaining five, one must be the unpaired heterotropic; but, as already stated, it cannot be positively identified by inspection. Closely similar groups may
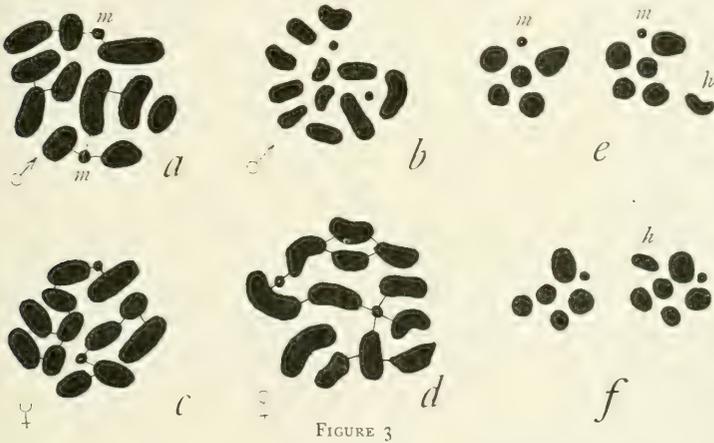
FIGURE 3

Alydus pilosulus.—*a*, Spermatogonial group; *b*, group from a dividing investing cell of the testis; *c*, oögonial group; *d*, from a dividing cell of an egg-follicle; *e, f*, two pairs of sister-groups, each from a single spindle, anaphase of second spermatocyte-division, in polar view.

occasionally be found in dividing cells of the enveloping cells of the testis (Fig. 3, *b*). Whether multiple groups occur like those described in Anasa, I cannot say.

The dividing oögonia and follicle-cells, of which a large number have been observed, always show fourteen chromosomes that may be arranged in seven equal pairs (Fig. 3, *c*, *d*). As in the spermatogonia, the largest and the smallest pair are usually at once recognizable, and also the four second largest. The remaining six, of nearly equal size, must of course include the heterotropic chromosome and its maternal mate.

d.   Harmostes reflexulus

My material of this species is much less abundant than that of the three preceding, and the preparations are not of the same excellence. They nevertheless show beyond doubt that the numbers are here the same as in Protenor and Alydus, viz., thirteen in the male and fourteen in the female. In my sections of both sexes the chromosomes appear less regular in contour than in the other species examined (probably owing to somewhat defective fixation). They show clearly, however, in both sexes a largest pair and a smallest (*m*-chromosomes), as in the other forms.

C.   Second Type.   Forms Possessing Unequal Idiochromosomes

The sexual differences of these forms have been worked out in Lygæus turcicus, five species of Euschistus (variolarius, ictericus, tristigmus, fissilis and servus), Cœnus delius and Podisus spinosus. In the last named species the unreduced number is sixteen, in the others fourteen. In all, the number of chromosomes is the same in both sexes, but while the males show a large and a small idiochromosome, the females show two large idiochromosomes that are equally paired. This difference clearly appears in all the species examined but is most conspicuous in Euschistus variolarius, E. ictericus and Lygæus turcicus, where the inequality of the idiochromosomes is most marked. The relative size of the idiochromosomes varies somewhat (perhaps owing to differences in the degree of extraction of the dye) but on the whole is characteristic of the different species, as described below.

In all of the species of Euschistus examined, and in Cœnus delius, a largest and a smallest pair of ordinary chromosomes (the latter marked *s* in some of the figures) are readily distinguishable. These give rise to corresponding large and small bivalents in the first mitosis, and are recognizable as single chromosomes in the spermatid-groups (Figs. 4, 5). The small chromosomes are in every case smaller than the large idiochromosome, and in Mineus bioculatus (Fig. 4, *p*, *q*) are actually smaller than the small idiochromosome. It is possible that this pair of chromosomes
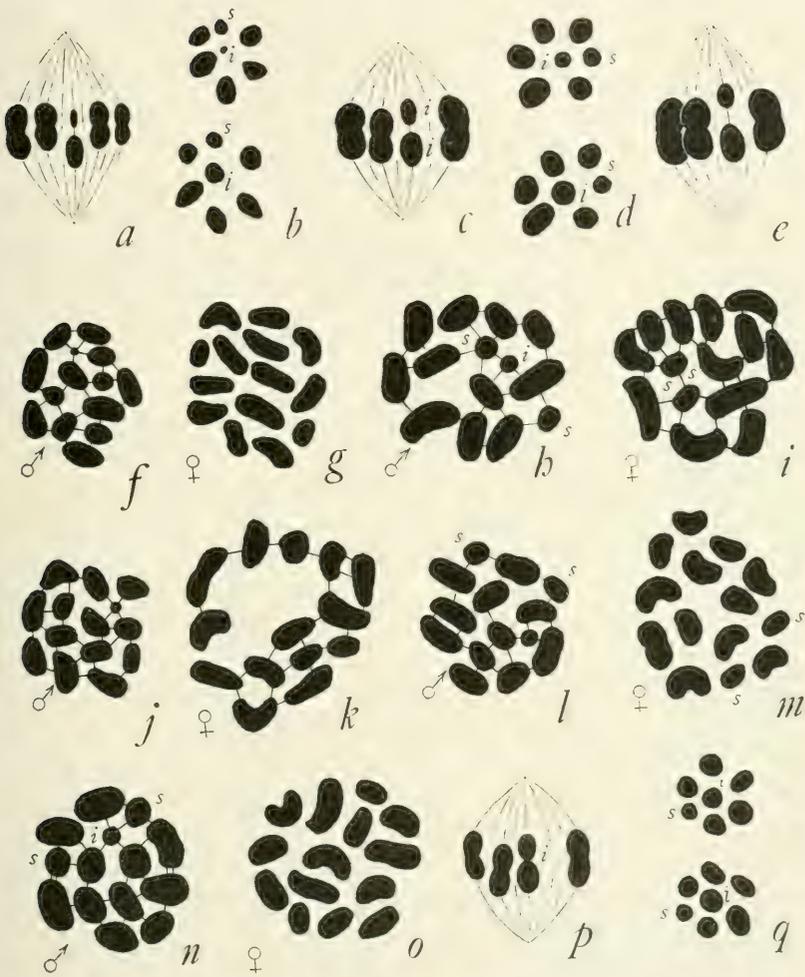
FIGURE 4

Euschistus, Mineus.—*a*, E. variolarius, second spermatocyte-division; *b*, sister-groups, second division; *c, d*, corresponding views of E. servus; *e*, second spermatocyte-division, E. tristigmus; *f, g*, E. variolarius, spermatogonial and oögonial groups respectively; *h, i*, corresponding views of E. servus; *j, k*, the same, E. ictericus; *l, m*, the same, E. tristigmus; *n, o*, the same, E. fissilis; *p*, Mineus bioculatus, second spermatocyte-division; *q*, sister-groups, from the same spindle, second division.

may correspond to the microchromosomes, or *m*-chromosomes, that are so characteristic of the first type (*m*, in Figs. 2, 3).

e. Euschistus

In E. variolarius the inequality of the idiochromosomes (Fig. 4, *a*) is greater than in any other of the observed forms excepting Lygæus turcicus. The sister spermatid-groups (Fig. 4, *b*) consist in each case of a ring of six ordinary chromosomes with the idiochromosome near its center. In the outer ring may be distinguished as a rule four or five different sizes of chromosomes, the largest and smallest (*s*) being always recognizable, and usually also a second largest and second smallest. The large idiochromosome is always distinctly larger than the smallest chromosome (*s*) of the outer ring, while the small idiochromosome is very much smaller than either, and in long extracted preparations looks exactly like a centrosome. The spermatogonial groups correspondingly show seven pairs of chromosomes (Fig. 4, *f*), of which the small idiochromosome, the smallest pair of ordinary chromosomes, and two large pairs are recognizable. The remaining seven include three equal pairs, while the seventh is the large idiochromosome, but it is impossible to identify this chromosome more nearly. The oögonial groups show fourteen equally paired chromosomes, as shown in Fig. 4, *g;* but my preparations do not show this so well in this species as in the others.

E. ictericus shows a similar spermatogonial group (Fig. 4, *j*) except that the small idiochromosome is relatively a little larger and the small pair of ordinary chromosomes but slightly smaller than the others. The oögonial groups (Fig. 4, *k*, an unusually open specimen) very clearly show the absence of the small idiochromosome, but the equal pairing of the chromosomes is less obvious than in the following species.

In E. tristigmus (Fig. 4, *e, l, m*) the small idiochromosome is relatively much larger than in the foregoing species, while in E. servus, it is usually a little larger still (Fig. 4, *c, d, h*). In both these forms the smallest pair of ordinary chromosomes are at once recognizable in the spermatogonia (*s*, Fig. 4, *h, l*) and the equal pairing of the others is evident. In E. servus the oögonial groups

show the equal pairing of all the chromosomes with equal clearness, the absence of the small idiochromosome being evident (Fig. 4, *i*). The small pair (*s*) evidently correspond to the small pair in the male (4, *b*) and the large idiochromosome-pair must therefore be represented by one of the larger pairs. Fig. 4, *n*, *o*, show the spermatogonial and oögonial groups of E. fissilis, showing the same relations as in E. servus, save that the small pair are relatively larger.

The above-described species of Euschistus, while agreeing precisely in the general relations, present individual differences so marked as to show that even the species of a single genus may be distinguishable by the chromosome-groups. In this case the most interesting feature is the series shown in the inequality of the idiochromosomes, which becomes progressively greater in the series (1) E. servus, (2) tristigmus, fissilis, (3) ictericus, (4) variolarius, the inequality in the last case being fully as great as in Lygæus. I may again mention the fact that in the opposite direction the genus Brochymena often shows the idiochromosomes less unequal than in E. servus; in Mineus they are sometimes of nearly equal size (Fig. 4, *p*, *q*), while in Nezara no inequality exists. Practically all intermediate conditions are therefore shown within the limits of a single family between the extreme inequality shown in E. variolarius and no inequality at all. It is quite clear from the observations here brought forward that this progressive differentiation has occurred only in the male sex, as I conjectured in my first paper.

f. Cœnus delius

The relations in this form are so closely similar to those seen in Euschistus servus or fissilis, as described above, as hardly to require separate description. Fig. 5, *b*, shows the spermatogonial metaphase-group; 5, *i*, the corresponding oögonial group. Both these preparations show very clearly the small pair (*s*) of ordinary chromosomes (not so well shown in the figure of the spermatogonial group in my first paper). Here, as in Euschistus, it is evident that the large idiochromosome is much larger than the members of the small pair.

g.   Lygæus turcicus

In this species the inequality of the idiochromosomes is nearly or quite as great as in Euschistus variolarius, but the differentiation of the chromosome-pairs is less marked than in that species, and the small pair cannot be distinguished with certainty in any of the stages.   In the spermatogonial groups, accordingly, only the small idiochromosome is markedly smaller than the others (Fig. 5, *c, d*); and hence its lack of an equal mate is rendered very conspicuous.   In the female the small idiochromosome is absent as usual and all the chromosomes are equally paired (Fig. 5, *f, g*). The idiochromosomes cannot be distinguished from the ordinary chromosomes.

h.   Podisus spinosus

In this species both sexes show sixteen chromosomes.   In the spermatogonial groups (of which I am now able to give a better figure than the one in my first paper) the small idiochromosome appears relatively larger than in any of the foregoing species, though still not more than half the size of any of the others (Fig. 5, *j*).   In the female (follicle-cells, Fig. 5, *k*) all the chromosomes are equally paired and the small idiochromosome is absent, but owing to the relatively large size of the latter in the male the chromosome-groups of the two sexes do not show so obvious a contrast as in the foregoing cases.

Resumé and Conclusions Regarding the Second Type

In all the forms described under this type the two sexes show the same number of chromosomes but differ in that the male groups include a large and a small idiochromosome while the female groups have two large idiochromosomes of equal size. This result agrees with that already reached by Stevens ('05) in the case of the beetle Tenebrio, and involves the same conclusions that she has indicated.   Since all the chromosomes of the oögonial groups are equally paired, it is evident that all the matured eggs must contain half such a group, one of the chromosomes being the maternal representative, or mate, of the large idiochromosome
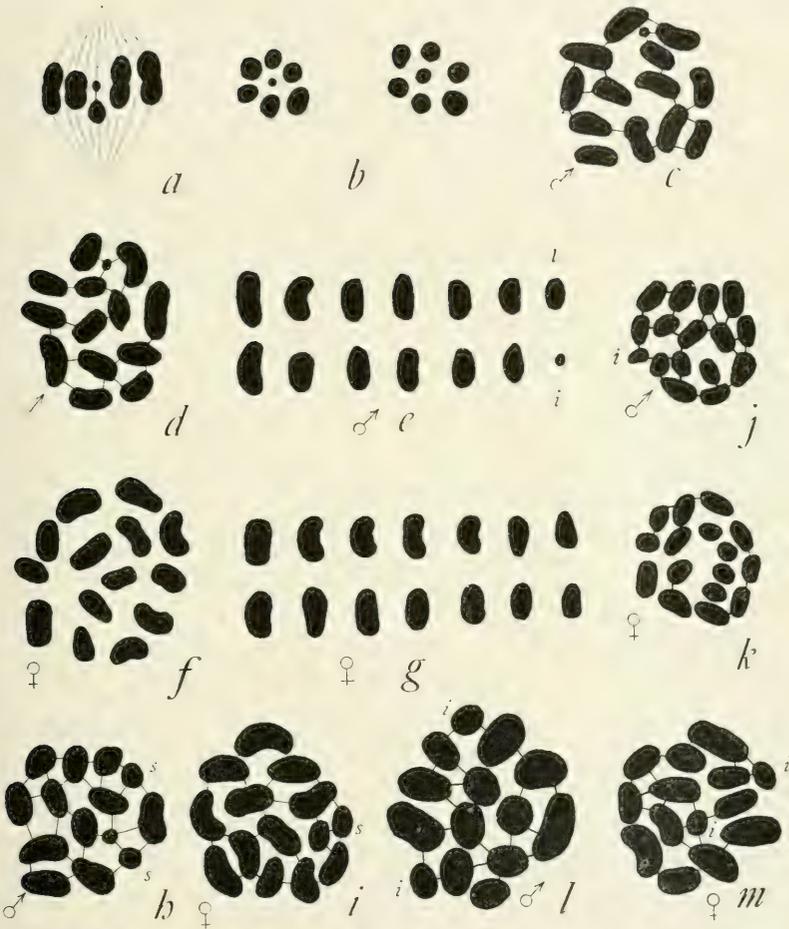
FIGURE 5

Lygæus, Cœnus, Podisus, Nezara.—*a*, Lygæus turcicus, second spermatocyte-division; *b*, sister-groups, second division; *c*, *d*, spermatogonial groups; *e*, the chromosomes of *d* arranged in pairs; *f*, oögonial group; *g*, the same in pairs; *h*, *i*, Cœnus delius, spermatogonial and follicle-cell groups; *l*, *m*, Nezara hilaris, spermatogonial and oögonial groups respectively.

of the male.   Fertilization of such an egg by a spermatozoön containing the small idiochromosome will produce a group identical with that occurring in the male; fertilization by one containing the large idiochromosome will produce the characteristic female group.   This result is thoroughly consistent with that obtained in the first type; for if the small idiochromosome be supposed to disappear in the male, the phenomena become in every respect identical with those occurring in the first type.   The large idiochromosome is therefore undoubtedly homologous with the heterotropic chromosome, and the latter owes its unpaired character to the fact that its former paternal mate has vanished, as I conjectured in my first paper.

It is further evident that in synapsis, in both sexes, the members of each chromosome-pair become coupled to form symmetrical bivalents, except in case of the idiochromosomes of the male. In this case alone do chromosomes of unequal size couple to form an asymmetrical bivalent; and it is a consequence of this coupling that the subsequent distribution allots the small idiochromosome to one-half of the spermatozoa and the large one to the other half.

D.   Third Type.   Forms in which the Idiochromosomes are of Equal Size

Of these forms I have been able to examine only a single case, namely, that of Nezara hilaris; and in the course of a whole summer's collecting I obtained but a single female in the proper stage to show the oögonial divisions.   Fortunately both ovaries show a considerable number of division-figures which demonstrate the facts with perfect clearness.

A particular interest attaches to this form on account of the fact, described in my first paper, that the idiochromosomes are of equal size and hence give no visible differential between the two classes of spermatozoa.   This form gives therefore a test case concerning my general conclusion that the differentiation of the idiochromosomes has occurred only in the male; for since these chromosomes are here alike in all the spermatozoa, it might with some plausibility be assumed that the differentiation had in this

species taken place in the female. The facts conclusively show that such is not the case.

The spermatogonial groups (Fig. 5, *l*) show fourteen chromosomes, all of which may be symmetrically paired. The smallest pair, *i, i,* (as I showed in my first paper) are the idiochromosomes as is shown by their characteristic behavior during the growth-period and in the maturation-divisions. In synapsis the twelve larger chromosomes couple to form six bivalents, while the idiochromosomes divide as separate univalents in the first spermatocyte-division. Their products then conjugate as usual to form the idiochromosome-dyad, which differs from all the forms hitherto observed in being composed of two equal members. All the spermatid-nuclei are accordingly exactly similar in appearance and no visible dimorphism exists (*cf.* Fig. 4 of my first paper, Wilson, '05, 1). We should accordingly expect to find the oögonial groups exactly similar to the spermatogonial; and such is clearly shown to be the fact by the preparations, the oögonial groups showing fourteen equally paired chromosomes among which the idiochromosomes are readily recognizable by their small size (Fig. 5, *m*).

In this case, therefore, alone among all those examined, no visible differences are shown by the nuclei of the two sexes. One pair of the chromosomes are, however, different in nature from the others, as is shown by their different behavior in the male in the growth-period and in synapsis; and it is quite clear that the two members of this pair are always assigned to different spermatozoa. In respect to this chromosome, therefore, the spermatozoa fall into two classes as truly as the other forms, though they cannot be distinguished by the eye. It is hardly necessary to point out how important this case is in giving a firm basis of comparison with the more usual forms in which, if we can trust the existing accounts, all of the functional spermatozoa are exactly alike in appearance, and no sexual differences of the chromosome-groups are apparent.

*E.   The Differential Chromosomes in the Synaptic and Growth-
periods*

I will now briefly consider a very marked difference between
the sexes in respect to the behavior of the differential chromosomes
during the contraction-phase of synapsis and the succeeding early
growth-period.[1]   In the male, as was fully described in my last
paper, both the heterotropic chromosome and the idiochromo-
somes condense early in the growth-period (usually as early as the
contraction-phase of synapsis) to form rounded, condensed,
intensely-staining chromosome-nuclei.   In this condition they
persist throughout the whole growth-period of the spermatocyte,
without ever assuming the looser texture and more elongate form
of the other chromosomes.   In the earlier part of this period they
are as a rule closely associated with a large pale plasmosome, but
later become separated from it.

In the female no trace of such a chromosome-nucleus can be
found in the contraction-figure of the synaptic period.   My best
preparations of this stage are from the ovaries of the larval Anasa,
which show a distinct synaptic zone of oöcytes intervening between
the zone of multiplication and the growth-zone; but I have
observed the same condition in the ovaries of recently emerged
adults of Harmostes, Alydus, Euschistus, Cœnus and Podisus.
In all these forms the contraction-figure is very similar to that of
the spermatocytes, the chromosomes being in the form of deeply
staining, ragged, and apparently longitudinally split loops that
are crowded into a spheroidal mass toward the center or one side
of the nucleus and surrounded by a large clear space.   The nuclei
at this time occasionally show one or two small deeply-staining
nucleolus-like bodies (probably plasmosomes); but these are
much smaller than the chromosome-nuclei of the spermatocytes
at this period, and in many of the nuclei are absent.   The contrast
between these nuclei and those of the male at the corresponding
period is so striking as to be at once apparent.   In later stages the
chromosomes spread through the nuclear cavity, become looser
in texture and finally give rise to a fine reticular structure.   In

[1]A fuller presentation of observations on these phenomena is reserved for a subsequent paper.

these stages a variable number of deeply-staining nucleoli make their appearance; but their true nature can only be determined positively when the whole ovarian life of the egg has been followed and the process of maturation observed. I can, therefore, only state that no chromosome-nucleolus is present in the contraction period of synapsis, or in the early growth-period; and even though it be present in later stages, which I think is very doubtful, a wide difference between the sexes would still exist in respect to the earlier period.

F. General Resumé

The foregoing results may be given a general formulation as follows: If n be the unreduced number of chromosomes in the female, the matured eggs in all cases contain half this number ($\frac{n}{2}$). The males are of three types. In the first, one of the chromosomes (the heterotropic or "accessory") is without a mate, and the unreduced number is accordingly one less than that of the female. Half the spermatozoa possess, and half lack, the heterotropic chromosome, the first class having the same number as the matured eggs ($\frac{n}{2}$), the second class one less ($\frac{n}{2}-1$). In the second type the male has the same number of chromosomes as the female, but possesses one large and one small idiochromosome while the female possesses two large ones. In maturation half the spermatozoa receive the small and half the large idiochromosome. The third type differs from the second in that the idiochromosomes are of equal size in both sexes, and no visible differences exist between the two classes of spermatozoa or the somatic groups of the two sexes. Designating the large and small idiochromosomes as I and i respectively, the relations in fertilization and sex-production are as follows:

TYPE I

(Protenor, Anasa, Alydus, Harmostes)

Egg $\frac{n}{2}$ + spermatozoön $\frac{n}{2}$ (including heterotropic) $= n$ (female).

Egg $\frac{n}{2}$ + spermatozoön $\frac{n}{2} - 1$ (heterotropic lacking) $= n - 1$ (male).

TYPE II

(Lygaeus, Euschistus, Coenus, Podisus)

Egg $\frac{n}{2}$ (including I) + spermatozoön $\frac{n}{2}$ (including I) $= n$ (including II) (female).

Egg $\frac{n}{2}$ (including I) + spermatozoön $\frac{n}{2}$ (including i) $= n$ (including Ii) (male).

<div align="center">

TYPE III

(Nezara)

</div>

Egg $\frac{n}{2}$ + spermatozoön $\frac{n}{2} = n$ (male or female, including in each case two equal idiochromosomes).

These relations are graphically shown in the following diagram (Fig. 6) in which the differential chromosomes are black and the ordinary ones unshaded (only two pairs of the latter shown). For the sake of simplicity only the final result of synapsis (second column) and the ensuing process of reduction (third column) are shown, without regard to variations of detail. The matured eggs (*ov*) are represented with a single polar body (the result of the reduction-division) which is greatly exaggerated in size. The female-producing and male-producing spermatozoa (*sp*) are lettered *a* and *b* respectively. It will be evident from an inspection of this diagram that the second type may readily be derived from the third, and the first from the second by the reduction (second type) and final disappearance (first type) of one of the differential chromosomes. This I believe to represent the actual relations of the three types.

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II. GENERAL.

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In recent years evidence has steadily accumulated to strengthen the view that the general basis of sex-production is given by a predetermination existing at least as early as the fertilized egg, but there is a wide divergence of opinion in regard to the conditions preëxisting in the gametes prior to their union.[1]

The fact that in some organisms (such as Dinophilus, Hydatina or Phylloxera) the unfertilized eggs, sometimes even in the ovary, are visibly distinguishable as male-producing and female-producing forms, has led a number of recent writers to deny that the spermatozoön can play any part in sex-determination. Beard, for example, asserts that "The male gamete, the spermatozoön, has and can have absolutely no influence in determining the sex

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[1] The general question of sex-determination, with its literature, has within the past five years been so ably and thoroughly reviewed by Cuénot, Strasburger, Beard, von Lenhossék, O. Schultze and others, that I shall here limit myself in the main to an analysis of the new observations brought forward.
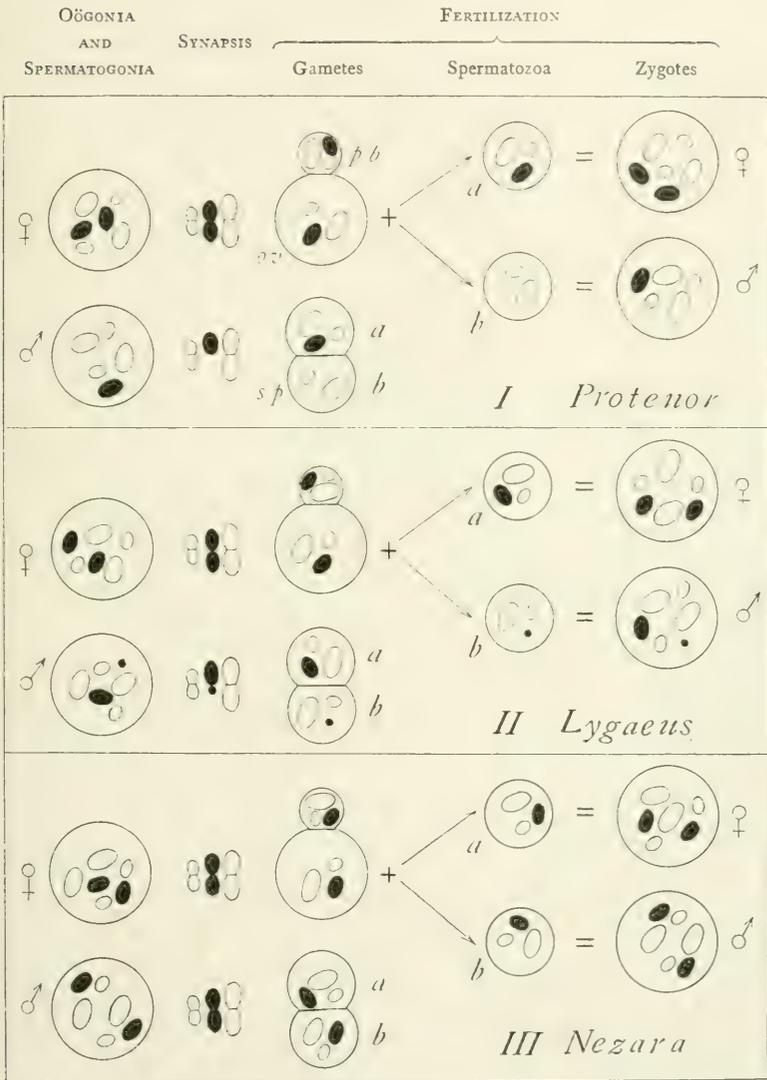
Fig. 6.

of the offspring" ('02, p. 712); and a similar conclusion, though less dogmatically stated, is reached in the general reviews of Lenhossék ('03) and O. Schultze ('03). The opposite view that the spermatozoön alone is concerned in sex-determination (which like the preceding one, is of very ancient origin) has, however, been maintained by some recent writers, for instance, Block (whose work I know only from Cuénot's review) and McClung, as already mentioned.[1] On the other hand, both Cuénot ('99) and Strasburger ('00) in their able reviews, have argued that both gametes may be concerned in sex-determination; and the last named author urged the view, afterward recognized as probable by Bateson and developed in detail by Castle ('03), that sex-production takes place in accordance with the Mendelian principles of inheritance.

The observations here brought forward, together with those of Stevens on Tenebrio, establish the predestination (in a descriptive sense) of two classes of spermatozoa, equal in number, as male-producing and female-producing forms. Though indistinguishable to the eye in their mature state, these two classes differ visibly in nuclear constitution at the time of their formation; and since this occurs in the same order of insects as Phylloxera, where the eggs are visibly distinguishable (by their size) as male-producing and female-producing forms, it is evident that a substantial basis now exists for the views expressed by Cuénot and Strasburger, and for the Mendelian interpretation of sex-production worked out by Castle. Whether in the Hemiptera that form the subject of this paper the eggs are, like the spermatozoa, predestined as male-producing and female-producing forms can at present be a matter of inference only. I have not been able to distinguish such classes by their size, and the data show, almost with certainty, that if they exist they do not exhibit any visible nuclear differences like those present in the spermatozoa. But this gives no ground for denying their existence. No visible nuclear dimorphism of

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[1] "By exclusion then, it would seem that the determination of this difference (the sexual one) is reposed in the male element" (McClung, '02, p. 78). McClung nevertheless maintained the existence of a selective power on the part of the egg such that "the condition of the ovum determines which sort of spermatozoön shall be allowed entrance into the egg substance" (*op. cit.*, p. 76).

the spermatozoa exists in Nezara, yet this condition is con-
nected, by an almost continuous series of intermediate forms,
with one in which a conspicuous difference of nuclear con-
stitution is to be seen. It seems hardly open to doubt that
sex-production conforms to the same essential type throughout
this series. At least a possibility is thus established that in
organisms generally both eggs and spermatozoa may be pre-
destined as male-producing and female-producing forms, whether
they are visibly different or not. In any case, it is evident
that in the Hemiptera the chromosome-combination characteristic
of each sex is established by union of the gametes and is a result
of fertilization by one or the other of the two forms of spermatozoa.
Sex must therefore already be predetermined in the fertilized egg,
and it is difficult to conceive how it could subsequently be altered
in these animals by conditions external to the egg or embryo.
Since the idiochromosomes or heterotropic chromosomes form the
distinctive differential between the nuclei of the two sexes, it is
obvious that these chromosomes are definitely coördinated with
the sexual characters. We must therefore critically inquire into
the causal relation between sex-production and the chromosomes,
of which this coördination is an expression.

That sex-production *may* be interpreted as the result of a
Mendelian segregation, transmission and dominance of the sexual
characters has been shown by Castle ('03). The history of the
differential chromosomes in synapsis and reduction evidently
affords a concrete basis for such an interpretation in the terms of
the Sutton-Boveri chromosome-theory. Analysis of the facts now
known will, however, show even more clearly than the more
general considerations adduced by Castle, that this interpretation
is only admissible under the assumption that a selective fertiliza-
tion occurs, such that eggs containing the female-determinant are
fertilized only by spermatozoa containing the male-determinant
and *vice versâ*. Until I had read Cuénot's recent interesting
paper ('05) on the breeds of mice and their combinations, the
necessity for making this assumption seemed to me an almost
fatal difficulty in the way of the interpretation, but if Cuénot's
conclusions be well founded the *à priori* objections to such a

selective fertilization are in large measure set aside. I therefore think that the possibility of a Mendelian interpretation of sex-production should be carefully examined, though as will be shown, an alternative interpretation is possible.

I. In such an examination the distinction between sex-determination and sex-inheritance should be clearly drawn;[1] for it is well known that each sex may contain factors capable of producing the characters of the opposite sex, and it may well be that the patency or latency of the sexual characters is determined by factors quite distinct from those concerned with their transmission from parent to offspring. For the purpose of analysis it will, however, be convenient to speak of the idiochromosomes or their homologues as "sex-determinants," this term being understood to mean that these chromosomes are the bearers of the male and female qualities (or the factors essential to the production of these qualities) respectively. They may also be designated (whenever it is desirable to avoid circumlocution) as sex-chromosomes or "gonochromosomes." As a basis of discussion the Mendelian interpretation may be taken to postulate, further, that the two sex-chromosomes, which couple in synapsis and are subsequently disjoined by the reducing division, are respectively male-determinants and female-determinants in the sense just indicated. The most convenient approach to the question is offered by the heterotropic chromosome, since its unpaired condition in one sex renders its mode of transmission more clearly obvious than that of the idiochromosomes. The facts (especially as observed in Protenor) clearly prove that this chromosome alternates between the sexes in successive generations, passing from the male to the female in the production of females, and from the female to the male in the production of males (Fig. 6). The important bearing of this on both sex-inheritance and sex-determination will appear beyond.

Since the heterotropic chromosome is without a fellow in the male it must, if it be a sex-determinant at all, be the male-determinant, which exerts its effect uninfluenced by association with a female-determinant. But since the spermatozoa that contain

[1] *Cf.* Watase, '92.

this chromosome produce only females, it must be assumed that the maternal mate or fellow, with which it becomes associated on entering the egg, is a dominant female-determinant. Further, since males result from fertilization by spermatozoa that do not contain the heterotropic chromosome, the latter must in male-producing eggs be derived from the egg-nucleus (*cf.* the diagram, Fig. 6). The general interpretation, therefore, must include the assumption that there are two kinds of eggs (presumably in approximately equal numbers) that contain respectively the male- and the female-determinant,[1] and that the former are fertilized only by spermatozoa that lack the heterotropic chromosome (*i. e.*, the male determinant) and *vice versâ*,[2] giving the combinations (*m*)*f* (female) and *m*—(male). Such a selective fertilization is therefore a *sine qua non* of the assumption that the heterotropic chromosome is a specific sex-determinant.

A nearly similar, though somewhat more complex, result follows in the case of the idiochromosomes. In respect to sex-production the large idiochromosome is identical with the heterotropic chromosome, and the morphological evidence is nearly or quite decisive that the heterotropic chromosome is actually a large idiochromosome, the smaller mate of which has disappeared. The small idiochromosome may therefore be regarded as a disappearing, or even vestigial, female-determinant that is recessive to its larger fellow (the male-determinant); and its reduction in size may plausibly be regarded as an atrophy resulting from its invariably recessive nature (this chromosome being strictly confined to the male). Precisely as in case of the heterotropic chromosome, the large idiochromosome of the male (male-determinant) must be derived in fertilization from the egg-nucleus (Fig. 6); and, as before, it must be assumed that eggs that contain this chromosome are fertilized only by spermatozoa that contain the small idiochromosome, those that contain the female-determi-

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[1] This would follow from the coupling of the two sex-chromosomes in synapsis to form the bivalent (*m*)*f*, and its division in such a way as to leave in the egg either the male- or the female-determinant indifferently.

[2] Otherwise the combinations *mm* or *f*— might result, which is contrary to observation, since the sex-chromosomes are in this type never paired in the male or unpaired in the female.

nant only by spermatozoa containing the large idiochromosome. In this type, accordingly, it is clear that the large idiochromosome (like the heterotropic chromosome to which it corresponds) passes alternately from one sex to the other, while the small one never enters the female; and this would remain true even did selective fertilization not occur (Fig. 6). The same interpretation may finally be extended to Nezara, where the idiochromosomes are of equal size in both sexes, the relations of dominance being the same as before.

The two vital points in this result are first, the assumption of selective fertilization, and second the relations of dominance and recession in the two sexes. As regards the first point, until the appearance of Cuénot's paper, referred to above, almost no definite evidence had been produced of an infertility between particular classes of gametes in the same species; though it has long been known that many plants are in a greater or less degree infertile to their own pollen, and an analogous fact has been more recently demonstrated in Ciona by Castle ('96) and Morgan ('04). Correns ('02), in his study of hybrid maize, was led to suggest that in this case there might be a somewhat diminished fertility between the gametes bearing the recessive character (thus accounting for a relative deficiency of extracted recessives in the second generation of crosses, F_2). In studying the breeds of mice Cuénot has found it impossible to obtain pure or homozygous yellow forms. Yellow mice are invariably heterozygotes (the yellow being dominant over gray, black or brown) and when crossed with a pure race of a different color (*e. g.*, gray) give the typical Mendelian result, yellow and gray offspring appearing in equal numbers. This proves that a complete Mendelian disjunction of the yellow and gray determinants takes place in maturation. When yellow mice of known constitution (*e. g.*, Y(G)) are paired with like forms, the first offspring include pure gray forms (extracted recessives) slightly in excess of the normal ratio of 25 per cent., and yellow forms; but contrary to the Mendelian expectation the latter, when paired with one another, never give pure dominants (YY), but again produce pure grays (GG) and heterozygous yellows (Y(G)). Cuénot therefore concludes that although complete segregation of both the gray and yellow

characters takes place in the gamete-formation, and the resulting yellow-bearing gametes unite freely with those bearing the recessive color, they do not unite with each other: "Ceux-ci (the yellow heterozygotes) forment bien des gamètes de valeur CJ ou AJ, mais ces gamètes ne peuvent pas s'unir les uns aux autres pour donner des zygotes ayant les formules CJCJ, AJAJ ou CJAJ; par autre, ils s'unissent facilement a tous les autres gamètes que j'ai essayés pour former avec eux des hétérozygotes mono- ou dihybrides" (*op. cit.*, p. cxxx). This conclusion is sustained by the fact that the combination Y(G) x Y(G) (CYCG x CYCG in Cuénot's terminology) produces a relative deficiency of yellows in the offspring, as is to be expected.[1] In pairing Y(G) with Y(G), accordingly, the Y-bearing spermatozoa unite only with the G-bearing eggs, and *vice versâ*, which is exactly analogous to the selective fertilization assumed in case of the sex-bearing gametes. Perhaps it may be possible to find a different explanation of the facts; but if Cuénot's interpretation be well-founded the case goes far to remove the scepticism which I think one must otherwise feel in regard to a selective fertilization of the gametes in sex-production.

An examination of the question of dominance involved in the Mendelian interpretation leads to some interesting conclusions. In forms possessing unequal idiochromosomes the sexual formulas would be for the female (m) f and for the male m (f) (f being the small idiochromosome). Applying the same interpretation to Nezara, where the idiochromosomes are of equal size, the corresponding formulas are (m) f and m (f), giving the gametes (m), f, m and (f). Assuming likewise a selective fertilization the facts would be:

Eggs		Spermatozoa		
(m)	$+$	(f)	$=$ (m) (f), producing a male, $m(f)$.
f	$+$	m	$=$ mf, producing a female (m) f.

[1] The deficiency, though constant, is very slight. Cuénot himself seems to consider this a difficulty, but I believe a very simple explanation may be given. With equal numbers of the gametes of both sexes the ratio of yellows to grays should be two to one, instead of three to one as in the typical Mendelian case (since the class YY is missing). If, however, the spermatozoa be in large excess, as they undoubtedly are, all or nearly all the Y-bearing eggs will be fertilized by G-bearing spermatozoa, and *vice versâ*, thus bringing the ratio of yellows (Y(G)) to grays (GG) more or less nearly up to three to one.

Now it is clear that if the relations of the chromosomes to sex-production be the same here as in the second type, the chromosome m must alternate in successive generations between the male and the female (like the large idiochromosome or the heterotropic chromosome to which it corresponds), and hence also shows an alternation of dominance, being dominant in the former sex and recessive in the latter. If, therefore, dominance and recession be inherent in the chromosomes, there must be such a relation between them that m is always dominant to the chromosome (f) of the male, and always recessive to the chromosome f of the female, and that the latter two chromosomes (f and (f)) are never interchanged between the sexes. This last assumption is not so improbable as it may at first sight appear; for in the second type it is certain, as already pointed out, that the small idiochromosome $((f)$ under the general assumption) never enters the female, while the large idiochromosome, m, like the heterotropic, alternates between the two sexes in successive generations.

A strict Mendelian interpretation of sex-production may unquestionably, I think, be constructed upon the foregoing assumptions. But an interesting suggestion for a somewhat modified Mendelian interpretation is given by the possibility that the dominance of the sex-chromosomes is determined by extrinsic factors, namely, by conditions in the protoplasm of the zygote. If this were the case it is evident that the idiochromosomes could not be considered as sex-*determinants* in the strict sense of the word. The determination of sex would in this case be due to factors preëxisting in one or both of the gametes, irrespective of the sex-chromosomes, and the latter could only be considered as a means by which the sex-characters are transmitted or inherited. The possibility is here clearly offered that either or both forms of gametes may be predetermined as males or females (or at least male-producing and female-producing) prior to fertilization and irrespective of the chromosomes; and thus an interpretation of the ordinary forms of gametes would be reached in harmony with such cases as Dinophilus and other forms in which male-producing and female-producing eggs are distinguishable in size prior to fertilization. Such an interpretation would further be perfectly consistent with

the modification of sex-production in some cases by external conditions, and with the production of both males and females in parthenogenesis (though this may be otherwise explicable); and it might also give the explanation of selective fertilization.

II. It has not been my intention to advocate the foregoing interpretation, but only to set forth as clearly as possible, the assumptions that it involves. It is nevertheless my opinion that the analysis places no insuperable obstacles in its way, and that, however dominance be determined, the Mendelian interpretation may in fact give the true solution of the problem. I have, however, endeavored to seek for a different interpretation that may escape the necessity for assuming a selective fertilization; and although I have to offer nothing more than suggestions, some of which undoubtedly encounter serious difficulties, I shall make them in the hope that they may afford some clue to further inquiry. Some of these suggestions are equally applicable to the Mendelian interpretation considered above, but for the purpose of discussion this interpretation may for the time be laid aside.

It seems possible that the differential chromosomes may perform a definite and special function in sex-production without being in themselves specifically male-determining and female-determining or even qualitatively different save in the degree of their special activity (whatever be its nature). This suggestion is given by the fact that the presence of one heterotropic chromosome or large idiochromosome is associated with the production of a male, while if two such chromosomes are present a female is produced. This very obviously suggests that the same kind of activity that produces a male will if reinforced or intensified produce a female; and with this would accord the production of males from unfertilized eggs, and females from fertilized ones, in the case of the bee. In these cases the decisive factor may be a merely quantitative difference of chromatin between the two sexes. But it is obvious that such a difference cannot give the basis for a general explanation, since in Nezara, and presumably in many other organisms, both the number of chromosomes and the quantity of chromatin is the same in both sexes. And yet

the existence of a quantitative difference in some cases raises the question whether it is not the result or expression of some more deeply lying nuclear difference which may still be present in those cases where no quantitative difference exists. I find it altogether incredible that two animals as nearly related as Nezara and Euschistus should differ fundamentally in the relation of the chromosomes to sex-production; and if there is any reason to conclude that sex-determination is effected by the idiochromosomes (or by the combination of which they form a part) in the case where they are visibly different, I cannot avoid the belief that this conclusion applies with equal reason to the case in which they appear to the eye alike in all the spermatozoa. It therefore seems to me an admissible hypothesis that a physiological or functional factor may be present that differentiates the spermatozoa into male-producing and female-producing forms irrespective of the size of the differential chromosomes; and further, that the morphological difference that has arisen in some forms may have been a consequence of such an antecedent functional difference. If we could assume for instance that the differential chromosome-pair in the male includes a more active and a less active member (the latter having in many cases become reduced in size or even having entirely disappeared) the suggestion might be greatly extended in application. Under this assumption the facts might receive a general formulation in the statement that the association of two more active chromosomes of this class produces a female, while the association of a more active and a less active one (or the absence of the latter, as in case of the heterotropic chromosome) produces a male. Reduction of the less active member to form a small idiochromosome would introduce a quantitative difference of chromatin as well as a qualitative one. Its complete disappearance in the male, leaving only the active member as the heterotropic chromosome, would reduce the difference to a merely quantitative one. The assumption of such a physiological difference is admittedly a purely speculative construction, and may seem *à priori* very improbable. But from the *à priori* point of view it would seem equally improbable that a morphological dimorphism of the spermatozoa, affecting

only one pair of the chromosomes, should have arisen; yet this is an observed fact. I therefore think the suggestion is worthy of serious consideration. If it could be adopted the necessity of selective fertilization would be avoided, for the observed results would follow from the fertilization of any egg by any spermatozoön.

But even if in accordance with fact the suggestion is still obviously incapable of direct application to cases in which sex is determined independently of fertilization—for instance, sex-production in parthenogenetic development or in hermaphrodites, and in forms (such as Dinophilus) where male-producing and female-producing eggs are distinguishable in size before fertilization. It is possible that these cases may be explicable (under either general interpretation) as a result of some forms of differential distribution of the chromosomes occurring at the time of the formation of the polar bodies (parthenogenesis) or at some earlier period in the cell-lineage of the germ-cells; and this possibility should of course be tested by a close cytological study of the facts. On the other hand, there is nothing in the facts to negative the assumption that in some cases the chromosome-combination, established at fertilization, may be in something like a balanced state that is capable of modification by conditions external to the nucleus (as already suggested in the case of dominance).

Boveri's interesting observations on the dispermic eggs of Ascaris ('04) have given direct evidence that the chromosomes react to their cytoplasmic surroundings; and the same fact is even more clearly shown by the difference of behavior of the differential chromosomes in the two sexes of Hemiptera during the synaptic and growth-periods. Hence, even though a preëstablished basis of sex-determination be given in such a physiological dimorphism of the spermatozoa as I have suggested, the sex of the fertilized eggs may in many cases be only a matter of greater or less predisposition and not an immutable predetermination. The nuclei, and hence the primordial germ-cells, may in such cases be in a state of approximate equilibrium, and still retain the power of response to varying conditions in the cellular environment. The production of eggs or spermatozoa in hermaphrodites may thus be explicable as a result of greater or less nuclear

activity in the two cases, incited by intra-cellular conditions that are external to the chromosome-groups; and a similar explanation may apply to the related case of the formation of visibly different female-producing and male-producing eggs in the same organism.

It would not, I think, be profitable to speculate further in regard to these special cases, but I have wished to indicate that a hypothesis of sex-production which recognizes in some cases a fixed predetermination in the chromosome-groups of the fertilized egg is not inconsistent with the control of sex-production in other cases by conditions external to the nucleus. The constant chromosomal differences of the sexes existing in many Hemiptera, therefore, by no means preclude experiments on the modification or control of sex-production.

I have intentionally excluded from the foregoing suggestions any discussion of the specific nature of the activities of the differential chromosomes, since we are almost wholly ignorant of the functions of chromosomes in general. But although we here enter upon still more debatable ground, I think we should not hesitate to consider such possibilities in this direction as the facts may suggest.

One of the principal, or at least most obvious, differences between the germ-cells of the two sexes is their great contrast in constructive activity, evinced by the enormous growth of the primary oöcyte as compared with the primary spermatocyte. This growth of the oöcyte involves the production of a mass of protoplasm (including under this term the yolk or metaplasm as well as the active protoplasm) thousands of times the bulk of the spermatocyte; and although the latter also increases noticeably in size during the growth-period, the accumulation of protoplasm is almost insignificant as compared with that which takes place in the female. Now, as described above, the idiochromosomes and heterotropic chromosome remain during this period in the male in a relatively passive condition as compared with the other chromosomes, while this is not the case in the female. The thought cannot be avoided that there is a definite causal connection between the greater activity of these chromosomes in the

oöcytes and the great preponderance of constructive activity in these cells; and it is especially this coincidence that leads me to the general surmise that one of the important physiological differences (I do not say the only one), between the chromosome-groups of the two sexes, may be one of constructive activity. I have elsewhere (The Cell, Chapter VII) reviewed at some length the evidence pointing toward the conclusion that the nucleus (more specifically, the chromatin) is especially concerned with the constructive processes of cell metabolism; and while I no longer hold the view that the nucleus can be considered as the actual formative center of the cell, it still seems to me very probable that the formative processes are directly or indirectly under its control, as has been advocated by many students of cell-physiology. If this view be well-founded, the facts observed in Hemiptera give a very definite and concrete basis for assuming a greater constructive activity in the cells of the female generally, which reaches a climax in the growth-period of the oöcyte.[1] It seems possible that some of the specific differentiations that take place in the later history of the germ-cells may be directly trace-able to the primary difference in the growth-process. It is well known that the young oöcytes and spermatocytes show a very close similarity, not only in size but also in many details of struc-ture. The enormous accumulation of cytoplasm in the oöcyte as compared with the spermatocyte leaves the latter with a great relative excess of the kinoplasmic or archoplasmic material in which the most characteristic differentiations of the spermatozoa—such as the acrosome, middle-piece, axial filament and tail-envelopes—take their origin. Perhaps a direct causal relation here exists.

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[1] This suggestion recalls the theory developed by Geddes and Thomson, in their well known work on the "Evolution of Sex," that "the female is the outcome and expression of relatively preponderant anabo-lism, and the male of relatively preponderant katabolism" (*op. cit.*, revised ed., 1901, p. 140). As de-deloped by these authors, this theory has always seemed to me to have too vague and general a character to have much practical value, though it expresses a certain physiological contrast between the sexes that undoubtedly exists. My suggestion is only remotely connected with that theory, since it refers the differ-entiation of the sexes to a functional difference that preëxists in the cells of the male, and involves no contrasted processes of anabolism and katabolism. Nevertheless, the observations here brought forward may harmonize with that side of the theory which lays stress on the preponderant constructive activity of the female cells.

III. Though I have found it convenient to consider the two foregoing interpretations separately, they evidently have many points of agreement, and perhaps may be reduced to a common basis. Both assign to the differential chromosomes a specific function in sex-production, both recognize the possibility of a determination of sex (as opposed to its transmission), by conditions external to the chromosome-groups, and both assume, in one sex, a specific difference in the sex-chromosomes, followed by a Mendelian disjunction in the formation of the gametes. The essential point in which the second interpretation diverges from the first is that the sex-chromosomes are not conceived as bearing the male or female qualities respectively but as differing only in the degree of their activity, and this difference is assumed to exist in the male only (owing to the relation of fertilization to sex-production). It must be admitted that each interpretation involves a considerable element of pure conjecture, and that each includes assumptions which without additional data must be considered as serious difficulties. The principal one involved in the first interpretation is the assumption of selective fertilization; but if this assumption be granted I believe that it may give an adequate solution of the problem of sex-production in the sexual reproduction of diœcious organisms. The second interpretation avoids this difficulty; it may explain the primary difference between the gametes of the two sexes, the latency of female characters in the male, and the development of such secondary female characters as may be regarded as an exaggeration or intensification of corresponding characters in the male. It seems conspicuously to fail to explain the reverse case of characters that are more highly developed in the male; and to many this will doubtless appear a fatal difficulty. But we are still ignorant of the action and reaction of the chromosomes on the cytoplasm and on one another, and have but a vague speculative notion of the relations that determine patency and latency in development. Additional data will therefore be required, I think, to show whether the difficulty in question is a fatal one, and in what measure either of the two general interpretations that have been considered may approach the truth. The positive result of the

observations of Stevens and myself is to demonstrate the existence of a constant and definite correlation between the chromosomes and the sexual characters, which is visibly expressed in the relations of a single pair of chromosomes. These relations unquestionably afford a concrete basis for an interpretation of sex-production that assumes a Mendelian segregation and transmission of the sex-characters and to this extent they accord with the general assumption of Castle. The validity of both this and the alternative interpretation suggested must be tested by further inquiry.

Zoölogical Laboratory of Columbia University,
December 8, 1905.

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AN EXAMINATION OF THE EFFECTS OF MECHANICAL SHOCKS AND VIBRATIONS UPON THE RATE OF DEVELOPMENT OF FERTILIZED EGGS

BY

DAVID D. WHITNEY

It has been shown in recent years by several investigators that mechanical shocks and vibrations may start the development of *unfertilized* eggs of certain animals.[1] It has also been stated by Meltzer[2] that the early development of *fertilized* eggs of the sea urchin is greatly accelerated when the eggs are subjected to mechanical shocks and vibrations. Furthermore, Mathews and Whitcher[3] believe they have obtained results which show that such influences may cause the embryos of the sea urchin to be either larger or smaller than those of the control eggs, or abnormal in shape, or to develop more slowly than normal eggs.

Assuming these results to be well founded, I undertook, during the summer of 1905, some experiments to determine the influence of shaking at different stages of development. In order to exclude the obvious possibility, that the results of Meltzer, and perhaps those of Mathews and Whitcher, were due to differences in temperature, care was taken to make this factor the same in the shaken eggs and in the control. In some cases this was done by means of a water jacket around both sets of eggs. It soon became apparent that no difference at all *in the rate* occurred when this precaution was carried out; so that the work resolved itself into testing the claims of these investigators respecting the influence of shock on the early stages of development.

At the suggestion and under the kindly supervision of Prof.

[1]Mathews: Amer. Jour. Physiol., 1901, vi, p. 142. Fisher: Amer. Jour. Physiol., 1902, vii, p. 301.

[2]Meltzer: Amer. Jour. Physiol., 1903, ix, p. 245.

[3]Mathews and Whitcher: Amer. Jour. Physiol., 1903, viii, p. 301.

T. H. Morgan, I have studied the influence of mechanical agitation and vibration upon the fertilized eggs of Arbacia, Asterias, Fundulus heteroclitus and Ctenolabrus.

Of the fertilized eggs of Arbacia twenty-five lots from six individuals were shaken from one to forty-five minutes. Some were shaken by hand and others by means of a small hot-air engine. The eggs were placed in a test-tube half full of sea water, which in some cases contained small pebbles about the size of an ordinary pin head; in other cases water alone was present. The tube was then shaken back and forth one hundred to three hundred times per minute.

Six other lots from two other individuals were placed in a thick walled test-tube which swung vertically, and, by the use of the small engine, the bottom of the tube was made to strike quite hard against the side of a board from one hundred and fifty to four hundred times a minute.

In all cases the temperature of the control was the same as that of the shaken eggs. When the engine was employed for shaking, the test-tube was kept under running sea water, but when shaken by hand it was wrapped up in wet cloths. The eggs of the controls were also kept under similar conditions. When taken from the test-tube, the eggs were all kept at room temperature, 23°–26° C. Thus the temperature of the shaken and non-shaken eggs was always exactly the same.

After fertilization, the eggs were usually allowed to stand three to five minutes before using. In some cases, however, they were shaken immediately after fertilization. In other cases they were allowed to stand from five to twenty-five minutes.

In all these thirty-one sets of shaken eggs from eight individuals no acceleration of cleavage was noted in the earlier stages, nor were the plutei ever larger than those of the controls. In many cases the majority of the embryos of the shaken eggs were small and abnormal in shape, and were swimming upon the bottom of the dish, thus corroborating some of Mathews' and Whitcher's observations. This was especially true if the eggs were shaken in the test-tube with the small pebbles. Usually a few large plutei were found in both the controls and the shaken lots; but in no one

of these lots did there seem to be more large plutei than in the other.

The following experiments will illustrate the general character of the results obtained from Arbacia:

Experiment I. July 11.—Temperature of sea water 23° C.; of room 25° C. All lots of eggs, after fertilization, were kept at 23° C. for forty-five minutes, and were then placed in finger bowls of water at room temperature.

Lot A. Control. Great care taken in handling the eggs so as to prevent any jarring or shaking.

Lot B. Three minutes after fertilization the eggs were placed in a test-tube the bottom of which struck for forty-five minutes against the side of a wooden box two hundred and seventy-five to three hundred and fifty times a minute.

Lot C. Ten minutes after fertilization. Shaken five minutes back and forth two hundred and fifty times a minute in a test-tube half full of water plus some small pebbles.

Lot D. Immediately after fertilization. Shaken five minutes in same manner as lot C.

The early stages and the plutei of lots B, C and D were like those of the control.

Experiment II. July 11.—Conditions and results the same as in I.

Experiment III. July 12.—Temperature the same as in *I* and *II*.

Lot A. Control.

Lot B. Shaken thirty minutes back and forth two hundred and forty times per minute, in a test-tube half full of water. The tube was in a jacket of running water.

Early cleavages and the plutei of B were the same as A.

Experiment VI. July 13.—Temperature of sea water 23° C.; of room 26° C.

Lot A. Control.

Lot B. Shaken five minutes back and forth two hundred times a minute in test-tube half full of water plus some small pebbles.

Lot C. Shaken one minute back and forth three hundred times per minute in same manner as B.

Early segmentation of all three lots the same.

July 16.—Lot A. Normal plutei swimming through water in the dish.

Lot B. Normal plutei swimming upon the bottom of the dish.

Lot C. Normal plutei. A few swimming through the water, and the remainder swimming upon the bottom of the dish.

Experiment VIII. July 13.—Lot A. Control.

Lot B. Shaken three minutes by hand back and forth three hundred times per minute in a small vial half full of water plus small pebbles.

Lot C. Shaken five minutes in same manner as B.

B and C were one-half hour later than A in showing the first cleavage.

July 17.—Lot A. Normal plutei swimming through water in a dish.

Lot B. Very few normal plutei. Mostly gastrulæ, and many of them abnormal. Both plutei and gastrulæ were swimming upon the bottom of the dish.

Lot C. Normal and abnormal plutei swimming upon bottom of dish.

Experiment XII. July 17.—The sea urchins were kept in an ice chest at a temperature of 9° C. from five to six hours. Then the ovaries were quickly removed to water at 9° C. and fertilized by the sperm that had been shed by the males while in the ice chest.

Lot A. Control at 9° C. for thirty minutes.

Lot B. Shaken thirty minutes back and forth two hundred and ten times per minute, in a test-tube half full of water. The tube was in an ice water bath at 3° C.

Lot C. Shaken one minute back and forth three hundred times, at a temperature of 11° C. They were allowed to remain at this temperature for thirty minutes. At the expiration of this time, the three lots were placed in room temperature of 25° to 26° C.

The early segmentation and normal plutei of the three lots appeared at the same time respectively.

Some eggs were placed at room temperature—25° to 26° C.—

and others from the same lot were put under running sea water at a temperature of 23° C. The eggs at room temperature developed in the early segmentation one stage in advance of those which were under running water.

In all the experiments great care was taken in handling the control eggs, both before and after fertilization, so as to prevent jarring which might influence the rate of development of the control eggs in such a way that it would be identical with the eggs intentionally shaken.

Meltzer shook the eggs of Arbacia by hand and also by means of the piston of a stationary engine. He also placed them in dishes on the vibrating part of the same engine. He does not state whether the eggs of the control were kept at the same temperature as those that were being shaken, but only states that the temperature of the room was slightly higher than the outside temperature. I have made careful records of the temperature of the places in the engine house where he performed his experiments. If the conditions then were the same as they are now, all his results can be explained by the different degrees of temperature to which the eggs were subjected.

The eggs that he shook by hand in small vials containing small glass beads developed faster. The temperature in such a case would be increased by the friction of the beads and water, and by the warmth of the hand, in a remarkably short space of time.

I found that the temperature in the test-tube which struck against a board, when not kept under running water, was raised two to four degrees if the experiment was continued for fifteen to twenty minutes.

Meltzer may have avoided all these difficulties and have kept all the eggs of a series at the same temperature, but he does not state this; and from what he does say, he seems to have paid little attention to temperature and consequently concluded that the acceleration of development which he obtained was due to vibration and shaking. I find on the contrary, that by keeping all the eggs of a series at the same temperature no acceleration of development can be obtained by subjecting them to a slight or even to a great amount of mechanical agitation or vibration.

Fertilized eggs of Asterias subjected to mechanical shocks gave only negative results. About thirty-five lots from twelve individuals were used. The apparatus employed for producing the shocks was a thick glass test-tube, the bottom of which struck against two boards from two hundred to three hundred and fifty times per minute—the same that was used in connection with the eggs of Arbacia.

The eggs were kept in this tube, which was constantly in motion, from fifteen minutes to seven hours. Some were subjected to shock immediately after fertilization, and others remained undisturbed from five to thirty minutes before being placed in the tube. The temperature was kept uniform in all lots of eggs of the same experiment.

When many of the eggs had reached the eight-cell stage, about two hundred of them were selected at random, and the percentage of the eggs in the various segmentation stages was determined by counting. The shaken eggs usually varied from 1 to 3 per cent. either above or below the percentage of the same stages in the non-shaken lots. As the percentage varied so slightly, sometimes above and sometimes below that of the control, I concluded that the segmentation was neither hastened nor retarded by mechanical shocks.

The eggs of Fundulus heteroclitus were also placed in the test-tube of this apparatus and subjected to slight and to severe shocks, from a few minutes to ten hours. About forty lots—from twenty to twenty-five individuals—were used. As the early development of these eggs was found to take place normally in a damp chamber, some of them were placed in a test-tube, the air of which was kept moist by a piece of wet filter paper, and subjected to shocks. Similar results were obtained from both methods.

A few lots were placed in a test-tube half filled with water and the tube was made to move back and forth in a horizontal position from two hundred to three hundred and fifty times per minute.

In none of these experiments did the early cleavage stages of the shaken eggs appear earlier than in those of the controls.

As the eggs of Ctenolabrus float upon the surface of the water,

the test-tube was inverted, and its upper end made to strike against boards, as in the former experiments. In some of the experiments the tube struck against the boards gently, and in others it struck severely enough to kill many of the eggs. Intermediate shocks were also tried.

About thirty lots—from fifteen to twenty individuals—were used and were subjected to shocks from five minutes to several hours. In all these experiments the early cleavages appeared at the same time both in the controls and in the shaken eggs.

From the foregoing observations it appears that mechanical shocks and vibrations are not effective in accelerating the early segmentation of the fertilized eggs of Arbacia, Asterias, Fundulus and Ctenolabrus.

These observations were made at the Marine Biological Laboratory at Wood's Hole, while occupying one of the tables of the Carnegie Institution.

MORPHOLOGY OF THE PARTHENOGENETIC DEVELOPMENT OF AMPHITRITE [1]

BY

JOHN W. SCOTT

WITH FOUR PLATES AND FIVE FIGURES IN THE TEXT

INTRODUCTION

1. General Statement as to Object of Work and Results Obtained

The subject for the following investigation was suggested to me by Dr. F. R. Lillie early in the summer of 1902. Some recent statements by Loeb and others, in regard to the effects of certain salt solutions upon the development of the eggs of some marine Annelids, had led Lillie in the previous year to make a series of observations upon the egg of Chætopterus. Lillie's chief purpose in making such a series of experiments was, "To test what was the significance of cleavage in the egg, and what was the rôle of cell division in development." He arrived at the following general conclusion: "The process of cell division, as such, is necessary neither to growth, differentiation, nor to the earliest correlations; but it is accessory, in Metazoa, to all three as a localizing factor, often from the earliest stages." Lillie made the suggestion that it would be well to test the questions raised in his paper in some other marine Annelids.

My experiments upon the eggs of Amphitrite, however, have involved conditions not met in the egg of Chætopterus and necessarily other questions have come up for solution. In starting out I had in view, among others, the following considerations: 1. Using the methods adopted by previous investigators to produce artificial parthenogenesis, was it possible to produce differen-

[1] A dissertation submitted to the faculty of the Ogden Graduate School of Science in candidacy for the degree of Doctor of Philosophy, Department of Zoölogy, The University of Chicago, June, 1904.

tiation without cleavage in the unfertilized eggs of Amphitrite?
2. May such differentiation be produced in the fertilized eggs?
3. Can normal trochophores or normal adult worms be raised
from unfertilized eggs? 4. If so, how are abnormalities appear-
ing in the early stages regulated to produce a normal embryo?
In other words, my object was to learn if possible how this sort
of parthenogenetic development was related to the normal.

From observations on living eggs and from material preserved
during the summer of 1902, I succeeded in demonstrating:
1. That differentiation without cytoplasmic cleavage may occur
in the unfertilized eggs of Amphitrite. (Scarcity of material
prevented testing this question for fertilized eggs.) Cleavage,
when present, is usually abnormal and is always so in the later
stages. 2. Strictly speaking, this kind of development cannot
be termed parthenogenesis, for the differentiation so resulting
never leads either to normal or to abnormal self-sustaining
organisms. However, I shall use in this paper the term partheno-
genesis in a restricted sense to indicate the development that is
initiated in the eggs of Amphitrite by treatment with salt solutions
and by agitation. 3. There is no correlation or regulation of
organs in the later development under the conditions of the experi-
ment. 4. No specific solution was necessary to produce partheno-
genesis as was claimed by Fischer ('02), though some solutions
apparently do not have any effect upon the egg. 5. The egg was
found to be extremely susceptible to agitation or shaking, espe-
cially at certain periods after it had been removed from the body
cavity. 6. It was learned from a study of the preserved eggs
that the parthenogenetic development was closely connected with
the method and extent of the nuclear division and the chromatin
distribution and that much depended upon the ripeness of the egg.

Considering these results, I found it necessary during the fol-
lowing summer to make an examination of the very early
development, both normal and parthenogenetic. In the normal
egg this included a study of the origin and number of chromo-
somes, the maturation, and fertilization, none of which had been
described for Amphitrite. Corresponding stages in the parthen-
ogenetic eggs were also preserved and studied. In general, it

has been found that the early development of the fertilized Amphitrite egg conforms to that of the typical Annelid, while in the later stages, so far as they were studied, I have confirmed the observations of Mead ('97). With the unfertilized eggs, on the other hand, the form of development varies considerably under different conditions. The polar bodies may or may not be expelled, and when expelled they may be normal or abnormal. Cleavage likewise may be present or absent, and is always abnormal in late stages. I have found also that the principal conditions which produce variations in the form of development are the state of ripeness of the egg and the kind and strength of the solution used.

2. *Methods*

For a normal series the usual method of adding sperm to the dish of eggs was employed. The care and precautions necessary in manipulating and handling the unfertilized eggs have been described in a previous paper. Boveri's picroacetic (dilute) and Kleinenberg's picrosulphuric were used as killing fluids, the former giving the better results. The eggs were preserved in 80 per cent alcohol until used.

From each lot of eggs whole mounts and sections were prepared. For whole mounts Conklin's hematoxylin method gave fairly satisfactory results. This solution was prepared by adding 20 parts distilled water to 5 parts Delafield's hematoxylin; to each 5 cc. of this mixture was added one drop of Kleinenberg's (undiluted) picrosulphuric acid. The best results were obtained when eggs were stained for twenty minutes and afterward washed for about one hour in frequent changes of 50 per cent alcohol. Several stains were used for sections, but the best results were obtained by staining three to five minutes in strong Delafield's hematoxylin followed by from thirty to fifty seconds in Orange G. The iron-alum method proved unsatisfactory on account of the great amount of yolk present. For the same reason it was found necessary to cut the sections 7 to 10 micra in thickness.

NORMAL HISTORY OF THE EGG

1. Periods of Maturation and First Cleavage

I have tried to determine just when and where the germinal vesicle breaks down under normal conditions; whether this occurs after the egg is deposited in sea-water or while the egg is still floating in the body cavity. The latter view is correct as shown by the following facts: Eggs from the ripest female that I obtained in two seasons' work were deposited between 6.15 and 6.50 p. m., while I was away from the laboratory. At 6.54 p. m. some of these eggs were fertilized and a part of the lot was preserved. All of those preserved were in the metaphase, with a few exceptions that showed more advanced development. This could not have happened if the vesicle breaks down only after the egg is in the sea-water, for the eggs are deposited a few at a time at each rhythmic peristalsis of the worm's body. In other experiments where the eggs were cut out of the body of the female and left unfertilized, I have noted that the germinal vesicle may break down within a few minutes, but frequently does not until several hours later. In any case a peri-vitelline space forms and the egg flattens or undergoes collapse at the animal pole, the spindle rests in metaphase and sperm are very rarely found within the cytoplasm until after this phase. In fertilized eggs the first polar body makes its appearance about ten minutes later. This is followed after an interval of about eight minutes by the second polar body. I have seen the second polar globule thrown off within twenty-four minutes after the eggs were deposited and the first cleavage occurs eighteen to twenty minutes later. The periods here given are minimal; they may be much longer and are affected by ripeness of the egg, fertilization, agitation, certain chemical agents, and probably other causes.

2. Orientation of the Egg. Origin and Number of the Chromosomes

The rather large germinal vesicle in the ripe egg of Amphitrite has a slightly eccentric position, near the animal pole. The yolk

next the vegetative pole is somewhat denser than it is in the region above the nucleus. This eccentric position of the germinal vesicle is present in very immature eggs just after they become free in the body cavity, when the egg consists simply of a germinal vesicle and a surrounding thick layer of cytoplasm in which no trace of yolk can be found. The nucleolus has no definite position within the nucleus.

As the egg increases in size by the growth of the nucleus and the deposition of yolk, the denser portions of the latter substance are found in the immediate neighborhood of the nucleus; the yolk found nearer the surface of the egg is composed of small granules and there is not so much deposited in the upper side of the egg as at other places. At this stage dark masses or patches that present a fiber-like appearance are frequently found in the cytoplasm; judging from the staining reaction these are probably portions of the undifferentiated reticulum. The germinal vesicle now shows a definite reticulum with small micromeres at the intersections. When the egg is near the mature condition chromatin granules, or chromomeres, are found collected in various groups. The reticulum of the nucleus has nearly disappeared. In eggs which seemed to be ripe and in which the germinal vesicle was on the point of breaking down, chromatin groups were found present in the nucleus and the chromatin granules were apparently fusing to form the chromosomes.

Peculiar difficulties have interfered with getting the stages immediately succeeding those just mentioned, stages that show the exact origin of the chromosomes and asters. The next stage found in my preserved material, where the eggs had been deposited as a result of the rough handling of the female, is represented in Fig. 1. One distinct aster is present which seems to have arisen within the nuclear area, and a few radiating fibers point to the origin of the other aster somewhat nearer the animal pole. The germinal vesicle has apparently just broken down. A part of the contracting, fading nucleolus is still visible, and the chromosomes or probably chromomeres are in groups or strings. In the egg of Fig. 2 both asters are well formed, the chromosomes are found in scattered groups, and the nucleolus is still intact.

The process next observed is the collecting of chromatin to form the prophase of the first maturation division. While this is in progress the asters enlarge, the centrospheres make a rapid growth, and a symmetrical spindle develops and rotates to take its definitive position. The chromomeres or chromosomes have become quite numerous and there are strong indications that they are arranged in eleven groups of four each. In Fig. 3 the clear area shows the original position of the nucleolus which has disappeared; some of the chromosomes are fusing. In Fig. 4 the spindle is better formed, the chromomeres are collecting near the equator, and some show appearances of fusing.

In describing the maturation and fertilization of another marine annelid, Arenicola, Child figures and describes a "nuclear cavity." I have found the same appearance in the eggs of Amphitrite but my sections have enabled me to prove that the clear area, or "cavity," represents the original position of the nucleolus. The latter in disappearing gradually shrinks (Fig. 1), grows irregular in shape, and apparently goes into solution leaving a clear area (Fig. 3).

In early metaphase it is usually possible to count eleven chromosomes in the spindle (Fig. 5). A polar view of the chromosomes in the early prophase shows that the chromomeres have not all fused. The reduced number of chromosomes I have found to be eleven; this count was made at a stage shortly after the expulsion of the second polar globule (Fig. 9). At a later stage in one of the cleavage cells twenty-two were counted as the entire number.

3. *Nuclear Changes in Maturation*

The nuclear phenomena of maturation in Amphitrite are in general like those of Chætopterus as described by Mead. The first aster makes its appearance after the germinal vesicle breaks down and is first seen in a position that represents a lateral portion of the upper hemisphere of the nuclear area. The second aster appears a little later, near the surface of the egg, and lies directly under the animal pole (Fig. 1). Both asters are now growing rapidly, though the second aster always remains behind

the first in size. The spindle forms and the chromosomes arrange themselves in prophase; but before this process is complete the spindle swings around, and when the metaphase is reached it has migrated to the surface where it rests in its definitive radial position (Fig. 5). At this time the spindle is long and tapering. If not disturbed in any way, practically all eggs will remain indefinitely in this condition unless fertilized. But if slight agitation or certain chemical stimuli be applied further development may occur. When fertilized other changes make their appearance within five or six minutes. The chromosomes quickly pass through the anaphase, and at the time of early telophase the asters as well as the spindle fibers begin to fade while the spindle itself has become shorter and barrel-shaped (Fig. 6). In later telophase when the first polar body is being constricted off, the chromosomes of the inner group have approached still nearer the surface and remnants of the spindle and inner aster have almost entirely disappeared (Fig. 7). There are stages just later than this when no traces of this aster can be discovered. Very soon the second maturation spindle arises nearly parallel to the surface, and the chromosomes which have receded a little arrange themselves in prophase. Frequently at this time the pole above this region shows a flattening or slight concavity and as the spindle turns the chromosomes pass into metaphase. The rest of the process in throwing off the second polar globule is similar to that described for the first. Differences, however, have been observed. The spindle is smaller than the first and the rays of the aster remaining in the egg, instead of fading out in telophase, extend further into the cytoplasm, persisting until a later stage (Figs. 8 and 9).

4. *Relation of Cytoplasmic to Nuclear Phenomena in Maturation*

The ripe egg with germinal vesicle intact gives no indication of the animal pole if judged simply by its shape. But when the germinal vesicle breaks down a rapid and remarkable change takes place in the shape of the egg, during which the egg undergoes collapse or flattening in the polar diameter. Unless fertilization is accomplished most eggs remain indefinitely in this condition,

with the first maturation spindle in metaphase.   However one or
both polar bodies may be thrown off and, as Mead has pointed
out, some eggs may pass through the early cleavage stages.   The
rapid change just mentioned in the shape of the egg is interesting
when considered in connection with the coincident movement of
the spindle to the surface.   The camera lucida drawings of Text–
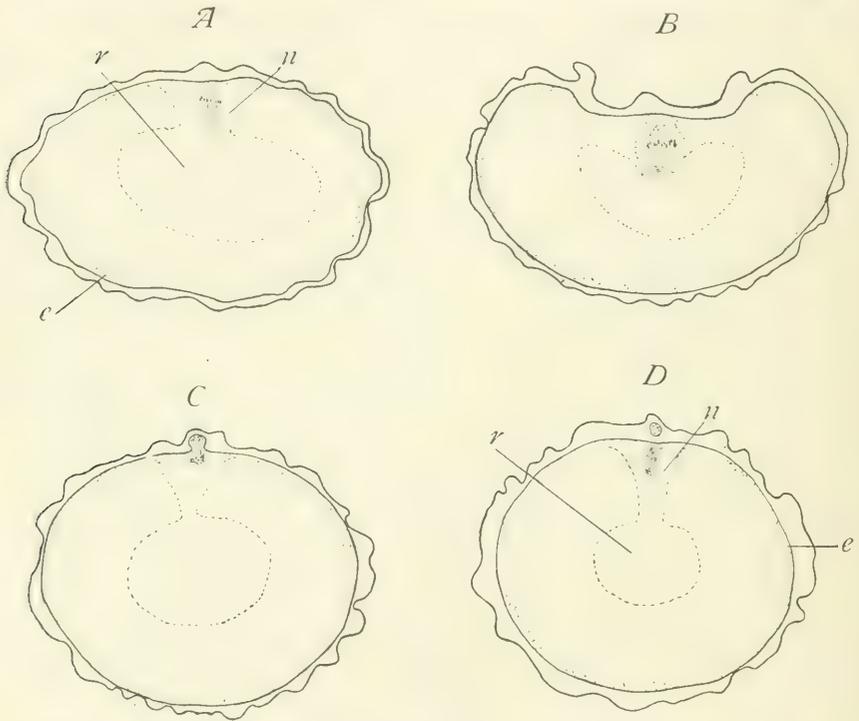
Fig. I.   Diagrams to show changes in shape of eggs during expulsion of polar globules.   Collapse
in the polar diameter to show how first maturation spindle comes to the surface, in *A* (typical), and *B*
(rather infrequent).   First polar body being expelled in *C*, egg regaining spherical shape; *D*, anaphase
of the second maturation spindle, the egg nearly spherical.   Camera lucida.   Leitz oc. 1, obj. 1–12.

Fig. I show conditions found at this time.   The typical condition
is shown in Fig. I, *A*, where the polar diameter is to the transverse
diameter about in the proportion of three to five.   An extreme but
frequent condition is shown in Fig. I, *B*.   In both figures there is
represented a thin ectoplasmic layer; an oval or elongate area,
marking the region of the germinal vesicle; the spindle in meta–

phase occupying a radial position, lying within a differentiated area in which there is very little yolk; the area in question being in direct communication with or lying partly within the original nuclear area.

The explanation of these phenomena is apparently as follows: After the germinal vesicle breaks down, the nuclear sap begins to diffuse out through the cytoplasm in which are many yolk granules, and staining reactions show that it very soon reaches the surface of the egg. However, owing to the slightly eccentric position of the egg nucleus and probably also to the structural organization of the cytoplasm, this material comes to the animal pole of the egg earlier than to the rest of the surface (Figs. 1 and 2). The action of the nuclear diffusion seems to render the cytoplasm more fluid and therefore less viscous, especially at the animal pole, as is shown by the fact that the cytoplasm usually flows out into and takes the shape of the inequalities of the enclosing egg membrane. The result is what we should expect if the nuclear sap were withdrawn from the nuclear area, *i. e.*, the egg flattens or undergoes collapse at the animal pole. In this way the surface is carried down to the region of the first maturation spindle that up to this time has moved very little or not at all from the place of its origin. This state of affairs is shown clearly in Text–Fig. I, *A* and *B*. The general mass of the cytoplasm now becomes more uniformly affected by the nuclear sap, as the stains seem to indicate, and under the influence of surface tension the egg begins to slowly assume a globular shape; the spindle remains near the surface and so is carried away from its original position. When the anaphase is reached the typical condition is shown in Fig. I, *C;* the shape of the egg has become a rounded oval.

Leaving out of consideration the causes which separate the chromosomes into two groups, the idea that the nuclear material tends to liquefy the cytoplasm suggests a hypothesis which may possibly explain the formation of the polar globules. When the chromosomes which are to form the first polar body very nearly touch the surface of the egg (Fig. 6), a sort of papilla-like projection pushes out ahead of them; soon these chromosomes surrounded by a small amount of clear cytoplasm are pinched off in

the form of a little droplet, the first polar globule. Now this is just what we should expect if the region immediately surrounding the chromosomes is rendered more liquid while the general mass of the egg is undergoing slow contraction in resuming the globular condition. The formation of the second polar body, which soon follows the first, may be explained in the same way. Fig. I, *D*, shows the second maturation spindle in metaphase. The egg has apparently resumed its original shape by the time the second polar body is completely constricted off.

5. *Fertilization. Fusion of Germ-nuclei*

By the time the second maturation spindle is in anaphase the sperm head has penetrated one-third to one-half the distance to the center of the egg. It lies slightly in advance or to one side of the centrosphere which is beginning to be recognized by its characteristic appearance. The sperm head has begun to enlarge and its more pointed end is directed toward the centrosphere. During the telophase of the second maturation the male pronucleus (as we may now call the sperm head) continues to enlarge and to advance toward the center of the egg. There is evidence to show that it breaks up into a number of chromosomes, each of which forms a vesicle, and that these vesicles rapidly fuse to form a larger vesicle. At any rate, two, three, or four lobes or vesicles are frequently found which fuse at a later period. The sperm aster is clear cut and growing; the centrosphere which lies in advance of the pronucleus is still hazy without definite outlines. Just after the expulsion of the second polar body the male pronucleus has traversed approximately two-thirds of the distance to the center of the egg and appears as a medium-sized vesicle with deeply-staining reticulum. There is a rather large vanishing aster and the centrosphere has increased in size and clearness. About this time the egg chromosomes begin to swell, while the small aster, somewhat enlarged and persisting from the maturation spindle, has at its center a clear area that has the appearance of a centrosphere. By the time the male pronucleus reaches a region near the center of the egg it takes the form of a clear vesicle with deep-staining and sometimes diffusely-staining reticulum.

The female pronucleus is moving toward the center with each chromosome forming a little vesicle. The female centrosphere is small and ill-defined, the male centrosphere is large with well marked boundaries (Fig. 10). The male pronucleus increases in size, but it remains almost stationary near the center awaiting the approach of the female pronucleus. The female chromosome vesicles now fuse to form a vesicle which rapidly enlarges. The female aster and centrosphere have in the meantime entirely disappeared and only traces of the male aster may be seen. Fig. 11 shows the germ-nuclei in contact, approximately of the same size and appearance. Soon the limiting walls break down and the chromatin quickly collects to form the primary stage of the first cleavage.

6. Cleavage

An early spireme stage of the first cleavage presents a typical appearance. When the fully-formed tapering spindle takes its characteristic position it lies in or very nearly in a plane perpendicular to the polar axis slightly nearer the animal pole with the chromosomes in metaphase (Fig. 12). While the clear-staining area at each end of the spindle (centrosphere) becomes larger, the spindle itself becomes broader, blunt at both ends, and decidedly shorter. In Amphitrite the centrosome, whatever may be its function, is not a permanent organ of the cell; at least it is not differentiated at all times by the fixation and stains I have used. In Fig. 13 is seen the condition of the egg in telophase. The chromosomes with faint outlines are passing into the vesicular condition. These collect and fuse in the region of the centrosphere to form the resting nucleus of the daughter-cell. The asters are undergoing degeneration, and the cytoplasmic division is well under way. Fig. 14 shows the chromatin collecting in the vesicular nucleus in preparation for the second cleavage; the asters have already divided.

The cell-lineage of Amphitrite has been described by Mead; and it is not necessary to describe later nuclear changes, for the same processes are repeated as have been described for the first cleavage. These processes may be stated briefly as follows: the

chromatin, contained in a clear vesicle with a sparsely-staining .
reticulum, collects into the form of a number of threads or spireme
which breaks up into a number of chromosomes; these collect,
divide and pass into a vesicular condition as they approach the
centrosphere; here their outlines become indistinguishable and
they fuse to form the vesicular nucleus of the daughter-cell.
No yolk lobe is formed in cleavage as is the case with the egg of
Chætopterus.

The first cleavage is unequal and in the 4-cell stage one cell,
D, is larger than any of the others. The sagittal plane of the
future embryo passes through this cell and the one diagonally
opposite. The cleavage is now oblique and alternates in direction
up to the 64-cell stage when the germ layers are completely sepa-
rated. At this stage one cell is mesoderm, seven cells are ento-
derm, and the rest ectoderm. The complete invagination of the
mesoderm is shown in Fig. 15. The mesoderm in this egg con-
sists of four cells, one small cell from each large one not being
shown. The nuclei of certain cells are moving away from the
periphery, the first indication of the invagination of the entoderm.
There were 104 nuclei present in this egg.

7. *Structure of the Trochophore at Various Stages*

By the time the 64-cell stage is reached, the primary prototroch
is composed of four separate groups of cells of four cells each;
these become flattened down, never divide again, and soon become
ciliated. The completed prototroch, formed by the addition of
certain other cells, is composed of twenty-five cells which may all
be recognized after the larva begins to elongate. The paratroch
is composed of four cells differentiated at a comparatively early
period; these persist as a ring of ciliated cells around the body
until the larva has developed five or six metameres. The cilia
are at first numerous and fine; later they become larger, stronger,
and have more vigorous movements. The apical tuft is large and
well developed in the young trochophore but it slowly atrophies
when the body begins to elongate. The normal round blastulas
always swim up toward the surface of the dish in which they are
kept; this is due to the location of the cilia, and the presence of a

segmentation cavity no doubt plays a part. The movement at first is chiefly rotary and gradually increases in rate. When the trochophore begins to increase in length, the altered shape causes it to swim in a more or less winding path with rapid movements that are evidently produced by definitely correlated forces. The regular shape of the trochophore and the correlated movements of the symmetrically arranged cilia are the controlling factors of the path through which it swims.

The shifting of areas in the lower hemisphere—the subumbrella region—is the chief means by which the one-layered blastula becomes metamorphosed into the three-layered larva. In this process the contour of the egg is not very much altered. The mesoderm and entoderm sink in, filling the segmentation cavity and diminishing the surface area. The cells of the somatic plate become thinner and spread over the surface of this region. After the blastopore closes the subumbrella region elongates rapidly due to the cleavage of cells just in front of the paratroch. The formation of metameres, the differentiation of the alimentary tract, of the mesoderm, of the mucous glands, of the problematical bodies, and of the chief part of the nervous system need no further mention for our comparison.

The rate of early development in Amphitrite is quite rapid. The time required for maturation and first cleavage has been mentioned. Cell division continues with equal swiftness, cilia are developed by the time the 64-cell stage is reached, and under favorable conditions the blastula swims in from four to five hours after the egg is fertilized. By the time the trochophore is twenty hours old it is approximately pear-shaped, a stomadeum has developed, a large enteron is very noticeable, vacuoles are found in the region of the prototroch, and the brownish-black pigment of the eye spots is to be seen in the pretrochal region (Fig. 16).

The figure just mentioned represents a camera lucida drawing of a normal, living gastrula twenty-three hours old. Its shape was slightly altered by pressure of the cover-glass used to hold it in position. Some of the more characteristic details of structure are given and in one region the cells lining the enteron are shown.

DEVELOPMENT OF THE UNFERTILIZED EGGS

A. Description of the Living Material

1. Methods Used in Producing Parthenogenesis, and Their General Effect upon the Eggs

As soon as the worms were brought into the laboratory, each female was washed thoroughly from one to two minutes in fresh water to rid it of any chance sperm and then placed in a dish of sterilized sea-water until ready for use. The Amphitrite was usually washed several times in fresh water, though this was found unnecessary as demonstrated by the control eggs. The body cavity of the worms was opened in sterilized sea-water by snipping the thin body-wall over the region of the egg pouches. In this way very little blood or fluid was mixed with the eggs; they did not develop so well if much superfluous material was in the solution. The eggs were next placed in the desired solutions, usually at once, but frequently after various intervals. Care was taken to avoid shaking or squirting the eggs unless agitation effects were desired.

Many solutions with different dilution and time of exposure were employed, but the following methods have proved most successful in producing differentiation in unfertilized eggs of Amphitrite; the eggs were never all in the same stage of ripeness and the percentages give the best results found:

No. of Method.	Solution (Method) Employed.	Time Exposed.	Percentage of Active Swimmers.
1	2– 5 parts ($\frac{N}{8}$ N)Ca(NO$_3$)$_2$ + 98–95 parts s. w.	Permanently.	25 per cent.
2	5–10 " ($\frac{N}{8}$ N)Ca(NO$_3$)$_2$ + 95–90 " s. w.	1 hour.	15 per cent.
3	5 " ($2\frac{1}{2}$ M)KCl + 95 " s. w.	1 hour.	10 per cent.
4	5 " ($2\frac{1}{2}$ M)KNO$_3$ + 95 " s. w.	1 hour.	15 per cent.
5	5 " ($2\frac{1}{2}$ M)CaCl$_2$ + 95 " s. w.	1 hour.	5 per cent.
6	By agitation.		20 per cent.

Of the methods mentioned above the surest and most satisfactory is number 1, though number 2 is almost as good. The percentage of swimmers varies in different experiments from 5–25 per cent. The highest percentages of swimming embryos found in any of my experiments were obtained by using methods

3 and 4; in the former 30–40 per cent, in the latter 50 per cent. However this was from a single experiment and the results given were due to two causes, for these solutions were used in connection with the disturbance and agitation incident to handling the eggs in changing the solutions. Even the results given in the table for methods 3 and 4 may have been affected slightly by agitation, as the control seemed to show. In another paper I have shown how extremely sensitive the egg of Amphitrite is to agitation. This is a very important but uncertain method of producing parthenogenesis in these eggs. Usually not more than 2 or 3 per cent of the eggs will develop in this way, occasionally 5 to 10 per cent, and in one case 20 per cent. In the paper mentioned I have called attention to periods of susceptibility to agitation; further experiments show that the time of susceptibility to such a stimulus varies within wide limits, depending chiefly upon temperature and the ripeness of the particular lot of eggs.

A general effect of the calcium nitrate solution is partially to inhibit cytoplasmic cleavage and to stimulate a division of the nucleus. If the eggs are nearly ripe when removed from the body of the female, the weaker solutions do not seem to interfere with the expulsion of normal polar bodies or the first few cleavages. In the stronger solutions there is sometimes a tendency for the eggs to adhere or stick together when in contact, but I have never found giant embryos. In the potassium chlorid solution cleavage of cytoplasm is more aided than interfered with, especially if the eggs have reached the right degree of maturity. Consequently cleavage of the cytoplasm frequently keeps pace with that of the nucleus until quite a late stage. The first polar body is not formed except in very rare cases. The effect on cleavage of the potassium nitrate solution is much the same as that of the potassium chlorid solution. The effect of the calcium chlorid is more like that of the calcium nitrate solution.

The eggs that develop after agitation usually do not form polar bodies and nearly always are unsegmented. The nuclear divisions are as extensive and take place in the same manner that they do in eggs treated by the salt-solutions. Occasionally an egg will show a considerable amount of very irregular and abnormal cleavage

(Fig. 39).   Cleavage of the cytoplasm without previous cleavage of the nucleus has not been found in developing eggs.

2.   Early Stages.

It would be a needless task to undertake the description of individual experiments where much was repeated again and again in order to verify results and obtain comparisons of development.
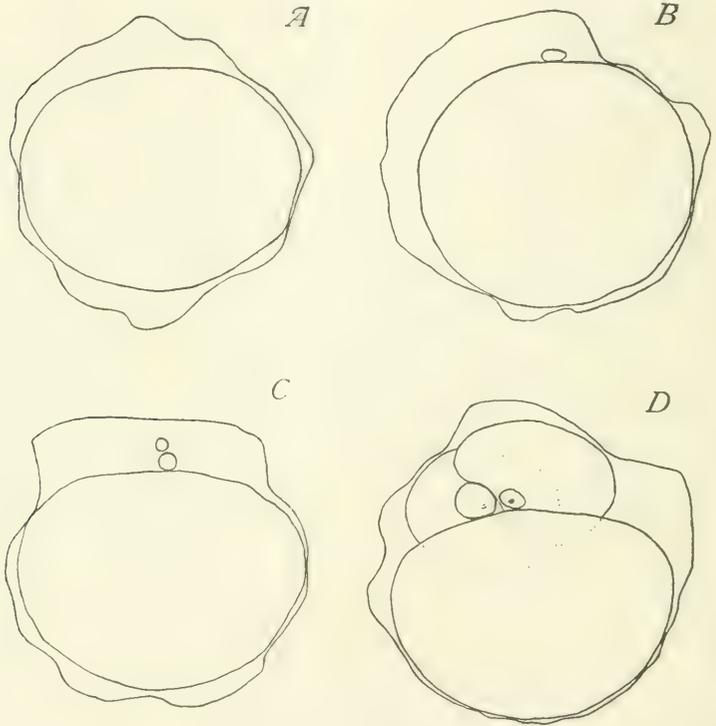
Fig. II.   Diagrams to show early development.   Method No. 1 used.   *A, B* and *C* were types forty-one minutes after beginning the experiment; *D,* another egg sixty minutes later.   Camera drawings from whole mounts.   Leitz oc. 1 (15), obj. 7.

Hence a general description will be given.   The germinal vesicle breaks down, sometimes within a few minutes, sometimes several hours after the eggs are subjected to the solutions.   It has been found that *the riper the eggs are, the more rapidly does the ensuing differentiation take place and the nearer it approaches the normal development.*

One or both polar bodies may be normal, or both abnormal, in both size and appearance; or only one is formed, which is always abnormal. Most of these abnormal polar bodies can be readily distinguished in the living egg by their size which varies greatly (Figs. II, III; 44–46). When normal polar bodies are found in the solutions the time required for this process is also normal. But it takes longer to complete the process of throwing off the abnormal polar bodies, and the longer the process the more abnormal it becomes. When both polar bodies are formed, here as under normal conditions the egg undergoes flattening or collapse in the polar diameter. When no polar bodies are thrown off, the change in shape is never so striking and may not be noticeable (Figs. 40–41, 55).

Cleavage usually begins within one and one-half hours after the eggs are placed in the given solution, though I have seen eggs in the process of cleavage within forty minutes after the beginning of an experiment. The first two blastomeres of the egg are frequently abnormal in size and appearance, but a study of the living material shows that an apparently normal cleavage may continue for several divisions. In every case ameboid movements, or abortive attempts at cleavage, occur at this time, even in eggs in which no distinct cleavage plane is ever formed; the egg often shows two or three lobes, a condition which is more common in the potassium chlorid cultures. The cleavage cells of an egg treated with calcium nitrate are always more compact and fit closer together than the normal, while the potassium chlorid eggs have the opposite tendency. The different types of cleavage can be best understood by consulting the diagrams (Figs. II, III; 17, 49, 50, 58–61). In six to twelve hours the cilia become strong enough to cause the rotation of the eggs. Up to this time cytoplasmic cleavage, when present, occasionally keeps pace with the nuclear divisions, but later it seems to lag behind more and more and often entirely ceases.

Certain unripe eggs after being treated with potassium chlorid solution tend to break up rapidly into many small spherules (Fig. IV). Occasionally this effect is produced by some other solutions if the concentration is sufficient and I have found it rarely in the controls. This phenomenon was noticed by Fischer, who says, "Sometimes the eggs go into the morula stage. The
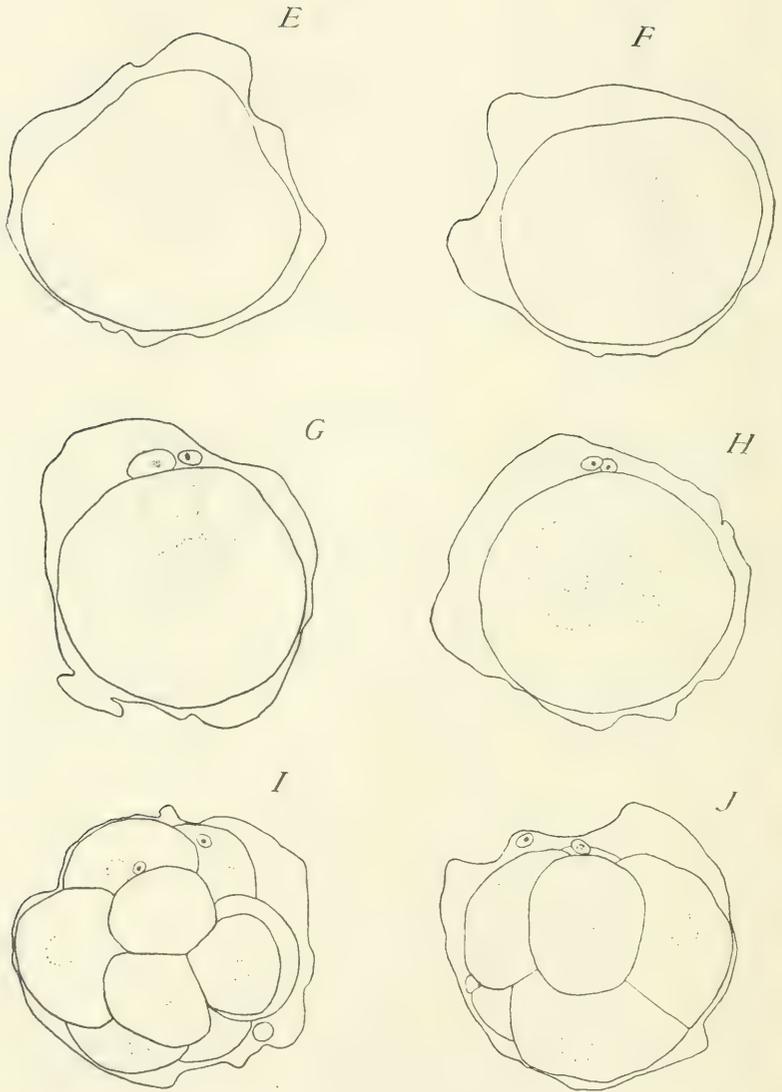
Fig. III. Diagrams to show types of early development. From whole mounts, same magnification as Fig. II. *E*, one hundred minutes, and *F-J*, two hundred and twenty-five minutes after beginning the experiment. Dotted lines enclose nuclei.

origin of the morulas is somewhat obscure. The fact that they appear in solutions which have at no time contained any black eggs would suggest that nucleated eggs can, in a very short time, give rise to morulas without any external signs of cleavage." Fischer thus raises the question whether nucleated eggs may not pass into the "morula" stage without any external signs of cleavage and so give rise to swimming blastulas or later stages. But these eggs do not develop, as we shall see. In one of my experiments 95 to 99 per cent of the eggs showed this condition. At 11.30 A. M. the eggs were removed from the body of a large female and placed for one hour in a potassium chlorid solution. Before they were returned to sterilized sea-water, the germinal vesicle had broken down in nearly all eggs and a few showed a very small peri-vitelline space. Thirty minutes later, at 1 P. M., the eggs showed protuberances over their surfaces as though *cells* were being constricted off. At 2.15 P. M. nearly all the eggs were in a multi-spherular condition; when pressed down slightly with a cover-glass no segmentation cavity was found and the central portion of the egg was still unsegmented. The eggs were examined at frequent intervals until 8.45 P. M., when the spherules were not more than half the size of those found earlier in the afternoon.

Fig. IV. A typical multispherular egg six hours old from a potassium chlorid solution. Camera drawing. Leitz oc. 1 (16), obj. 7.

The next morning no swimming eggs were to be found; many were apparently bordering on dissolution and all seemed full of small vacuoles. Sections of these eggs showed that the germinal vesicle had broken down in most cases and had diffused out into the cytoplasm before spherulization began. Very infrequently an egg was found with the germinal vesicle intact and surrounded by spherules. There was never any mitotic division of the nucleus. In eggs where the nuclear sap diffused out into the cytoplasm, there was found a more or less diffuse achromatin stain in each spherule. It is very evident that

this peculiar change in some eggs is not one of differentiation, but is due to the effect of the potassium chlorid in altering the viscosity and lowering the surface tension of the egg-cytoplasm. Since the spherulization continues for some hours after it begins, it seems that the cytoplasm may undergo a ripening process, somewhat as the normal egg must do in order that cleavage into small cells may occur; and inasmuch at the process of spherulization continues longer in eggs where the germinal vesicle has previously broken down, it indicates that the diffused nuclear sap may have something to do with this ripening process. Treadwell ('02) when working upon Podarke noticed that some of the control eggs broke up into a great many "small globules" with no trace of nuclear division, the nucleus lying in one cell which was usually larger than the others. He found no ciliated embryos among these eggs.

3. Description of Swimming Embryos, Eighteen to Twenty-five Hours Old

At this age the various cultures as a rule show their best development, though the swimming eggs frequently show wide differences in structure and appearance. To illustrate these differences we may cite a few examples. The egg in Fig. 20, from a potassium chlorid solution, was found swimming near the surface of the water about twenty-four hours after the beginning of the experiment. A clear ectoplasmic layer is differentiated, in marked contrast to the yolk which lies throughout the central portion of the egg. The cilia projecting from the entire surface are large, stiff and more strongly developed in certain regions. Segmentation is extensive; scarcely ever are eggs found with a greater number of cells than shown here, although the cells are not so numerous as in the normal egg of fifteen hours. The cell boundaries are not clearly defined, indicating a tendency to fuse, and certain regions do not show cell structure. Clear spots show the position of nuclear areas in the cells. This egg was compressed slightly under a cover-glass until it could be examined with a high power, but this treatment failed to reveal the segmentation cavity which can be easily demonstrated in the same manner in the

normal egg (Fig. 16). Vacuoles are sometimes present as shown in Fig. 20. Another egg, raised under the same conditions and nearly the same age, was taken from the bottom of the dish where the great majority of the swimming eggs are found (Fig. 21). The size and distribution of cilia and the arrangement of cytoplasm and yolk are practically the same as in the previous case, but there is not a trace of segmentation and only two nuclear areas are found. We may take as another typical example an egg from a calcium nitrate solution (Fig. 28). Here we find the cilia arranged irregularly in three distinct groups, a well differentiated ectoplasmic layer, the yolk pretty evenly distributed throughout the interior of the egg, traces of segmentation, numerous nuclei, and some limited patches of brownish pigment.

Eggs as a rule do not show such uniform distribution of cilia as these examples, or such regular shape (Figs. 22, 23, 26, 29, 39). The long *apical tuft of cilia* so characteristic of the normal trochophore is *always absent*. In shape the eggs may be comparatively globular, oval, or pear-shaped (Figs. 29, 24, 35); ordinarily these contours are represented in the active swimmers. On the other hand abortive cleavage may produce an irregular outline (Figs. 22, 36), and cleavage may stop though nuclear division proceeds. The number of cells may vary from one to many, and where the egg is manifestly composed of more than one cell the boundaries of these cells may be indicated by mere indentations, or by complete cleavage planes; both conditions are found frequently in the same egg (Fig. 22). The great majority of the swimming eggs have no true segmentation at all and in these unsegmented eggs the nuclei are usually very numerous (Figs. 29, 32, 33), but are sometimes few in number (Fig. 37); the nuclei also differ in size (Fig. 33). In eggs with considerable cytoplasmic segmentation there is as a rule one nucleus to each cell though two or three may be present. In all cases of swimming embryos the ectoplasmic layer is pretty well differentiated and this differentiation seems to bear some relation to the distribution of the cilia. For where the cilia extend around the entire circumference of the egg there is a correspondingly well developed ectoplasmic layer, and where the yolk lies close to the surface the cilia are noticeably absent.

However there may be an ectoplasmic layer where no cilia develop. The yolk may be broken up into groups in the case of segmentation or be scattered by nuclear division (Figs. 29, 32), and very frequently it exhibits a tendency to collect near one side of the egg, a region which I believe indicates the position of the vegetative pole.

Another important and significant differentiation at this period is the development of a brownish pigment. If one may judge from color and appearance this pigment is undoubtedly homologous with the eye spots of the normal embryo. In the normal trochophore this pigment is definitely localized in two small brownish or reddish-brown spots (Fig. 16). In the parthenogenetic eggs the pigment is never localized in spots. Occasionally two or more pigmented areas are found (Figs. 26, 28, 31), but more frequently the pigment is found in a single diffuse mass scattered along near one side of the egg (Figs. 32, 35, 37). Rather infrequently swimming eggs are found which have an oblong or blunt pear-shape; to a casual observer they might not appear dissimilar to the normal trochophore (Figs. 21, 23, 25, 32). There appears to be such a differentiation in shape in the egg shown in Fig. 35, but I think the form is due mainly to an early segregation of cleavage materials (yolk, cytoplasm) in which the cleavage division had the most prominent effect, this being followed by a fusion of the blastomeres.

From what has been said about the unsymmetrical distribution of cilia, the lack of an apical tuft, and the irregular shape of these swimming structures, it is not surprising to find that their swimming movements are distinctly different from those of the normal trochophore. They do not swim so rapidly, they do not move in such a definite path, nor do the movements of the cilia appear so well correlated.

4.   Later Stages and Fate of the Parthenogenetic Eggs.

The maximum percentage of swimming eggs is found between the twelfth and twenty-fifth hours, depending upon the temperature and the rate of development of a given lot. After the twenty-fifth hour they die rapidly and in most experiments all are dead

at thirty-six or thirty-seven hours, although I have raised them until over forty-eight hours old. Death is not due to the *environment*, for the normal eggs are readily raised under the same conditions. Typical conditions of eggs from the calcium nitrate solutions are shown in Figs. 29 and 31. In the various cultures the amount of segmentation tends to diminish in these later stages, due to fusion, wholly or in part, of adjacent blastomeres. Frequently, however, eggs retain a condition of definite segmentation which was reached at a much earlier period (Fig. 30). Fig. 32 is typical of the oldest eggs that came under my observation. There is no apparent segmentation of the cytoplasm, a great many light areas are present some of which may be vacuoles; the yolk is irregularly distributed, and no pigment can be seen. In the same experiment quite a large per cent of the eggs were still swimming at the age of forty-four hours. Some of these were round, some oblong, some with irregular outlines, and all were distinctly different from the normal. Later the vacuoles increased in number.

The fate of the parthenogenetic eggs may be summed up as follows: Segmentation of the cytoplasm remains until very late in substantially the same condition that it reached before the development of the cilia. Those eggs which have extensive and strongly developed cilia survive those on which the cilia are limited in extent or weakly developed; these surviving eggs also have a shape nearest to the normal. After swimming actively for some time the cilia do not move so rapidly, apparently shrink in size and seem to disappear; the protoplasm assumes a lighter appearance, vacuolization sets in, and just before dissolution the cells, if present, tend to flow together and the egg as a whole tends to assume a more roundish shape. The egg-wall breaks down at some point and soon dissolution is complete. I have seen cilia still vibrating after the egg-contents began to flow out.

I have mentioned in the first part of this paper the definite change in shape that occurs in eggs at the time of maturation and I have referred to the ameboid movements of the cytoplasm that are found in early cleavage stages of both fertilized and unfertilized eggs. In the normal eggs these ameboid movements possess

a definite character and relate to cleavage. Moreover they take place slowly in all eggs. In eggs with no segmentation these ameboid movements are very noticeable, for every nuclear division that takes place is within the limits of one cell and consequently the whole egg is influenced by each division of the nucleus. It is for this reason that ameboid movements in the unsegmented egg are so much more noticeable than in those eggs in which the cleavage is complete. In contrast with these movements of the egg connected with segmentation phenomena, there are other ameboid
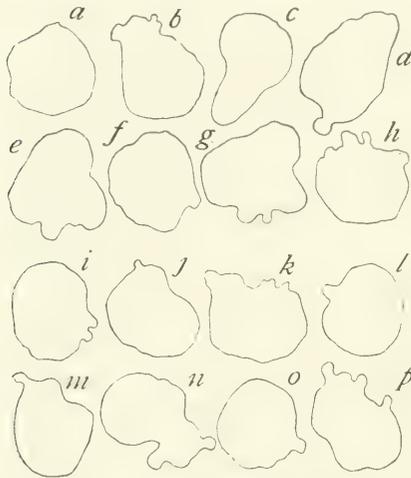
Fig. V. Camera drawings to show rapid ameboid movements. From a calcium nitrate solution, forty-three hours old. Sketches made at one-half minute intervals, *a–h*; sketches at one-quarter minute intervals, *i–p*.

movements of a different kind that are sometimes found in the salt-solution eggs at a comparatively late stage of differentiation. An egg is occasionally found thirty to forty-five hours after the beginning of the experiment that shows no segmentation and no ciliation but is undergoing extraordinary ameboid activity. Such an egg has a general opaque appearance but the border is clearer than the rest of the egg. The small pseudopodia sometimes form and disappear very quickly. In Text-Fig. V are shown camera lucida drawings of an egg of this kind forty-three hours after the beginning of the experiment. In the first two rows, *a–h*, are eight successive sketches made at one-half minute intervals; in the third and fourth rows, *i–p*, are sketches made eleven minutes later of the same egg at one-fourth minute intervals. These drawings do not show more than half the forms that were taken by this egg during the time the sketches were being made. The peculiar differentiation just described may be correlated, I believe, with processes found in the normal larvas of about the same age. By

the time the trochophore is forty hours old it has developed three or four well-defined body segments. During this development in structure a physiological differentiation has occurred so that the larva is now able to make comparatively quick, jerky and wriggling movements. I think without doubt that this is the sort of differentiation that takes place in these eggs treated with salt-solutions. The protoplasm presents the same medium-opaque appearance and the movements possess the same general character.

B. Description of the Preserved Material

1. Early Stages

If the eggs are quite ripe, that is, in a condition to be fertilized, a study of the sections of unfertilized parthenogenetic eggs discloses no abnormalities until after the expulsion of the polar bodies. This applies as a rule only to the weaker calcium nitrate solutions. In strong salt-solutions the process of development is hindered or considerably modified. Unripe eggs when treated with any of the solutions always give abnormal results. Since any given lot of eggs removed from the body cavity usually contains many stages of ripening, there are always many forms of differentiation in each experiment. Fig. 45 gives a type in which the polar bodies depart slightly from the normal (*cf.* Figs. 7, 8, 9). It will be noticed that the first polar body is slightly larger than the normal due to additional cytoplasm and that the second polar body is still further increased in size. The chromosomes in the second polar body are noticeably ragged in appearance. In Fig. 44 these differences are still further accentuated. By referring to Fig. 46 we find a very abnormal type of both polar bodies. The first polar body is in practically the same condition as the second polar body of Fig. 44, while the second polar body has increased enormously in size by the addition of yolk granules.

The state of affairs where only one polar body is formed is represented fairly well by Fig. 47. The remaining chromosomes have retreated to near the center of the egg and are in a stringy, fragmentary, decidedly abnormal condition which probably corresponds to a stage just previous to the first cleavage. It will be

noticed that the egg membrane is very thick, a condition which is produced by the calcium nitrate solutions in unripe eggs and which has served to distinguish certain types of differentiation at a later period. I have also observed, after the first polar body was thrown off, the chromosomes of the second polar body lying in an isolated group in the cytoplasm near the animal pole; the remaining chromosomes retreat to the center of the egg to take part in certain phenomena of cleavage. Sometimes the chromosomes of the first polar body are thrown off in a separate group which lies somewhere near the surface as in Fig. 42.

It frequently happens that no polar bodies are expelled (Figs. 40, 41). In this case the egg does not collapse in its polar diameter, the spindle lies in or very near its original position, the chromosomes, spindle and asters all show slight abnormalities, and the stain indicates that the egg is immature. The chromosomes are not usually separated permanently by this kind of maturation process and frequently do not separate at all. Another abnormal condition of the first maturation spindle is shown in Fig. 43. In eggs where both polar bodies are expelled the spireme for the first cleavage is formed in the same manner as under normal conditions (Figs. 44, 45, 46).

I have mentioned that observations on the living egg sometimes failed to disclose any abnormal process in the early cleavage stages and an examination of the sections shows that the same is true of the nuclear phenomena. However there is a tendency for the chromatin division to be fragmentary or unequal and the astral radiations do not possess the distinct, definite outlines of the normal condition (Fig. 48). Very rarely there is found a tripolar mitotic figure and occasionally the first division results in three nearly equal cells (Fig. 50). Even when the nuclear division is approximately normal and complete the cytoplasmic cleavage is quite often incomplete or abortive. Frequently therefore more than one nucleus is found within the limits of one cell, confirming observations made upon the living egg.

The development of eggs when treated with potassium chlorid solutions does not begin until after the eggs have been returned to sea-water. In extremely rare cases the formation of one abnormal

polar body has been observed in cultures from this solution. Though no polar bodies are formed certain peculiar phenomena take place within the egg at this time, the chromosomes pass into the vesicular condition and greatly enlarge. The vesicles are less numerous at first and some have faint outlines, but later there are from 16 to 22 in each egg. Maturation asters form in the proper position and a faint spindle may be present, but the spindle-rays do not seem to have any important influence upon the chromosome vesicles (Figs. 55, 56). Sometimes the spindle and vesicles move up to a region indicating the position of the animal pole. The maturation asters are rather delicate in appearance and fade out before the chromosome vesicles fuse in preparation for the first cleavage division. Very commonly the maturation asters and spindle do not form at all, though the chromosome vesicles enlarge in the manner described above. Fig. 57 shows an early condition in the formation of the first cleavage spindle; the cleavage asters are distinctly different in size and appearance from the maturation asters just described.

The cleavage phenomena in the potassium chlorid cultures show more variability than in solutions where the calcium nitrate salt was used. This variability seems to be correlated with the fact that an excessive amount of chromatin remains within the egg. At the first cleavage the amphiaster is similar but distinguishable from the normal; the chromatin-division takes place in the normal manner, but with certain minor irregularities which for the most part consist in scattered and fragmentary chromatin-granules or chromosomes (Figs. 58, 61). Later cleavage stages show more abnormal conditions. Frequently a triple aster is found at the first cleavage and three-celled eggs or triple groups of chromosome vesicles are very much more common in this than in the calcium solution. I have seen four, five, six, and in one case, seven asters all taking part in the chromatin division, and in some of these eggs there was no sign of cytoplasmic cleavage. In Fig. 61 is shown an egg which may be taken as a type; the part that each of five asters is taking in the distribution of the chromatin is readily seen, and one is not surprised to find in later cleavage that each cell may contain one or many nuclei and that these nuclei

may be of various sizes (Figs. 62, 63). When cleavage of the cytoplasm occurs the nuclear division is most nearly normal. In general a potassium chlorid culture after five or six hours contains a few eggs with many cells having one nucleus each, a great many more with a smaller number of polynuclear cells more or less incompletely divided, and some eggs undivided but multinuclear. The unequal size of the nuclei in some of the cells indicates that the chromosome vesicles in telophase may fuse to form two vesicles instead of one. I believe that some of the nuclei may arise in this way though I have made no direct observations to prove it.

Some interest attaches to the number of chromosome vesicles to be found in potassium chlorid eggs in the late anaphase and telophase of the first cleavage. A constant number cannot be counted owing to the fact that some usually begin to fuse before others have distinct and definite outlines. In the egg of Fig. 59, in the left-hand group of vesicles, 18 (+4?) could be counted, while in the other group 20 (+4?) were found. The usual number that can be definitely counted is from 15 to 20. At a somewhat later stage, in an egg where the cleavage of cytoplasm was normal, eleven vesicles were counted in each of the two cells (Fig. 60). With these conditions may be compared Fig. 49, which represents an egg treated with calcium from which both polar bodies have been expelled; it is interesting to note that we have here 10 (+1?) vesicles in one group and 11 in the other (not all shown). In the calcium nitrate solutions where both polar bodies are formed the reduced number of chromosomes, eleven, appears again in the daughter-cells of the first cleavage. This number no doubt continues in the later cleavage-cells. In the potassium chlorid egg (Fig. 59), there is evidently the full number of chromosomes in each of the two cells, and as no polar bodies were expelled this number can be accounted for on the theory of the individuality of the chromosome, since these chromosomes had no maturation division; still the number must have been reduced from 44 to 22 by fusion before division. But if we take a potassium chlorid egg, like that shown in Fig. 60, the eleven vesicles of each daughter-cell indicate a bivalent character retained throughout division; or

they have assumed this character after division by fusion in twos, a view that is improbable from lack of any direct evidence. Certain difficulties prevent a thorough analysis of these phenomena but the above conditions may be accepted as typical.

2. Condition of the Eggs as Shown by Sections of Later Stages

In a general way my observations on the living egg are completely confirmed by a study of the sectioned material, which also furnishes additional and interesting data. Usually cleavage is more extensive within the egg than at the surface, probably indicating fusion at the surface subsequent to cleavage. Some eggs are multinuclear without any signs of cytoplasmic cleavage, and cleavage of both cytoplasm and nucleus falls farther and farther behind the normal as development proceeds. More nuclei are uniformly found in the calcium than in the potassium cultures, especially in the unsegmented egg, but the potassium chlorid solution shows eggs with more distinct and more complete cleavage planes. I have been able to discover cilia along the edges of the prepared material of all such types of eggs as are represented in my drawings of late stages and there is no doubt that such eggs represent typical swimmers. The killing fluids used injured or destroyed most of the cilia, hence no attempt has been made to figure them in the drawings and such ciliation has not been found of any benefit in localizing regions of the eggs.

One of the most advanced types of cleavage in the potassium chlorid solution at eight to nine hours is represented by Fig. 64, and Fig. 65 is a type near the other extreme with very little cleavage. In the segmented eggs mentioned there were found portions of a segmentation cavity and evidences of an attempt at invagination (*cf.* Fig. 15). In the egg with very little cleavage the nuclei vary in size and the largest one has evidently not divided for some time since no nuclei of a similar size were found near it. This fact, that a nuclear vesicle in an unsegmented egg enlarges extraordinarily unless mitosis occurs, is in accord with Lillie's findings for the egg of Chætopterus and indicates that the size and growth of the nucleus are independent of mitotic division. In all developing eggs, whether segmented or not, the early differentiated ectoplasmic

layer is retained. Fig. 51 represents an egg taken at fourteen hours from a calcium nitrate culture; the cleavage planes are incomplete and there is a clear attempt at invagination.

No further very important developmental changes occur in most of the swimming eggs. However, conditions are occasionally found that throw additional light upon parthenogenetic development or upon causes connected with the cessation of development. Fig. 67 is an egg from a potassium chlorid culture at the age of twenty hours which may be taken as typical of the most advanced differentiation that I have discovered in eggs from this solution, although other eggs with more cells and more nuclei have been found. While manifest irregularities are noticeable, the invagination of the mesoderm is easily made out and is well under way. Why the development has advanced no further than where it should have been some twelve hours earlier, if fertilized, seems traceable to the following causes: The number of cells is too small to complete invagination in the normal manner; only small portions of the segmentation cavity were formed, probably due to the action of the solution upon the surface tension of the cytoplasm and perhaps to slow and imperfect cleavage; and incomplete cleavage may also have hindered the invagination. In another egg of the same age from the same solution (Fig. 66), there was no trace of segmentation, and vacuoles, which characterize the fertilized eggs at about this age, were found.

The conditions in the calcium solutions at about twenty hours are also interesting. Unsegmented eggs frequently show a large number of small nuclei (Fig. 52); this fact verifies observations on the living egg. In Fig. 53, the amount of cleavage, the number and distribution of nuclei, the lack of any segmentation cavity, the ectoplasmic differentiation, and the remnants of the region of the germinal vesicle are all characteristic of eggs from this solution at this age.

Very rarely I have found an egg that showed a striking resemblance to the normal, as in Fig. 54. Although this egg is twenty and one-half hours of age, it is at a stage that it should have reached twelve hours earlier under normal conditions. The invagination of the mesoderm is complete; and in each of the

two mesoderm cells there has been nuclear division without division of the cytoplasm. The nuclei of the entoderm cells (partly shown) are migrating toward the segmentation cavity and are thus taking the first steps toward the invagination of the entoderm. Large vacuoles are found which are not present in fertilized eggs of the same number of cells but are found in fertilized eggs of the same age. There is little essential difference between this and a normal egg (Fig. 15). In the normal egg the cleavage of the cytoplasm is a little more definite and complete, the mesoderm consists of four different cells (two small ones not shown), and this egg is more symmetrical in shape.

From the study of sections of a few well-developed unfertilized eggs, it would seem not impossible to raise normal gastrulas and even later stages from unfertilized eggs. But I have not succeeded in doing so, though I used the same and even better precautions than those that gave me success in raising the normal embryos.

THEORETICAL CONSIDERATIONS

1. Brief Review of Some Previous Papers

Before taking up the theoretical discussion it is desirable to give a short resumé of some papers relating to the subject under consideration.

Mead ('98, 2) tried the effect of a weak potassium chlorid sea-water solution upon the unfertilized eggs of Chætopterus. He found that the formation of the polar bodies and the yolk lobe took place in a normal manner and noticed abortive attempts at cleavage. The abnormal phenomena began after the maturation mitoses and the reconstruction of the egg nucleus. He came to two conclusions in regard to the effect produced by the potassium chlorid: "First, it is of the nature of a stimulus, compatible with the continuance of the normal developmental processes, and is not of the nature of a poison or an irritant setting up irregular, abnormal, and inconstant changes; second, the stimulus must be referred to the specific properties of the salt and not to a change in the density of the water in which the eggs are placed." Morgan ('00) repeated and verified Mead's experiments. By increasing

the concentration of the sea-water (NaCl, MgCl$_2$), he found that development may proceed in much the same manner, but no polar bodies were extruded. Loeb ('01) produced trochophores and claimed that "increase in the osmotic pressure or the loss of water on the part of the egg is the cause of the parthenogenetic development of the egg." He also believed that potassium chlorid possessed a specific effect upon the eggs of Chætopterus.

Fischer ('02) succeeded in raising the unfertilized eggs of Amphitrite to the "trochophore stage" by adding a small amount of calcium salt to sea-water and by agitating mechanically the sea-water in which the eggs were contained. Though he noticed that the developing eggs "present a totally different appearance" from the fertilized eggs, his observations were incomplete and superficial. In the same paper he states that "the appearance of the swimming trochophores, the ciliary activity, and the general behavior of the parthenogenetic larvas is exactly that of the normally fertilized larvas."

Treadwell ('02) used a potassium chlorid solution with high osmotic pressure to produce artificial parthenogenesis in Podarke. No polar bodies were formed; the nuclei were much larger and stained more intensely than the normal ones; ciliated structures were produced without cleavage; and he concluded, though his experiments were "incomplete," that the cleavage which included both nucleus and cytoplasm was not normal, *i. e.*, was not karyokinetic. It seemed "as if one of the cells is to be regarded as a lobe of protoplasm which contains some of the nuclear material, and may later become completely divided by a membrane from the other cell." It seems probable to me, however, that he was wrong in this conception.

Recently Bullot ('04) has worked upon the eggs of the annelid Ophelia with the view of solving the problem of the relation between differentiation and cleavage. Though his results are inconclusive they are entitled to a brief mention here. He asks the question, "Do the swimming blastulas found after ten hours in the cultures arise from the segmented egg, or do they originate from the unsegmented eggs?" By placing shallow dishes under the field of his microscope, he was able to make camera lucida

drawings of individual eggs at various intervals. For one experiment he gives a series of eight drawings the last one made nine and one-fourth hours after the eggs were placed in the solution. Out of forty-eight eggs sketched in this series sixteen were swimming at nine and one-fourth hours and these had arisen from eggs that had previously shown segmentation. His drawings show, however, that the segmentation in these eggs was not always the same; yet he concludes, "These experiments show conclusively that in the annelid of the genus Ophelia which was used in these experiments the parthenogenetic larvas originate from *regularly* segmenting eggs" (italics mine). This conclusion is also open to certain objections. First, he says nothing about the later fate of the thirty-two eggs which were not swimming at nine and one-fourth hours. A study of the individual eggs figured by Bullot shows that the segmentation does not proceed in all at the same rate and therefore it is unlikely that all should begin swimming at the same time. In Amphitrite the maximum number of swimmers is sometimes not reached until ten hours after the first swimming eggs are seen; the earliest swimming structures always show more or less segmentation, but some hours later swimming eggs are found with little or no cleavage. This latter condition is partly due to fusion of blastomeres but more frequently to the fact that the egg has not divided or that cleavage has been abortive. It is also difficult to understand what is meant by the phrase, "regularly segmenting eggs." He cannot mean the definite, determinate cleavage characteristic of the fertilized Annelid egg, for his figures show otherwise. From his own experiments Bullot attempts to cast doubt upon the results obtained by Lillie. It is readily seen that no weight should be attached to this objection.

I shall take the privilege of referring to the important paper of Lillie on Chætopterus ('02) as the discussion demands.

2. *Effects of the Salt-solutions and of Shaking*

There are several reasons why the eggs of Amphitrite are favorable subjects for experimental studies of this kind. In the fertilized egg the characteristic changes in shape at the time when the polar bodies are expelled, the determinate type of cleavage

resulting in an early segregation of the germ-layers, the rapid development, and early appearance of important differentiations, all furnish favorable points for comparison. The unfertilized egg responds not only to treatment with certain salt solutions but is also highly susceptible to mechanical agitation. A specific salt, calcium nitrate, may be used but the egg develops nearly as well, especially if slightly agitated, in solutions where the osmotic pressure has been raised (potassium chlorid, potassium nitrate). *These conditions applied separately to the egg give some important modifications in development, but the indirect, or general, effect of any one method is to bring about certain differentiations found in the fertilized egg.* It is not claiming too much then to say that these differentiations are independent of the particular method used, *i. e.*, they depend upon the organization of the egg. We may also speak of the direct or specific effects upon the unfertilized egg produced by each particular method.

Herbst has shown that cells in calcium-free sea-water tend to separate from each other, and he ('04) regards their tendency to "join together" in calcium-containing sea-water as a reversible coagulation process. This effect of calcium nitrate then would ultimately hinder cleavage and gastrulation as in the eggs of Amphitrite. But when the osmotic pressure is increased either with the calcium or potassium salt, the polar bodies are no longer thrown off; the egg does not collapse when the germinal vesicle breaks down, thus preventing the maturation spindle from getting to the surface. It is probable that an excessive absorption of water tends to prevent the collapse of the egg and so prevents the formation of the polar bodies. Herbst ('04) has shown that potassium is important for growth and that it leads to the taking up of water. The appearance of Amphitrite eggs after treatment with potassium chlorid is in this way accounted for if we consider the blastomeres as swelling up with absorbed water (Fig. 17). The strong solution also destroys, or weakens, some of the processes of maturation (asters, spindle) and at the same time causes the chromosomes to pass into a vesicular form and grow to an unusual size. The question arises, Is the suppression of the maturation asters due to the chemical effect of the salt, or to an increased

osmotic pressure? I am inclined to the latter alternative. The effect is only temporary and this would probably not be the case if the chemical nature of the egg had been changed. Another argument in favor of this view is that in Chætopterus eggs weak solutions of potassium chlorid do not hinder the process of maturation while strong solutions do. There is also evidence to show that differentiating processes are taking place at the same time though they may not be expressed in the normal morphological form; for we find in proper sequence a reconstitution of the egg nucleus by a fusion of the enlarged chromosome vesicles and the ordinary phenomena of mitotic cleavage division. Besides I have shown in an earlier paper that "there are processes of differentiation going on in the unfertilized eggs of Amphitrite which may be started into activity at definite intervals by mechanical agitation."

After agitation the unripe eggs do not expel the polar bodies for the reasons which have been mentioned. In ripe eggs shaking causes such a disturbance in the organization of the cytoplasm that cleavage is usually absent, and when present is very irregular (Fig. 39). It is probable that this treatment so coagulates or at least so alters the protoplasm in the ripe egg that it fails to flatten in the polar diameter, and the physical conditions are then unfavorable for the expulsion of the polar globules.

The action of potassium chlorid on cleavage cells and in producing spherules may be applied in explanation of certain phenomena described by Lillie and Treadwell, who worked with strong concentrations of this solution. Treadwell noticed in Podarke eggs, a great variety of cytoplasmic cleavages. Lillie ('02) describes as a very common occurrence a sort of pseudo-cleavage which was due to the separation of pseudopodia. But he also found in the two cell-stage, where the nucleus passed into one cell, that the direction of the cleavage plane was normal. In other words we have here two factors at work, one a direct effect of the solution in altering the viscosity or consistency of the egg cytoplasm, the other a process connected with the differentiation of the egg. It seems apparent that pseudo-cleavage and the production of spherules are independent of cytoplasmic differentiation; these conditions are the results of a general effect of the salt upon the

egg protoplasm and have nothing to do with development. I may add that I have repeated some of Lillie's experiments, have confirmed his results for the living egg, and find further support for this view.

3. Is this Development Parthenogenesis?

In discussing whether this sort of development should be termed parthenogenesis or not, this much is clear: we find certain recognizable differentiations that are found normally in fertilized eggs. In the fertilized egg these differentiations are closely correlated in time and place, in the unfertilized egg the correlation is less complete. In the early development of the calcium-treated unfertilized egg of Amphitrite there are found all the processes (as indicated by mitotic division, cleavage, cilia, etc.), that are characteristic of normal development. In so far then we may use the term parthenogenesis, and if we wish to distinguish it from parthenogenesis that occurs under normal conditions we may call it "artificial." But on the other hand we must consider that physiologically self-sustaining organisms are not produced; we must remember that these processes become more and more divergent as this pseudo-development proceeds and that a normal larva is never produced even under favorable circumstances. Therefore we cannot speak of this development as parthenogenesis, meaning the production of a normal embryo from an unfertilized egg. The end result is always abnormal.

4. Relation of Differentiation and Cleavage

We may speak of differentiation as possessing a morphological or a physiological character, and in a wide sense the problem of the origin and process of differentiation is the problem of development. But in this discussion the term will be used to mean any specific morphological characters or physiological processes that are clearly homologous to characters or processes in the development of the fertilized egg. The definition has no reference to the accurate localization of organs and substances or to the correlation of processes necessary to normal development. Used in this sense the important differentiations that I have found in the unfertilized

egg of Amphitrite are the following: The early development of a
layer of ectoplasm and with it a tendency for the yolk to collect
centrally, the formation of cilia, the development of a brownish-
black pigment, the appearance of vacuoles that are found in the
fertilized egg of the same age, ameboid movements that are con-
nected with cleavage, and ameboid movements at a late stage that
are independent of cleavage. We have seen that these forms
of differentiation are relatively independent of cytoplasmic cleavage
and the expulsion of polar globules. What then is the nature of
these differentiations? The growth of cilia shows that there has
arisen in these regions a new specific composition of the cytoplasm.
The development of a pigment likewise indicates a chemical com-
position which was not present before. The rapid ameboid move-
ments occurring at a late period suggests a functional difference
in the cytoplasm, and the slow ameboid movements connected
with cleavage depend upon processes of differentiation which
modify the viscosity of the cytoplasm. The presence of vacuoles
at a definite period after development begins, points to the con-
clusion that they are caused by processes which have their basis
in the organization of the egg. The same may be said of the
early differentiation of a layer of ectoplasm and the processes
involved in the transformation of the asters and the mitotic
division of the nucleus. In all of these differentiations we find
evidence of preceding morphological organization or chemical
activity, a fact in agreement with the generally accepted idea that
morphological differentiation usually involves and is preceded by
differentiation of a chemical character. The nature of differentia-
tion therefore depends ultimately upon the organization of the egg,
which has its basis in cytological structure and specific chemical
composition. That the unfertilized egg of Amphitrite gives
evidence of both morphological and physiological organization is
shown by the following facts. Polarity is present from a very
early stage and the yolk is deposited in relation to this polarity.
The cytoplasm shows a difference in permeability and the definite
way in which the ripe egg collapses preceding maturation is cer-
tainly dependent upon the structure of the egg. But in addition
to these morphological characters we can prove the presence of

conditions in the egg which, if disturbed by mechanical agitation, lead to certain kinds of differentiation and development. The organization in many other eggs is well known; for example, the sea-urchin (Boveri, '01), Unio (Lillie, '01), Crepidula (Conklin, '02), the Ctenophore (Fischel, '03), and the Ascidian (Conklin,'03).

These phenomena of differentiation are relatively independent of each other and, since they are equally independent of cleavage, Lillie ('02) has pointed out that the cleavage, therefore, cannot be considered the cause of any of these differentiations, and we may add that the same is true of the processes involved in throwing off the polar bodies, unless one excepts the fact that the extra amount of chromatin is conducive to the formation of multipolar spindles. The development of cilia is relatively independent of nuclear division, for cilia are found on eggs that have many or very few nuclei. A similar relation holds for the development of vacuoles. On the other hand considerable nuclear division seems always to be present whenever pigment is found. In like manner transformation of the asters is always accompanied by division of the nucleus, and both processes are followed by more or less effectual attempts at cleavage. In regard to the rapid ameboid movements at a late stage, I have studied such a small number of eggs that it would be unsafe to draw general conclusions. Whenever observed these eggs had none of the other differentiations mentioned and were without cleavage, but the cytoplasm possessed a light appearance which is characteristic of normal development at about the same age.

It is well known that nuclear without cell division may take place normally in the eggs of Arthropods. Various methods have been used with success to produce the same process by artificial means; we have also seen how the ripeness of the egg determines whether the two processes shall occur together, the treatment being the same in each case. The study of enucleated, fertilized egg-fragments has added further data to this question. The multiplication and transformation of asters in such egg-fragments has been found to occur independent of the nucleus; it has been shown further that this may be found without (Boveri, '97), or with division of the cytoplasm (Zeigler, '98; Wilson, '01; Boveri,

M. '03). Boveri ('97) and Driesch ('98) have contributed the additional fact that the rhythm of cleavage in hybrid-fertilized egg-fragments depends upon the egg-cell and is not controlled by the sperm. It is probable then that the rhythm of cleavage depends upon the cytoplasm and not upon the nucleus. Conklin ('02) concluded that the position of the spindle is the result of movements and stresses in the cytoplasm and that the position of the spindle and direction of division are functions of the cytoplasm. He has also called attention to the importance of cytoplasmic movements in causing early differentiations in cell-divisions. Lillie ('98) demonstrated in the egg of Unio that the size of the cells and the rate and direction of cleavage possess a prospective significance and he concluded that these phenomena were explainable, "by the hypothesis of differentiation of the cytoplasm into materials of different qualities and positions." It will be remembered also that he found cytoplasmic without nuclear division in the egg of Chætopterus. Treadwell ('98) in comparing an egg with equal (Podarke) with another with unequal cleavage (Amphitrite), says, "There can be no question that in Podarke there is as great differentiation as in any Annelid of the unequal type." Wilson ('01) found that the nuclear area may give rise to a monaster which passes through transformations parallel to normal division. During telophase the egg frequently becomes ameboid and later it may actually divide into a number of irregular masses, one of which contains a nucleus. From this study of the parthenogenetic egg of Toxopneustes he drew the conclusion, "that any or all of the asters, whether connected with the nucleus or not, may operate as centers of cytoplasmic division, though if unconnected with the nuclear material the activity ordinarily goes no further than an abortive attempt to divide." Having mentioned some of the facts in regard to these phenomena let us return once more to the conditions in Amphitrite.

My observations show that the more nearly normal the asters and the astral radiations are, the nearer the cytoplasmic cleavage approaches the normal. That is, when the centrospheres develop into the typical large, clear areas and the astral radiations reach far into the cytoplasm with straight, strong, and clearly defined

rays, the cytoplasmic cleavage is normal. But when the centro-
sphere and asters are poorly developed, the cleavage of the cyto-
plasm is more or less incomplete or abortive, whether the egg be
fertilized or not. The same thing may be said in regard to the
relation between abnormalities in the asters and the division of
the chromatin, only in this case the fragmentary and unequal
division of the chromatin is apparent in late metaphase before the
aster has reached its full growth.

From a study of these facts it is seen that the development and
transformation of the asters is the most general of all these phe-
nomena. And since this is the process that invariably precedes
cleavage, we may consider it the expression of the active forces
which so alter the viscosity and surface tension of the egg-cell that
cleavage is the result. This view does not in any way conflict
with the conditions found in Unio and Crepidula, where the *place*
of cleavage is prearranged in the cytoplasm. Looked at from this
point of view cleavage is an incident correlated with certain
phenomena of differentiation, and its purpose is adaptive to the
needs of the organism. And if we regard the egg as a simple
mosaic, as all recent works seem to indicate, then cleavage is a
tendency to localize or isolate processes of differentiation by
separating them with cell walls. When each new cell wall becomes
established greater differentiation becomes possible. The form of
cleavage results from organization and differentiations in the egg,
and is not itself a process of differentiation.

5.· *Causes of the Cessation of Development*

In our description and discussion of the unfertilized egg, we
noted various conditions which hindered or stopped further
development. But the facts given, instead of being causes for no
further development, were in most cases simply a statement of the
conditions within the egg after it had become so abnormal that it
was incapable of regulating or correlating further processes.
Let us see if we can trace these conditions to more immediate
causes. From our understanding of the relation of cleavage to
differentiation, it is easy to see why there is no further develop-
ment in unripe eggs. For in these eggs the viscosity of the cyto-

plasm is unfavorable to cleavage and lack of cleavage prevents the localization of various differentiating processes. Even in the fertilized eggs where the cytoplasm is not quite ripe we find the same retarding effects and ultimately the same cessation in development. The interesting question for us then is, Why does the development of the unfertilized eggs stop though they are apparently in a ripe condition? In such eggs polar bodies are formed in quite the normal manner, the early cleavages have the same outward appearance and practically the same rhythm as the fertilized egg. That development ceases is not from a lack of nutritive material for this is sufficient in quantity and in a condition suitable for metabolism. It is not from a lack of formative material for the entire egg is present and the cytoplasm is of a proper ripeness. But whenever abnormalities appear they are first found in connection with the asters, and following or accompanying them we find unequal and fragmentary divisions of the chromatin. Abnormal phenomena in all other respects appear later. It is therefore logical to conclude that the cessation of development has some intimate connection with the developmental processes concerned with the growth and transformation of the asters, and that the latter process is in close relation with the periodic nuclear changes. Let us examine still other conditions found in the egg.

The entrance of the sperm certainly causes profound changes in the cytoplasm since no other sperm then finds it possible to enter the egg. That the effect of the various salts I have used is not the same as that of the sperm is proved by the fact that swimmers may be developed by these methods from eggs that are too unripe to be fertilized by the sperm. In the one case the sperm is kept out by the unripe cytoplasm, in the other the solution penetrates or alters the cytoplasm and causes the germinal vesicle to break down, thus setting free forces that lead to nuclear division and other phenomena of development. But the fact that some of these eggs may be started in development by mechanical agitation indicates that simply a change in the state of the protoplasm is sufficient to start development. Mathews and Whitcher ('03) have concluded that, "For artificial parthenogenesis nothing else

is necessary than that this change be produced." Nevertheless we have seen that something else is necessary in order to insure the development of normal larvas, and that this something has to do with asters and chromatin. It is probable that agitation in some way causes the walls of the germinal vesicle to break down and thus permits reactions between nucleus and cytoplasm. Now it is clear that the effect of the methods I have used to produce artificial parthenogenesis is not to cause a cessation of development for the end result is practically the same in each case. Primarily of course the egg lacks the elements introduced by the sperm, the most important being the male chromosomes and the sperm centrosome. But inasmuch as normal mitosis may take place without the sperm, and on account of the inconstant presence of a distinguishable centrosome in eggs of Amphitrite, it is evident that we may neglect these factors so far as the question under consideration is concerned. In any case they can bear only a subsidiary relation to cleavage and differentiation.

Perhaps no other fact is so evident, and at the same time so little understood, as the reaction of nucleus and cytoplasm in development. When these relations are solved there is little doubt that we shall obtain thereby at least some of the causes of morphogenic metabolism. We know that by supplying chemically-known food constituents we may produce the growth and reproduction of the yeast-cell, and in its development it follows ordinary laws of chemical reaction. There are many reasons to believe that similar processes take place in the animal cell. But here the chemical complexity hides from us at present the exact nature of many of the processes of development and differentiation which could be easily understood. All my experiments indicate in a most striking manner the intimate relation that exists between cytoplasmic and nuclear differentiation; the correlation in development between these two factors is very complete where a normal organism results. And inasmuch as the cessation of development is a culminative process, that is, the abnormalities appear in successive transformations of the asters and nucleus, we must look upon the cessation of development as due to incomplete reactions between the nucleus and the cytoplasm, each suc-

cessive reaction depending in some measure at least upon the preceding one. In applying this conception to Amphitrite, the question is still left open as to whether the cessation of development is a qualitative or quantitative divergence from the normal course of events. While it is probably true that the nuclear material derived from the male is qualitatively different from that derived from the female, as is shown by inherited characters, so far as the phenomena found in the unfertilized eggs of Amphitrite are concerned, the abnormality reveals itself first as a quantitative and not as a qualitative difference. I refer to eggs where only half the number of chromosomes are present. Now, where two chemical substances are combined in definite proportions to produce a series of chemical phenomena, if only one-half the required amount of one of these be taken, it is evident that the series of reactions will be incomplete, and the process will come to an end sooner than under normal conditions; we may suppose the reaction in the beginning differs simply in quantity from the normal, and that qualitative differences may possibly appear later due to reactions set up with the substance found in excess. This conception then applies to the egg treated with calcium nitrate where both polar bodies are expelled; the first mitotic division does not differ in kind from the normal but only in the number of chromosomes that undergo splitting at metaphase.

The conditions of my experiments have been such that in ripe eggs either both or no polar bodies were expelled. If a means could be devised to retain the chromosomes of the second polar body within the ripe egg, it would be interesting to note if normal development occurred. For we should then have the normal number of chromosomes. In the potassium chlorid more than the normal number are present; connected with this is a tendency to form multipolar asters as in double-fertilized eggs, though the normal number of chromosome vesicles or only half this number may be present in the telophase of the first cleavage division. The frequent occurrence of the multipolar asters might be explained as due to a tendency of the excess of chromatin to collect in more than one vesicle during the telophase of the preceding division. Or, it may be a tendency for the chromosomes that

normally constitute the polar bodies to maintain their individuality in groups. But in all cases the development stops, and it cannot always be due to an insufficient amount of chromatin. While the suggestion of Boveri ('02) that the chromosomes are qualitatively different, may be accepted as a possibility, it seems probable that in Amphitrite the sperm chromosomes are a means of producing morphogenic processes which the normal number or half the normal number of female chromosomes may start but find it impossible to continue for any length of time. However there is still the possibility that the strong solution so affects the cytoplasm that it is incapable of reacting on the nucleus in the normal manner.

SUMMARY OF RESULTS

Normal Egg

1. In general, the early development (maturation, fertilization) conforms to the typical Annelid type. For cleavage and later development, so far as my observations go, I have verified the work of Mead.

2. Under normal conditions the egg is retained in the body cavity until the first maturation spindle is in metaphase. When deposited and left undisturbed the egg rests in this condition. If fertilized the formation of the polar bodies and the ensuing development follows.

3. The ripe egg shows a distinct polarity as evidenced by the eccentric position of the germinal vesicle. This polarity is found before any yolk is laid down in the cytoplasm and the deposition and arrangement of the yolk is apparently determined in relation to the nucleus. There is thus indicated a definite structural organization of the egg at a very early period.

4. Peculiar and perfectly definite cytoplasmic changes occur in connection with the formation of the polar globules.

5. The reduced number of chromosomes is eleven, the somatic number twenty-two. The eleven chromosomes found at the metaphase of the first maturation division are probably derived from eleven groups of chromomeres (chromosomes) of four each.

6. The rate of development is comparatively rapid. The primary germ layers are entirely separated at the 64-cell stage, and blastulas swim within four to five hours after fertilization.

Unfertilized Eggs

1. Under the conditions of the experiments certain forms of development occur with or without cleavage and with or without the formation of polar bodies in the unfertilized egg of Amphitrite. Such development takes the form of nuclear divisions, the early differentiation of a layer of cytoplasm, the growth of cilia, the appearance of vacuoles that are found in the fertilized egg of the same age, the development of a brownish pigment, the ameboid movements of the cytoplasm that are connected with cleavage, the ameboid movements at a later stage of development that appear entirely independent of cleavage, and the change in shape of the egg which in most cases at least is connected with incomplete, arrested or abortive division of the cytoplasm. The apical tuft of cilia which is characteristic of trochophores from fertilized eggs is always absent.

2. The means employed for producing this development were, (*a*) the use of certain salt solutions and (*b*) some method of agitating the eggs.

3. The calcium nitrate solution used stimulated the formation of polar bodies, produced nuclear division, and tended to cause a fusion of the cleavage cells. The potassium chlorid prevented the formation of the polar bodies, stimulated division of the nucleus, and where cell division was complete tended to separate the blastomeres.

4. Though some eggs would occasionally stick together no actual fusions took place.

5. Cleavage when present is usually abnormal; it tends to grow more abnormal as development proceeds and I have never seen a perfectly normal type of cleavage at a late stage. The segmentation cavity is therefore wanting or only partially developed. There was no cytoplasmic without preceding nuclear division.

6. The cleavage asters, especially the astral radiations, fre-

quently show imperfections at the first cleavage. The rays tend to be weak and fragmentary and do not take such a clear, definite stain as the normal.

7. Nuclear division is always mitotic. It may be equal at first but sooner or later becomes unequal and fragmentary. In the potassium chlorid solutions multipolar mitoses are common; this is undoubtedly associated with the extra amount of chromatin retained in the egg. In later stages a nuclear vesicle may enlarge extraordinarily unless mitosis occurs.

8. Normal polar bodies are expelled in the weaker calcium nitrate solution if the eggs are in a ripe condition. If the cytoplasm is not ripe the egg does not alter its shape; consequently the maturation spindle though normal in other respects does not come to the surface and the polar bodies are not expelled. Solutions with high osmotic pressure prevent entirely or permit only partial formation of very weak asters and spindle that are incompetent to cause maturation division. Moreover the chromosomes swell up into large vesicles at this time.

9. The "morula" of Fischer is in all probability not a form of development, since no differentiations characteristic of normal development are ever found.

10. Amphitrite eggs are very susceptible to agitation, especially at certain periods of development.

11. Results obtained from any experiment depended much upon the ripeness of the given lot of eggs. The riper the eggs, the more rapidly does differentiation take place and the nearer it approaches the normal development.

12. The rate of development is always slower than for the fertilized eggs. The germinal vesicle breaks down sometimes within a few minutes, sometimes several hours after being treated with the solution. The first swimmers are usually noticed seven to ten hours after the beginning of the experiment, and ordinarily the maximum number of swimming eggs is found between the twelfth and twenty-fifth hours.

13. It has not been found possible to produce physiologically self-sustaining organisms by these methods. The differentiation always diverges more and more widely from the normal and con-

sequently we do not find in the late stages any regulation of abnormalities or any true correlation.

14. That death is not caused by environment is proved by the fact that fertilized eggs may be raised under the same conditions.

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DESCRIPTION OF FIGURES.

The essential features of all figures were drawn with a camera lucida. The tube length used is written in (cm.) immediately after the size of the ocular.

Reference letters.—*a*, Apical tuft of cilia; *c*, chromomeres (chromosomes); *d*, egg membrane; *e*, brownish pigment; *f*, female chromosome vesicle (vesicles); *g*, polar globules; *l*, layer of ectoplasm; *m*, male pronucleus; *me*, mesoderm cells; *n*, nucleus, nuclear areas; *nu*, nucleolus; *p*, peri-vitelline space; *pr*, prototroch; *pa*, paratroch; *r*, region affected by nuclear sap; *s*, segmentation cavity; *v*, vacuoles; *x*, clear spot left by nucleolus; *y*, yolk granules (yolk).

Plate I.

All figures to illustrate the normal development.

Fig. 1. Unfertilized egg after breaking down of germinal vesicle. Shows collapse of the egg, origin of asters, formation of chromosomes, dissolution of nucleolus, and a region differentiated by escape of nuclear sap. Leitz oc. 1 (20), obj. 1–12.

Fig. 2. Unfertilized egg; asters better developed but other conditions perhaps not quite so advanced as in Fig. 1. Leitz oc. 1 (20), obj. 1–12.

Fig. 3. First maturation spindle forming; nucleolus has dissolved leaving a clear spot. Leitz oc. 4 (20), obj. 1–12.

Fig. 4. First maturation spindle has rotated and now lies in the polar axis; chromosomes in early prophase. Leitz oc. 4 (20), obj. 1–12.

Fig. 5. Metaphase of first maturation spindle; peri-vitelline space formed. Leitz oc. 4 (20), obj. 1–12.

Fig. 6. Late anaphase, first maturation. Leitz oc, 4 (20), obj. 1–12.

Fig. 7. Condition just after expulsion of the first polar body. Leitz oc. 4 (20), obj. 1–12.

Fig. 8. Late anaphase, second maturation spindle. Fifteen minutes after fertilization. Leitz oc. 4 (20), obj. 1–12.

Fig. 9. Shortly after expulsion of the second polar body. Fifteen minutes after fertilization. Leitz oc. 4 (20), obj. 1–12.

Fig. 10. Age, after fertilization, twenty minutes. The male pronucleus in central position; female chromosomes passing into the vesicular condition and retreating toward the center of the egg. Leitz oc. 1 (18), obj. 1–12.

Fig. 11. The greatly enlarged pronuclei just before fusion. Age, twenty-five minutes. Leitz oc. 4 (20), obj. 1–12.

Fig. 12. Metaphase of first cleavage division; age thirty minutes. The asters affecting a large area enclosed within a dotted line. Leitz oc. 1 (18), obj. 1–12.

Fig. 13. Early telophase of first cleavage division. Age, forty-one minutes. Leitz oc. 1 (18), obj. 1–12.

Fig. 14. Asters dividing for second cleavage division. Age, forty-eight minutes. Leitz oc. 1 (18), obj. 1–12.

Fig. 15. Sagittal, horizontal (nearly) section of early gastrula; cilia not shown. The mesoderm consists of four cells, two small ones not shown in this section. Dotted lines enclose nuclei. Nuclei of some cells moving centrally, a characteristic of invaginating entoderm. Leitz oc. 3, obj. 1–12.

Fig. 16. Normal gastrula twenty-three hours old; camera drawing from living embryo; only some of the more characteristic details shown.
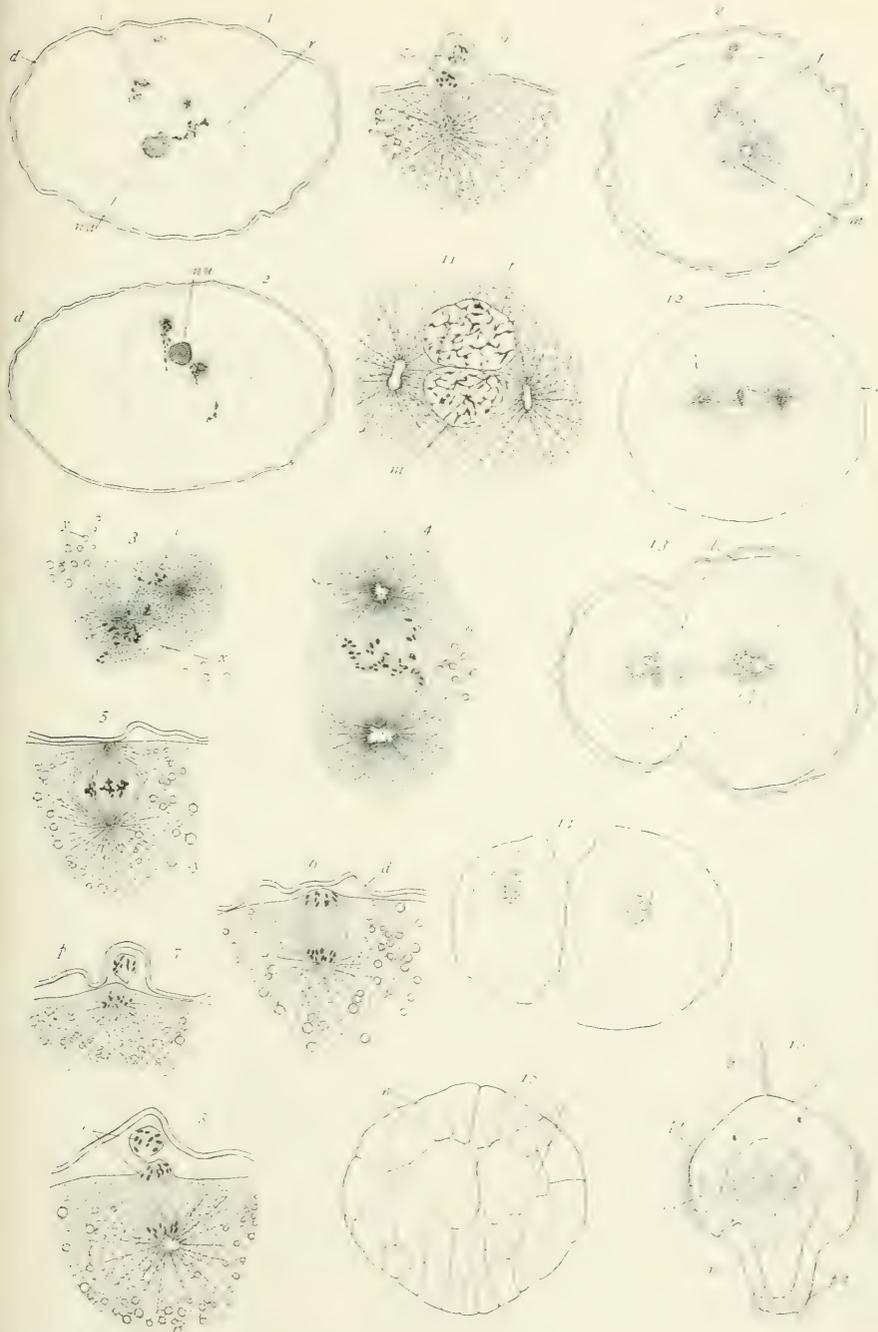
PLATE II.

All figures, except 33 from a whole mount, are from living material. Figs. 17–22 and 30 are from eggs treated with potassium chlorid, method 3 of text; Figs. 23–29 and 31–33 are from calcium nitrate cultures, method 1; 34–38 from calcium chlorid cultures, method 5; 39 from swimming egg produced by shaking. For the age of an egg, the time after the beginning of the experiment is given.

Fig. 17. Age, four hours and fifty minutes. Loose arrangement of cells; nuclei shown in dotted lines.

Fig. 18. First one seen to move in this experiment; age, six hours and twenty-eight minutes.

Fig. 19. Egg taken from the bottom of the dish; age, nineteen hours. Ectoplasm well differentiated from the yolk.

Fig. 20. Age, twenty-three hours and twelve minutes. Swimming at surface. Unusual amount of cleavage, one nucleus to each cell; vacuoles present. Type of more active swimmers.

Fig. 21. Age, twenty-three hours and twenty minutes; from bottom of dish. No cleavage; two large, clear spots (nuclear areas) were to be seen.

Fig. 22. Age, twenty-one hours and twelve minutes. A type intermediate between Figs. 20 and 21; egg composed of four cells, but incompletely divided; cilia on one cell.

Fig. 23. Age, twelve hours. No cleavage; bluntly pear-shaped. Leitz oc. 1, obj. 7.

Fig. 24. Age, twenty-three hours and twenty minutes. Comparatively little cleavage, cells not completely separated. Leitz oc. 1, obj. 7.

Fig. 25. Age, nineteen hours and thirty minutes. Incomplete cleavage. Leitz oc. 1, obj. 7.

Fig. 26. Age, twenty hours. Common type; no cleavage; several nuclei; brownish pigment in two regions; moderately active. Leitz oc. 1, obj. 7.

Fig. 27. Age, twenty hours. Four cells; many nuclei in the large cell in which pigment is developed. Leitz oc. 1, obj. 7.

Fig. 28. Age, twenty-two hours and thirty minutes. Common type; incomplete and probably partly fused cleavage planes; many nuclei; pigment in four regions. Leitz oc. 1, obj. 7.

Fig. 29. Age, twenty-eight hours. One type of most active swimmers; no cleavage; many nuclei; pigment in two regions. Leitz oc. 1., obj. 7.

Fig. 30. Age, thirty-seven hours and twenty-five minutes. Comparatively few cells and nuclei, but these are distinct; from bottom of the dish.

Fig. 31. Age, forty-one hours. Swimming actively; no cleavage, but with an attached yolk lobe; many nuclei; pigment in four regions. Leitz oc. 1, obj. 7.

Fig. 32. Age, forty-eight hours. Oldest actively swimming embryo found; indications of one cleavage plane; very many nuclei (comparatively); diffuse pigment in one region. Leitz oc. 3, obj. 5.

Fig. 33. Age, fourteen hours. From stained whole mount; no cleavage; nuclei vary greatly in size; one or more nucleoli in most of the nuclei. Leitz oc. 1 (15), obj. 1–12.

Fig. 34. Age, nine hours. Intermediate type of cell and nuclear division.

Fig. 35. Age, twenty hours. Much pigment; bluntly pear-shaped, due to early cleavage conditions.

Fig. 36. Age, twenty hours. From same experiment as preceding; three incompletely divided cells, with one nuclear area in each.

Fig. 37. Age, twenty-five hours. Very active swimmer; no segmentation; three very large nuclear areas; much pigment.

Fig. 38. Age, twenty-three hours. No cleavage; considerable nuclear division.

Fig. 39. Produced by shaking. Swimming near surface at about twenty hours.
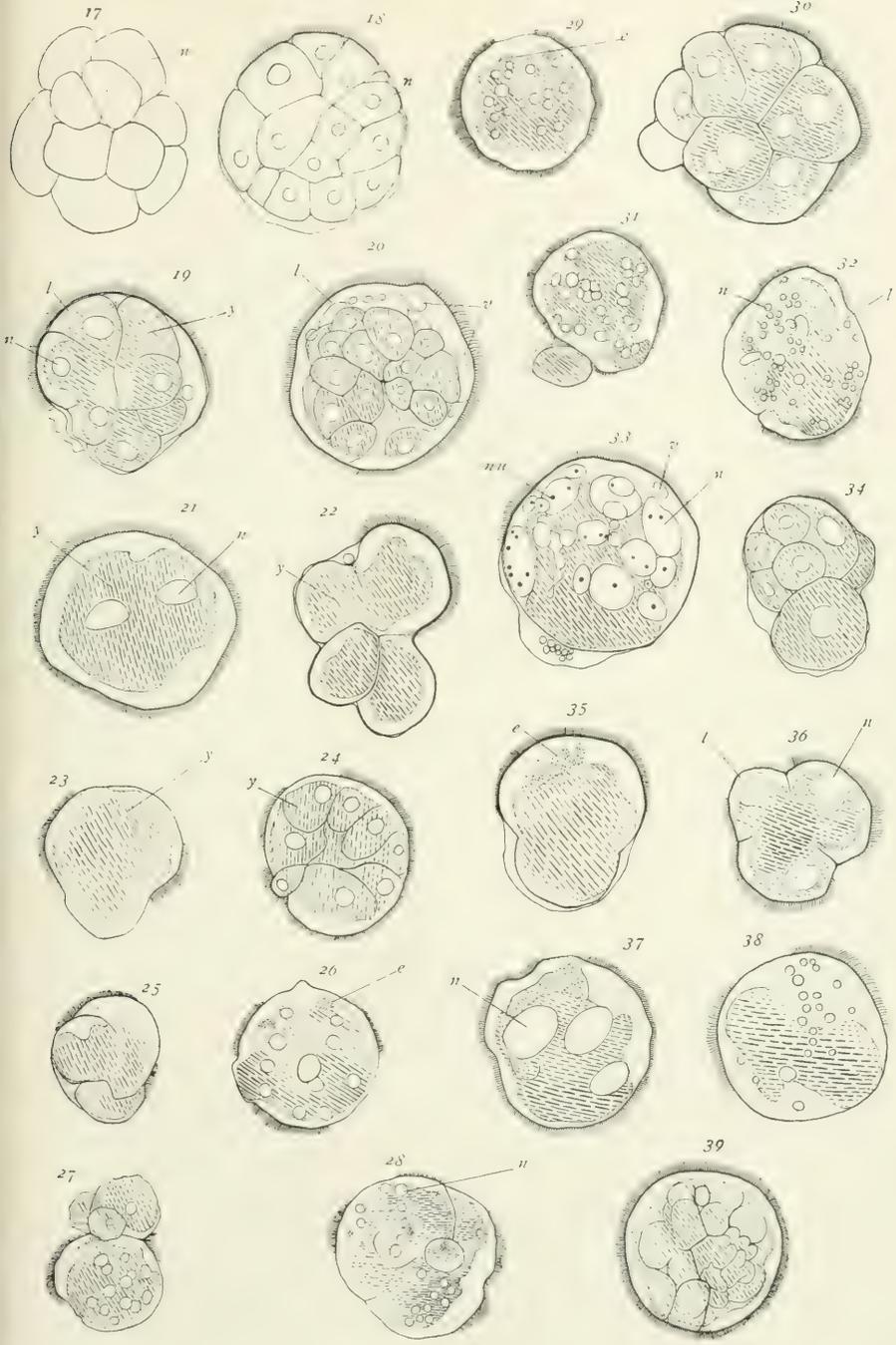
PLATE III.

All figures of this plate are from sections of eggs treated with calcium nitrate. The age is dated from the time the eggs were put in the solution.

Fig. 40. Unripe egg. Age, fifty-six minutes. No collapse in polar diameter; first maturation spindle in metaphase, undergoing mitosis without moving to the surface; thick egg membrane characteristic of unripe condition. Leitz oc. 1 (20), obj. 1–12.

Fig. 41. Unripe egg. Same age as preceding; in late anaphase.

Fig. 42. Unripe egg. Age, two hours and twenty-six minutes. First maturation spindle in anaphase has advanced part way to the surface; unequal division of chromosomes. Leitz oc. 4 (20), obj. 1–12.

Fig. 43. Very unripe egg. Age, three hours and fifteen minutes. Development very slow; very abnormal division of chromosomes; asters still in position of origin. No polar bodies formed, Figs. 40–43. Leitz oc. 4 (20), obj. 1–12.

Fig. 44. Both polar bodies expelled, each one slightly abnormal; female chromosomes passing into vesicular condition. Age, forty-one minutes. Leitz oc. 1 (20), obj. 1–12.

Fig. 45. First polar body nearly normal, second polar body too large. Age, twenty-four minutes. Leitz oc. 4 (20), obj. 1–12.

Fig. 46. Polar bodies abnormal, the second very much enlarged with yolk granules; the female pronucleus in preparation for first cleavage division. Age, sixty-eight minutes. Leitz oc. 1 (20) obj. 1–12.

Fig. 47. Unripe egg. First polar body partly expelled; rest of very abnormal chromosomes have retreated to near center of egg. Age, three hours and fifteen minutes. Leitz oc. 4 (20), obj. 1–12.

Fig. 48. Division of the nucleus without cleavage; no polar bodies. Age, sixty-eight minutes. Leitz oc. 1 (20), obj. 1–12.

Fig. 49. Polar bodies (not shown); groups of chromosome vesicles removed less than a normal distance from one another; eleven vesicles in each group; abortive cleavage results. Age, sixty-eight minutes. Leitz oc. 1 (20), obj. 1–12.

Fig. 50. Three-celled egg (drawn from three sections). Age, one hour and forty minutes. Leitz oc. 1 (17), obj. 1–12.

Fig. 51. Typical incomplete cleavage. Age, fourteen hours. Leitz oc. 1 (20), obj. 1–12.

Fig. 52. Typical of condition with no cleavage; many nuclei, which vary greatly in size; some vacuoles. Age, twenty hours. Leitz oc. 1 (19), obj. 1–12.

Fig. 53. Typical of condition when cleavage is present; few cleavage planes complete; vacuoles. Age, twenty hours. Leitz oc. 1 (20), obj. 1–12.

Fig. 54. The most advanced and most nearly normal type of cleavage found in my sectioned material; the mesoderm has invaginated; vacuoles present. Age, twenty hours. Leitz oc. 1 (18) obj. 1–12.
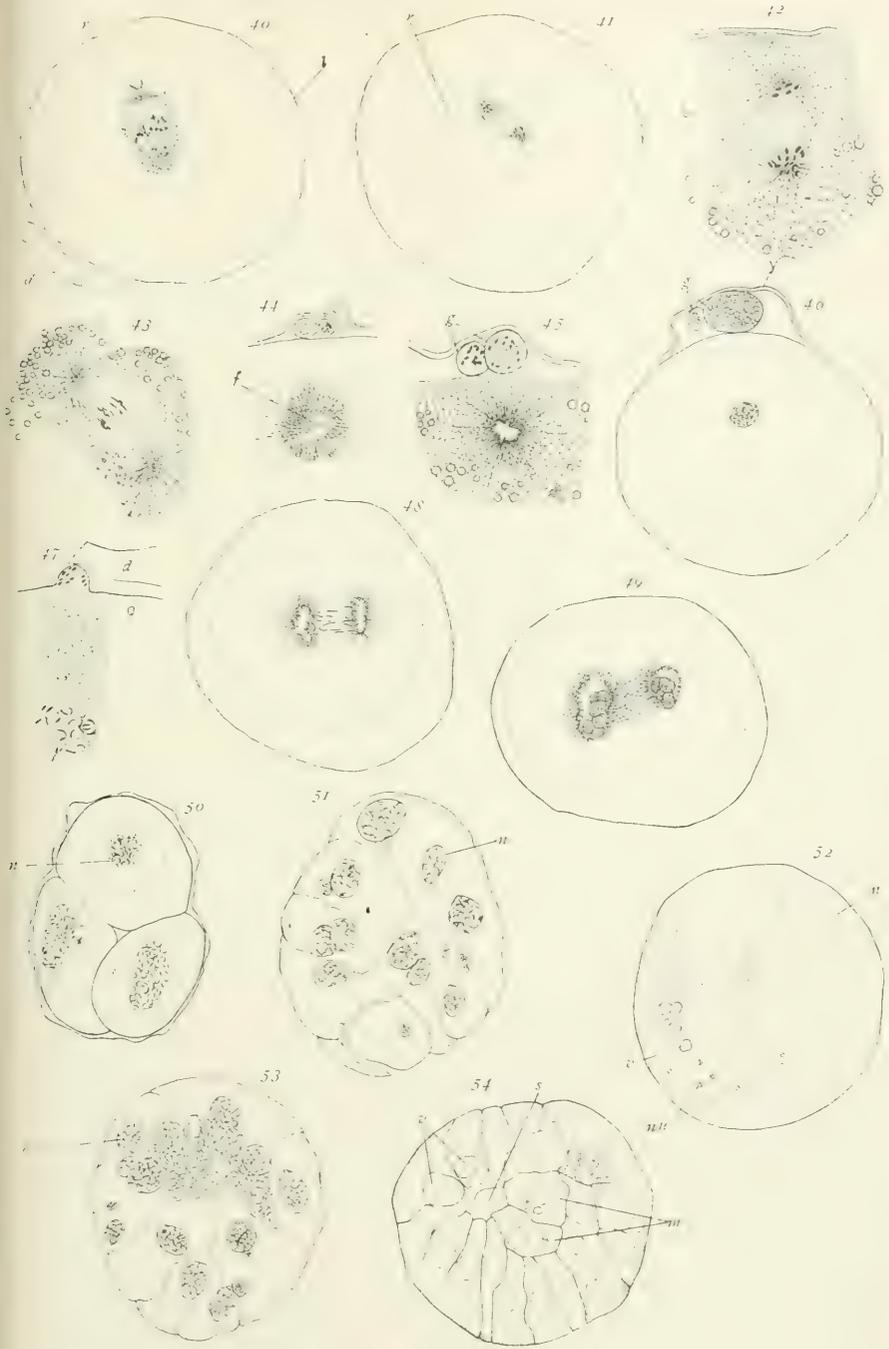
PLATE IV.

Figures of this plate are all from eggs treated with potassium chlorid.

Fig. 55. Condition during maturation; one aster present; chromosomes in vesicular condition and greatly enlarged; no collapse in polar diameter. Age, one hour and thirty-eight minutes. Leitz oc. 1 (18), obj. 1–12.

Fig. 56. Maturation phenomena; both asters and a faint spindle present; egg did not collapse. Age, one hour and thirty-eight minutes. Leitz oc. 1 (18), obj. 1–12.

Fig. 57. Prophase of first cleavage division. Age, two hours and forty-three minutes. Leitz oc. 3, obj. 1–12.

Fig. 58. Anaphase of first cleavage division. Cleavage in such eggs usually abortive. Age, two hours and seventeen minutes. Leitz oc. 3, obj. 1–12.

Fig. 59. Telophase of first cleavage divisions; 20 (+4?) chromosome vesicle in the right hand group, 18 (+4?) in the left hand group. Cleavage in such eggs is frequently complete. Age, two hours and twenty-five minutes. Leitz oc. 3, obj. 1–12.

Fig. 60. Two-celled stage; division complete; eleven enlarged chromosome vesicles in each cell. Probably indicates one origin of multinuclear cells. Age, three hours and fifty-five minutes. Leitz oc. 3, obj. 1–12.

Fig. 61. Multipolar asters; anaphase of the second division. Chromatin division irregular and unequal. One method of origin of multinuclear cells. Age, three hours and fifty-five minutes. Leitz oc. 3, obj. 1–12.

Fig. 62. Cleavage planes complete; irregular and unequal division of chromatin in one cell. some cells multinuclear; diffuse chromatin stain near center of egg indicates original position of the germinal vesicle. Age, four hours and forty-two minutes. Leitz oc. 3, obj. 1–12.

Fig. 63. Several vesicles in each cell; cleavage planes complete. Age, four hours and forty-two minutes. Leitz oc. 3, obj. 1–12.

Fig. 64. Egg showing typical cleavage. Mitotic figure in one cell; more than one nuclear vesicle in others; part of segmentation cavity present. Age, eight hours and forty-seven minutes. Leitz oc. 3, obj. 1–12.

Fig. 65. Egg typical of incomplete cleavage with probably later fusion. Nuclei vary greatly in size. Age, eight hours. Leitz oc. 3, obj. 1–12.

Fig. 66. Typical of eggs with no cleavage. A few large nuclei; large vacuoles. Age, twenty hours. Leitz oc. 3, obj. 1–12.

Fig. 67. One of the most advanced types of cleavage found in this solution. Evident invagination of the mesoderm. Age, twenty hours. Leitz oc. 3, obj. 1–12.
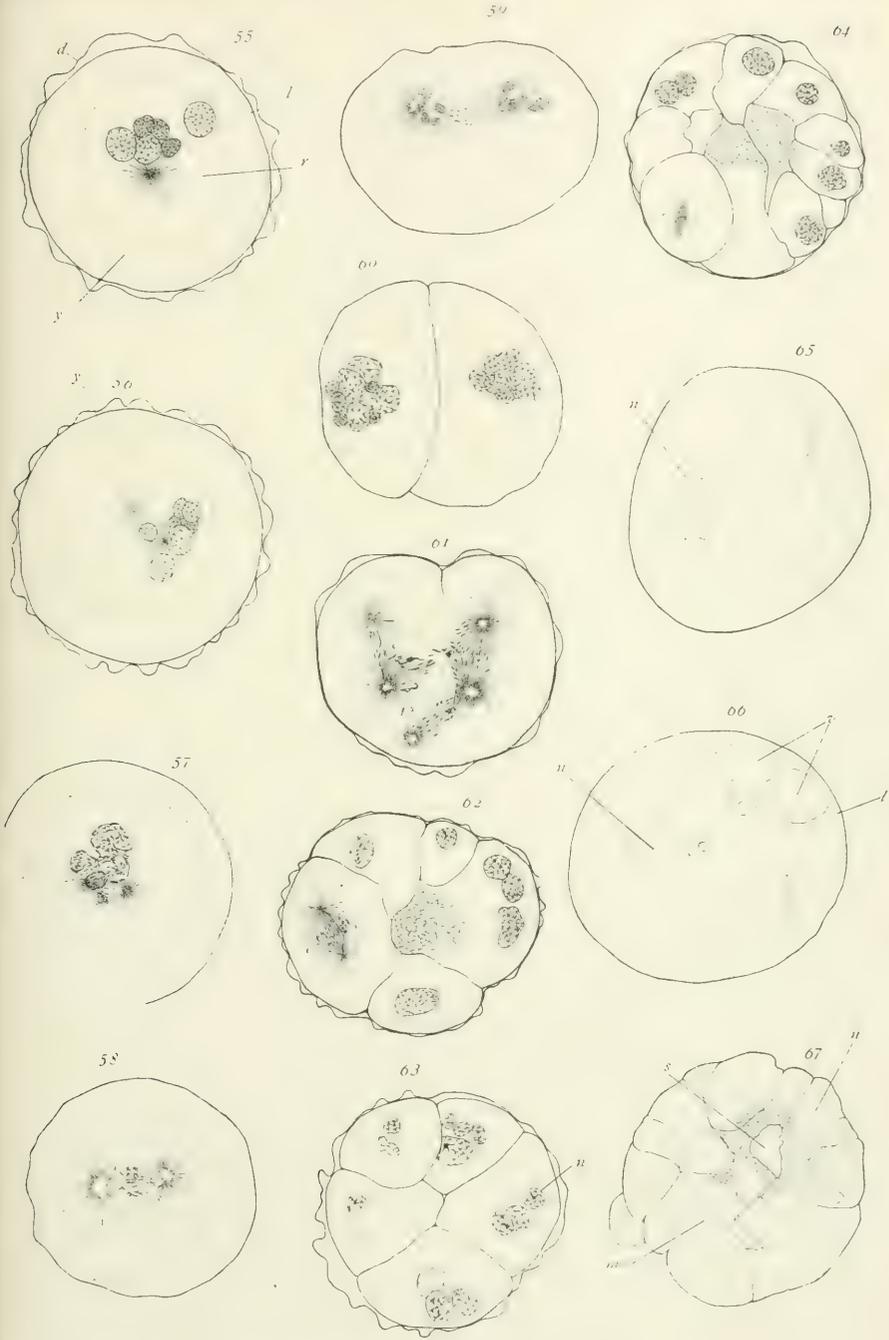
THE DEVELOPMENT OF FUNDULUS HETEROCLITUS IN SOLUTIONS OF LITHIUM CHLORID, WITH APPENDIX ON ITS DEVELOPMENT IN FRESH WATER

BY

CHARLES R. STOCKARD

WITH NINETEEN FIGURES

The eggs of Fundulus heteroclitus are hardy and, therefore, stand experimental treatment most satisfactorily. Since they are capable of development in both salt and fresh water it is possible to eliminate all of the physical effects of hypertonic solutions, for effective solutions of a salt may be applied in fresh water that have a final pressure lower than that of normal sea water. Mathews has stated that the eggs of this fish are peculiarly adapted to treatment with solutions because they appear to be easily penetrated by nearly all sorts of ions, and they are quite insensitive to variations in osmotic pressure of the solutions. Whether this statement is absolutely true or not there can be little doubt of the fact that the ions resulting from LiCl in solution do penetrate the membranes and give an effect that is certainly not due to osmotic pressure, since the same result is obtained with solutions of LiCl in both salt and fresh water, the one being hypertonic while the other is a hypotonic solution. Whether or not the membranes are easily or quickly penetrated is a question better answered by a study of the results.

The fact that lithium salts produce a marked and really specific effect on development was observed first in 1893 by Herbst while studying the eggs of the sea-urchin. The definite type of embryo thus produced was termed by him the lithium larva. Soon after this several investigators studied the action of lithium salts on the development of the frog's egg and found that it induced decided effects, yet they failed to show that a distinct type of embryo

resulted, although Gurwitsch concluded that his radial larva was the specific result of the lithium action. Morgan then in 1902 carried on experiments to test the effects of LiCl on frog's eggs, and again in the following spring he used a number of lithium salts along with other solutions for comparison. He finally concluded that lithium produced in the development of the frog's egg a typical larva just as Herbst had shown for the sea-urchin. Both of these workers claimed for similar reasons that the effect was due to the chemical action of the lithium ion and not to osmotic or pressure effects; hence the specific character of the embryos. Loeb, Mathews and others have subjected the eggs of the fish, Fundulus heteroclitus, to solutions of lithium salts, but only to observe the physiological effects of the solutions, ignoring the morphological changes induced.

With these results in view Prof. T. H. Morgan kindly suggested that I study, from a morphological standpoint, the effects of lithium salts on the fish's egg. It is, therefore, a pleasure to avail myself of this opportunity of expressing my indebtedness to Professor Morgan for his kindly advice and criticism throughout the progress of this work. The experiments were conducted during the past summer while occupying a table in the United States Fish Commission Laboratory at Wood's Hole, and I wish to thank the authorities of this laboratory, particularly the director, Dr. F. B. Sumner, for the courtesies extended me while there.

The Fundulus egg differs in its mode of development from both that of the sea-urchin and the frog, hence it is of interest to note whether or not an effect such as that produced by lithium is at all comparable in the three cases. Of course it is fully realized that no strict embryological comparison should be made among types of development so different as these, yet one may find little objection to a comparative study of the chemical effects produced in the three widely different forms by one and the same element, lithium. At present I am not in a position to state that the abnormalities which have been induced in the Fundulus egg by means of LiCl are specific for lithium, as a number of other salts must first be experimented with. The abnormalities described are not exceptional, but general, occurring in as large a per cent.

of the eggs as could be expected. All of the experiments were repeated frequently—some as often as a dozen times—and always with constant results.

METHOD

The following treatment and precautions were used throughout the experiments. It was decided to keep the eggs as nearly as possible under normal conditions by using solutions of the LiCl in ordinary sea water, and to run experiments with fresh and distilled water solutions merely as checks on these. Normal sea water controls were carried in each experiment and as a further check eggs were kept developing in ordinary fresh and distilled water. A series of solutions of LiCl in sea water was prepared in strengths $\frac{1}{8}$ n, $\frac{1}{4}$ n, $\frac{3}{8}$ n, $\frac{1}{2}$ n, $\frac{5}{8}$ n, $\frac{3}{4}$ n, $\frac{7}{8}$ n, and normal, a normal solution of LiCl being equivalent to about a 4.25 per cent. solution; the eggs were then subjected to these to ascertain the various effects of different strength solutions. It was found that those solutions weaker than $\frac{5}{8}$ n produced no apparent effect during the early stages of development. In the $\frac{3}{4}$ n, $\frac{7}{8}$ n, and normal solutions the eggs showed varying degrees of abnormalities, the ones in the stronger solutions dying after a few hours. A series was then prepared between $\frac{5}{8}$ n and $\frac{3}{4}$ n so as to obtain different degrees of abnormal development. These solutions were 2.62 per cent., 2.82 per cent., 3.02 per cent. and 3.22 per cent. LiCl or .62 n, .66 n, .71 n and .76 n. These four strengths were then used in all of the experiments. A fresh water series was also prepared and I found, as Mathews had, that a $\frac{1}{4}$ n solution was the minimal poisonous dose, that is, the weakest solution preventing the formation of an embryo; it will be noted that this is only about one-half the strength required to affect the eggs when in a sea water solution of LiCl. A $\frac{7}{20}$ n or 1.48 per cent. LiCl solution in distilled water was resorted to, though its effect was rather severe, being about the same as that produced by a 3.22 per cent. LiCl solution in sea water. Controls were always taken from the same bunch of eggs as those on which the experiments were performed. It is quite noticeable that Fundulus eggs withstand solutions many

times stronger than those used to affect the eggs of the sea-urchin and frog.

When Fundulus eggs are placed in LiCl solutions shortly after fertilization, or before the two-cell stage, a very constant modification in the early stages of development will be noticed. The protoplasmic cap or blastoderm becomes unusually prominent, bulging up in an arched fashion that is much more marked than in normal eggs. After reaching about the sixty-four cell stage and later, a clear bubble-like appearance is noted in the living eggs below the disc (Figs. 1 and 2). On sectioning and staining the blastoderms at this stage it is found that the bubble-like appearance is due to the greatly enlarged condition of the segmentation cavity (Fig. 13). The central periblast has been forced from its normal place close below the blastoderm (Fig. 14) and pushed down into the yolk mass at the same time causing the cap to arch more decidedly above on account of the strain thus induced about its periphery. This certainly looks like an osmotic effect but one fails to see how it could be attributed to such a cause when it is remembered that the same condition exists both in the sea water LiCl solution and in the distilled water solution (compare Figs. 1 and 2), the one being hypertonic while the other is hypotonic and thus in the two cases opposite, and not equal, effects would be expected.

Lithium chlorid in all cases most obviously delays the development (compare Figs. 1, 2, 3 and 4 with 11; 5 and 8 with 6). In the stronger solutions, eggs as old as forty-three hours, show the blastoderm still as a polar cap and in these older stages it presents a most interesting appearance. On examining such living eggs the blastoderm is found to be greatly raised and in some cases almost pinched away from the yolk. When one looks down on the top of this blastoderm it is seen to suggest very strongly a gastrula of some holoblastic egg such as that of a sea-urchin or starfish, the center appears thin while the peripheral curved surface has a much thicker appearance as if the center showed below it the blastopore and the sides represented the

folded double wall of the gastrula seen in profile. Stages were seen in which this "blastopore" appeared very large as if invagination was just beginning, and other stages showed various degrees of decrease in the size of the blastoporic appearance (Figs. 15 and 16). These eggs finally died, in many cases on account of the blastodermic edges becoming approximated, thus pinching the cap entirely away from the yolk. On studying sections of these peculiar forms it was found that as the segmentation cavity became abnormally large, thus pushing the blastodermic roof up into a more decided arch, the edges or periphery of the blastoderm were brought closer together at the same time becoming thicker and showing more cell layers than were seen in the crown or top of the disc (Figs. 15 and 16). The periblast seems more loosely connected with the blastoderm than is usually the case; thus it is readily understood how the latter may finally pinch away from the yolk mass—compare Figs. 13, 15 and 16. These figures also make clear the manner in which the blastopore-like image in the living egg seems to decrease in size—as if a blastopore was closing; this is merely the visual effect produced by the decreasing circumference of the blastodermic periphery as it becomes puckered together like the mouth of a sac. No dividing cells were found in these sections of the blastoderms, and when it is recalled how abundant such cells usually are in sections of fish blastoderms at this stage we are struck with the inhibition or decrease in division rate caused by the LiCl, and hence the slow rate of growth. Normally a forty-hour egg would have the young fish well formed. No indication of invagination was shown about the edges of these blastoderms, but it may be possible that if eggs in this condition were transferred from the strong solutions back to sea water that the slight recovery thus induced might cause them to continue development and perhaps to slightly invaginate as this appears to be the easiest direction of growth. Lack of material during the latter part of the season prevented the trial of such an experiment.

Many eggs die when the blastoderm becomes confined to the polar region as is indicated above, but in rare cases they survive and develop to the extent that the blastoderm thins out and

All were drawn from camera sketches of the living eggs except figures 5, 6, 8 and 9.

Fig. 1. Egg from a normal $\frac{1}{20}$ LiCl distilled water solution when twenty-two hours and twenty minutes old. *sg*, Segmentation cavity; *od*, oil drops.

Fig. 2. When twenty hours old in 3.22 per cent. LiCl in sea water.

Fig. 3. Twenty-three hours old in 2.82 per cent. LiCl in sea water. *gr*, Germ ring; *es*, embryonic shield.

Fig. 4. Twenty-two and one-half hours old in 2.82 per cent. LiCl sea water.

Fig. 5. Nine days old, taken from 3.02 per cent. LiCl when twenty-eight hours old and placed in pure sea water. *h*, Head; *t*, tail; *f*, fin.

Fig. 6. Control embryo nine days old. *ht*, Heart.

Fig. 7. In 2.82 per cent. LiCl fifty-four hours old.

Fig. 8. Embryo with no eyes, nine days old, from 2.82 per cent. LiCl into sea water at thirty-two hours old. *ht*, Heart.

Fig. 9. Cauda bifida normal $\frac{5}{8}$ LiCl forty-eight hours. *t*, Tail.

Fig. 10. In 2.82 per cent. fifty-four hours old.

Fig. 11. Control twenty-four hours old. *em*, Embryonic thickening.

Fig. 12. In 3.02 per cent. thirty-one and one-half hours.
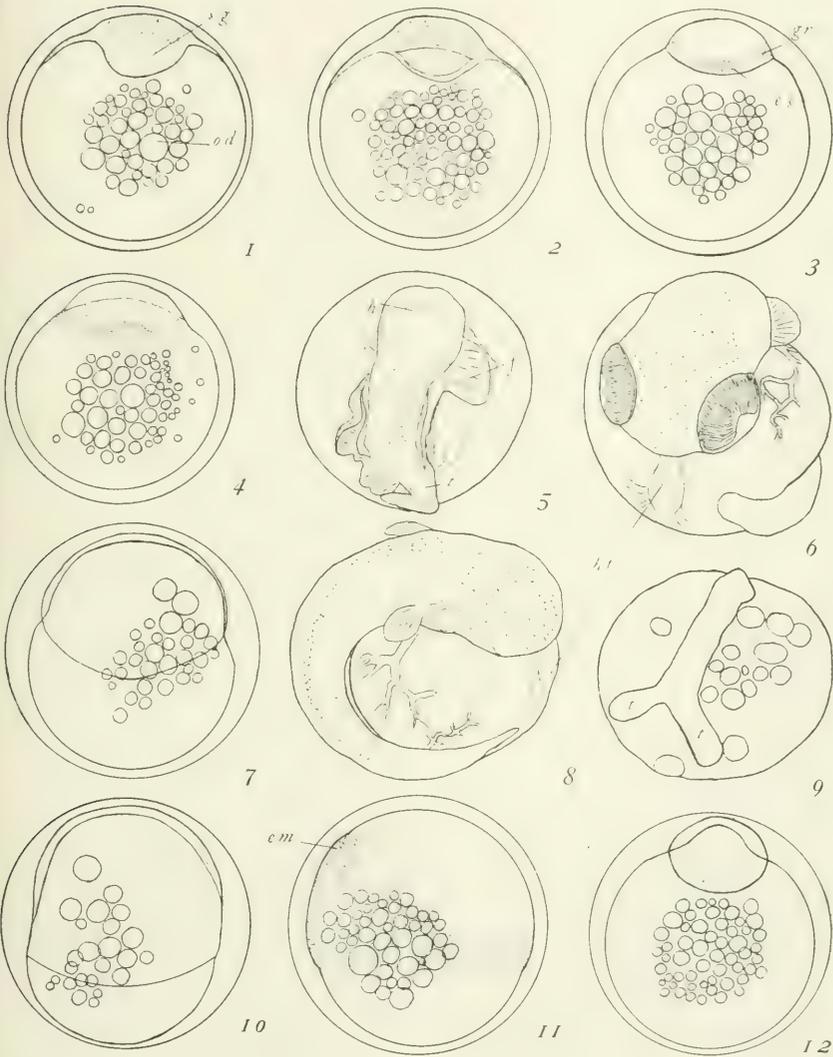
flattens slightly, and in some cases forms an embryonic shield in this
polar position.   Fig. 18 shows such a shield which, forming in a
cap forty-six hours old, extended no further over the yolk than a
normal high segmentation stage would or than the germ area
shown in Fig. 1 or 2 does.   A longitudinal section of this shield
is shown in Fig. 17 and it is seen that the folding and cell arrange-
ments are more or less normal, in this case little or no indication
of the germ ring was found beyond the edges of the shield.   Fig. 19
shows a section through a blastoderm with the embryonic shield
thickening well marked.   In Figs. 3 and 4, embryos about
twenty-three hours old, the germ area is still high up on the yolk,
but the germ ring and embryonic shield have reached a stage of
development commonly seen when the germinal area has descended
about one-third of the way down the yolk.   In these eggs the
embryonic area never completely covers the yolk, the blastopore
always remains open and through it the yolk is seen to protrude.
*These instances show most clearly that a second effect of lithium
chlorid is to prevent the down-growth of protoplasm in the egg.*

The embryos that result from the eggs just mentioned are
peculiar, being necessarily short as the embryonic material only
extends part of the way down the yolk.   They are also poorly
formed in the head region and the tail seems to dwindle out
suddenly as a small pointed end.   Figure 5 shows such a monster
in position on the yolk when nine days old; a normal control
embryo of the same age is shown in Fig. 6.   These short embryos
were cleared and stained and finally studied in sections.   They
usually lacked all indications of eyes, either optic vesicles or lens,
but the ears were being formed and the trunk organs were present.
As is seen in the figure the fins are poorly developed.

When weaker solutions of LiCl were used, such as 2.62 per
cent. and 2.82 per cent., the formation of the embryo approached
nearer the normal, but even in the 2.62 per cent. no embryo was
ever developed in an entirely normal way.   In all cases the rate
of development was retarded, the heart beat was slower, and the
blood was colorless, seeming to lack the hemoglobin constituent.
The latter condition must have lessened the respiratory efficiency to
quite an extent, a fact which would also contribute to their slow

rate of development. Embryos in these weaker solutions often showed cauda bifida (Fig. 9), and others that were more nearly normal in form had rather irregular and twisted outlines, some of these having the tail end bent at right angles to the body axis. These malformations of the caudal end are no doubt due to the slow descent of the protoplasmic mass over the yolk and in some cases to the entire failure of this mass to completely enclose the yolk. This causes the embryo to be short, crowded into less space, and twisted in appearance.

A most noticeable feature in these living embryos was the apparent absence of eyes (Fig. 8 a nine-day embryo). When one looks at a batch of developing fish eggs after the embryos have formed, a most conspicuous feature is the large black eyes on each egg—the lack of such an appearance is striking in the lithium embryos—and the entire mass of eggs has, therefore, a pale, unhealthy look. Sections of the heads of these larvas show that in many cases no indication whatever of eyes existed, while in some a small deep-seated poorly-formed eye was found, which, on account of its probable paleness, was entirely unnoticeable in the living embryos.

The germinal area in some eggs advanced over the yolk in a very peculiar manner. Figure 10 shows a thin cellular sac that extends more than half way down the yolk, constricting it slightly about the equator and giving the entire egg a rather unusual shape. At the border of the protoplasmic sac little or no indication of germ ring or embryonic shield could be distinguished, though these structures must have existed to some extent since at later stages short malformed embryos would arise appearing in life as if their tail ends were entirely lacking. Figure 7 shows a circular cap which does not extend so far down the yolk as in the last case, but the very strong line of demarcation which often exists between yolk and protoplasm is well shown. Figure 12 shows a rare case in which a small part of the yolk is pulled up into the blastodermic cap as a small polar cone.

The rapidity of action of the LiCl solution, the degree of abnormalities and death rate produced by solutions of different strengths as well as the periods in development at which the solutions seemed

most and least effective may best be treated by referring directly to notes taken while testing these several questions.

As was mentioned before the rapidity or ease with which metallic ions penetrate the membranes of Fundulus eggs is uncertain. But the examples cited below seem to show that lithium ions, at any rate, enter with comparative readiness and produce abnormalities which in these experiments, at least, can not be attributed to other causes. Eggs that were fertilized "dry" and then placed in sea water LiCl solutions of 2.82 per cent., 3.02 per cent. and 3.22 per cent. showed characteristic lithium effects after a period of only three hours, while in the eight-cell stage, the blastoderms bulging up abnormally. The control from the same lot of eggs failed to show any such modifications. Again on applying a series of solutions of LiCl in sea water of strengths of 1.59 per cent., 2.12 per cent., 2.65 per cent., 3.18 per cent., 3.71 per cent. and 4.25 per cent. and examining after sixteen hours the eggs in the three weaker solutions presented a normal appearance, while those in the three stronger solutions contained a large per cent. of abnormal forms, all in the strongest solution being very abnormal. The blastoderm was arched and the segmentation cavity appeared as a bubble below. After being in the solutions forty-eight hours the 2.65 per cent. lot contained twenty-six per cent. undeveloped eggs and many abnormally-shaped embryos, some with cauda bifida and irregular outlines. The 1.59 per cent. and 2.12 per cent. lots also showed malformed embryos though not so many as in the 2.65 per cent. lot. Thus it is shown that weak solutions do not affect the development in its early stages though they later cause the production of deformed embryos. In another experiment eggs were subjected to 2.82 per cent. LiCl when twenty-two hours old, and others when twenty-five hours old were put into 3.02 per cent. LiCl. When they were all forty-four hours old, the ones which had been in the stronger solution, though for three hours less, showed more marked modifications than those in the weaker. These facts seem to show that LiCl does enter the egg with sufficient readiness for all experimental purposes.

The degree of effectiveness for the different strength solutions may be illustrated as follows: When forty-six hours old many eggs

in 2.62 per cent. LiCl show a faint line indicating the embryo, and there are also a large per cent. of dead eggs present.   In the 2.82 per cent. solution at this time there are still more dead ones; the
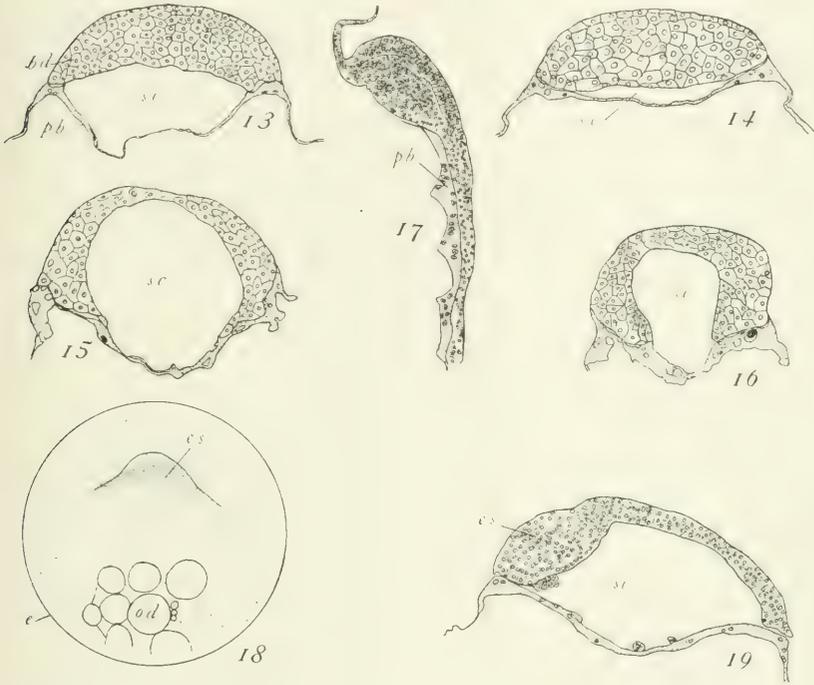
Fig. 13.   Seventeen hours old in LiCl normal solution.   *bd*, Blastoderm;   *pb*, periblast;   *sg*, segmentation cavity.

Fig. 14.   Control ten hours old.   *sc*, Segmentation cavity.

Fig. 15.   In 3.02 per cent. LiCl forty-three hours old.   *sc*, Segmentation cavity.

Fig. 16.   In 3.02 per cent. LiCl forty-three hours old.   *sc*, Segmentation cavity.

Fig. 17.   Longitudinal section embryonic shield from Fig. 18.   *pb*, Periblast.

Fig. 18.   Embryonic shield in polar cap forty-six hours old in 3.22 per cent. LiCl.   *es*, Embryonic shield;   *od*, oil drop;   *e*, border of blastoderm.

Fig. 19.   Section of polar cap twenty-two hours old in 3.22 per cent. LiCl.   *es*, Embryonic shield;   *sc*, segmentation cavity.

germ ring has not grown completely over the yolk except in rare cases, but the embryo is formed in the embryonic shield.   Among those in 3.02 per cent. none of the blastopores have closed and the

embryos are all short and abnormal, with a still larger number of dead eggs.   In the 3.22 per cent. LiCl less than half are still alive; all of the blastopores are unclosed; some of the embryos are fairly well developed in the embryonic shield, while some of the embryonic caps are still at the upper pole with the embryos forming in a thickening, like an embryonic shield, which often lies immediately over the pole of the egg (Fig. 18); others have blastoderms which may finally pinch away from the yolk and die (Figs. 15 and 16).

In another experiment the control embryos are distinctly formed when fifty-four hours old; while in the 2.62 per cent. LiCl the blastopores are only about two-thirds closed and embryos are forming in the embryonic shields.   In 2.82 per cent. LiCl blastopores are still wide open; in 3.02 per cent. caps, with slight embryonic shields, are present; and in the 3.22 per cent. LiCl all seem to have stopped developing and many have the blastoderms broken away from the yolk and floating freely within the egg membrane.

When the same eggs are sixty-nine hours old forty-five are alive in the 2.62 per cent. LiCl solution. The embryos are all well formed, though a few have failed to close the blastopore.   In 2.82 per cent. LiCl only seven out of thirty-five are still alive, or 20 per cent., and only one of these has a definite embryo formed.   In 3.02 per cent. LiCl only five out of fifty-three eggs are living, or 9.5 per cent., and these are all deformed.   Of those in the 3.22 per cent. LiCl solution sixteen are dead and one is living, or 6 per cent. alive. In this one the cap is scarcely half way down the yolk.

At ninety-six hours old these eggs are in the following condition: The 2.62 per cent. LiCl lot have thirteen out of fourteen still living, all with well formed embryos though far behind the control in development.   The 2.82 per cent. ones have not fared so well; only three out of seven are still alive but these, also, have well formed embryos.   The one survivor of the 3.22 per cent. LiCl solution is now dead, the cap has pulled back off of the yolk, which on the day before it had half covered, and is now a dead mass of cells at the upper pole.

The embryos in the 2.62 per cent. LiCl solution when nine days

old are far behind the control in development; the heart beat is only about one-half as fast as normal, and the yolk is badly shrunken so that the entire embryo swings about freely in the egg membrane. The 2.82 per cent. LiCl embryos are still alive, though they also have much shriveled yolks and are otherwise like the ones in the 2.62 per cent. solution.

Eggs of various ages were subjected to the LiCl solutions to ascertain whether or not there were periods in development when they appeared more sensitive than at other times. As a further means of deciding this question, eggs were also removed at intervals from the LiCl solutions and placed in pure sea water. Eggs which were sixteen hours old (early germ-ring stages) were placed in 3.02 per cent. LiCl and when they were forty hours old they had become very abnormal and many were dead. Eggs put into 3.02 per cent. LiCl when seventeen hours old are described thus, when eight days old: The pigment is confined almost altogether to the yolk area and is largely of the black type, little if any of the brown being present; the blood is colorless and the heart beats slowly; the eyes have little or no color and in most cases seem absent, and the yolk has decreased greatly in size. Of the eggs put into 2.82 per cent. LiCl when nineteen hours old almost the entire lot died, the remaining ones resembling those which had been in the same solution since fertilization. If put into 2.62 per cent. LiCl when twenty hours old they appear, when forty-four hours old, similar to those that have spent their entire life in a solution of this strength. Those put into 2.82 per cent. when twenty-two hours old appear at forty-four hours almost normal though a little behind in their development. The extreme sensitiveness of the nineteen-hour and twenty-hour stages as thus indicated is noteworthy. Figure 11 shows a normal egg somewhat beyond this age, and it is seen that the germ ring has just turned the equator of the egg. Other eggs were put into 3.02 per cent. LiCl when eighteen, nineteen, twenty, twenty-one and twenty-two hours old, respectively, and kept in this solution for three days. Then they were transferred to pure sea water again. Seventeen days later they were still alive but developing very slowly and seemed far from being ready to hatch while the control

ones had hatched from three to four days previous. Thus it seems that LiCl leaves a lasting effect on these embryos and that they are unable to recover, though under normal conditions, for as long as seventeen days after.

Eggs sixty-five hours old with well-formed embryos were placed in 3.02 per cent. LiCl solution; twenty-eight hours later on examining the living eggs no variations from the normal could be detected; fifty-four hours after being in the solution no change was observed, though when nine days old they were behind the control in their development at least two days.

To test the time of greatest sensitiveness in another manner, eggs were taken from the LiCl solutions after being in them only thirty minutes, while in the two-cell stage. The LiCl in this case left no trace of any effect that could be detected in their later development. Again eggs taken from 3.02 per cent. LiCl after six hours treatment were put into sea water, their age being then seven and three-quarter hours, they appeared normal when twenty and twenty-four hours old, but when forty-four hours old they had fallen behind the control in their development. Eggs were taken from 2.82 per cent. LiCl when fifteen and one-half hours old, having been in the solution fourteen hours and were placed in sea water, twenty-four hours later the eggs showed no improvement over those still in the solution. When examined after being out of the solution for sixteen days they still showed little if any recovery. In another case eggs were placed in sea water after being in 2.82 per cent. LiCl for eighteen and one-half hours and others in a 3.02 per cent. solution seventeen and three-quarter hours. When forty-four hours old they were still abnormal but far ahead of those remaining in the lithium solutions. After being in 3.02 per cent. LiCl for twenty-six and three-quarter hours eggs were placed in sea water where they failed to recover, though their death rate was materially lowered; after two days 89 per cent. still were living while of those left in the solution only 9.5 per cent. remained alive. This lowering of the death rate was noted in many instances where morphological recovery from the lithium effect seemed lacking. It appears as though the eggs gain in power of resistance, or rather resistance is no longer required

after being transferred to the sea water and hence though they may not recover in one sense, they are better able to survive than they would be under the depressing effects of the lithium.

During one experiment a very strange result was noted: The entire series of LiCl solutions failed to give any visible effect for more than the first day of development, but later the characteristic effects were manifested and those eggs that were removed from the solutions and placed in sea water during the day of apparent non-effectiveness later showed the effects of the LiCl on their subsequent development in the sea water, although no effect was observable when they were removed from the lithium solutions.

The above experiments were all repeated several times and the particular instances cited merely serve as examples to illustrate the general results obtained. From such responses to the LiCl treatment one may be justified in drawing the following conclusions.

CONCLUSIONS

The rapidity with which lithium chlorid in solution affects the egg varies directly as the strength of the solution, eggs being very slow to manifest the effects when placed in dilute solutions, while a response was obtained on one occasion within three hours in rather strong solutions.

The degree of abnormality and the death rate also vary directly as the strength of the lithium chlorid solutions, striking abnormalities being found in 3.22 per cent. solutions while similar malformations in a less marked degree with a much lower death rate were noted in the 2.62 per cent. solutions.

The lithium chlorid solution affects the development of the embryo at any stage in which the eggs may be placed in it. But the extent of the abnormalities seem to vary inversely with the age at which the eggs are subjected to the solution, showing merely a lessened rate of development with other minor effects if placed in the solution during late stages.

When eggs are once affected by the lithium chlorid solution—which is found to be the case if they remain in it as long as six hours—

they are unable to completely recover from this effect even though they be placed in pure sea water during the remaining period of their development.

REVIEW OF LITERATURE

Herbst, in '92, '93 and '96, conducted extensive experiments to test the action of a large number of salts on the developing eggs of the sea-urchin. He found that of a number of salts used, those of the metal lithium produced the most decided results, and further that with the different salts of lithium the results were the same. Thus the conclusion was reached that the action of these salts was not due to their several acid radicals but to their common lithium ion. Herbst, therefore, stated that the abnormalities induced were typical lithium effects, not being obtained by the use of salts of other metals. He used the term "lithium larva" to designate the peculiar monster that resulted. The effect of lithium on the sea-urchin's egg seemed chiefly to cause "exogastrulation," that is, the endoderm instead of becoming infolded by invagination became really inverted and formed a sac connected with the ectoderm or outer gastrula wall by a short stalk, thus producing a three-part larva, two sacs, the gastrula wall and the primitive gut, joined by a short tube or stalk. From the end opposite the stalk the primitive gut sac in many instances formed another small sac representing, as Herbst claimed, the vasoperitoneal pouch. The ectoderm sac at times became smaller and occasionally finally disappeared leaving the entire blastula wall endodermal. The larva recovered slightly so as to form the calcium framework when removed from the lithium solutions; the amount of recovery depending upon the length of time it had spent in the solution. This briefly is the main action as found by Herbst in three genera of sea-urchins treated with, in all, thirteen salts of lithium. The features of especial interest to us are the marked inversion of the layers and the resulting enlarged embryonic cavities.

Gurwitsch, in '95 and '96, treated developing frog's eggs with LiCl and other salts, finding that sodium and lithium salts were most effective. His lithium solutions were very weak (o.3 per

cent., 0.4 per cent. and 0.5 per cent.), only about one-half the strength required to affect the Fundulus egg when applied in fresh water, and about one-third or one-fourth the concentration necessary to give a response when used in sea water. Frog's eggs under the influence of LiCl showed a retardation in the segmentation of the white area, in rare cases this area failing entirely to divide. Thus they became marked into upper and lower hemispheres and the cells at the sides of the floor of the segmentation cavity seemed to push in tending to fill this cavity. At this period Gurwitsch claimed that his embryos exhibited a radial symmetry but Morgan contradicts this and holds that they are really bilateral although the bilaterality may be less evident than that shown in the normal egg. Gurwitsch also finds that the downgrowth of the substance constituting the upper hemisphere is lacking, as indicated above, and this is without doubt a marked characteristic of the action of LiCl on the fish's egg. This also gives in the two cases an abnormally high position to the developing embryo and may, along with the failure of the blastopore to close, cause the malformed caudal areas of many individuals. Abnormalities in the position of the blastopore and brain, particularly of the former, were found to be the most decided effects of the LiCl solutions.

Madame Rondeau-Luzeau conducted an extensive series of experiments to test more especially the action of several chlorids on development. She found that a double action exists, namely, a physical action due to "hypertonicity" of the solutions and a chemical action depending upon the kind of salt employed. Only in the case of LiCl did the chemical action predominate when weak solutions were tested. In my work the fact that solutions in both fresh and sea water were used seems to preclude, in this case, all chances for the physical or hypertonic effect and leaves the chemical effect alone responsible for the abnormal development observed. Except in the case of LiCl Mm. Rondeau-Luzeau found that death was much oftener due to the osmotic pressure of the solutions than to chemical poisoning. The teratological action of the chlorids appeared to be due to their chemical action in the case of eggs treated after fertilization. The effects of the solutions

were also found to be more marked at some stages than at others, particularly so at the time of closure of the blastopore and of the formation of the medullary folds. LiCl was found to exert a more powerful chemical action than any other salt tried. The minimal amount of this salt that will effect the development of the frog's egg may be represented by an osmotic pressure of 169 cm. (0.3 to 0.4 per cent.); while for KCl, it it 405 cm. (1.2 per cent.); for NaCl, 459 cm. (1.9 per cent.); for $MgCl_2$, 485 cm. (1.3 per cent.); and for $CaCl_2$, 484 cm. (1.5 per cent.).

Morgan, in '02 and '03, subjected frog's eggs at various stages in their development to 0.4 per cent., 0.5 per cent., 0.6 per cent., 0.7 per cent. and 0.8 per cent. solutions of LiCl. Most of these solutions would be too weak, even though prepared in fresh water, to affect Fundulus eggs. Morgan found that in the frog's egg LiCl did produce a specific effect which he attributed to the chemical action of the lithium ion. Delayed development was noted as being a most obvious condition and this is equally true of the fish's egg. Eggs in two-cell and four-cell stages were more affected than those in later segmentation, and those beginning to gastrulate were least affected. Stronger solutions were necessary to give an equal effect when applied in later stages. Similar facts are also indicated in my notes above. But it was found that the Fundulus eggs placed in the solutions during cleavage and at the beginning of gastrulation up to the sixteenth or seventeenth hours were equally affected without regard to the stages at which they were subjected to the action of the salt. Another very noticeable effect of this salt was to prevent the downgrowth of protoplasm from the upper hemisphere of the frog's egg, as Gurwitsch also found. This makes gastrulation difficult, so that embryos in the stronger solutions do not pass beyond this stage. The absence of the downgrowth of the protoplasm in the fish's egg has been emphasized above and it will be remembered that in the stronger solutions the blastoderm folded into a ball upon the upper pole and finally pinched itself away from the yolk and died. One type of the frog embryo showed a complete inversion of the layers, the black area folding down into the yolk cells. The complete accomplishment of such a feat by the fish's egg could hardly be imagined,

but it seems possible that it may attempt a similar process, when it is recalled to what extent the periblast area may be forced down into the yolk when the segmentation cavity becomes unusually enlarged as it sometimes does. In the frog the black area becomes more or less spherical and sinks into or is engulfed by the yolk-cells of the white area. The frog eggs often form a sharp line between the black and white hemispheres, usually above the equator. This line, Morgan states, corresponds to the inturned edge of a circular blastopore whose position at or above the equator of the egg is explained by the lack of downward move-ment of the material of the upper hemisphere. The segmenta-tion cavity of the upper cells was obliterated. This is a very different state of affairs from that seen in the fish where the seg-mentation cavity becomes so enormously exaggerated. Many short and folded embryos were found in later stages, some also showed cauda bifida; short embryos and forked tails are also common among the late effects of LiCl on the fish embryo.

After considering these investigations upon the effects of lithium one is strongly inclined to conclude that the salts of this metal do exert a specific influence on developing eggs. But I am not now in the position to state that these effects on the Fundulus eggs are really specific for lithium, though they have been so repeatedly and constantly obtained that one is led to believe that they are at least characteristic of lithium action on eggs of this form. Other chemical or physical stimuli might possibly pro-duce similar abnormalities; this could be definitely stated only after the study has been continued with a large number of salts of various metals.

SUMMARY

1. Lithium chlorid delays development to a most obvious degree.

2. Eggs seem equally affected by the solution when placed in it at any time during cleavage stages; at other times the effects vary. They seem most sensitive about the period when the germ-ring circles the equator; though they are always affected to a greater or less degree; the rate of development is slower, the em-

bryo presents a pale appearance, since the blood lacks color and the pigment spots are fewer than normal.

3. After having remained for as long a time as six hours in LiCl solutions of sufficient strengths to cause abnormalities the eggs are incapable of complete recovery when placed in pure sea water during the remainder of their development.

4. In LiCl solutions the blastoderm is usually prevented from growing downward over the yolk, it therefore bulges up as a cap on the upper pole of the egg. This cap in the stronger solutions constricts its border, thus folding its periphery, and finally pinches itself away from the yolk and dies.

5. The segmentation cavity is enormously enlarged since the central periblast pushes down unusually far into the yolk mass while the blastoderm bulges up giving the cavity a more arched roof.

6. In many eggs the blastoderm never completely encloses the yolk; thus the blastopore remains open and short, peculiarly formed, and often cauda bifida embryos result.

7. In the late embryos the heart beats slowly, the eyes often fail to develop, the blood is colorless and, therefore, appears to lack hemoglobin. These characters taken with the inability to recover from the lithium effect, seems to prove without doubt that such an effect is due to chemical, and not to physical, causes. The fact that similar abnormalities are induced by LiCl solutions prepared with sea water and fresh water, therefore, giving both hypertonic and hypotonic solutions show further its chemical rather than its physical, action.

APPENDIX

Notes on the Development of Fundulus heteroclitus in Fresh Water

At Cold Spring Harbor, Long Island, in the summer of 1904, I collected material for comparing the development of the eggs of Fundulus heteroclitus in fresh water with their development in sea water, which is their normal medium. I wished also to ascertain whether or not those embryos hatched in fresh water would

show a higher degree of adaptability for living in this medium than the normally hatched fish. The fresh water at this place, as indicated by later experiments, evidently contained some substance that affected the embryos very strangely, as almost without exception the late embryo assumed a peculiarly twisted position upon the yolk the tail bending up in a circular fashion and striking the body about half way from the head. Almost all of these eggs died before hatching and those embryos that lived to hatch were unable to straighten their bodies and died within a few hours. The control embryos began hatching three and one-half days before these fresh water ones.

During the past summer at Woods Hole this experiment was repeated with the extra precaution of running a distilled water control. Here the results were entirely different in regard to the form of the embryo. Those fish that hatched in the fresh and distilled water exhibited perfectly normal shapes having also occupied the usual position on the yolk. But again the fresh water embryos were late in hatching, being in the earliest case two days behind the control and some were five days late in coming out.

The fresh and distilled water embryos gave an interesting mortality record for different periods of their development. During the first ten to twelve days these eggs were about as hardy as those in the sea water, though from this time until hatching began they died at a rate of over 5 per cent. per day. In one case where there were two hundred and twenty-five eggs when hatching began, thirty-nine, or $17\frac{3}{5}$ per cent. died that night and only six individuals were hatched. During the next twenty-four hours forty-three, or $23\frac{1}{9}$ per cent. died, leaving only one hundred and forty-three still alive. The following day, or three days after hatching had begun, only eighteen were alive, $87\frac{1}{3}$ per cent. having died that night, five dying after hatching. None lived in fresh water longer than ten hours after hatching out. From this it is seen that the fish become peculiarly sensitive to the unusual medium shortly before the hatching time and only a few survive to break through the egg membrane.

The fish that did hatch in the fresh water were certainly no better fitted to live in this medium than those hatched in sea water.

When embryos that had hatched in fresh water were transferred directly to either one-half sea and one-half fresh water, or to pure sea water, they began at once to show quicker movements, and always lived normally in these media.

Thus it is seen that although the eggs of Fundulus heteroclitus will develop in an apparently normal fashion in fresh water, so far as form is concerned, they are slower in hatching. The eggs die in large numbers during the hatching period, and those that do hatch are unable to survive unless transferred to sea water. At this period of late development they probably die from the same cause that kills the mature fish if they are put into fresh water.

Zoölogical Laboratory, Columbia University,
　　　October 16, 1905.

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PARTIAL REGENERATION OF THE SPERM-RECEP-TACLE IN CRAYFISH

BY

E. A. ANDREWS

WITH ELEVEN FIGURES

In American crayfish of the genus Cambarus there is a small pocket in the shell of the female that receives sperm from the male and subsequently frees it when the eggs are laid. This is not found in other crayfishes, and as Cambarus is the most specialized of the group the sperm-receptacle, or annulus ventralis as it has been called, seems to be a new acquisition. At the same time it is used as one of the necessary reproductive organs. From its character, as a dense part of the shell and from its small size and protected position, on the ventral side between the legs, it seems improbable that it has been subjected to very many injuries in the history of the genus. Yet at the time of shedding all parts of the shell are soft, and amongst several hundred females examined there were two cases in which the annulus had been injured, as if by the claws of some other crayfish pressing against the shell when soft. However, these were peripheral injuries and did not necessarily prevent the use of the organ.

That the organ has ever been removed in the history of crayfish without the death of the organism seems highly improbable as we have no knowledge of any enemy that could cut out that region without destroying the neighboring nerve trunk, at the least.

To determine what would happen if the organs were removed twenty-four females were operated upon in May and June, 1904, so that all, or most all, of the annulus was extirpated. These were adult females, some of which had laid in April and May. The telson-rostrum measurements of these were as follows:

One was 50 mm. long; one 55 mm.; three were 60 mm.; eight were 65 80 mm.; one 85 mm.; five 100 mm.; four 110 mm., and one 120 mm. in length.

In July five of these cast their shells; one 100 mm., and four 80 mm. in length. In each case there was something new formed of the nature of an annulus, but so imperfect as to be of no use as a sperm receptacle.

To understand these partial regenerations it is necessary to consider the anatomy of the normal annulus. The part of the shell so-called has in Cambarus affinis the form indicated in Fig. A, which is a ventral, or external view of the annulus from a female 110 mm. long, decalcified and made translucent. There are here two prominent elevations or knobs with a groove between, and a transverse depression posterior. Running across this depression is a zig-zag crack, represented by a heavy black line. Following the zig-zag in a general way is a wide dotted streak, intended to represent the tubular cavity that contains the sperm. This tubular cavity opens to the exterior by a rounded hole, partly concealed under the larger of the two knobs; it also opens along its whole length by the crack represented by the heavy line. The curved broken lines indicate the very thick, chitinous walls of the tubular cavity, showing through the translucent shell in this preparation.

All this, however, is only the shell of the annulus; under the shell there was a corresponding modification of the epidermis and connective tissue. The shell fits like a slightly exaggerated mask over the epidermis. The knobs, depressed area and thick-walled tube all have their correspondingly-shaped, underlying epidermal counterparts, upon which they were made.

The male puts the sperm into the hole at one end of the tube and it subsequently comes out of the zig-zag crack to meet the eggs as they pass over the annulus.

A comparative study of several species of Cambarus shows that the essential part of the annulus is the tubular cavity and that in one of the lower species this is more evidently a longitudinal groove or pocket with its edges nearly closed together. Also in the ontogeny of Cambarus affinis it is found that the annulus of the

young larva presents only a single longitudinal depression and
that in successive moults this becomes bent, almost closed, and
associated with external sculpturing like that of the adult.

The essence of the annulus is thus an inpitting of epidermis
which produces a chitinous pocket, opening only to the exterior.

In extirpating the annulus the shell represented in Fig. A was
cut away from the rest of the crayfish's shell and removed,
dragging with it a variable amount of live epidermis and con-
nective tissue that remained in the cavities of the knobs and
adhering to the walls of the tube and to other parts of the removed
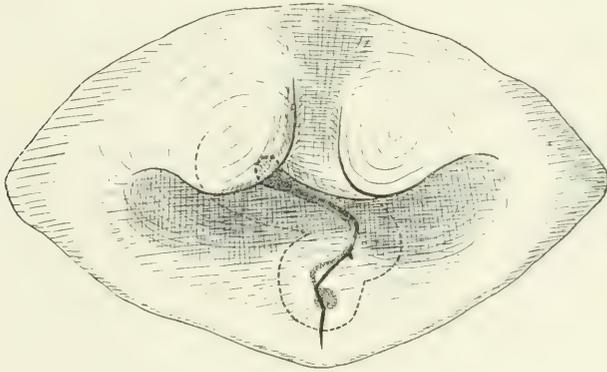
Fig. A.

shell. This left in place of the annulus a soft, bleeding, sponge-
like mass which the pressure of contained blood caused to bulge
out, more or less. The wounded surface soon became covered
over by a layer of material that hardened and turned brown and
then remained as a firm protection until the crayfish cast its shell.

The cast-off shells then showed in place of such an annulus
(Fig. A), which is seen in cast shells from normal crayfish, merely
a rough, brown membrane, continuous all about its edges with
the old shell of the crayfish.

The animals that cast their shells showed new annuli as stiff
bluish plates of the form of an annulus but without its sculpturing
and with very little to represent its pocket, or tube.

The regenerated annuli thus obtained are indicated in Figs. 1,

2, 3, 4, 5, drawn to the same scale as Fig. A and enlarged about 13 diameters. The part in each that seems to represent the essential sperm pocket is shown in Figs. 1′, 2′, 3′, 4′, 5′, each magnified about 100 diameters.

All the regenerated annuli are noticeably short, as in young crayfish; but the figures fail to give the full surface since these annuli are not flat but have a large posterior face as well as the ventral (and anterior) one shown in the figures. The posterior and ventral faces make a large angle where they meet in an elevated ridge, which ridge is the posterior boundary of the above figures. It is near this elevated ridge that the inpitting of epidermis and shell has taken place to form what is like the early stage in the sperm-pocket in the larva. In the larva also the inpitting takes place near the posterior edge of the ventral face.

In the largest female that regenerated, 100 mm. long, there was a peculiar abnormal outgrowth from the posterior face of the new annulus, Fig. 5. This was a soft protuberance bearing a rounded lobe and two papillæ that were slightly constricted, or jointed, somewhat suggesting rudimentary limbs. The smaller papilla was dorsal and is not represented in the figure. This annulus was again abnormal in having some ten pits, or infoldings scattered over its ventral surface as well as the median lengthwise pit that seemed to be homologous with a sperm pocket. On the posterior, or dorsal, face there were also nearly as many supernumerary ridges and depressions as upon the ventral face. Most of these were, in general, transverse, and upon the dorsal face more were upon the right of the median line, while upon the ventral face, Fig. 5, more were upon the left.

The median pits that are thought to represent the beginnings of sperm pockets had the following characters in the five specimens studied.

In the first, Figs. 1 and 1′, there was a very simple lengthwise groove, or pit, with closed lips and near it elevations that converged as indicated by the lines in Fig. 1. In Fig. 1′ the central line represents the closed lips of the groove as seen at the surface, while the cavity of the pit is represented by shading and the very thick shell walls of the pit are indicated by the broken lines.

Fig. 1

Fig. 1'

Fig 2
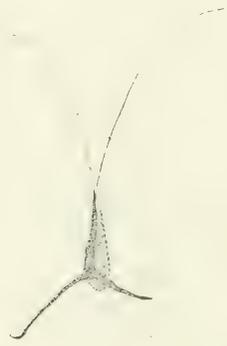
Fig. 2'
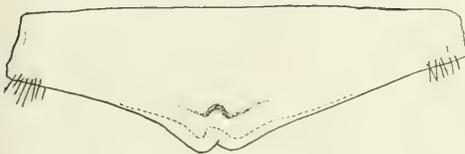
Fig. 3

Fig. 3'

Optical sections of this preparation showed that the epidermis was turned in as a deep groove in which the epidermal cells abutted against the shell lining the pit.

In another specimen the lengthwise pit was associated with an anterior depression indicated by the lines in Fig. 2, and with a sharp transverse ridge suggesting the posterior curved edge of a normal annulus. The pit, Fig. 2', opened anteriorly into the depressed area and its open mouth branched posteriorly right and left. The bottom of the pit, shown by lighter shading, ended bluntly posteriorly. The very thick inturned shell is indicated by broken lines indicating its parallel lamellæ.

In the third specimen the pit was at the posterior edge and, as in Fig. 3, the preparation showed beneath the shell a wide open epidermal groove drawn away from the shell, artificially. The pit, Fig. 3', was open along its length, ended sharply in front and opened out behind between two prominent ridges. The annulus was much elevated at its posterior edge and from a more posterior view the above ridges looked not unlike the knobs of a normal annulus, Fig. A, while posterior to them there was a transverse ridge similar to that seen in Fig. 2 and again suggesting the posterior rim of a normal annulus.

In specimen four there were two pits close together and somewhat involved with one another, Figs. 4 and 4'. Both were wide open, ended bluntly at their anterior ends and expanded posteriorly into a wide depression. The more dorsal pit was nearer to the median plane, the other more to the animal's left side.

In the fifth specimen, Figs. 5 and 5', the median pit was much like that in a very young larva. A depressed area near the posterior edge of the annulus leads forward into a deep groove, the walls of which are very thick inturned shell. In this specimen there was a slight pouch on each side where the cavity of the groove was wider than the open mouth of the groove. In a vague way these side pouches suggest the "recess" of the normal annulus.

These imperfect annuli, laid bare at the time of shedding, would doubtless have continued outwardly in that shape until the next shedding. Whether they would have progressed, during
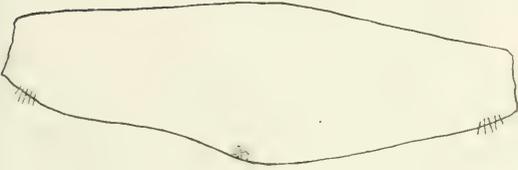
Fig 4

Fig. 4′

Fig. 5
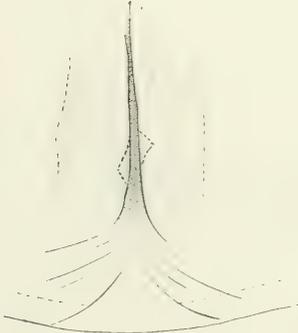
Fig. 5′

successive moults, toward the normal adult form must be determined by the results of future experiments.

In the normal growth of crayfish the annulus has a new shell formed over it at each period of shedding and these successive shells are more and more complex from the early larva to the adult stage.

These experiments show that when in the adult the shell over the annulus was removed with more or less of the epidermis and connective tissue (removed, probably, for the first time in the history of the organ), a new pit and also a new shell, comparable to an early larval annulus in complexity, but to an adult annulus in size, were formed.

Thus there may be a partial restoration, in a few months' time, of an organ that is historically new, though essential, and that is moreover a single organ without fellow or metameric homolog and found only in one sex.

An ability in the crayfish partially to regenerate this special adult organ, to begin to retrace the ontogeny of this organ, would seem of use to the species only if it were carried far enough to complete a functioning sperm receptacle. Experiments may show this to be the case.

That this special ability could have been evolved by natural selection seems difficult to conceive since accidental extirpation is but a remote possibility. The ability to start this organ a second time in one generation would seem comparable to the fundamental ability to make the organ again and again in successive generations.

November 8, 1905.

EXPERIMENTAL STUDY OF LIGHT AS A FACTOR IN THE REGENERATION OF HYDROIDS

BY

A. J. GOLDFARB

In a paper entitled "The Influence of Light on the Development of Organs in Animals," '95, Loeb states "that light favors the development of polyps in Eudendrium (ramosum); that no polyps, or only very few, are developed in the dark." At the suggestion of Prof. Morgan, I undertook to determine the minimum amount of light required by this hydroid to regenerate its polyps. The experiments were conducted during the summer of 1905 at the Marine Biological Laboratory, at Wood's Hole, Mass., while occupying a room of the Carnegie Institution.

EXPERIMENTS ON EUDENDRIUM RAMOSUM

Preliminary Experiments

Vigorous colonies of Eudendrium ramosum were selected. These consist of a main stem or stock and its branches. These primary branches in turn give rise to secondary and tertiary branches bearing polyps at their distal ends. These polyps—and gonads when present—were separately cut off. The decapitated colony was placed in a glass bowl containing about 200 cc. of normal sea water. These bowls were allowed to remain in the light for fifteen, thirty, sixty minutes, respectively. At the expiration of each of these periods the bowls were placed in a dark chamber, from which all light was carefully excluded. Other series, of six bowls each, prepared in the same way were placed in the dark immediately after the removal of the polyps. After forty-eight and seventy-two hours, respectively, each series was exposed to the light as above mentioned.

In the following tables, the numbers indicate the regenerated hydranths which have free and distinct tentacles.

Tables A, B and C indicate results of these experiments.

A

EXPERIMENT BEGUN JULY 14, 1905, 12.30 M.
(*Exposed before being placed in dark*)

No.	Time of Exposure.	July 15	July 16	July 17	July 18	July 19	July 21
1	15 min.	o	11	10	8	o	o
2	30 min.	o	13	22	18	1	o
3	45 min.	o	14	18	8	o	o
4	1 hr. 15 min.	o	19	16	7	o	o
5	2 hrs. 15 min.	o	11	13	13	1	o
6	3 hrs.	o	7	11	11	o	o

B

EXPERIMENT BEGUN JULY 12, 1905, 12.30 M.
(*Exposed after forty-eight hours in dark*)

No.	Time of Exposure.	July 13	July 14	July 15	July 16	July 18	July 19	July 21
1	5 min.	o	27	18	20	3	o	o
2	10 min.	o	13	15	18	9	1	o
3	10 min.	o	18	23	11	3	1	o
4	10 min.	o	14	15	11	2	o	o
5	15 min.	o	27	24	18	7	o	o
6	15 min.	o	10	10	10	3	o	o

C

EXPERIMENT BEGUN JULY 13, 1905, 4.30 P. M.
(*Exposed after seventy-two hours in dark*)

No.	Time of Exposure.	July 14.	July 15.	July 16.	July 18.	July 19.	July 21.	July 22.	July 23.	July 24.	July 25.	July 26.
1	3 min.	o	o	o	4	4	3	1	o	o	o	o
2	5 min.	o	o	o	4	3	2	o	o	o	o	o
3	8 min.	o	o	o	3	3	3	3	o	o	o	o
4	10 min.	o	1	1	6	8	6	4	2	2	1	o
5	15 min.	o	4	5	7	7	5	4	o	1	1	1
6	25 min.	3	6	5	9	9	5	5	2	o	o	o

It appears from these tables, that the brief exposure of three minutes was probably not the minimum required by this hydroid for the stimulation of its hydranth formation.   It is further to be observed, in those colonies kept in the dark forty-eight hours or more after removal of polyps, and then exposed, that regeneration of hydranths occurred before such exposure.   Further, the number of newly-formed hydranths may or may not be increased after an exposure.

The appearance of hydranths, formed in the dark, may be explained: (1) As the result of the exposure from the moment when the hydranths were removed to the moment when the decapitated colony was placed in the dark.   (2) Or, what seemed less probable, that hydranths were regenerated normally in the dark.

First Cycle

To avoid a source of error, all Eudendrium colonies were hereafter prepared as follows: The room was converted into a photographic dark room.   Light was admitted only through a pane of red glass about six inches square.   Particular pains were taken to keep out all white light.

In the preceding experiments each hydranth was separately removed and the main stem with all its branches was used.   In the following experiments each primary branch bearing other branches and hydranths was cut off near its point of attachment to the main stem or trunk of the colony.   The trunks with the stumps of the primary branches only, were used.   The number of primary branches so removed was noted in each case.   The colonies were separately exposed, then placed in dark chambers. These were made of double boxes, one within the other, painted black within, and edges protected by bands of black cloth.   One colony of nearly every series was kept in shaded but continuous light of the room.   This colony will be termed the "Control."

In series D—more or less typical of the results obtained in three different series of experiments—the colonies were exposed soon after removal of the branches.   In series E, F and G the colonies were at once placed in the dark, and exposed one, two and three days, respectively, after removal of the branches.

D

EXPERIMENT BEGUN JULY 21, 1905, 2.00 P. M.

(Colonies exposed before being placed in dark)

No. of Branches Removed.	Time of Exposure.	July 22.	July 23.	July 24.	July 25.	July 26.	July 27.	July 28.	July 29.	July 30.	July 31.	Aug. 1.	Aug. 3.	Aug. 4.	Aug. 5.	Aug. 6.	Aug. 7.
15	Not exposed	0	9	10	6	6	4	3	3	3	1	1	0	0	0	0	0
15	1 min. light	0	11	9	9	9	8	5	1	2	2	1	1	1	0	0	0
15	1 min. sunlight	0	7	4	3	3	4	4	1	1	1	1	1	1	0	0	0
15	2 min. light	0	8	5	5	5	5	5	3	1	3	3	2	2	2	0	0
15	2 min. sunlight	0	11	6	7	6	7	2	1	1	1	1	1	1	0	0	0
15	4 min. light	0	14	11	8	9	6	5	5	0	0	0	0	1	5	5	0

E1

EXPERIMENT BEGUN JULY 22, 1905, 3.00 P. M.

(Colonies exposed to light twenty-one hours after removal of branches)

No. of Branches Removed.	Time of Exposure.	July 23.	July 24.	July 25.	July 26.	July 27.	July 28.	July 29.	July 30.	July 31.	Aug. 1.	Aug. 3.	Aug. 4.
21	Not exposed	0	0	1	1	1	1	2	1	1	0	0	0
26	1 min.	0	5	13	9	5	5	4	0	0	0	0	0
28	2 min.	0	5	7	10	7	8	4	1	0	0	0	0
23	4 min.	0	14	13	8	13	11	13	8	6	5	4	4
22	6 min.	0	2	18	13	13	9	4	1	1	1	0	0
26	8 min.	0	11	14	7	9	10	4	1	0	0	0	0
22	Control	0	0[1]	14	11	7	7	5	1	1	0	0	0

[1]Six buds formed.

F

EXPERIMENT BEGUN JULY 22, 1905, 3.00 P. M.

(Colonies exposed to light forty-eight hours after removal of branches)

No. of Branches Removed.	Time of Exposure.	July 23.	July 24.	July 25.	July 26.	July 27.	July 28.	July 29.	July 30.	July 31.	Aug. 1.
19	Not exposed	o	o	1	1	2	2	1	o	o	o
23	1 min.	o	8	11	8	11	13	11	7	4	o
27	2 min.	o	4	7	4	5	4	4	2	2	o
24	4 min.	o	o	2	4	4	4	o	o	o	o
21	1 min. sunlight	o	o	o	1	4	3	2	1	o	o
20	2 min. sunlight	o	4	4	9	11	10	8	4	2	o
23	Control	o	3	13	14	14	8	7	1	2	8

G

EXPERIMENT BEGUN JULY 22, 1905, 3.00 P. M.

(Colonies exposed to light seventy-two hours after removal of branches)

No. of Branches Removed.	Time of Exposure.	July 23.	July 24.	July 25.	July 26.	July 27.	July 28.	July 29.	July 30.	July 31.	Aug. 1.
17	Not exposed	o	o	o	2	4	5	6	4	3	3
19	1 min.	o	2	4	5	7	6	4	o	o	o
15	2 min.	o	o	o	4	11	10	8	4	o	o
17	4 min.	o	1	5	3	1	2	3	2	1	o
14	1 min. sunlight	o	1	o	1	9	12	10	5	2	2
17	2 min. sunlight	o	o	3	9	8	9	6	4	o	o
15	Control	o	o	4	7	7	2	3	3	1	o

It will be observed: 1. About forty-eight hours after removal of the hydranth-bearing branches, new hydranths were formed. 2. These hydranths may appear as follows: (*a*) Single hydranths may appear at the oral ends of the branches that have been cut; (*b*) single hydranths or group of hydranths may appear at the aboral end of the main stem or trunk; (*c*) one of the branches may greatly elongate, give rise to new secondary and tertiary branches, each bearing a hydranth; (*d*) the aboral end of the main stem may greatly elongate, give rise to many short branches, each bearing a

hydranth; (*e*) any combination of (*a*), (*b*), (*c*), (*d*), may occur. 3. No record was kept of the history of the individual hydranths. This is to be regretted. For, hydranths on a given colony may be regenerated yet will not be recorded if other hydranths on the same colony should disintegrate or be absorbed. Such occurrences are probably rare, and do not seriously affect the conclusions. 4. The tables reveal a large degree of variability No one table can correctly be said to be typical in all its details of the phenomena in any given experiment. The conclusions are not based on the "typical" tables but on all the collected data.

From the foregoing experiments we may draw the following conclusions:

1. Colonies that had not been exposed to the light during or after removal of the branches, regenerate their hydranths.

2. The difference in the percentage of hydranths regenerated, on colonies exposed for brief periods and those not exposed is in many cases negligible.

3. In most cases, there is but little difference in the percentage of hydranths regenerated on colonies kept in the dark but exposed for brief periods, and on the "control" colonies.

It seemed desirable to determine under what conditions these hydroids would thrive best, and to determine if it were possible to keep all the colonies under practically uniform conditions.

Colonies were prepared as previously mentioned.

(*a*) Some were kept in shaded light of the room, cooled by evaporation from wet cloths; the water in the bowls was not changed. (*b*) Conditions the same, but water in bowls changed daily. (*c*) Bowls partly immersed in running water. The water in bowls was not changed. (*d*) Colonies placed in the direct rays of the sun. Water in bowls changed daily. (*e*) The hydranths were separately cut off, leaving main stem, primary branches and a large number of secondary and tertiary branches. Water in bowl was not changed.

This experiment showed that the direct rays of the sun or the heat caused by them, were injurious; as no polyps were developed in (*d*). Colonies from which the hydranths had been separately

removed in (*e*) regenerated very few hydranths.   Colonies placed in the diffuse light of the room, with the temperature of the water lowered by means of wet cloths, and the sea water changed daily, throve the best, and regenerated the largest number of hydranths.

Hereafter the sea water in all bowls was changed daily or every other day, and all bowls were covered with wet cloths.

There was the possibility that the pane of red glass admitted too much light into the dark room, and probably such light was not monochromatic.   Partially to overcome these defects, a large sheet of red paper—used in photographic work—was so arranged that it could be made to cover to a greater or less extent the red glass. This paper covered the red glass completely on bright days, or uncovered a very narrow slit of the glass on cloudy days.

In the following experiments every precaution was taken to keep the Eudendrium colonies in the most vigorous condition and to admit as little red light during observations as circumstances permitted.   Tables H and I may be considered typical of results obtained in experiments in which colonies were exposed immediately after or twenty-four hours after removal of branches.   But on observing that hydranths were regenerated in colonies not exposed to the light, experiments were then undertaken in which none of the colonies of the series was exposed.   Table J is typical of results thus obtained.

H

Experiment Begun Aug. 14, 1905

(Colonies exposed immediately after removal of branches.   Two colonies in each bowl)

No. of Branches Removed.	Time of Exposure.	Aug. 15.	Aug. 16.	Aug. 17.	Aug. 18.	Aug. 19.	Aug. 20.	Aug. 21.	Aug. 22.	Aug. 23.	Aug. 24.	Aug. 25.
44	Not exposed	0	10	16	16	19	13	9	7	6	1	5
44	5 sec.	0	0	3	6	9	10	11	11	11	9	7
44	10 sec.	0	1	4	4	3	3	5	·6	15	15	7
42	½ min.	0	1	0	0	0	2	2	2	4	3	4
42	1 min.	0	0	0	1	2	8	12	13	10	4	3
42	5 min.	0	0	0	3	7	10	10	12	14	3	2
86[1]	Control	0	3	6	13	28	40	50	40	—	11	3
Temperature °C............		20	20	18	19	18	19	20	23	—	23	22

[1] 3 colonies in bowl.

I

Experiment Begun Aug. 8, 1905, 2.00 p. m.

(Colonies exposed twenty-four hours after removal of branches. Three colonies in each bowl)

No. of Branches Removed.	Time of Exposure.	Aug. 9.	Aug. 10.	Aug. 11.	Aug. 12.	Aug. 13.	Aug. 14.	Aug. 15.	Aug. 16.	Aug. 17.	Aug. 18.	Aug. 19.	Aug. 20.	Aug. 21.	Aug. 22.	Aug. 24.	Aug. 25.
60	Not exposed	0	4	4	1	0	2	2	2	3	7	7	5	3	1	0	0
60	15 min.	0	16	23	14	8	5	4	3	3	4	4	4	4	2	0	0
60	30 min.	0	1	9	11	13	6	4	2	1	1	0	1	2	2	5	4
60	60 min.	0	16	17	7	6	6	0	1	2	2	3	3	5	2	2	2
54	3 hours.	0	26	20	14	14	2	0	0	0	0	0	0	1	1	4	5
54	Control	0	19	44	47	24	11	12	13	14	18	17	23	21	17	6	0
Temperature °C........		—	25	25	24	21	20	20	20	18	19	18	19	20	21	23	22

J

Experiment Begun Aug. 14, 1905, 4.00 p. m.

(No colonies exposed to light during or after removal of branches. Two colonies in each bowl)

No. of Branches Removed.	Aug. 15.	Aug. 16.	Aug. 17.	Aug. 18.	Aug. 19.	Aug. 20.	Aug. 21.	Aug. 22.	Aug. 23.	Aug. 24.	Aug. 25.
54	0	2	5	5	2	1	0	0	0	0	0
54	0	4	13	15	8	6	4	1	0	0	0
54	0	3	14	14	5	2	0	0	0	0	0
50	0	7	15	14	11	7	6	6	3	0	1
50	0	5	8	12	7	6	5	2	1	0	0
50	0	10	20	14	6	6	3	6	6	1	0
Temperature °C. .	20	20	18	19	19	19	20	21	—	23	22

From all of the foregoing experiments[1] we may conclude that:

1. Hydranths are regenerated about forty-eight hours after removal of hydranths or branches.

2. The largest number of hydranths observed at any one time is recorded from the second to the fourth day after removal of the hydranth or branch, or on the first or second day after the appearance of the regenerated hydranths.

[1]The total number of hydranths and branches removed exceeded 2,391.

3. Thereafter there is a gradual decline in the number of hydranths.

4. Finally no hydranths are recorded on any of the colonies constituting a series, and the close of the first "cycle" is reached. It may take from nine to twenty-two days before all the hydranths of a series are gone. The average time is thirteen days.

5. There seems to be no causal relation between the number of regenerated hydranths of a colony and the time during which such colony had been exposed. Colonies exposed for one minute may form an equal, greater or less per cent. of hydranths than those colonies exposed for two minutes.

6. In four experiments the per cent. of hydranths formed on colonies kept in the dark but exposed for brief periods, was equal to or greater than the per cent. recorded in the control colonies. In but two experiments was the per cent. less than in the control colonies.

7. Colonies kept in the dark forty-eight and seventy-two hours, respectively, after removal of the branches, and then exposed, regenerate their hydranths before such exposures.

8. Colonies that had at no time during the experiment of fifteen days been exposed, nevertheless regenerated a great many hydranths.

9. The percentage of regenerated hydranths on colonies not exposed was in four series equal to or greater than in those colonies exposed for brief periods. In four other series the per cent. was less than in the colonies that had been exposed. Furthermore, in two series the percentage of regenerated hydranths in colonies not exposed was greater than in the "control" colonies.

We may safely conclude that during the first period or cycle of about thirteen days, *darkness does not prevent the regenerative processes in Eudendrium ramosum from taking place. Nor does darkness necessarily retard the development nor decrease the number of hydranths formed.* On the contrary exposure to the light for short periods may or may not increase the rate of growth nor the number of hydranths.

Second Cycle

The colonies of Eudendrium ramosum seemed to be, during this first period of about thirteen days, in a condition not unlike phototonus in plants, *i. e.*, under the influence of previous exposure to the light. "In darkness," Pfeffer[1] says, "a plant continues to grow normally as long as it remains in a condition of phototonus, *i. e.*, so long as the influence of the previous exposure persists. This period is frequently very prolonged. So long as the plant remains in a condition of phototonus, . . . small changes of illumination produce no perceptible effect." It seemed not improbable that the colonies that regenerated hydranths in the dark were under the influence of the light previous to cutting away the branches.

Acting on this supposition the following experiments were undertaken. After all the hydranths of a series had disappeared and the end of the first cycle was reached, *i. e.*, when the influence of the previous illumination was gone, all the colonies but one were subjected to the light for varying periods of time. This one colony was carefully kept in the dark. There was also in nearly every series one control colony placed in the light.

Tables E2, K, L and M are typical results obtained in nine series.

E2

(This Table is continuation of E1*)*

No.	First Exposure July 22. Time.	No. of Branches Removed.	Second Exposure Aug. 4. Time.	Aug. 5.	Aug. 6.	Aug. 7.	Aug. 9.	Aug. 10.	Aug. 11.
1	Not exposed	21	Not exposed	0	0	0	0	0	0
2	1 min.	26	¼ min.	0	2	4	1	0	0
3	2 min.	28	½ min.	0	0	0	0	0	0
4	4 min.	23	¼ min.	—	—	—	—	—	—
5	6 min.	22	1 min.	0	7	5	0	0	0
6	8 min.	26	4 min.	0	0	1	0	0	0
7	Control			0	0	0	0	0	0
Temperature °C........................				—	24	23½	23½	25	25

[1]Pfeffer: "The Physiology of Plants," vol. 2, ed. 1903. Trans. by Alf. J. Ewart.

K

DATE OF EXPERIMENT JULY 13, 1905

No.	First Exposure, July 16. Time.	Second Exposure, July 27. Time.	July 27.	July 28.	July 29.	July 30.	July 31.	Aug. 1.	Aug. 3.	Aug. 4.
1	3 min.	Not exposed	o	o	o	o	o	o	o	o
2	5 min.	1 min.	o	4	4	4	4	1	o	o
3	8 min.	2 min.	o	1	5	5	4	1	1	o
4	10 min.	4 min.	o	9	17	17	13	3	o	o
5	15 min.	6 min.	2	7	13	9	11	6	2	o
6	25 min.	1 min. sunlight	o	6	9	8	2	o	o	o

L

No.	No. of Branches Removed.	First Exposure, Aug. 14. Time.	Second Exposure. Time.	Second Exposure. Date.	Hydranths regenerated at end of 1 day	2 days	3 days	4 days	5 days
1	44[1]	Not exposed	Not exposed	Aug. 27	o	o	o	o	—
2	22	$1\frac{1}{2}$ min.	$1\frac{1}{2}$ min.	Aug. 27	o	o	o	o	—
5	21	$\frac{1}{2}$ min.	$\frac{1}{6}$ min.	Aug. 25	o	o	o	o	2
4	21	1 min.	$\frac{1}{4}$ min.	Aug. 25	o	2	2	4	4
5	21	1 min.	$\frac{1}{4}$ min.	Aug. 26	o	1	6	9	—
6	21	5 min.	1 min.	Aug. 26	o	o	2	3	—
7	86[1]	Control			3	3	1	2	o

[1]More than one colony in each bowl.

M

No.	No. of Branches Removed.	First Exposure, Aug. 8. Time.	Second Exposure, Aug. 25. Time.	Hydranths regenerated after 1 day	2 days	3 days	4 days
1	60[1]	Not exposed	Not exposed	o	o	o	o
2	60[1]	15 min.	$\frac{1}{6}$ min.	o	o	1	3
3	40[1]	30 min.	$\frac{1}{4}$ min.	o	o	o	o
4	40[1]	1 hour	$\frac{1}{2}$ min.	o	o	1	2
5	36[1]	3 hours	1 min.	o	o	1	o
6	56[1]	Control		1	1	3	9
Temperature °C..				18	18	17	18

[1]More than one colony in each bowl.

As in the first cycle hydranths are regenerated about forty-eight hours after exposure.

There is also no causal relation between the time of exposure and the number of hydranths developed.

Formation of new hydranths occurred with but two exceptions, on colonies that were exposed, even for such brief periods as five or ten seconds.

The maximum number of polyps observed at any one time is recorded on the third or fourth day after exposure, or on the second day after the appearance of the polyps. This is somewhat later than in the first cycle.

Thereafter there is a rapid decline in the number of hydranths, more rapid than during the first cycle.

Finally no more hydranths develop or are observed on any of the colonies comprising a series. The end of the second cycle is reached. It may take from seven to fourteen days before all the hydranths are gone. The average is ten days—much shorter than in the first cycle.

The five colonies not exposed at the beginning of the second cycle did not regenerate any hydranths during this cycle. In two colonies two hydranths were formed, however, though colonies were not exposed.

The control colonies bore no more hydranths by the end and in some cases by the middle of the second cycle.

From these observations we may infer that after the first cycle, *light was necessary for the regeneration of the hydranths. Without light no regeneration of hydranths, or very little, takes place.* It is surprising to note what a short exposure—in some cases one-twelfth minute—was sufficient to start the regenerative processes. These brief exposures, however, may so stimulate the development of hydranths that the maximum number of hydranths noted at any one time during the second cycle is often equal to, or greater than, the maximum number during the first cycle. Those colonies that had not been exposed but had nevertheless formed new hydranths during the second cycle, may have been stimulated by over-exposure to the red light. In one case at least, according to an observation made at the time, a colony which had not been

exposed was removed from the bowl. This was thoroughly cleaned to remove sediment that had collected. The colony was then replaced. All this was done in red light. Two days later a hydranth appeared on the colony.

Third Cycle

At the end of the second cycle the colonies of four series were again exposed. By this time several colonies were broken into two or more pieces, or stolons or branches broken off, or the colony was otherwise mutilated.

Tables E3 and N give tabulated results.

E_3

(*This Table is continuation of* E_1 *and* E_2)

No.	No. of branches removed.	First Exposure, July 23. Time.	Second Exposure, Aug. 4. Time.	Third Exposure, Aug. 11. Time.	Aug. 12.	Aug. 13.	Aug. 14.	Aug. 15.	Aug. 16.	Aug. 17.	Aug. 18.	Aug. 19.	Aug. 20.	Aug. 21.	Aug. 22.	Aug. 24.	Aug. 25.
1	21	0 min.	0 min.	2 min.	0	0	6	5	4	3	4	1	0	0	0	0	0
2	26	1 min.	¼ min.	1 min.	0	1	3	0	0	0	0	0	0	0	0	0	0
3	28	2 min.	½ min.	½ min.	0	0	1	2	2	3	4	4	3	3	3	1	0
4	23	4 min.	0 min.	¼ min.	0	0	2	0	0	1	1	1	1	1	1	1	0
5	22	6 min.	1 min.	⅙ min.	0	1	1	2	1	0	0	0	1	0	0	2	1
6	26	8 min.	4 min.	0 min.	0	0	2	1	1	0	1	0	0	0	0	0	0
7	22	Control			0	0	0	0	0	0	0	0	0	0	0	0	0
Temperature °C........................					24	21	—	20	20	18	19	18	19	20	22	23	22

1.   We observe that hydranths may be formed forty-eight or seventy-two hours after exposure. They may not, however, appear until four or five days after exposure.

2.   The largest number of hydranths that appeared at any one time was in four colonies greater, in two colonies equal to, and in twelve colonies less than, the largest number that appeared at any time during the preceding cycles.

3.   As in the first two cycles, the maximum number of hydranths was observed on the second or third day after exposure, or on the first or second day after the appearance of the hydranths.

No.	First Exposure, July 17. Time.	Second Exposure, July 28. Time.	Third Exposure, August 11. Time.	Aug. 12.	Aug. 13.	Aug. 14.	Aug. 15.	Aug. 16.	Aug. 17.	Aug. 18.	Aug. 19.	Aug. 20.	Aug. 21.	Aug. 22.	Aug. 24.	Aug. 25.	Aug. 26.	Aug. 27.	Aug. 28.	Aug. 29.
1	3 min.	½ min.	½ min.	1	1	1	2	3	2	2	2	1	1	0	0	1	2	2	3	3
2	5 min.	1 min.	1 min.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	7 min.	2 min.	2 min.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	10 min.	4 min.	¼ min.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
5	15 min.	1 min. sunlight	0 min.	0	1	1	0	1	1	1	1	1	2	3	4	2	1	0	0	0
6	Control			0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Temperature °C.	....	....	....	24	21	—	20	20	18	19	18	19	20	21	23	21	18	18	17	19

4. The number of hydranths formed bears no causal relation to the time of exposure.

5. The number of hydranths declines, but for no apparent cause may increase in number. So that the third cycle may last for five days or may last as long as eighteen days.

6. Colonies not exposed at the beginning of the third cycle may or may not regenerate hydranths. Out of the four colonies not exposed at the beginning of the third cycle, three had been exposed at the beginning of each of the preceding cycles.

7. The control colonies regenerated but one hydranth.

We notice how erratic the response to light has now become. Hydranths are developed on colonies not exposed at the beginning of the third cycle. The number of hydranths decreases, finally all are gone, yet for no apparent cause will increase again. Is it possible that at the beginning of the third cycle, arbitrarily chosen, the cycle had not actually come to a close, and the hydranths that had formed were influenced by the exposure at the beginning of the second cycle? Or, had the colonies become so sensitive that the slight exposure to red light necessitated by daily observations was sufficient to start regeneration? Could the colonies after a time dispense with light and thrive in the dark, except for slight red illumination? The evidence is not conclusive in support of any one of these suppositions.

Fourth Cycle

The evidence is practically identical with that obtained for third cycle. Table O is typical of results obtained.

Some control colonies also show periodic increase and decrease in the number of hydranths. There may be two, three or even four cycles observed in the history of one control colony. Such cycles are not explicable by a difference of temperature or change of sea water in the bowls, for these cycles occur apparently independently of such changes. A lapse of thirteen or fourteen days during which no hydranths appear may intervene between cycles.

No.	First Exposure, July 16. Time.	Second Exposure, July 26. Time.	Third Exposure, Aug. 4. Time.	Fourth Exposure, Aug. 10. Time.	Aug. 11.	Aug. 12.	Aug. 13.	Aug. 14.	Aug. 15.	Aug. 16.	Aug. 17.	Aug. 18.	Aug. 19.	Aug. 20.	Aug. 21.	Aug. 22.	Aug. 24.	Aug. 25.	Aug. 26.	Aug. 27.	Aug. 28.	Aug. 29.
1	3 min.	0 min.	1 min.	1 min.	0	0	0	0	3	4	4	4	2	1	0	0	0	0	0	0	0	0
2	5 min.	1 min.	0 min.	0 min.	0	0	0	0	0	2	2	2	1	0	0	0	0	0	0	0	0	0
3	8 min.	2 min.	2 min.	$\frac{1}{6}$ min.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	10 min.	4 min.	$\frac{1}{2}$ min.	$\frac{1}{4}$ min.	0	0	1	2	2	2	3	3	3	3	4	3	3	2	2	1	1	1
5	15 min.	6 min.	1 min.	2 min.	0	0	0	0	0	1	2	2	2	2	1	1	1	0	0	0	0	1
6	25 min.	1 min. sunlight	15 min.	$1\frac{1}{2}$ min.	0	0	1	1	2	2	3	4	1	2	1	0	1	1	0	0	0	0
Temperature °C					24	24	21	—	17	20	18	19	18	19	20	21	23	22	18	18	17	18

Exposure to Continuous Light

The colonies of four series were again exposed to the light as in the preceding experiments, but no formation of hydranths occurred even by the fifth day. It seemed not improbable that the colonies were spent. Did the protracted stay in the dark permanently injure the hydroids or rather did darkness permanently effect the regenerative processes in Eudendrium? This could be determined by permanently exposing the colonies to the light. The colonies of the four series were removed from their dark chambers and placed permanently in the light. Before examining the results we should bear in mind that:

1. Prior to this exposure the colonies bore no hydranths for periods of three to fifteen days.

2. This exposure occurred from twenty to twenty-five days after removal of the stems.

3. Many of the colonies were now badly injured or were but remnants of the original colony.

Tables P and Q are typical of results obtained.

It will be observed that hydranths are regenerated very frequently five days after exposure, though it may take nine and even twelve days before hydranths make their appearance. This is much longer than was necessary at any other time.

2. The number of hydranths formed is surprisingly large. In four colonies the maximum number of hydranths equaled, in five colonies was greater, and in fourteen colonies was less than the maximum noted on any colony at any other time.

3. The maximum number of hydranths for any one time was recorded from the seventh to the tenth day after exposure, or from the third to the fifth day after the appearance of the hydranths, much later than during any preceding period. During preceding cycles, hydranths regenerated by the second or third day after exposure and decreased in numbers thereafter. In these experiments hydranths were slow in making their appearance, but kept on regenerating new ones in some cases for the first six days.

4. The per cent.[1] of newly-formed hydranths on control colo-

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[1] The per cent. in this and previous cases is obtained by dividing the number of branches removed into the number of hydranths regenerated in a colony.

P

No. of Branches Removed.	First Exposure, July 24. Time.	Second Exposure, Aug. 1. Time.	Third Exposure, Aug. 10. Time.	Aug. 11.	Aug. 12.	Aug. 13.	Aug. 14.	Aug. 15.	Aug. 16.	Aug. 17.	Aug. 18.	Aug. 19.	Aug. 20.	Aug. 21.	Aug. 22.	Aug. 24.	Aug. 25.	Aug. 26.	Aug. 27.	Aug. 28.	Aug. 29.
19	0 min.	0 min.	0 min.	0	0	0	0	0	0	0	1	5	7	10	15	11	7	4	1	0	0
23	1 min.	½ min.	½ min.	0	0	0	0	0	0	0	1	3	8	9	8	7	5	3	0	0	0
27	2 min.	½ min.	½ min.	0	0	0	0	0	0	0	3	3	13	10	10	5	1	2	1	4	3
24	4 min.	1 min.	1 min.	0	0	0	0	0	0	0	1	8	7	9	10	7	5	4	2	0	0
21	1 min. sunlight	2 min.	2 min.	0	0	0	0	0	1	1	1	4	15	13	18	17	10	10	4	2	1
20	2 min. sunlight	4 min.	4 min.	0	0	0	0	0	0	0	–	3	4	7	9	7	6	0	1	1	1
23	Control			0	0	0	0	2	4	4	6	4	5	7	7	6	4	2	2	1	1
Temperature			....	24	24½	21	–	20	20	18	19	18	19	20	21	23	22	18½	18	17	18

(Note in the Aug. 14 column: "Permanently placed in light.")

2

No. of Branches Removed.	First Exposure, July 25. Time.	Second Exposure, Aug. 1. Time.	Third Exposure, Aug. 11. Time.	Aug. 12.	Aug. 13.	Aug. 14.	Aug. 15.	Aug. 16.	Aug. 17.	Aug. 18.	Aug. 19.	Aug. 20.	Aug. 21.	Aug. 22.	Aug. 24.	Aug. 25.	Aug. 26.	Aug. 27.	Aug. 28.	Aug. 29.
17	0 min.	0 min.	0 min.	0	0	0	0	0	0	0	0	0	1	3	4	4	0	0	0	0
19	1 min.	½ min.	½ min.	0	0	0	0	0	0	0	0	0	0	1	3	3	2	1	2	1
15	2 min.	1 min.	1 min.	0	0	0	0	0	0	0	0	0	0	3	1	1	2	2	2	1
17	4 min.	2½ min.	2 min.	0	0	0	0	0	0	1	1	1	4	0	2	1	0	1	2	1
14	1 min. sunlight	4 min.	¼ min.	0	0	0	0	0	0	0	0	0	0	2	0	2	2	2	1	1
17	2 min. sunlight	8 min.	⅓ min.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
15	Control			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Temperature.	....			24	21	—	20	20	18	19	18	19	20	21	23	22	18	18	17	18

Permanently placed in light.

nies is exceeded by the per cent. on colonies previously kept in the dark.

5. The regenerative processes were certainly not impaired by the almost continuous confinement in the dark.

6. After the first cycle light certainly stimulates the regeneration of hydranths in Eudendrium ramosum.

EXPERIMENTS ON PENNARIA TIARELLA.

The hydroid Pennaria tiarella is found abundantly on the same piles covered by Eudendrium ramosum. The environment for both these hydroids is practically the same, although Pennaria may also be found in exceedingly large numbers attached to floating eel grass. In the following experiments colonies of Pennaria were taken with one exception from the piles of a dock. Vigorous colonies were chosen. The branches were removed and the colonies treated in the same way as Eudendrium. A total of 2,672 branches were cut off.

Tables R and S are typical of results.

From these experiments we may conclude that:

1. If colony with its branches and hydranths be kept in the dark for forty-eight hours or more the hydranths are dropped off or disintegrated.

2. If these colonies be kept in the dark they do not regenerate any hydranths.

3. Exposure to daylight or direct sunlight for less than three hours did not result in the development of hydranths. Hydranths were formed after an exposure of three, four, four and one-half and five hours, respectively, only in Experiment R. The hydranths thus formed disappeared after forty-eight hours in the dark.

4. Exposure to diffuse light or to sunlight for periods varying from a few minutes to nineteen hours did not result—with the exceptions noted above—in the formation of hydranths. Exposures for two days invariably induced formation of new hydranths. Probably exposure to brilliant light for not less than two days is in most cases, needed for regeneration to take place.

5. Confinement in the dark for periods of thirteen to seventeen

R

DATE OF EXPERIMENT, JULY 31, 1905

(Three colonies placed in each bowl)

No.	No. of Stems Removed	Aug. 1.	Aug. 2.	Aug. 3.	Aug. 4.	Aug. 5.	Time of Exposure.	Aug. 6.	Aug. 7.	Aug. 9.	Aug. 10.	Second Exposure.	Aug. 11.	Aug. 12.	Aug. 13.	Aug. 14.	Aug. 15.	Third Exposure.	Aug. 16.	Aug. 17.	Aug. 18.	Aug. 19.	Aug. 20.	Aug. 21.	Fourth Exposure.	Aug. 22.	Aug. 24.	Aug. 25.	Aug. 26.	Aug. 27.	Aug. 28.
1	28, 22, 27	0	0	0	0	0	Not exp.	0	0	0	0	Not exp.	0	0	0	0	0	5 hours	0	1	1	0	0	0	7 hours	0	0	0	0	0	0
2	20, 21, 24	0	0	0	0	0	5 min.	0	0	0	0	1 hour	0	0	0	0	0	4 hours	0	1	1	0	0	0	5½ hours	0	0	0	0	0	0
3	25, 26, 27	0	0	0	0	0	10 min.	0	0	0	0	2 hours	0	0	0	0	0	3 hours	0	0	0	0	0	0	4 hours	0	0	0	0	0	0
4	20, 23, 25	0	0	0	0	0	15 min.	0	0	0	0	3 hours	0	2	2	0	0	2 hours	0	0	0	1	0	0	2½ hours	0	0	0	0	0	0
5	19, 27, 30	0	0	0	0	0	20 min.	0	0	0	0	4½ hours	0	2	5	0	0	Not exp.	0	0	0	0	0	0	Not exp.	0	0	0	0	0	0
6	Control (see S.)																														

EXPERIMENT BEGAN JULY 31, 1905

(Exposed forty-two hours after removal of branches. Three colonies in each bowl)

No. Branches Removed	Time of Exposure posture	Aug. 1	Aug. 2	Aug. 3	Aug. 4	Aug. 5	Second Exposure Time posture	Aug. 6	Aug. 7	Aug. 9	Aug. 10	Third Exposure Time posture	Aug. 11	Aug. 12	Aug. 13	Aug. 14	Aug. 15	Aug. 16	Aug. 17	Aug. 18	Aug. 19	Aug. 20	Aug. 21	Aug. 22	Aug. 24	Aug. 25	Aug. 26	Aug. 27	Aug. 28
90	Not exp.	0	0	0	0	0	Not exp.	0	0	0	0	Not exp.	0	0	0	0	0	0	0	15	18	23	22	19	12	10	4	4	3
86	½ min.	0	0	0	0	0	5 min.	0	0	0	0	1½ hours	0	0	0	0	0	0	0	11	19	19	20	19	5	5	3	2	8
93	1 min.	0	0	0	0	0	10 min.	0	0	0	0	3 hours	0	0	0	0	0	0	0	11	16	16	16	15	5	7	11	11	10
89	2 min.	0	0	0	0	0	15 min.	0	0	0	0	4½ hours	0	0	0	0	0	0	0	1	2	3	4	4	2	2	2	0	1
60	4 min.	0	0	0	0	o[1]	In light	8	10	5	2	In light	3	4	11	4	1	1	3	7	5	6	6	5	1	0	0	0	0
68	Control	0	0	0	0	1	Control	3	0	4	3	Control	2	8	7	0	0	1	0	0	1	0	0	1	0	0	0	0	0

Columns Aug. 15 – Aug. 28: Permanently placed in light.

[1] Were exposed permanently to light.

S

days does not permanently prevent regeneration, for, on continuous exposure after such confinement, hydranths are readily developed in large numbers.

6.   Pennaria colonies kept in the dark never developed any hydranths.   Yet these colonies were often literally crowded with living, thriving colonies of Bryozoa (species undetermined), with many small campanularian hydroids probably Clytia cylindrica, and with many young individuals of Eudendrium ramosum.

7.   Light is absolutely essential for the normal growth, development and regeneration of Pennaria tiarella.

SUMMARY

Eudendrium

In examining the effect of light on regeneration of hydranths in Eudendrium ramosum, we must distinguish between

1. Colonies kept in the dark but under the influence of previous illumination
2. Colonies kept in the dark and *not* under the influence of previous illumination.

1.   These colonies regenerate their hydranths two days after removal of their branches.   Such regeneration is independent of light.   That light is not essential to the regeneration of hydranths during the first thirteen days (during which influence of previous illumination obtains) is shown by the fact that, (*a*) colonies kept in the dark and not exposed develop a large number of hydranths; (*b*) colonies kept in the dark and not exposed may regenerate an equal or greater per cent. of hydranths than those exposed for short periods, or those kept in the continuous light, viz., the control colonies.

2.   In colonies not under the influence of previous illumination, *i. e.*, after the first thirteen days or thereabouts, the response to light is decidedly different.   Such colonies do not develop hydranths or very few hydranths *unless exposed*.   The surprisingly short exposure of one-twelfth minute may suffice to start the regenerative processes, but some exposure is essential.

The illumination prior to removal of branches will stimulate the

regeneration of hydranths on colonies kept in the dark, and maintain such hydranths for a long period or cycle of about thirteen days. The second cycle is initiated by a brief exposure which stimulates the colony to renewed activity. Similarly short exposures may rejuvenate the series of colonies during a third and fourth cycles. The per cent. of hydranths formed at any one time during the second, third or fourth cycle may be equal to, or greater than, the per cent. formed at any preceding time.

There is no causal relation between the number of hydranths formed and the time of exposure, *i. e.*, the per cent. of hydranths formed after exposure of one-half minute may be greater, equal to, or less than per cent. formed after exposure of one minute.

The minimum diffuse bright light required to cause hydranths to regenerate is probably one-twelfth minute. It is not impossible that under certain conditions a less exposure could bring about regeneration.

The regenerative processes are not impaired by almost continuous confinement in the dark for from twenty to twenty-five days. On continuous exposure the per cent. of hydranths formed may equal or exceed that during any preceding time.

Sometime after removal of hydranths, the colonies kept in the dark, but exposed for brief periods, are stimulated to regenerate hydranths more readily than those colonies kept in the light, viz., the control colonies.

Pennaria

Prolonged darkness is inimical to development and maintenance of hydranths in colonies of Pennaria tiarella. If kept in the dark colonies lose their hydranths within forty-eight hours, and do not develop new ones.

Exposure to brilliant light for three or four hours may stimulate the development of hydranths, but usually an exposure of not less than two days is necessary for such development.

As in Eudendrium, confinement in the dark for long periods does not permanently affect the regenerative powers of these colonies.

OBSERVATIONS AND EXPERIMENTS CONCERNING THE ELEMENTARY PHENOMENA OF EMBRYONIC DEVELOPMENT IN CHÆTOPTERUS[1]

BY

FRANK R. LILLIE

WITH ONE PLATE AND SEVENTY-EIGHT FIGURES IN THE TEXT

[1] The experimental parts of this study and the observations dealing with the living egg were carried on at the Marine Biological Laboratory of Woods Holl, Mass; the study of the preparations in the Zoölogical Laboratory of the University of Chicago.

I.   INTRODUCTION

This paper is based on the study of the egg of a single species of annelid, Chætopterus pergamentaceus Cuvier, and is a contribution to the study of the elementary phenomena of development. I have been able to extend my earlier observations on differentiation without cleavage (Lillie, '02) and to demonstrate homology of regions and cell-constituents between unsegmented eggs that have undergone differentiation and larvæ normally formed. It has been found in the course of the later study that the homology is dependent upon a very exact localization of germinal areas and specific substances in the unsegmented egg, which play essentially the same rôle whether they be divided by cell-walls or not. The microscopic composition of these substances and the relations of nuclear activity to differentiation have also been studied, and it has been necessary to include the ovogenesis, maturation, fertilization and cell-lineage in order to control the other data.

In the present paper the attempt will be made to present the substance of the entire study without too much detail, leaving the elaboration of certain parts for a later contribution. However, I shall make no apology for entering into details, because there is no other explanation of heredity than a complete account of development, and one cannot describe even a small part of so complex a thing without many words, unless one knows in advance what is essential and what is not.

The numerous cytological studies of germ-cells of the last few years have focused attention especially on the chromosome-complex, and the remarkably uniform results have established a series of propositions on so firm a basis that they seem destined

to form the working hypothesis of investigations in heredity and development for a considerable period of time. These are: (1) The constancy in number of the chromosomes in any species. (2) The persistence of a descendent of each chromosome in every cell throughout the series of cell-generations. (3) The composition of the chromosome-complex of the first cleavage-spindle of corresponding maternal and paternal groups, equal in number and in all other respects, save individual variation. (4) The biparental character of the embryonic nuclei, similar to that of the first cleavage nucleus. (5) That the individual chromosomes of both the maternal and paternal chromosome-complexes are probably qualitatively different. (6) That pseudo-reduction (synapsis) consists in union of corresponding maternal and paternal chromosomes. (7) That reduction in the number of chromosomes is effected by separation of the units of such bivalent chromosomes.

Though all of these propositions are not accepted by all cytologists, yet in the main they have become the current opinions. Enough, certainly, has been definitely established to put the hypothesis, that the chromosomes constitute the bearers of the hereditary qualities, on a definite basis. *The embryologist, therefore, has his problem defined, and it is no less an undertaking than to derive the entire body of the individual from the chromosome-complex as its germ. This is the embryological program.* It has been recognized as such in the more important recent theories of development; but hypothesis has far outstripped observation, and it is remarkable how very few observations have been recorded establishing definite morphogenic relations between nucleus and cytoplasm. Yet, if the hypothesis be true, such relations must be of the very essence of embryonic development.

The observations recorded in this paper have led me to some very definite opinions as to the relations of nucleus and cytoplasm in embryonic development. I am well aware that they cover only a very small part of this embryological program; but I venture to express the hope in putting them forward that others may be led to test and to extend them.

Our advance along these lines is very dependent on methods. The results of this study could not have been attained without the

methods employed. It is probable that centrifuging and staining intra vitam will prove to be very generally applicable, and I believe they will yield valuable results. The method of analyzing the developmental processes by suppressing the cleavage without prejudice to other morphogenic activities, has been the most valuable one on the whole. I believe that it is capable of quite a wide application even in cases where the differentiation does not proceed to the formation of cilia. Bastian ('04) seems to have witnessed such a process in rotifers, though, by an incomparable blunder, he interpreted the unsegmented ciliated ova as a definite genus of ciliate infusorian. It applies in other annelids than Chætopterus as Scott's ('06) and Treadwell's ('02) papers show, and I think there is every reason to believe that it will prove available in animals of different phyla.

II. MICROSCOPIC COMPOSITION OF THE PROTOPLASM OF THE EGG

1. The Living Protoplasm

The egg of Chætopterus is a spheroidal mass of a semifluid, transparent and homogeneous substance with a large number of granules of various sizes and optical properties suspended in it; more fluid droplets occur here and there. The living protoplasm shows no evidence of a filar, reticular or alveolar structure, except that granules or droplets may be so closely set in certain places as to produce the appearance of an emulsion. This, however, is a secondary character. The primary optical properties of the protoplasm are those of a transparent colloidal solution, the particles of which are ultramicroscopic; this may assume the most various configurations according to the number, size and arrangement of the suspended, microscopically visible, granules.

The ground substance is a suitable name for the fluid that contains and suspends all the granules and droplets; if all these were imagined removed it would preserve a faithful semblance of the egg. Thus it is regarded as forming the external pellicle and as continuous through the nuclear membrane with the nucleoplasm. Its optical properties are the same everywhere like a drop of water, but, unlike such a drop, it may exhibit different physical and chemical properties in different parts.

The Granules.—The varying microscopic character of regions of the egg is thus due entirely to the granules and droplets suspended in the ground substance. Droplets of a homogeneous transparent liquid, more fluid than the ground substance, are especially characteristic of the germinal vesicle and of the protoplasm in the neighborhood of the vegetative pole (Fig. 1). I have not, however, been able to follow their behavior very fully. The granules on the other hand are more solid bodies, though it is probable that some are semifluid; certainly all have considerable density.

By subjecting the living egg to considerable pressure one can examine it with the highest powers; my observations were made with a Zeiss 2 mm. homogeneous oil-immersion objective, apert. 130, and compensating oculars 6, 8 and 12. Under such magnifications one sees that the protoplasm of any part consists of the ground substance with granules of two kinds, viz: slightly or nonrefringent granules of relatively large size, which I shall designate spherules, and very minute (less than 1μ in diameter) highly refringent granules of approximately uniform size, the microsomes. The spherules vary in size from about 2.25μ to about the size of a microsome.

In the living protoplasm the microsomes and the smaller spherules are in a constant state of tremulous agitation vibrating in rapid rhythm with considerable amplitude. The range of these movements is considerably increased if the egg is actually crushed so that the protoplasm is broken into small fragments. No one who has studied these movements, as I have done for hours at a time, could believe that the microsomes are nodal points of a network, or are connected by filaments as they appear to be in the best stained sections. One is forced to conclude that they have freedom of movement in all directions, *i. e.*, that they are suspended in a fluid medium which has no filar, reticular or alveolar structure.

The description so far applies to the substance of the germinal vesicle as aptly as to the cytoplasm; though the spherules of the germinal vesicle are probably small droplets in the sense used above; they certainly differ in many respects from the spherules of the cytoplasm. But the substance of the germinal vesicle con-

tains as large a proportion of microsomes as does the cytoplasm, and these cannot be distinguished apart in the living condition, though they can be differentiated by fixation and staining, as described below. The germinal vesicle is of course characterized also by the nucleolus described in Part III; the chromosomes were not distinguished in the living germinal vesicle. There are also large spaces filled with fluid (Fig. 1).

Microsomes may occur separately or in conjunction with spherules; in the latter case a certain number appear to be inseparably united to the spherules; no matter how much the protoplasm is broken up, the union cannot be dissolved. I am not sure that this is characteristic of all the various kinds of spherules, but it certainly is of those of the ectoplasm. There are different kinds of spherules characteristic of different regions of the egg and related to different kinds of differentiation. Their differences, arrangement and properties are considered later.

The relation between microsome and spherule is more or less problematical. From the fact that spherules may grade down in size to approximately the dimensions of microsomes, one is tempted to assume that the spherules are produced by growth of microsomes, or by their agglutination and fusion, and the same conclusion follows from other facts considered beyond. The microsomes would thus be the primitive source of these larger granules; the spherules a farther step in the processes of differentiation.

According to this view the microsomes are the primitive formed elements of the cytoplasm. The source of the microsomes themselves becomes therefore a vitally important question. There are three possibilities: (*a*) That the microsomes arise from smaller ultramicroscopic particles in the ground substance; (*b*) that they are an original cytoplasmic element multiplying by fission; (*c*) that their primitive source is the nucleus, in which event they are to be regarded as chromatin derivatives, for all the formed elements of the nucleus are chromatin derivatives, or, at least, have their base in the chromosomes.

I have observed no phenomena that indicate either of the first two possibilities, but have on the other hand repeatedly observed the transformation of nuclear microsomes into cytoplasmic micro-

somes in sufficient quantity to supply all the microsomes found in the egg. *In addition to these direct observations several important considerations unite in support of the hypothesis, that microsomes are chromatin particles.*

The direct observations concerning the structure and fate of the residual substance of the germinal vesicle (pp. 172–178) and concerning chromatin distribution in uninucleated unsegmented eggs that undergo differentiation (pp. 230–232) conclusively prove the derivation of a large proportion of the microsomes from the nucleus. Now each daughter nucleus is built up from a particular chromosome-complex, and it is a commonplace cytological generalization that chromosomes arise by the union of chromatin particles (chromomeres) of approximately the size of cytomicrosomes; this is readily observed in the egg of Chætopterus. When the nucleus comes to rest the chromosomes resolve themselves again into these ultimate formed elements of the chromatin, after passing through specific form changes. Thus the chromosomes are composed of particles of about the same size as the cytomicrosomes. The staining reaction of the chromomere and cytomicrosome is the same, in certain stages at least; newly formed microsomes take the basic stain with nearly the same intensity as chromatin particles. In the ovogenesis of Chætopterus the main source of the microsomes is the structure described by Mead ('98, pp. 193, 194) as the paranucleus as follows: "Up to the time when the egg has attained about two-thirds its full size, only a part of the protoplasm presents the loose reticular appearance; the rest remains as large purple masses, which I consider to be equivalent to the *Nebenkerne* or paranuclei of various authors (Fig. 1, *c, d, f*). These masses are not homogeneous, but resolve themselves into a cytoplasmic network, of which the meshes are much compressed, and the strands usually parallel with the surface of the nucleus, though at the periphery of the masses they fray out and become continuous with the open network which contains the yolk." I would add that the paranucleus forms a kind of cap to the germinal vesicle, from which it probably originates. The reticulum, in my opinion, is an artefact, and the essential thing about the paranucleus is that it is composed of a dense aggregation of micro-

somes, that subsequently become distributed to all parts of the cytoplasm.

These various considerations have convinced me that the larger proportion of the microsomes are derived from the nucleus. It seems improbable that there should be any *radical* difference in the origin of any, for the morphological characters of the cytomicrosomes, such as size, density, color and refringibility in life, and staining reactions, are remarkably constant. I will not deny that they may multiply outside of the nucleus; but, except in the case of the centrosome, I know of no evidence in favor of such multiplication of bodies resembling microsomes. On the other hand it is certain that the chromomeres multiply within the nucleus. If the microsomes are *extra*nuclear chromomeres, as here suggested, the doctrine of the individuality of chromosomes must be understood, as Häcker has pointed out, not in the sense that the entire substance of a chromosome is handed on through successive generations of cells, but only in the sense of persistence of part of the substance in each cell-generation. The ultimate consequences of this hypothesis of the microsomes are far-reaching, and need not be considered here.

2. *Comparison of Living, and of the Fixed and Stained Protoplasm*

In general it may be said that the sections give an extraordinarily faithful picture of the protoplasm, with one exception, viz: the appearance of a reticulum in the ground substance (Fig. 1). Practically all methods of fixing and staining employed show this reticulum. I have already mentioned one reason for supposing it to be an artefact, viz: the rapid "Brownian" movements of the very particles (microsomes) that appear in the preparations to be in the nodes of the reticulum. A second reason for this belief is the fact that the spherules may be driven through the cytoplasm in any direction by centrifugal force without destroying its capacity for development (Part III, 1, *c*). A third argument is found in the formation of pseudopodia at certain stages in the experiments described in Part IV, of such exceeding tenuity that

an alveolar or reticular structure of the pseudopodial protoplasm in any way resembling that of the sections is impossible; but such delicate strands of protoplasm are living and exhibit characteristic movements, though they grade off into invisibility. When one adds that no reticulum can be seen by any of the usual devices in the living protoplasm, and that a similar reticulum has been demonstrated to be formed in colloid solutions known to be homogeneous by the usual methods of coagulation employed in the preservation of protoplasm, it certainly seems much more reasonable to conclude that the reticulum is an artefact in the preparations.

The preparations, however, in other respects greatly extend the observations that it is possible to make on the living protoplasm. Thus they enable one to distinguish different classes of microsomes and spherules by microchemical reaction, to differentiate the chromatin, and to obtain fixed pictures of stages that pass like a vision in the living egg. A proper correlation of observations on the living protoplasm and the fixed and stained sections greatly enlarges the scope of each and is indispensable for thorough interpretation.

It is not my intention to enter into a discussion of the various theories of the morphological composition of protoplasm; I desire only to meet one objection that will certainly occur to most cytologists, viz: as Wilson ('99) expresses it, "A continuous series of size gradations exists from the largest deutoplasm spheres down to the minutest 'granules,' and these bodies and the 'alveoli,' which form the middle terms of the series, arise in a sensibly homogeneous protoplasm." As I have already pointed out, the spherules in the protoplasm of Chætopterus grade down to the size of microsomes; it is also true that granules smaller than microsomes occur. Thus microsomes are terms in a series of *sizes*. This, however, does not prove that they may not be specific elements for the size-gradation may be interpreted in various ways. Microsomes are aggregations of molecules, and it is probable that, if we could trace their origin morphologically, smaller granules would be recognized as their precursors. It might, therefore, be better to use some other term than "microsomes" for the granules in question, but any

more specific term would be open to other objections. The word microsome is used in this paper only for a class of granules which are very numerous, very uniform in size, and with other specific characters described in the text and illustrated in the figures.

III. THE STRUCTURE OF THE EGG

1. Observations

a. Before Rupture of the Germinal Vesicle

The ovaries of Chætopterus occupy the bases of the enlarged parapodia in the posterior sexual division of the body, and have the form of convoluted tubes. The wall of the tubes is made up for the most part by the ova which extend from the lumen. The pole of the ovum farthest from the lumen may be called the free pole and that next the lumen of the ovarian tube, the attached pole. The epithelial arrangement of the ova remains until they are full grown, so that there is no difficulty in distinguishing free and attached poles at any stage. It may be stated at once that the free pole becomes the animal pole of the oösperm, and the attached pole consequently represents the vegetative pole. Thus it is possible to trace the germinal areas recognizable in the oösperm back to an early stage of the ovocyte.

By so doing the so-called period of growth of the ovogenesis assumes a new significance as a period in development, and it must be studied from this standpoint. This problem, however, will be left for later treatment, though certain broad features may be mentioned from time to time in the present paper.

The following account is based on a study of the living egg and of sections of material killed in picro-acetic acid, sublimate-acetic, Flemming's fluid and Zenker's fluid; sections from each kind of material were stained in the following ways: (1) Iron hæmatoxylin followed by orange G; (2) thionin and orange G; (3) neutral gentian (see Bensley '00). So that at least twelve different combinations were used, and all gave essentially similar results in differentiating the structures described. Thus it will not be neces-

sary to describe the results of particular methods in detail. Most of the features described were seen in the living egg and demonstrated also by experimental means.

The cytoplasm and germinal vesicle will be described separately.

1. *Cytoplasm.*—The main structural feature of the cytoplasm is its differentiation into two layers, an outer or *ectoplasmic* and an inner or *endoplasmic*.

The ectoplasm (Fig. 1) covers the free hemisphere and ends a short distance below the equator so that the endoplasm comes to
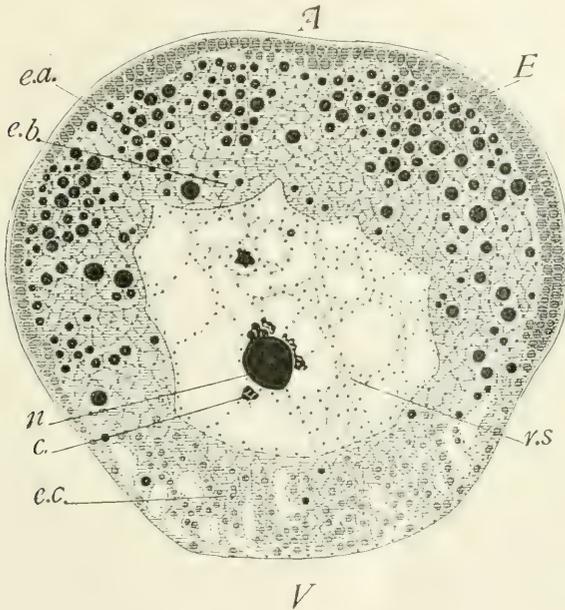
Fig. 1.   Axial section of a full-grown primary ovocyte, which still formed part of the ovarian epithelium.   Note that the ectoplasm is confined to the upper two-thirds of the egg.

A, Animal pole or free pole of ovarian ovocyte; *c*, chromatin; *E*, ectoplasm; *e.a.*, endoplasm *a*; *e.b.*, endoplasm *b*; *e.c.*, endoplasm *c*; *n*, nucleolus; *r.s.*, residual substance of the germinal vesicle; *s.n.*, sperm nucleus; *V*, vegetative pole or attached pole of ovarian ovocyte.

Note.—All figures of sections in this paper were drawn with the camera at a magnification of 1900 diameters (Zeiss comp. oc. 12 and obj. 2 mm. oil immersion).   They have been reduced to about 0.6 of original size.   Most of the drawings of living eggs were made with the camera at a magnification of about 400 diameters; these were subsequently redrawn, somewhat enlarged, in ink.

the surface at the vegetative pole. There is sometimes a defect in the ectoplasm in the center of the free or animal pole. This is particularly obvious after staining in thionin, for the spherules remain unstained and the microsomes stain intensely blue; thus is produced the effect of a blue plug in the center of the free hemisphere. The ectoplasm is covered externally by a very delicate membrane, and the vegetative pole where the endoplasm comes to the surface is naked. The constant characteristic of the ectoplasm that enables one to follow it throughout the development is the presence of a large number of spherules of very uniform size, closely set together so as to produce the effect of a pavement when one focuses on the surface of the entire egg. These are as large as the average spherules of the endoplasm, but they differ from them in many respects: (1) In the living egg they are colorless, whereas the endoplasmic spherules are yellow in mass; (2) their position and arrangement are characteristic in each stage; (3) they always stain differently from the endoplasmic spherules; thus the osmic acid of Flemming's fluid leaves them unstained, but stains the endoplasmic spherules from gray to solid black; a section of such an egg mounted in balsam appears to have a layer of vacuoles in the ectoplasm, which close examination shows to be the ectoplasmic spherules. After iron hæmatoxylin and orange G following either picro-acetic or sublimate-acetic fixation, the ectoplasmic spherules are intensely orange, while the larger endoplasmic spherules are black; this result is easily obtained by proper extraction of the iron hæmatoxylin, which at first stains all spherules black, but which washes out of the ectoplasmic spherules long before it does from the larger endoplasmic ones; if one stops the extraction at this point, and stains in orange G a very clear differentiation of the ectoplasm is obtained. Other methods and stains differentiate the ectoplasm in different ways, but as I am unable at present to reason from the microchemical reaction to the chemical nature of the spherules involved, it seems superfluous to describe the details.

There are from one to three layers of these spherules in the ectoplasm over the upper hemisphere (Fig. 1). These are embedded in the ground substance which likewise contains micro-

somes external to the spherules as well as between and on them. The layer of the ground substance external to the ectoplasmic spherules may be called the surface pellicle; during the maturation and cleavage this pellicle forms characteristic waves described later on.

The endoplasm is not so uniform in its composition as the ectoplasm. There is, in the first place, a striking difference between the upper and lower hemispheres (Fig. 1). In the upper hemisphere the protoplasm is dense, and contains an aggregation of the largest spherules, which stain black in the osmic acid or iron hematoxylin (endoplasm *a*); these are massed largely toward the periphery leaving a zone of protoplasm (endoplasm *b*); surrounding the upper half of the germinal vesicle, relatively free from the spherules. Thus thionin (which is an intense microsomal stain) shows a deep blue crescentic area surrounding the upper half of the germinal vesicle, and sending out processes among the spherules to the periphery. At the free pole these processes are strongest and may cause an interruption in the ectoplasm, where the endoplasm comes to the surface. There is a beautiful reticulum in the cytoplasm where it is not concealed by spherules, and the microsomes lie in the nodes; I have already given reasons for believing that the reticulum is an artefact. In the lower hemisphere the protoplasm is vacuolated, and contains smaller spherules that stain differently from the larger endoplasmic spherules[1] (endoplasm *c*).

Thus the endoplasm comes to the surface at two places, (1) a small area in the center of the animal pole, where the polar globules are later formed;[2] (2) over a large portion of the vegetative hemisphere where the spermatozoön usually enters.

2. *The Germinal Vesicle.*—The diameter of the germinal

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[1] The endoplasmic spherules have collectively a yellow tinge that is very bright in the eggs of some individuals and relatively pale in others. The spherule-bearing endoplasm is, therefore, often named in the following pages the yellow endoplasm, or yellow substance.

[2] I have not always been able to find this ectoplasmic defect in the primary ovocyte prior to the rupture of the germinal vesicle, and at the best it is relatively slight. But I believe it to be significant, for it marks the position of a constant exposure of the endoplasm that arises at the time of formation of the first maturation spindle, and persists throughout the development. The apical flagella arise from this exposed endoplasm.

vesicle is approximately half that of the entire egg (Fig. 1), and
thus comprises about one-eighth of the substance of the
latter. Only a small portion of it goes to form the chromo-
somes of the maturation spindles. There is also a large nucleolus.
The remainder is a special substance which I shall call the residual
substance of the germinal vesicle (Fig. 1, *r. s.*); it plays an impor-
tant rôle in the developmental processes and can be assembled,
even hours after the germinal vesicle has broken down, by the
action of centrifugal force; thus it is certain that it does not
"intermingle" with the rest of the cytoplasm. It is important,
therefore, to describe carefully the structure of the vesicle.

In the living condition one recognizes easily the membrane and
the large nucleolus; the peripheral part of the vesicle contains
more vacuoles than the central mass and thus appears less dense
(see Figs. 6–16). Apart from the droplets occupying the vacuoles,
the substance consists of an enormous number of microsomes and
small spherules embedded in a homogeneous ground substance.
The chromatin is not separately visible in the living germinal
vesicle.

In sections stained with iron hæmatoxylin and orange G the
germinal vesicle (Fig. 1) is seen to be occupied by a meshwork of a
few thick strands, bounding vacuoles. The strands are penetrated
through and through with microsomes that stain orange instead
of hæmatoxylin like the cytomicrosomes. But when the germinal
vesicle breaks down, these microsomes change their staining
reaction to that of cytomicrosomes. With thionin and orange the
cytomicrosomes stain an intense blue and the microsomes of the
germinal vesicle, orange, until the germinal vesicle breaks down,
when they, too, take the blue thionin stain. Thus the staining
reaction sharply differentiates the microsomes of the cytoplasm
and germinal vesicle. The membrane of the germinal vesicle
consists of a thin layer of the ground substance of the nucleus in
which the microsomes take the acid stain, and a thin layer of the
ground substance of the cytoplasm in which the microsomes take
the basic stain.

My study of the nucleolus and chromosomes is incomplete and
I shall defer a detailed description for another occasion. The

close association of certain chromosomes with the nucleolus is a very striking and constant character (see Figs. 1 and 4).

It will be seen, then, that the full grown primary ovocyte is highly differentiated: (1) Its polarity is expressed by, (*a*) the constant relation of free and attached poles; (*b*) the extension of the ectoplasm over the upper hemisphere mainly; (*c*) the exposure of the endoplasm at a small spot in the free pole and over a large area at the attached pole; (*d*) the contrast between the structure of the endoplasm in the free and attached hemispheres. (2) The existence of a sharply differentiated ectoplasm, of three kinds of endoplasm, and of the various substances of the germinal vesicle, gives a degree of orginal diversity that is certainly surprising.

Our problem is to trace these substances in development, to note the appearance of other substances, and from the behavior of all in the normal and experimentally modified development, to attempt to discover their respective rôles in the formation of an embryo. We should not attempt to prejudge the matter in advance by naming certain substances yolk or food-matters and others formative substances. If these are true categories, it should be possible to demonstrate it.

The following account will show that these substances are moved by internal forces to definite locations in the embryo and become parts of definite systems of organs. It will also be shown that the localization, and part of the differentiation, is independent of the process of cell division, though closely dependent upon interaction with nuclear derivatives.

b. The Period of Maturation and Fertilization

1. Polarization.[1]—As long as the germinal vesicle is intact the arrangement of the various substances in the egg does not conform in all respects to the position of the future embryonic areas. The rupture of the germinal vesicle initiates a series of movements of the substances by means of which they attain to their

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[1] The substances of the egg are polarized before this process, but have a radically different arrangement; a more exact term for the process would, therefore, be re-polarization, which is objectionable in other respects.

definitive positions during the prophases of the first maturation division. This rearrangement of the substances may be called the process of polarization, inasmuch as it takes place with reference to the polar axis of the egg. It produces a topographical arrangement of the substances that corresponds in many essential respects to the future embryonic areas. Thus the history of the maturation process is an important chapter in the development. Fertilization, which occurs during this period, will be considered only incidentally, as I have nothing substantially new to add to Mead's fine account.

If the eggs of Chætopterus be taken and allowed to stand in sea-water, the germinal vesicle breaks down, whether the eggs be fertilized or not; the first maturation spindle forms and moves to the predelineated animal pole; at the same time the various substances become polarized. Thus polarization is independent of fertilization. Unless the eggs be fertilized or stimulated in some other definite fashion, they remain in the metaphase of the first maturation division indefinitely (Mead '98), (Lillie '02). If the eggs be so fertilized or stimulated, the polar bodies are formed and the processes of development follow continuously.

The movements of the three classes of substances, ectoplasm, endoplasm, and the substances of the germinal vesicle will be described separately; they have reached practically their definitive positions by the time that the first maturation spindle becomes fixed at the periphery.

Ectoplasm.—Before the breaking of the germinal vesicle the ectoplasm covers the upper two-thirds of the egg; as soon as the germinal vesicle breaks down, it flows toward the vegetative pole, and, even before the first maturation spindle has become fixed at the animal pole, it has completely overflowed it (Figs. 2 and 4), thus covering up the exposed endoplasm. The membrane accompanies the ectoplasm and so covers the entire egg. This movement of the ectoplasm is clearly visible in the living egg as a series of waves of the transparent external pellicle; but, owing to the difficulty of orienting the living egg in this stage, I have not been able to follow the course of the waves satisfactorily. The sections, however, that enable one to trace the overflow of the vege-

tative pole, leave no doubt that they are associated with this process.

At the end of the polarization period the ectoplasm around the center of the vegetative hemisphere is as thick as anywhere else.
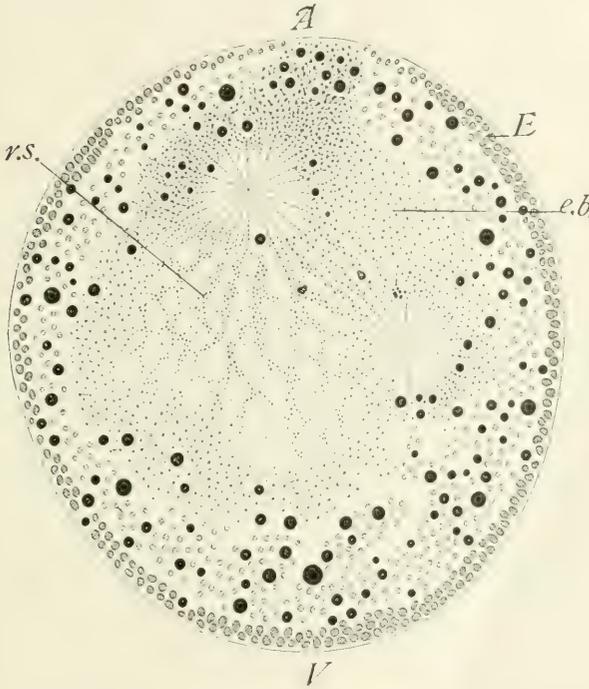
Fig. 2. Axial section through a primary ovocyte, killed about ten minutes after coming into sea-water, and three minutes after fertilization. The ectoplasm has already flowed to the vegetative pole, leaving an exposed area of endoplasm at the animal pole. Part of the *a* endoplasm has likewise flowed to the vegetative pole. The germinal vesicle has broken down and the maturation spindle is in process of formation between the two primary asters. The residual substance of the germinal vesicle is clearly seen. The chromosomes do not fall in the plane of the section. Letters same as in Fig. 1.

The original opening in the ectoplasm at the animal pole has become enlarged, and the outer end of the maturation spindle is fixed here (Figs. 2–5).

Endoplasm.—The endoplasm consists of three distinct parts as already described, (*a*) the uppermost part laden with large

spherules; (*b*) the non-spherular part overlying the germinal vesicle; (*c*) the vacuolated part containing small spherules situated below the germinal vesicle. In the process of polarization the endoplasmic substances, *a* and *b*, reverse their relations both in regard to one another and also with respect to the germinal vesicle. Substance *a* flows around substance *b* and the germinal vesicle toward the vegetative pole where it intermingles
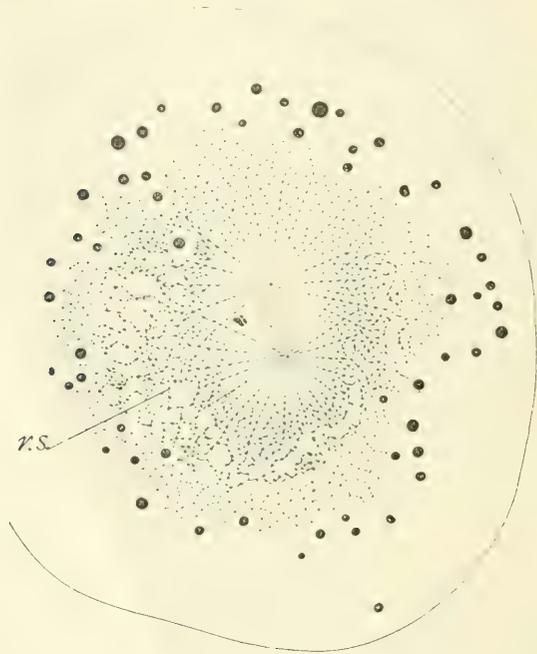
Fig. 3. A later stage of the same series, as in Fig. 2, killed twelve minutes after fertilization. It shows the entry of the first maturation spindle into the residual substance of the germinal vesicle. Orientation of section uncertain. Letters same as in Fig. 1.

with *c*, so that it is difficult for a time to distinguish them apart, except that the two kinds of spherules may be recognized intermingled (Figs. 2, 4 and 5). Substance *b* on the other hand maintains pretty nearly its original position, and the substance of the germinal vesicle passes up through it to the animal pole so as

to lie above it. The definitive positions of these substances is shown in Fig. 5. An intermediate stage is shown in Figs. 2 and 4.

Though endoplasmic substances a and c intermingle, they do not lose their identity. This is shown by their subsequent behavior; if, for instance, the eggs are allowed to remain unfer-
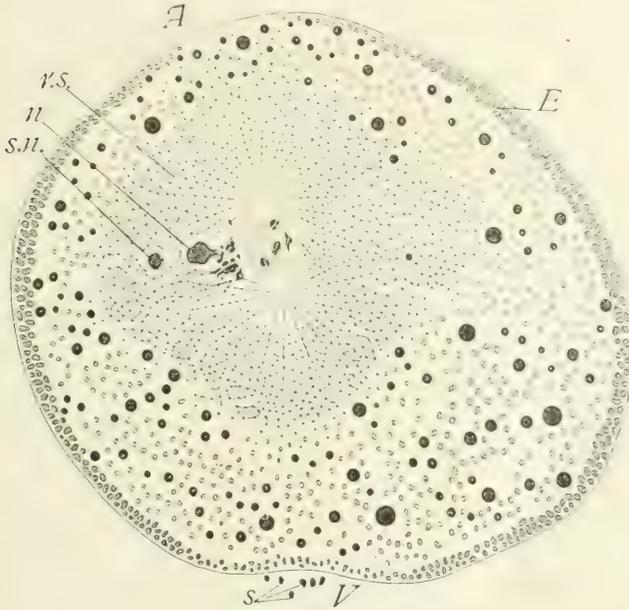
Fig. 4. Axial section of a primary ovocyte of the same series, as in Fig. 2, killed twelve minutes after fertilization. The maturation spindle is completely surrounded by the residual substance of the germinal vesicle, and the latter is directed toward the animal pole where the ectoplasm is wanting. The nucleolus has become smaller and some of the chromosomes appear to have arisen from it (compare Fig. 1). Letters same as in Fig. 1. *s*, spermatozoa.

tilized they will be found to have completely separated again in the course of about two hours (see Fig. 39, p. 208, representing a section of an egg that has stood two hours and seven minutes in sea-water). These substances also separate out in normally segmenting eggs and in unsegmented eggs that are undergoing differentiation.

The spermatozoön normally enters the egg through the exposed endoplasm of the vegetative pole, which usually appears slightly collapsed at this time (Fig. 4) and less vacuolated, as though there had been loss of fluid. That the spermatozoön may enter
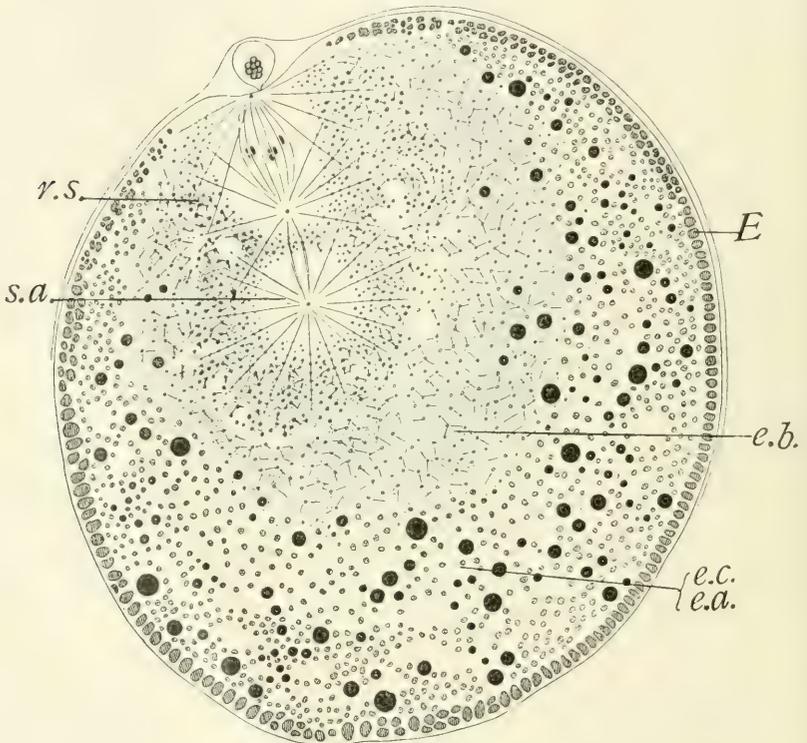
Fig. 5. Axial section of a secondary ovocyte thirty-two minutes after fertilization. *E.* Ectoplasm; *e.a.*, endoplasm *a*; *e.b.*, endoplasm *b*; *e.c.*, endoplasm *c*; *r.s.*, residual substance of the germinal vesicle; *s.a.*, sperm aster.

through the ectoplasm is shown by the fact that the eggs fertilize as readily after polarization as before.

Germinal Vesicle.—In the case of the germinal vesicle we have changes not only of position, but also in the character of the substances concerned:
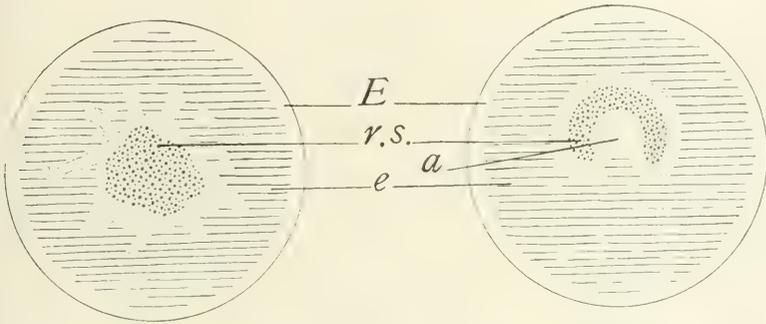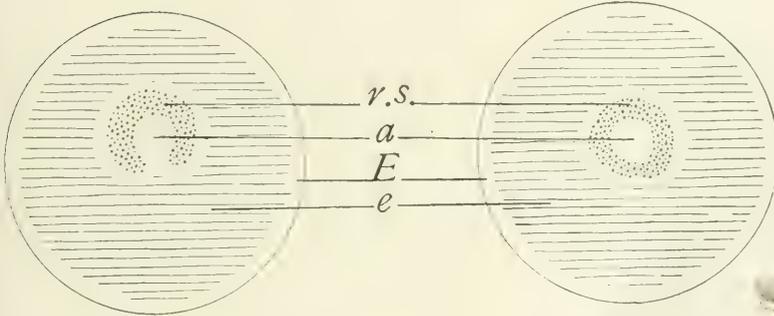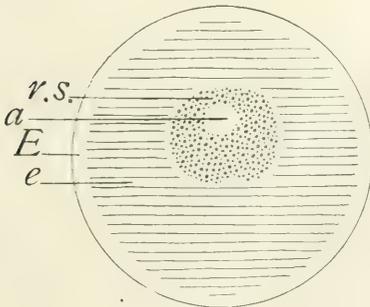
Figs. 6 to 10. See text for detailed description. Behavior of substances of the germinal vesicle as seen in a single living egg. Polar view. Fig. 6. 11 A. M. Rupture of the germinal vesicle. Outflow of cortical substance of the germinal vesicle. Fig. 7. 11.04 A. M. Fig. 8. 11.07 A. M. Fig. 9. 11.15 A. M. Fig. 10. 11.20 A. M. *a*, Aster or polar view of spindle; *E*, ectoplasm; *e*, endoplasm; *r.s.*, residual substance of the germinal vesicle.

As already described, one distinguishes readily in the living germinal vesicle a clearer cortical zone and a central more granular mass (Fig. 6). Soon after the eggs are in the sea-water the membrane weakens at a number of points at once and streams of the clear cortical substance can be seen to radiate out in all directions into the surrounding protoplasm (Fig. 6); these then form little islands of clear substance surrounding the germinal vesicle, and around two of these islands radiations begin to appear, and a spindle is formed between the two asters thus arising and the latter then approach one another until they are quite near together. In a polar view one can then see the granular substance of the germinal vesicle fold around the spindle (which contains the chromosomes as sections show) and finally form a complete envelope around it and the inner aster.

Figs. 6 to 10, drawn from the living egg, illustrate this process; the entire mass is moving to the animal pole during the process. At first the residual substance of the germinal vesicle lies at one side of the spindle (Fig. 7, 11.04 A. M.) and it is surrounded by a clear zone proceeding from the original cortical layer of the germinal vesicle; the residual substance of the germinal vesicle is horseshoe shaped, and the spindle occupies the opening. Fig. 8 is a sketch of the same egg three minutes later (11.07); the limbs of the horseshoe are bending toward one another so as to enclose the spindle. Eight minutes later (Fig. 9, 11.15) the limbs of the horseshoe have almost met around the spindle. At 11.20 (Fig. 10) the limbs have fused completely and a slight indentation and corresponding projection of the endoplasm mark the place of union. The polar view of the forming spindle is always the same.

If one has a side view instead of a polar view (Figs. 11 to 16), some aspects of the process can be seen better; thus the fact that the inner end of the spindle is completely surrounded by the granular substance comes more clearly to view. Figs. 11–15 show the spindle entering the residual substance. They are drawings of a single living egg taken at about two-minute intervals (compare the section shown in Fig. 3).

Thus the germinal vesicle is transported practically intact to the animal pole; during the process a considerable quantity of fluid
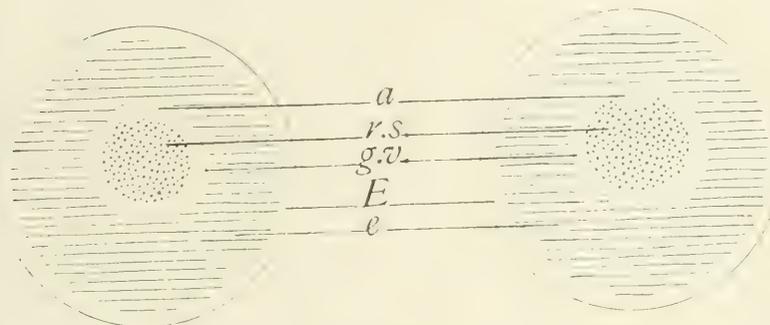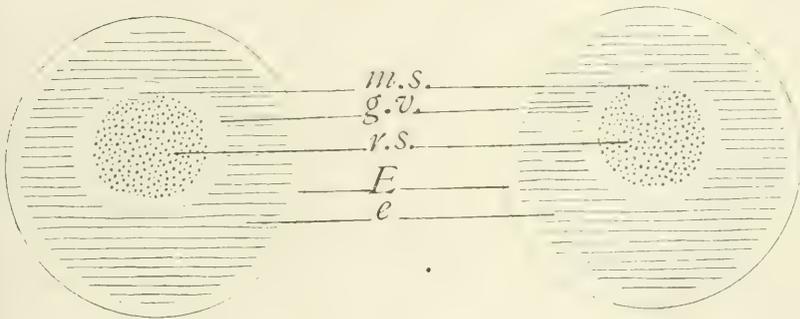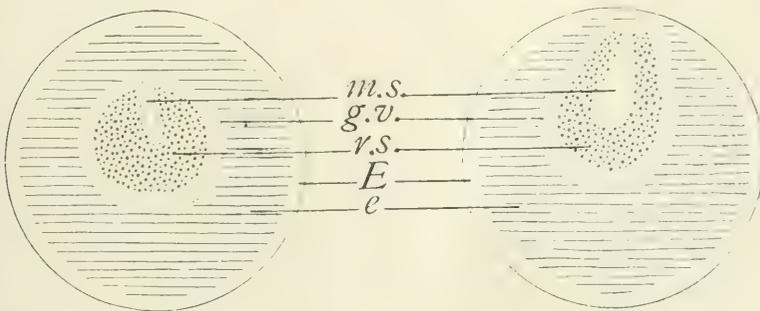
Figs. 11 to 16. For detailed description, see text. Behavior of substances of the germinal vesicle, side view. Figs. 11 to 15. Five views of the same living egg drawn with the camera, showing the origin of the maturation spindle and behavior of the cortical layer and residual substance of the germinal vesicle. Fig. 11. 9.46 A. M. Fig. 12. 9.48 A. M. Fig. 13. 9.51 A. M. Fig. 14. 9.53 A. M. Fig. 15. 9.54 A. M. Fig. 16. A later stage drawn from another living egg. It will be observed that the spindle arises between two separate primary asters and gradually sinks into the residual substance of the germinal vesicle, which finally forms a mantle completely surrounding it with the exception of the external end. The clear cortical layer of the germinal vesicle retains its original relation. Compare also Figs. 6 to 10. *a*, Aster; *E*, ectoplasm; *e*, endoplasm; *g.v*, germinal vesicle; *m.s.*, maturation spindle; *r.s.*, residual substance of the germinal vesicle.

has diffused throughout the cytoplasm, but this is the only loss. The end-condition is similar in some respects to the "secondary germinal vesicle" of Cyclops according to Häcker's description ('02).

The sections enable one to follow many details that are obscure in the living egg and to trace chemical transformations, obvious as changes in staining reaction in the residual substance of the germinal vesicle.

In the first place it is shown by the sections that the polarization of the spherules begins immediately after the rupture of the germinal vesicle. In the second place the chromosomes begin to separate from the surface of the nucleolus as soon as the wall of the germinal vesicle is ruptured, and the nucleolus (in consequence?) appears shrunken and vacuolated (Fig. 4). In the third place the staining reaction of the microsomes of the germinal vesicle begins to change from acid to basic, first at the periphery and later toward the center. Thus with a thionin and orange stain one finds eggs in which the peripheral microsomes of the germinal vesicle stain blue, and the central ones still stain orange; with hæmatoxylin and orange the peripheral microsomes at this stage take the hæmatoxylin and the central ones the orange. This cannot be satisfactorily shown in a figure without the use of color. This change in staining reaction rapidly embraces all the microsomes of the germinal vesicle, and at the same time the whole mass shrinks considerably owing to the diffusion of the original droplets of the germinal vesicle.

During this period of diffusion of the fluid substance of the germinal vesicle and the ensuing polarization of the ectoplasm and endoplasm, the protoplasm as a whole possesses a much higher degree of fluidity than before. The evidence for this, apart from the observed diffusion of the fluid of the germinal vesicle, is found in the change of contour of the sectioned eggs; before the rupture of the germinal vesicle, and again after the polarization is complete, the contours are perfectly regular in practically every egg. But during the period of polarization the contours are usually quite irregular, although the method of preparation is exactly the same. Moreover, the demarcation of the various endoplasmic substances is no longer clear (Figs. 2 and 4).

The strongest evidence for greater fluidity at this time is found in the fact that the ectoplasmic spherules are much more numerous and smaller than they were previously or than they are subsequently (compare Fig. 1 with 2 and 4). Evidently there is a reversible process of coagulation concerned, the spherules breaking into smaller particles as the fluidity increases, and setting or coagulating again by a process of fusion.

Mead ('98) has given a very careful account of the origin of the asters of the maturation spindle in Chætopterus. According to his account a large number of asters arise around the ruptured germinal vesicle; two of these, which he calls primary asters, become large, and form the asters and create the spindle of the maturation divisions. My observations confirm those of Mead in almost every particular; though I have not found numerous asters in the sections. Indeed in my preparations Mead's secondary asters appear simply as fluid droplets from the germinal vesicle with very slight indication of radiations. But as, according to my observations on the living egg, the primary asters likewise arise around fluid droplets from the germinal vesicle, it seems quite possible that Mead's interpretation, that there is no essential difference between primary and secondary asters, is correct. The primary asters certainly arise separately; the observations on the living egg and on sections are perfectly conclusive in this respect. They may at first be near together or very far apart, but they soon exert an influence on one another and a spindle arises between them (Figs. 11 and 12).

Figs. 1, 2, 4 and 5 illustrate sufficiently well for our present purpose the history and composition of the chromosomes. They show at the same time the microscopic structure of the residual substance of the germinal vesicle as it appears in sections, and confirm the observations of its migration to the animal pole along with the spindle. Thus Fig. 3 shows the spindle entering the residual substance; Fig. 4 a slightly later stage, and Fig. 5 the positions, structure and relations of the spindle and residual substance after they have attained the pole. The microsomes are distinguishable from ordinary cytomicrosomes only by their slightly larger size. Before the germinal vesicle breaks down the residual substance is represented by the coarse reticulum of the

germinal vesicle studded with innumerable microsomes that take the acid stain. When the germinal vesicle breaks down the reticulum shrinks together with the partial escape of the contained fluid and the staining reaction of the microsomes changes to basic like the cytoplasmic microsomes; all the fluid, however, is not squeezed out and it retains fluid droplets of considerable size that give it a permanent reticular structure. The meshes are thick and the microsomes are larger than the cytomicrosomes. It can now be traced by virtue of these structural peculiarities.

The nucleolus is carried along, with the chromosomes that are attached to it, by the residual substance, and it is therefore found at one side of the equatorial plate of the first maturation spindle (Fig. 4), where it has been figured by Mead. It gradually disappears and is lost from view.

The topography of the polarized ovocyte is shown in Fig. 5. It will be seen by comparison with Fig. 1 what a complete rearrangement of the substances of the unpolarized ovocyte has taken place; however, no new substances have been formed, though the substances of the germinal vesicle have undergone considerable changes.

2. *The Later Processes of Maturation and Fertilization.*—The formation of the polar globules and the presence of the sperm nucleus in the egg do not involve any important changes in topography of the substances, though the retreat of the egg nucleus to the center of the egg after the formation of the second polar body involves a more central localization of the residual substance of the germinal vesicle. Thus the topography of the oösperm is essentially the same as that of the polarized ovocyte.

The problems of the nature of the maturation divisions, behavior of the sperm and egg nuclei and origin of the cleavage centrosomes do not fall within the scope of this paper. So far as my observations go they confirm in most respects the account of A. D. Mead ('98). I expect to take up the behavior of the chromatin in a subsequent paper. It should be noted here, however, as bearing on the later portions of the present paper that the number of chromosomes in the maturation spindle is constantly nine, arranged usually in the form of a circle of eight with one central chromosome; the same number was found by Mead.

c. Action of Centrifugal Force in the Unsegmented Egg[1]

If the eggs of Chætopterus be taken and allowed to stand in sea-water without fertilization the germinal vesicle breaks down, the substances become polarized as already described, and the first maturation spindle is formed and moves to the animal pole, where it remains in metaphase indefinitely (unless the egg be fertilized or stimulated in some definite fashion). This condition is reached in from fifteen to thirty minutes, depending on temperature and some unknown factors.

If, at any later time before their death, the eggs be submitted to a fairly strong centrifugal action (1500 to 2000 revolutions in one minute) and then examined, it will be found that the endoplasm is arranged in three distinct layers, viz: a small gray cap, a clear band and a yellow hemisphere (Photographs A–H, and Figs. 17–22). The gray cap and clear band together take up about one hemisphere. The ectoplasmic layer is not visibly affected (see photographs and Fig. 24) and forms, as before, a continuous layer with an aperture at the animal pole. The maturation spindle is usually found fixed at the periphery immediately after centrifuging, so that it is obvious that it has not been moved by the centrifugal force.

These aggregations of substances bear no definite relation to the polarity of the egg, for the maturation spindle may be found either in the gray cap, which is then ring-shaped (Photograph H and Fig. 19), or in the center of the yellow hemisphere, or in any intermediate position. It is, however, most frequently found in the hemisphere containing the gray cap.

If now the eggs be fertilized, the polar bodies are formed at the original animal pole, and thus may appear in the gray cap, or at the center of the yellow hemisphere, or in any intermediate position; though in this case, again, they are found more frequently in the hemisphere containing the gray cap than in the yellow hemisphere (see various conditions illustrated in Photographs A–H and in Figs. 17–22).

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[1] The use of the centrifuge for the purpose of studying the composition of the protoplasm was suggested to me by Dr. E. P. Lyon, who had already obtained results, about to be published, on the ova of sea-urchins by this method.

Figs. 17 to 23. Showing the effects of centrifuging upon the living unsegmented egg. Compare Photographs A–I. *E*, Ectoplasm; *e*, yellow endoplasm; *c.b.*, clear band; *g.c.*, gray cap; *g.v.* germinal vesicle; *n*, nucleolus; *p.l.* polar lobe.

Fig. 17. Eggs in sea-water at 8.55 A. M. Centrifuged at 10.45 and fertilized. Drawn with camera at 12 o'clock noon. The gray cap lies in the vegetative hemisphere.

Fig 18. Eggs in sea-water at 1.55 P. M. Centrifuged 2.30 P. M. Fertilized 2.45 P. M. Drawn 3.40 P.M. The gray cap extends into the polar lobe. Beginning of the first cleavage.

Fig. 19. Eggs in sea-water 8.50 A. M. Centrifuged 9.40 A. M. Fertilized 9.45 A. M. Drawn 9.55 A. M. The gray cap surrounds the maturation spindle and is thus ring-shaped.

Fig. 20. Eggs in sea-water 1.55 P. M. Fertilized 2.03 P. M. Centrifuged 2.50 P. M. Drawn 3.00 P. M. The gray cap has passed entirely into the larger cell *CD*.

Fig. 21. Eggs in sea-water 8.50 A. M. Centrifuged 9.40 A. M. Fertilized 9.45 A. M. Drawn 10.20 A. M. The gray cap lies opposite to the animal pole.

Fig. 22. Drawn 10.29 A. M. History otherwise the same as No. 21. Shows intermediate position of gray cap.

Fig. 23. In sea-water 9.08 A. M. Centrifuged at same time before rupture of the germinal vesicle. No stratification. Note the position of the nucleolus within the germinal vesicle.
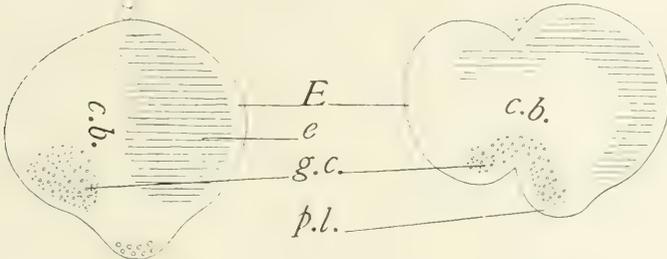
FIG. 17   FIG. 18
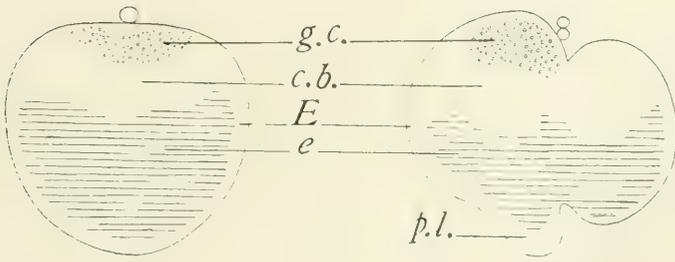
FIG. 19   FIG. 20
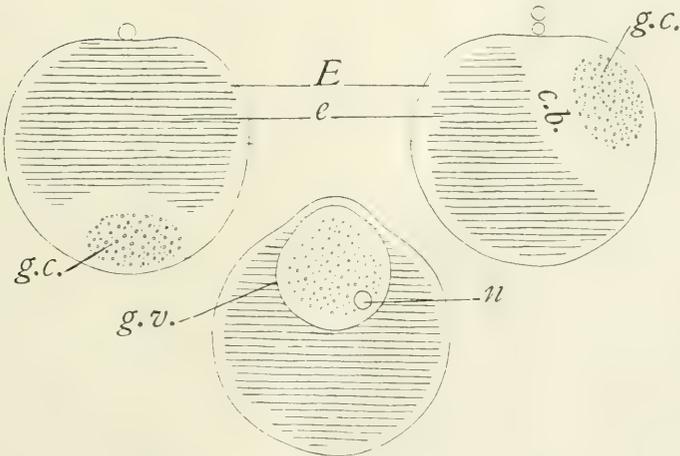
FIG. 21   FIG. 23   FIG. 22

The polar lobe appears at the usual time preceding the first cleavage, and always lies opposite to the polar globules, whatever be the position of the yellow substance (Photograph G and Figs. 17, 18 and 20). Thus, if the gray cap lies at the animal pole, the yellow substance extends into the base of the polar lobe, as normally; or, in the opposite case, the polar bodies lying at the center of the yellow hemisphere (Fig. 21), the gray cap may extend into the base of the polar lobe (Fig. 18). The structure of the peripheral part of the polar lobe is the same in either case, and does not differ from the normal.

It is therefore clear that the yellow and the gray substances are extremes as regards their specific gravity; they move freely through the ground substance of the protoplasm, and are thus distributed with reference to the direction of the centrifugal force, and so may occupy any position with reference to the polarity of the egg.

However, polarity is the force that governs the position of these substances in the normal development, causing aggregation of the yellow substance in the vegetative hemisphere and of the gray in the animal hemisphere. It is thus a stronger force than gravitation, for these substances aggregate in the same polar sense whatever be the position of the egg, but not so strong as the centrifugal force employed. It is now clear why the gray cap is thrown to the animal hemisphere more frequently than to the vegetative, for the force of polarity has already caused a partial aggregation of the substances before the centrifugal force is applied, and the eggs, therefore, tend to rotate with the heavier pole in a distal direction; but in a large percentage of the eggs, various causes combine to overcome this, the principal one being no doubt the crowding of the eggs in the bottom of the tube.

The process of cleavage takes place with reference to the polarity of the egg (Photograph I and Figs. 18 and 20) _and its fundamental form is not affected by the chance disposition of the endoplasmic substances though minor abnormalities result from certain forms of distribution._ Thus the first two cleavages are always meridional whatever the position of the yellow substance and the gray cap, and the third cleavage is equatorial and spiral in the usual sense.

The appearance of stratification given by centrifugal action is extremely striking, and it is so similiar to the banded condition described for echinoderms by Boveri ('01a and '01b) and for Dentalium by Wilson ('05), that one cannot fail to be impressed by the resemblance. Lyon has found that similar stratification may be produced in the eggs of echinoderms, annelids and tunicates by centrifugal action. So there can be but little doubt that it is a fundamental feature of egg-organization in the ova of bilateral animals.

It is a condition normally governed by the polarity of the egg, and does not become obvious under normal conditions in the egg of Chætopterus simply because the separation is not complete and precise, and thus the strata shade into one another, the animal pole being relatively light and grayish in hue and the vegetative relatively dense and yellow.

By the aid of the centrifuge one can also demonstrate that the substance of the gray cap is the residual substance of the germinal vesicle. If the eggs are centrifuged as usual immediately after they come into sea-water, the germinal vesicle being still intact, one does not get a gray cap or any banded appearance, but instead the germinal vesicle is thrown to the surface with such force that it may produce a protuberance of the cortical layer (Fig. 23), and the remainder of the endoplasm appears yellow and not strongly polarized by the centrifugal force; the cortex remains as before. The nucleolus always occupies the inner end of the germinal vesicle, showing that it is undoubtedly heavier than the remainder of the contents of the vesicle. Within the germinal vesicle there is no stratification, the granular substance preserving its earlier distributed arrangement. These eggs then mature on standing, and the maturation spindle moves to the periphery of the egg; in doing so it may take the longest course through the egg and become fixed at the pole opposite to that occupied by the germinal vesicle, or it may move to any other position. Thus it becomes practically certain that the polar force governs the migration and leads it to the animal pole. The only other assumption would be that the spindle determined polarity at whatever point on the surface it happened to be fixed, and then it would be perfectly incomprehensible why it sometimes migrates through the entire diameter of the egg.

If the eggs are allowed to stand eight to fifteen minutes in sea-water after being taken from the female, so that in some the germinal vesicle is still intact, and in others broken down, the latter always show the stratification more or less pronounced after centrifuging, and the former never show it, but instead the germinal vesicle is always peripheral and contains a granular mass similar to the substance of the gray cap.

Thus neither the gray cap nor the clear band are formed by centrifugal force unless the germinal vesicle is broken down, and the conclusion is inevitable that the entire gray cap is derived from the germinal vesicle. The yellow substance does not aggregate so closely before as after the germinal vesicle has broken down. Hence it is probable that the appearance of the clear band is due very largely to concentration of the yellow spherules at one pole. It would seem that the endoplasm has become less viscid as a result of the diffusion of substance from the germinal vesicle, so as to permit closer aggregation of the yellow granules. The structure of the gray cap completes the identification for it is the same as the residual substance of the germinal vesicle (Fig. 24; compare figures of normal maturation).

The maturation spindle is usually found fixed at the periphery immediately after centrifuging, whatever the direction of the centrifugal force may have been; when, however, it is torn loose, as sometimes happens, it is found in the clear band. Thus it is intermediate between the gray cap and yellow endoplasm in specific gravity. It is clear, therefore, that when it is situated in the region of the gray cap or yellow endoplasm, the centrifugal force must cause it to exert traction on the cortex of the ovum. That this is actually the case is shown by the form of the section of many eggs killed immediately after centrifuging, in which there is a very pronounced depression at the animal pole where the maturation spindle is attached (Fig. 24).

These sections are very interesting in two other respects: (*a*) When the spindle is torn loose from the periphery, it moves as a whole and carries the chromosomes with it—chromosomes and spindle are never found separated by the centrifugal force. This bears witness to the extreme viscosity of the spindle area, for it

can hardly be possible that chromosomes and spindle are of exactly
the same specific gravity; moreover the spindle preserves its form
intact, and is not bent or broken in any manner so far as I have
observed, though it must be subjected to considerable strain.
(*b*) If the eggs be fixed (killed) immediately after centrifuging, and
sectioned, stained and mounted in the usual manner, it is found
that the asters are almost entirely or entirely wanting, though
sections of the same eggs prepared without centrifuging show
well-developed asters at both poles of the spindle. The cyto-
plasm surrounding the spindle is dense and filled with microsomes
more numerous and smaller than usual.
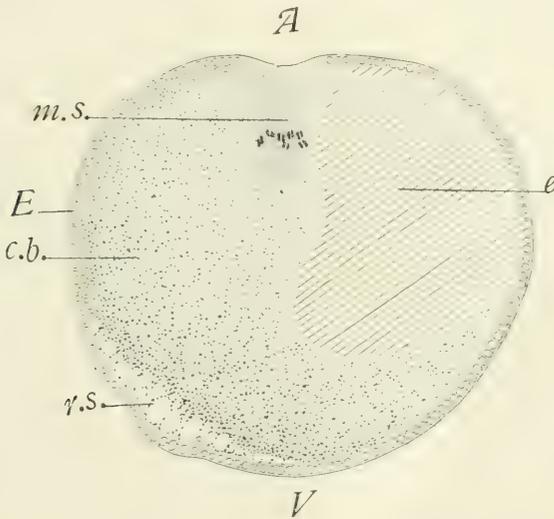
Fig. 24 Section of an egg killed in picro-sulphuric acid immediately after centrifuging. The clear
band *c.b.* is seen to be densely microsomal. The ectoplasm retains its original arrangement. The
yellow endoplasm and the residual substance of the germinal vesicle are at opposite sides of the
egg. The maturation spindle retains its original position. *A*, Animal pole; *c.b.*, clear band; *E*,
ectoplasm; *e*, endoplasm; *m.s.*, maturation spindle; *r.s.*, residual substance of the germinal vesicle;
V, vegetative pole.

*The residual substance of the germinal vesicle has been stripped
off the spindle and has given place to more finely microsomal pro-
toplasm.* The sphere and centrosome, however, remain with the
spindle (Fig. 24). If the eggs are permitted to stand after centri-

fuging the radiations appear; but their character is different from those of the normal egg, owing to the absence of the residual substance of the germinal vesicle around the spindle.

The most obvious interpretation of the clear band is that it represents the pure ground substance cleared of spherules by centrifugal force. This was, indeed, the interpretation that I gave it when working on the living material. The results obtained by staining, in hæmatoxylin, entire eggs killed in picro-sulphuric or picro-acetic acid, shows that this interpretation is only partially correct; for in these eggs the original clear band appears intense violet in color, and was not, for this reason, at first recognized; in such preparations the gray cap is stained relatively lightly and is seen to be highly vacuolated. The yellow mass is unstained and dense. Sections of such eggs (Fig. 24) show that the clear band contains most of the endoplasmic microsomes (which, as we have seen before, have strong affinity for basic dyes), embedded in ground substance. *Thus the centrifugal force has separated the microsomes and spherules of the endoplasm, the former then appearing as the clear band and the latter as the yellow mass.* This appears to me to furnish important evidence for the specific nature of the microsomes as argued throughout this paper. The result is very clear-cut and striking. The microsomes are thus seen to differ from the spherules in specific gravity as well as in the other respects already noted. Moreover, when the spindle is torn loose from the periphery, it is invariably found with the contained chromosomes in the clear band, thus showing that the microsomes have the same specific gravity as the chromosomes. This observation should be correlated with others described in this paper, showing origin of microsomes from chromosomes.

The definiteness and fixity of polarity is one of the most striking results brought out by centrifuging the eggs. The direction of polarity is not altered by any arrangement given to the endoplasmic substances, and it asserts its power over the maturation spindle under all the various circumstances of the experiments; similarly it determines the location of the polar lobe under all conditions, and, under normal conditions, regulates the distribution of the ectoplasm and the endoplasm.

The following statements may be made concerning the polarity of the egg of Chætopterus:

1. Polarity may be traced from the stage of the youngest ovocyte continuously.

2. In the ovocyte the free end of the egg is the animal pole and the attached end the vegetative pole.

3. It is not determined whether the oögonia are polarized or whether the polarity arises in the ovocyte owing to the relations of free and attached poles, or from some other external cause. My own view is that the polarity is inherent and is a property of the nucleus.

4. In the full-grown ovocytes and oötid the polarity is unaltered by any change of distribution of the granules of the endoplasm; it must, therefore, be a property of the residual protoplasm.

5. Polarity, however, determines the normal distribution of the granules in the egg, and is thus a force stronger than gravitation, because the distribution of the spherules is normally fixed with reference to polarity, but may bear any relation to the direction of gravitation.

6. A centrifugal force may overcome the polarity and distribute the spherules correspondingly.

The effects of polarity in the subsequent development will be onsidered in Part IV.

2. *Literature and Discussion*

a. The Axis of the Ovarian Ovocyte and its Relation to Polarity

It appears to be the rule among annelids and molluscs that the attached pole of the egg in the ovary becomes the vegetative pole of the oösperm. It is true that the number of observations bearing on this point is relatively small (Stauffacher '93, Lillie '95, Conklin '02 and '03).

On the other hand Boveri's observations ('01a and '01b) make it probable that in Strongylocentrotus the conditions are reversed. There is no theoretical objection to such a contrast. On the contrary, as Conklin has argued in his very suggestive paper on

"Inverse Symmetry" ('03), reversal of polarity would furnish a very simple and complete explanation of the condition of inverse symmetry. The considerations that Conklin brings forward in this paper, together with the actual observations in the literature, combine to render reasonably certain the hypothesis, that in all animals the axis of the epithelial ovarian ova becomes the primary axis of the segmenting ovum. It seems reasonably certain also that the poles of this axis may be interchangeable, owing probably to inverse polarization of the formative stuffs of the ovocyte.

b. The Ectoplasmic Layer

I have been surprised to find, since my observations on Chætopterus were completed, what a large number of definite observations on the existence of an ectoplasmic layer in the eggs of various animals were to be found in the literature. These have not been recently collated and their importance is, therefore, not fully realized by many embryologists. When brought together these observations bring into one category the "polar rings," "yolk-lobe" and certain other phenomena described by various authors; I therefore give rather full citations.

In Clepsine (Whitman '78) the "polar rings" evidently correspond to the two divisions of the ectoplasm of Chætopterus (see Part IV, 1, *a*, of the present paper). These rings appear after the formation of both polar globules and are situated near the upper and lower poles, respectively. Each consists of a "transparent fluid substance," radiating lines of which at first extend on the surface from each toward the equator of the egg; in my opinion these indicate the origin of the rings from an ectoplasmic layer, as in Rhynchelmis, though Whitman expresses no opinion concerning the origin of this substance. The upper ring contracts around the animal pole, but retains its central opening, which is usually eccentric, thus agreeing exactly with Chætopterus (see Photograph H). The substance of the lower ring aggregates at the lower pole in the form of a disc. Subsequently the substances of both rings plunge deep into the egg, and the upper ring substance is transmitted to the left posterior macromere, D, as in

Rhynchelmis; what becomes of the lower ring substance is uncertain.

The conditions in the egg of Rhynchelmis (Vejdovsky '88–'92) are most like those in Chætopterus. The condition before the rupture of the germinal vesicle is thus described by Vejdovsky (p. 32): "Unter der äusserst feinen Dottermembran, die man künstlich von der Eisubstanz überhaupt nicht abheben kann, erstreckt sich eine 0.003 mm. hohe Schicht des peripherischen Plasma, welches sich in Pikrocarmin intensiver färbt und deshalb ohne grosse Schwierigkeit wahrzunehmen ist (Holzschnitt Fig. I, A). Sie besteht aus einer hyalinen Grundsubstanz, in welches äusserst feine, aber doch deutliche, in Pikrocarmin intensiv sich färbende Plasmakörnchen, eingebettet sind. Bei starken Vergrösserungen ist es nicht schwierig sicher zu stellen, dass diese Körnchen regelmässig schichtenweise und concentrisch in der Grundsubstanz angeordnet sind, in einigen Fällen scheint es aber, dass sie hier unregelmässig zerstreut sind. Diese Körnchen sind denjenigen gleichzustellen, die man viel deutlicher an den Fasern des Cytoplasmanetzes wahrnimmt. Im Leben ist diese Protoplasmaschicht braun." According to this account it would appear that the ectoplasmic layer covers the entire ovum before the rupture of the germinal vesicle.

After the formation of the polar globules the ectoplasmic layer ruptures near the equator of the egg and flows to the two poles. Cases were observed where the accumulation took place entirely at the animal pole. The defect at the animal pole produced by the maturation spindles appears to be covered up in some cases, though it is always indicated for some time by a thinner spot. Some eggs have no ectoplasmic layer, and these do not develop beyond the stage of the first cleavage spindle. The two ectoplasmic discs are transmitted entirely to the larger cell of the two-cell stage. In the four-celled stage they are confined to the large posterior macromere, and during this stage they sink into the interior and surround the sphere containing the nucleus.

The behavior of the upper accumulation of ectoplasm is thus strikingly different from the condition in Chætopterus (see IV, I, *b*). But the transmission of the lower accumulation to the large

posterior macromere agrees with Chætopterus. Enough has been said to show what a remarkable similarity exists; the differences will no doubt be explained at some future time.

In both Clepsine and Rhynchelmis the area of the egg between the upper and lower accumulations of the ectoplasm is many times larger than in Chætopterus. This is correlated with the relatively very small size of the entoderm cells in Chætopterus. Differences in the functions of the ectoderm may also be a factor; thus all the ectodermal cells in Chætopterus are ciliated in contrast to Clepsine and Rhynchelmis where cilia are lacking. In Chætopterus the presence of ectoplasm seems to be essential for the formation of cilia.

Wilson ('04, *a*) describes an ectoplasmic layer in the egg of Dentalium; he finds that it is continuous with the "lower protoplasmic area" (substance of the polar lobe) and with the upper "protoplasmic disc." "As the egg, still unfertilized, lies in sea-water, the ectoplasm in the region of the upper disc slowly increases in amount, and in some cases this region shows a faintly radiating appearance around its periphery as if clear hyaloplasm were flowing into it from the surrounding region." It is evident that Wilson's "upper disc" corresponds to my "ectoplasmic defect" at the animal pole; the author calls "attention to the fact that the original disc is composed of very dense homogeneous protoplasm that differs markedly in character from the alveolar protoplasm of the ectoplasmic thickening that afterward extends over the whole upper surface of the egg." The resemblances between the ectoplasmic layers in Dentalium and Chætopterus will come out more clearly as the description proceeds. At present we may note as points in common (1) the "alveolar character" (Wilson) or presence of specific spherules. (2) Connection with the polar lobe and upper hemisphere. (3) Severance between the ectoplasm of the polar lobe and upper hemisphere later. (4) Existence of an ectoplasmic defect (upper protoplasmic disc of Wilson) at the animal pole. Vejdovsky's observations on Rhynchelmis agree in all these respects, and Whitman's on Clepsine in most.

Mead ('98) finds in the egg of Chætopterus "immediately inside the outer pellicle a narrow zone containing a single row of yolk-granules regularly arranged." This applies to the ovocyte of the

second order, and, at that stage, I also usually find the spherules arranged in a single layer (Fig. 15); it will be observed that Mead calls them "yolk-granules," not having recognized their true nature. Wheeler ('97) figures an ectoplasmic layer in the egg of Myzostoma; it is characterized by the presence of granules larger than microsomes, and like them staining very deeply in iron-alum hæmatoxylin. "Their number and distribution are quite variable, and they may even be entirely absent in some batches of eggs." Wheeler believes that they are chromatin granules derived from the disintegrating nuclei of nurse-cells. It is interesting to notice in his figures that the ectoplasmic layer is absent at the outer end of the maturation spindles, as in Chætopterus, Dentalium, Clepsine and Rhynchelmis. Conklin finds ('05) in the eggs of the ascidian Cynthia a "peripheral layer of deeply-staining protoplasm in which the test cells were formerly embedded and which contains no yolk, but numerous refractive spherules much smaller than those of the yolk." The layer contains yellow pigment "which seems to be associated with these small refractive spherules." As Conklin points out, Sobotta has observed and described a similar layer in the egg of Amphioxus. Conklin has traced its fate in Cynthia with the greatest exactness, and has found that it forms a yellow crescent on the posterior side of the egg, and that its substance enters into the composition of the mesoblast cells.

The existence of so similar a layer in such widely separated animals and its great morphogenic importance constitute strong reasons for believing that it is likely to be found in all the principal phyla. As will be shown in another place the assumption of its existence in certain forms, in which it has not been described, helps to explain other phenomena actually observed; for instance, the presence of the polar lobe in many mollusca, and the absence of flagella in abnormally differentiated eggs of Amphitrite (Scott) .

c. Residual Substance of the Germinal Vesicle

There are many observations in the literature bearing on the existence of a large quantity of residual matter derived from the

germinal vesicle after the formation of the first maturation spindle, though in most cases the observations are fragmentary and no definite interpretation is given. Figures by Wheeler ('99), Mead ('98), Coe ('99), Wilson ('04a), Vejdovsky ('88–'92) and others show a large amount of substance of the germinal vesicle remaining after the formation of the first maturation spindle; but none of these authors has traced its behavior in later stages. According to Häcker ('02) the entire substance of the germinal vesicle in Cyclops passes to the animal pole where it forms his "secondary germinal vesicle." This is like the condition in Chætopterus; but Häcker fixed his attention on the chromosomes and did not follow the residual substance farther. Without going into the details of the various accounts it is clearly shown in the literature that, as a rule, only a small proportion of the substance of the germinal vesicle enters into the formation of the first maturation spindle.

Conklin's observations ('05) are the most complete ones on the subject of the residual substance of the germinal vesicle. He finds, in Cynthia, that "as soon as the nuclear membrane has dissolved the chromosomes, nucleolus and a granular mass from which the spindle fibers are formed gather together into the center of this area of nuclear protoplasm." As the spindle forms the entire area moves to the surface of the egg, and the clear protoplasm spreads out "into a cap or peripheral layer (Ciona) or may form merely a somewhat flattened disc (Cynthia)." After fertilization the clear substance of the germinal vesicle flows to the vegetative pole, "leaving the first maturation spindle surrounded by only a small amount of protoplasm." Here it receives the sperm-nucleus and aster, and subsequently moves to the posterior side of the egg and up to the equator; "finally, after the meeting of the germ-nuclei near the posterior pole of the egg, these nuclei and the clear protoplasm surrounding them move inward to the center of the egg." "At the close of the first cleavage the nuclei and clear protoplasm move into the upper hemisphere, and thereafter, throughout development, this hemisphere contains most of the clear protoplasm and gives rise to the ectoderm." As will be seen from the subsequent description, the behavior of the residual substance in Chætopterus shows many points of similarity.

It seems probable, therefore, that the residual substance of the germinal vesicle represents a specific formative stuff of essentially the same character in different phyla.

d.   Polarization

The phenomenon that I have termed polarization of the formative stuffs likewise appears to be a universal one.   Here again Conklin's observations on Cynthia are the most complete; he has described definite flowing movements of the ectoplasm, residual substance of the germinal vesicle and of the endoplasm, beginning with the rupture of the germinal vesicle, by virtue of which the topography of the ovocyte is radically changed.   There is first a movement of the ectoplasm and the residual substance of the germinal vesicle to the lower pole of the egg, which corresponds to the polarization described in this section.   This is followed by a bilateral arrangement which corresponds to what I describe in Part IV, 1, *a*, as bilateral polarization.   The rearrangement of substances in the eggs of Ctenophores described by Fischel ('03), and in Echinids described by Boveri ('01a and '01b) appears to correspond to the bilateral polarization in Chætopterus (Part IV, 1, *a*), for it does not immediately follow the rupture of the germinal vesicle but is a result of fertilization.   Some of the observations of Whitman, Vejdovsky and Wilson, already considered, belong here.   Many authors have described or figured a polar segregation of the yolk in the lower hemisphere following the rupture of the germinal vesicle which is undoubtedly only a part of the polarization processes taking place at this time.

Wilson, Yatsu and Zeleny have found that there is a progressive limitation of potencies of parts of the egg of nemertines beginning with the rupture of the germinal vesicle.   Assuming that the various formative stuffs have limited potencies this would be the natural consequence of such a process of polarization as I have described for Chætopterus, because the new topography is more precise than the original one (compare also Conklin '05).

IV.   CLEAVAGE AND DIFFERENTIATION

The problem now becomes to determine the distribution of the
various substances in the early development, to follow the forma-
tion of others and to investigate the relations of all to the various
forms of differentiation.   To determine the normal distribution
of these substances I have studied the cell-lineage up to the time
of the formation of the mesoblast cell, and with the aid of sections
have determined the distribution of the various substances to the
different cells.   The use of intra-vitam staining has likewise con-
tributed to the study of the distribution of substances and to
the identification of new ones.   For the purpose of ascer-
taining the relations of the substances to the various forms of
differentiation, I have made a renewed study of the differentiation
of unsegmented eggs, which I described in an earlier paper
(Lillie '02).   By the action of centrifugal force an abnormal
distribution of the endoplasmic substances has been produced,
and the effect of this on the form of the cleavage has also been
studied to a certain extent.

A statement of the more general conclusions may make the point
of view of the entire description clear.   In the first place the
observations on cell-lineage show that the process of cleavage
does not produce an essentially different distribution of substances,
and that the parts of the trochophore are clearly mapped out in the
segmented ovum of sixty-four cells.   The topography of the
unsegmented egg is, therefore, essentially similar to that of the
trochophore.   In the second place it will be shown that some of
the substances (spherules) have in all probability a specific mor-
phogenic value, while others appear to have simply a nutritive
value, though a clear separation of the substances (spherules) into
these two categories has not been possible.   The morphogenic
significance of the substances is shown especially well by the
observations on the differentiation of unsegmented eggs, in which
clear regional homologies with the trochophore are found includ-
ing ectodermal, entodermal and mesodermal substances.   In the
third place it will be shown that the activation of these morpho-
genic substances is accompanied by and probably dependent upon
interaction with nuclear derivatives.

1.   Formative Stuffs in the Early Normal Development

Mead has given a good account of the cell-lineage up to sixty-four cells, and I have been able to confirm his results in practically all particulars. His description, however, was written from a different point of view, and, though complete and accurate as to the form of the cleavage, does not deal at all with the distribution of substances, which indeed he did not distinguish. It is necessary, therefore, to describe some parts in considerable detail, while for others I can rely on Mead's excellent account.

a.   The First Cleavage

The period of the first cleavage is meant to include the entire time from the dissolution of the membranes of the germ-nuclei to the two-celled stage. It is characterized by the appearance of the polar lobe involving division of the ectoplasm into two parts, by the appearance of bilateral symmetry, and by formation of new substances.

1.   *Polar Lobe and Division of Ectoplasm.*—At about the metaphase of the first cleavage the ovum begins to elongate in a polar direction and a slight constriction appears in an equatorial plane considerably below the equator (Photograph G and Fig. 26, p. 205); thus the ovum becomes decidedly pear-shaped, the stem of the pear being at the vegetative pole. In the region of the constriction the ectoplasm is divided so that there is a gap between the part situated below and that above it (Figs. 25 and 26); this gap is permanent, and a vegetative polar group of the ectoplasmic spherules is thus finally separated from the remainder. The constriction separating this group deepens as the first cleavage furrow begins to appear at the animal pole (Fig. 25); this furrow lies to one side of the polar globules and on the same side of the egg it passes into the constriction, so that the division becomes decidedly unequal and the polar group of ectoplasmic spherules (Fig. 25, *p.l.*), passes entirely into the larger cell (*CD*). The polar furrow deepens during the later phases of the first cleavage and thus produces a pedunculated lobe, the polar lobe, which contains all the polar ectoplasmic spherules (see Photograph I) and also

some endoplasmic spherules in its base.   At the height of forma-
tion of the polar lobe the ectoplasmic waves become extremely
pronounced all over it.   During the completion of the first cleav-
age the constriction around the polar lobe gradually disappears
and the substance of the polar lobe forms that part of the larger
cell next the cleavage furrow, where the group of ectoplasmic
spherules keeps a superficial position.
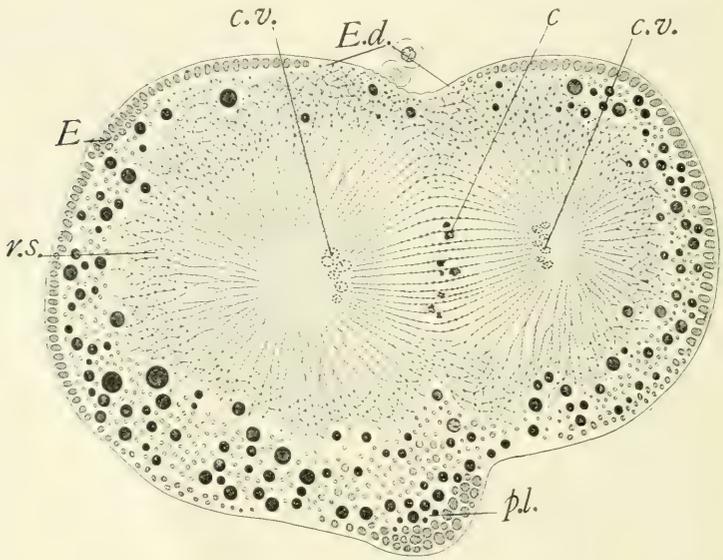
Fig. 25.   Longitudinal section, first cleavage, late anaphase.   Posterior end to the left, anterior
to the right.   The ectoplasm of the polar lobe has been separated from the remainder.   *c*, chroma-
tin masses cut off from the chromosomes; *c.v.*, chromosomal vesicles of the daughter nuclei.   *E.d.*,
Ectoplasmic defect; *p.l.*, polar lobe; *r.s.*, residual substance of the germinal vesicle.

Mead has given a good description of the form changes of the
first cleavage, and has described the polar lobe, which he calls the
yolk-lobe, following the older terminology.   The term polar lobe,
introduced by Wilson, seems preferable to the name yolk-lobe,
and I therefore employ it.

The polar lobe is fundamentally an ectoplasmic formation.
The proof of this is experimental.   We have seen that the endo-
plasmic substances may be given any arrangement with reference

to polarity by the centrifuge; but that the ectoplasm is unaffected and that the polar globules appear at the original animal pole even though this be the center of aggregation of the large endoplasmic spherules. Now the polar lobe invariably forms opposite to the polar bodies, so that in the extreme case, reversal of position of the endoplasm, the gray cap extends into the base of the polar lobe; or the protoplasm of the clear band may extend into its base in case of intermediate position of the endoplasmic substances (Photographs G and I and Figs. 17, 18 and 19). Thus the position of the polar lobe is independent of the position of the endoplasmic substances, and is determined by the polarity of the egg and the distribution, with reference to the polar axis, of the ectoplasm. Inasmuch as the animal pole is devoid of ectoplasm the ectoplasmic substance of the polar lobe may be termed briefly the polar ectoplasm.

2. *Bilateral Symmetry.*—The second striking feature of the first cleavage is the appearance of bilateral symmetry, which comes out unmistakably and permanently during the formation of the first cleavage spindle. Prior to this time I have been unable to detect any positive evidence of bilateral symmetry; thus it comes to expression in a few minutes. Like polarity itself the bilateral symmetry is independent of the distribution of the endoplasmic substances, and, in my opinion, can only be conceived as due to a force like that of polarity. The evidence for this follows:

The first cleavage spindle forms approximately in the center of the egg, but a little above the equatorial plane. Before this time the egg shows a perfect radial symmetry so far as the distribution of substances is concerned. In a horizontal section the germ-nuclei lie in the center of a mass of non-spherular protoplasm, which is surrounded by a ring of endoplasm of even thickness, and this again by an ectoplasmic ring of even thickness. As the spindle forms, however, the endoplasmic ring becomes broader at one end and narrower at the opposite end, as though the non-spherular protoplasm containing the spindle had moved toward one side of the egg; this side is the anterior, and the opposite side the posterior face of the egg, and the axis of the spindle lies in the plane of symmetry thus indicated, and therefore in the longitudinal axis

of the embryo. Thus in the early anaphase the first cleavage spindle lies eccentrically in a mass of non-spherular protoplasm, and the center of the endoplasmic spherules has been shifted toward the posterior end (Figs. 25 and 26).

At the same time the centrosphere at the posterior end becomes much larger than that at the anterior end (Fig. 26); this is very marked at the end of the prophase, and is, indeed, the first clear indication of the posterior end.

Treadwell ('97) has shown that, even when the first cleavage is equal in annelids, it bears the same relation to the axis of the embryo. But it should be noted that, when the first cleavage is unequal, as in Chætopterus, we have demonstrative evidence that the bilaterality thus established involves more than the mere determination of an axis; it involves also certain embryonic proportions of prospective significance. The cell *CD* is not only posterior in position, but it is larger and different to a certain extent in its composition, and behaves radically differently from the cell *AB;* in fact, the first cleavage predelineates the proportions and properties of the anterior and posterior ends of the embryo.

The gradual determination of the plane of bilateral symmetry furnishes a fascinating problem. It is difficult or impossible to say which of the phenomena observed to be involved are primary and which secondary; it seems probable that the primary determining factor has altogether eluded observation, and that the orientation of the spindle, the enlargement of the posterior centrosphere, and the shifting of the endoplasm and of the spindle are all consequences of some more remote cause, viz: a second process of polarization[1] analogous to that following the breaking of the germinal vesicle, but with reference to a new axis placed at right angles to the polar axis—the sagittal axis of the embryo.

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[1] I have called the shifting of substances that defines the bilateral symmetry a second process of polarization because, so far as I can see, there is no immediate explanation of the phenomenon. Certainly the unequal cleavage (and the determination of bilateral symmetry that goes with it) is not due to any *chance* distribution of the substances of the egg; for on no theory of chances can one explain the uniformly normal relation of composition and of mass between the cells *AB* and *CD*.

In centrifuged eggs the first cleavage is invariably normal with relation to the polarity, and usually unequal whatever the positions of the endoplasmic substances (see Photograph I, and Figs. 18 and 19). Thus the cell *AB* may receive most, or in other cases practically none, of the endoplasmic spherules. There is certainly a tendency in the former case toward equality in size of the two cells, but the tendency toward inequality is usually markedly the stronger of the two. Thus, just as the polarity of the egg is independent of the distribution of the endoplasmic substances, so also is the bilaterality. And conversely just as polarity determines the polar distribution of these substances so does the force of bilateral polarization acting at right angles to polarity normally determine their bilateral arrangement.

This conclusion is in full agreement with my earlier opinion on the organization of the egg of Unio ('01) where I distinguished polarity and bilaterality as fundamental primitive features of the egg organization. In his fine study of the organization and cell-lineage of the Ascidian egg, Conklin ('04) likewise states his opinion that the median plane and the posterior pole are determined by the "structure of the egg" and not by external incidents such as the path of the spermatozoön. I have chosen the terms polarity and bilateral polarization as preferable because they express the opinion, which, I believe Conklin also had in mind, that they represent conditions antecedent to the distribution of substances in a polar or bilateral sense, and hence more fundamental.

We know polarity and bilateral polarization only as conditions or forces that determine definite distributions of certain substances. Beyond this they can be defined only in a negative manner; thus they are not due to any visible aggregation of unlike substances; they are not determined by the path of the spermatozoön, nor by chance location of germ-nuclei. They might be conceived to be electrical or magnetic phenomena, but all the evidence of the influence of electricity and magnetism on development runs counter to this idea. But however obscure the nature of such forces may be they are clearly demonstrated to exist (see General Discussion, "Principle of Unity").

The first division is a differential one with reference to the substance of the polar lobe, and to the amounts of other substances. Thus *CD* receives the entire area surrounding the polar globules that is devoid of ectoplasm (Fig. 29), it also receives all of the substance of the polar lobe and the larger amount of the endoplasmic spherules. On the *AB* side the ectoplasm and the endoplasmic spherules come up to the first cleavage furrow at the animal pole; whereas on the *CD* side they stop some distance from it (Fig. 29). These points are of importance with reference to the subsequent development.

3. *Residual Substance of the Germinal Vesicle and Nuclear Derivatives.*—There remain yet two features of the first cleavage to consider, viz: the fate of the residual substance of the germinal vesicle and the formation and distribution of new nuclear derivatives.

The residual substance of the germinal vesicle forms a mantle around the first cleavage spindle (Fig. 25, *r.s.*) and is thus divided approximately equally between *AB* and *CD*. After the first cleavage is complete it is difficult to distinguish it by the usual means. But by means of the centrifuge it can readily be shown that it is still a distinct substance, and that certain of its properties, at least are still the same. If the two-celled stage be centrifuged a segregation is produced in the same manner as in the unsegmented polarized egg; that is, a stratification of three substances appears in each cell—a gray cap, a clear band and a yellow mass. These may be placed in a polar direction or in any other direction. If the centrifugal force acts at right angles to the axis of the egg, the gray substance goes to the surface in the upper cell, and lies against the cleavage wall in the lower cell; while the yellow endoplasm lies against the cleavage wall in the upper cell, and against the most distal surface of the lower cell (Fig. 27). Other variations need not be described. It will be seen that it is possible in this way to demonstrate the continued separate existence of the residual substance of the germinal vesicle.

Similarly one can demonstrate it in the four-celled and eight-celled stages; Fig. 28 illustrates this for the eight-celled stage. Later cleavage stages were not centrifuged but I have other evi-

dence of its independent persistence to a later stage. In centrifuged eggs in which it passes originally entirely into one cell it is possible to follow it, on account of its massed condition, up to late cleavage stages in which its position is just internal to the nucleus. Finally, if the results of staining intra-vitam with neutral red are to be trusted, we have a method that enables one to follow it step by step with perfect accuracy into the trochophore.
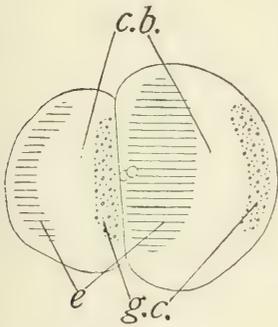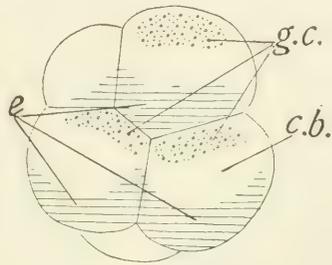

Fig. 27.   Fig. 28.

Fig. 27. To show the effects of centrifuging in the two-celled stage. Drawn from the living material. *c.b.*, clear band; *e*, endoplasm; *g.c.*, gray cap.

Fig. 28. To show the effects of centrifuging in the eight-celled stage. Drawn from the living material. Explanation of letters same as Fig. 27.

That the nuclei of cleavage stages set free certain substances at each prophase is a familiar idea, that has been best set forth by Conklin. It has, however, been impossible to follow these substances far by cytological methods, so that their fate is largely a matter of conjecture. In the egg of Chætopterus such substances, "oxy-chromatin," are similarly set free, and, in addition to these, there is liberated at each mitosis a group of large granules that can be followed through at least one cell-generation in each case. These are the bodies that Mead described in the first cleavage as nucleoli.

Their history in the first cleavage is briefly as follows: They arise in connection with the chromosomes from the segmentation nucleus and separate from them in the prophase of the first division. So that the equatorial plate consists of a certain number

of chromosomes with intermingled granules. The latter are hardly larger at this time than the ends of the chromosomes, and they stain in iron-hæmatoxylin even more intensely than the chromosomes themselves. Only the chromosomes divide and in the
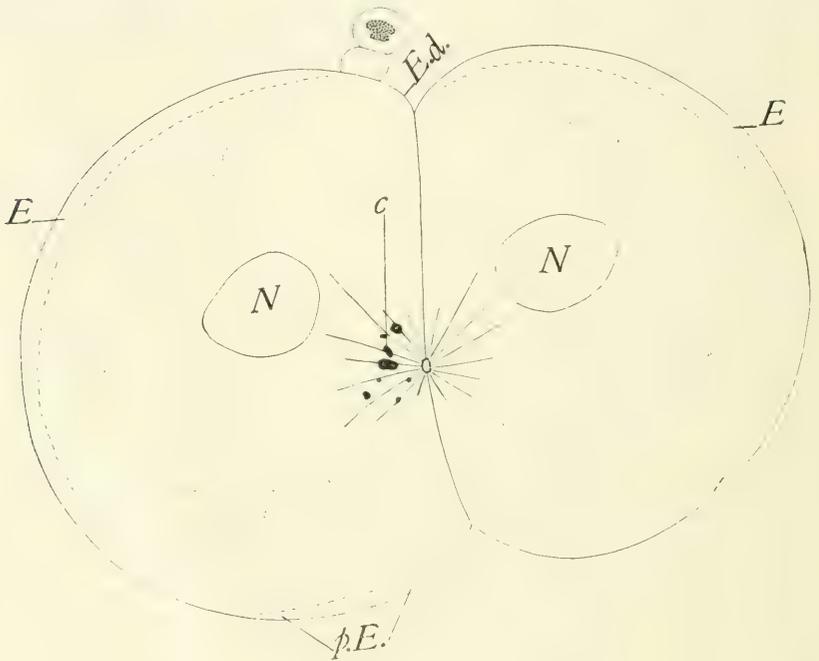

Fig. 29. Outline of longitudinal axial section of the completed two-celled stage. To show the transmission of chromatin masses cut off from the chromosomes exclusively to the larger cell. The fine dotted outline shows the boundary of the spherular endoplasm. *E*, Ectoplasm; *E.d.*, ectoplasmic defect; *c*, chromatin masses cut off from the chromosomes; *N*, nucleus; *p.E.*, polar ectoplasm.

anaphase the granules lie midway between the daughter chromosomes among the interzonal fibrils (Fig. 25, *c*). During the anaphase they become fewer and larger, no doubt by fusion with each other. In the telophase the cleavage plane passes anteriorly to them, thus leaving them invariably in the larger cell *CD*. So far as I could see, not one of them is left in the smaller cell. Fig. 29 showing the mid-body of the first cleavage is a characteristic view

of this stage, and shows with what certainty these granules may be traced into the larger cell. The origin of the bodies from the chromosomes and their differential distribution to one cell are suggestive of an important rôle in differentiation.

Thus a new group of granules arises during the first cleavage from the chromatin. A similar process is repeated in later cleavages, but whether or not it occurs to the same extent in all the cells I am unable to say. These granules attain the size of spherules by fusion; they then mingle with the *a* group of spherules of the endoplasm, and can no longer be distinguished. The repetition of this process up to sixty-four cells would produce a group of spherules staining in hæmatoxylin as numerous, probably, as the original *a* group of the endoplasm. It is possible, therefore, that spherules of the *a* group are continually disappearing and being replaced. Certain appearances in spherules of the *a* group during the cleavage favor this view; many are found that appear eroded or wasted to a crescent, a condition that I have not observed prior to the beginning of cleavage. It is impossible, therefore, to say in the late cleavage, how many of the spherules of the *a* habitus are original members of this group, and how many have been derived from the chromosomes during cleavage.

The observations are instructive as bearing on the question of differentiation of nuclei. The assumption that chromosomes may *divide* differentially has not the slightest foundation in the observed facts of karyokinesis, excepting in the maturation divisions. On the other hand the hypothesis that cytoplasmic differentiation is dependent on nuclear differentiation appears to me a necessary corollary of our growing knowledge of the characters of the chromosome complex. The above observations on differential distribution of nuclear derivatives shows a method by which not only may nuclear determination be realized, but also by which differentiation of nuclei may be obtained. If, for instance, this process of chromatin diminution takes place in certain cells and not in others, differentiation of the nuclei might result; when, on the other hand, the nuclear derivatives receive a differential distribution in daughter cells of different prospective tendencies, the problem of the determination of these cells seems simplified.

Thus nuclear determination and differentiation may exist even though every chromosomal fission be integral or equational.

I have described the first cleavage in considerable detail, because it includes part of the process of segregation of substances, particularly the bilateral polarization, and also illustrates the origin of new substances during the cleavage period. The account of the subsequent cleavages may now be divided into two parts. In the first will be given a very brief account of the form of the normal cleavage (cell-lineage) and the fate of the cells. In the second part the distribution of the substances in these cells will be traced.

b. The Form of Cleavage

The cleavage follows the usual annelid type (Figs. 30–37), *i. e.*, the ectoderm is derived from three quartets of micromeres formed by alternate dexiotropic and læotropic equatorial cleavages of the four macromeres, A (left anterior), B (right anterior), C (right posterior) and D (left posterior). The mesoblast comes from the cell $4D$; the entoderm from the cells $3A$, $3B$, $3C$, and $4D$. The somatic plate is derived from the cell $2D$ or X.

Certain special features of the cleavage deserve particular attention. In the four-celled stage D is much the largest of the cells, C comes next, A and B are the smallest (Figs. 30 and 31). The differences between the cells A, B, and C are, however, relatively slight. In the second cleavage the polar lobe is indicated by a protuberance of the vegetative pole of the cell CD toward the left side; its material passes entirely into the cell D. Again in the third cleavage there is usually a protuberance at the vegetative pole of D (Fig. 32) indicating the location of the substance of the polar lobe, which may, therefore, be followed readily into the cell $1D$. The polar globules are attached a little posterior to the first cleavage furrow on the line of the furrow between C and D, a position that is retained throughout the cleavage (Figs. 31 and 35). The ectoplasmic defect is transmitted to the cell $1d$ (Figs, 30, 33, 36 and 38).

The third cleavage is dexiotropic and approximately equal, though the ectomeres $1a$, $1b$, $1c$, and $1d$ are slightly smaller than the "macromeres" $1A$, $1B$, $1C$ and $1D$. Thus in the upper
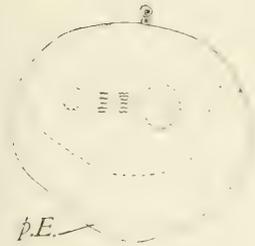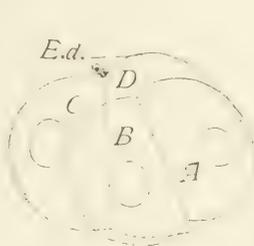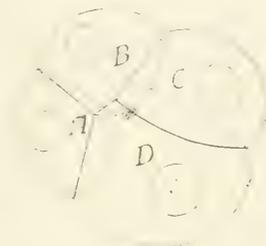
FIG. 26   FIG. 30   FIG. 31
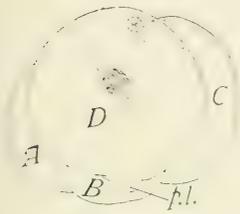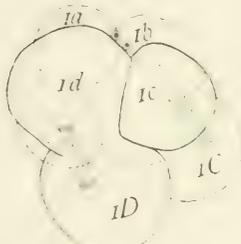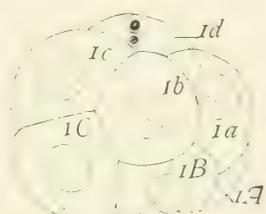
FIG. 32   FIG. 33   FIG. 34
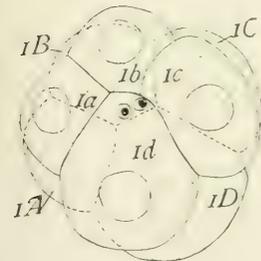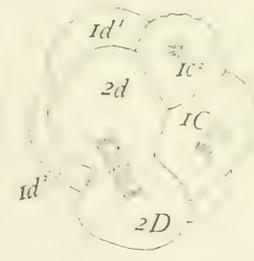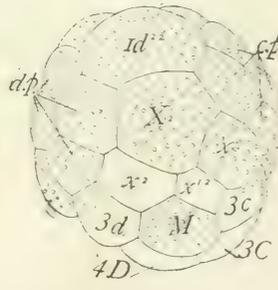
FIG. 35   FIG. 36   FIG. 37

Figs, 26, 30 to 37.   To illustrate the form of the normal cleavage in Chætopterus.   Drawn from preparations.

Fig. 26.   Anaphase of first cleavage.

Fig. 30.   Four-celled stage from in front.   Note that the ectoplasmic defect is confined to the quadrant *D*.

Fig 31.   Four-celled stage from the animal pole.

Fig. 32.   Four-celled stage from behind.   Early anaphase of third cleavage showing polar lobe in the *D* quadrant.

Fig. 33.   Formation of the first generation of micromeres, of approximately the same size as the macromeres.

Fig. 34.   Eight-celled stage from anterior end.

Fig. 35.   Eight-celled stage from animal pole.

Fig. 36.   Division of eight to sixteen cells to show large size of *2d*.

Fig. 37.   Approximately sixty-four-celled stage seen from behind.   Outline copied from Mead ('97).   The small circles in the cells represent the ectoplasmic spherules (diagrammatic).

In all of the figures on this plate except the last, the ectoplasm is indicated by the dotted contour line.   The relation of this line to the animal and vegetative poles should be noted.

quadrant the cell *1d* is much larger than its mates, similarly in the lower quadrant *1D* is the largest cell (Figs. 33 and 34).

In the fourth cleavage (eight cells to sixteen cells) the cell *1D* divides unequally so that its upper product *2d* is larger than the lower, the "macromere" *2D* (Fig. 36). The division of *1A*, *1B*, and *1C* are approximately equal; thus *2d* is by far the largest cell of the second quartet. In the sixteen cell-stage the cells *1d* and *2d* are much the largest cells in the egg. The orientation of the egg
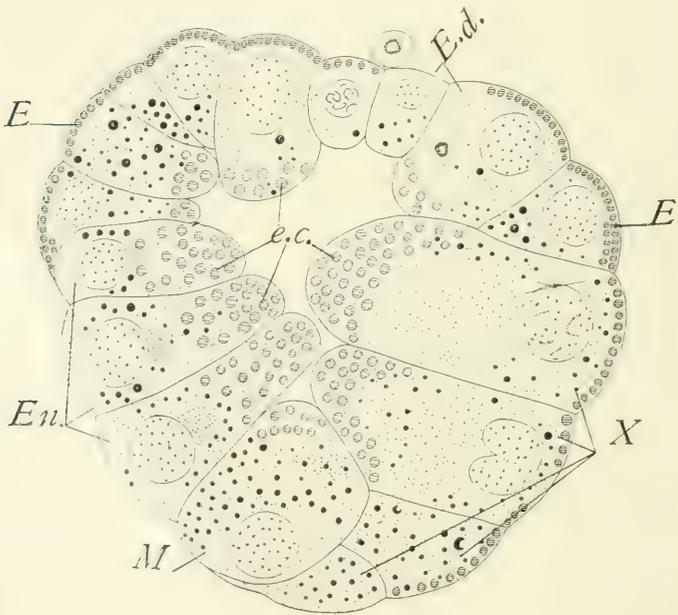

Fig. 38. A sagittal section through a stage of about sixty-four cells. The small upper cells are the apical cells. The ectoplasmic defect will be noted in the posterior apical cell to the observer's right. *E*, Ectoplasm; *e.c.*, endoplasm *c*; *En.*, endoderm cells; *E.d.*, ectoplasmic defect; *M.*, mesoblast cell. *X* derivatives of first somatoblast.

and the identification of the cells is thus a relatively simple matter. Fig. 37 (outline of cells after Mead, '97) illustrates the arrangement and prospective significance of the cells of the sixty-four cell-stage. Only the superficial part of each cell is indicated and this

is not always a good measure of relative size. The distribution of the ectoplasmic spherules is indicated by the small circles in the cells with approximate accuracy.

c. Distribution of Substances in the Cells

No cell is pure in regard to the formative stuffs it contains, but each cell, up to a late stage at least receives both ectoplasm and endoplasm, with the exception of the entoderm cells which appear to receive no ectoplasm (Fig. 38). The arrangement of the substances is the same in all ectodermal cells: (1) Externally a layer of ectoplasmic spherules; (2) the nucleus in a mass of microsomal cytoplasm; (3) just internal to the nucleus a mass of non-spherular substance; (4) within this a group of endoplasmic spherules staining black in iron hæmatoxylin (endoplasm *a*), and (5) next to the segmentation cavity a group of large spherules staining in orange G formed by segregation and fusion of the smaller endoplasmic spherules (endoplasm *c*) already described. The arrangement is similar in the mesoderm cell (though the polar ectoplasm was not distinguished in the section figured), and, with the exception of the absence of the ectoplasm, in the entoderm cells also.

This arrangement of substances is invariable; each cell is polarized; the axis of polarization of each is a radius of the egg, and the central ends are homologous. Thus each cell exhibits a stratification of substances similar to that of the entire egg.

There is, however, one difference in the arrangement of the substances in the single cells and in the entire ovum. In the latter the *c* endoplasm lies above the *a* endoplasm, whereas in the former the *c* endoplasm is most distal. It must be remembered that there is a polarization of the whole segmented egg as well as of the individual cells; the *c* and *a* endoplasmic substances retain the same relative position in the entire segmented egg as in the unsegmented. It is as though a cavity had been formed in the center of the *c* endoplasm of the unsegmented egg (Fig. 39) and nuclei placed around the periphery just within the ectoplasm, and cleavage planes cut through between nuclei to the cavity. Thus the

polarity of the cells is directly derived from the entire ovum. Nevertheless it is immanent in each cell, for, however much the arrangement of the cells may be disturbed, the arrangement of substances in the individual cells remains the same.

We have seen that the polar ectoplasm passes entirely into the D quadrant. Between the two and the four-celled stage it forms a polar lobe that lies at first against the A quadrant, but shifts to the B quadrant as the cells come to rest. When the spindles are formed for the third division a very small polar lobe appears in D (Figs.
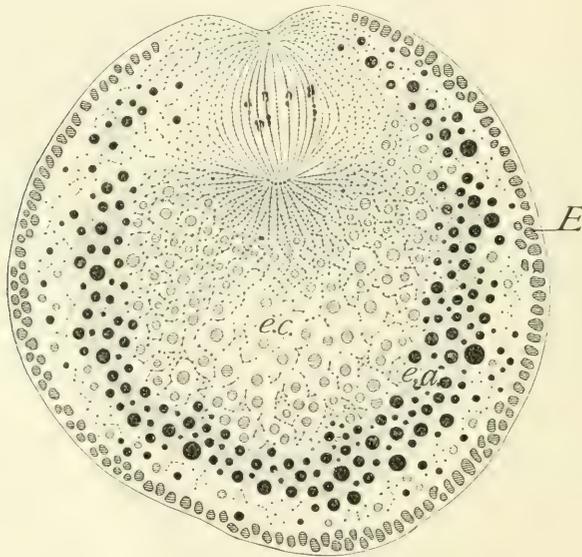

Fig. 39. Axial section of an unfertilized egg that had stood in the sea-water two hours and seven minutes before killing. The a and c spherules of the endoplasm have segregated out (*e.a.*, and *e.c.*; compare Fig. 5). A clear layer has arisen between the ectoplasm (E) and the a endoplasm; the outer centrosome of the maturation spindle has divided once, and the inner one several times.

32 and 40). Sections taken at this time show that part of the polar ectoplasm plunges into the interior of the egg (Fig. 40, *p.E.*). This is very conspicuous in the four-celled stage containing the spindles for the next division. A tongue of endoplasm then extends across, severing the portion lying internally from the portion that remains superficial in position (Fig. 40). It is possi-

ble ·that the upflow of the polar ectoplasm may carry some of it
above the line of the next cleavage furrow, so that a certain amount
may go into $1d$.   An upflow of endoplasmic spherules goes on at
the same time in all the cells both along the cleavage planes and
also next the surface.
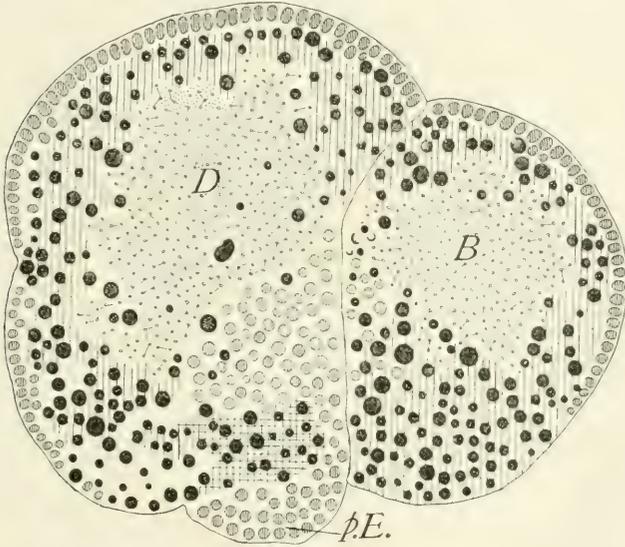
Fig. 40. Sagittal section through the cells B and D of the four-celled stage. The section passes
a little to one side of the middle line; in the median section the spindles of the third cleavage were
formed in metaphase, and the ectoplasmic defect was confined to D. The small polar lobe is con-
fined to the D quadrant and contains all of the polar ectoplasm, $p.E.$ Part of the latter is passing
into the interior of the cell along the plane between B and D. Most of the striated spherules in the
interior of the cells, however, belong to the c endoplasm, and it is impossible to distinguish exactly
the boundary line between them and the polar ectoplasm.

The substance of the polar lobe (polar ectoplasm) is thus far
from being pure in its distribution, for a small portion of it may
pass into the cell $1d$, and the remainder is divided in two parts of
which the central part is probably transmitted to the first somato-
blast ($2d$ or X), and the superficial part, to the second somatoblast
($4d$ or M).   I am not, however, perfectly certain in regard to
these points, though it is probable from the position of the
material; both Wilson ('04a) and Crampton ('96), moreover,

have shown experimentally that substance of the mesoblast is contained in the polar lobe.

These observations agree closely with Wilson's interesting experimental results on Dentalium; among other things he found that removal of the first polar lobe involved absence of the apical organ which normally arises in the D quadrant; the fact that some of the polar endoplasm probably enters into the composition of the cell rd might explain this curious result. Wilson, moreover, observed directly that substance of the polar lobe entered into the formation of both first and second somatoblasts.

It has been a difficult task to determine the fate of the residual substance of the germinal vesicle in the normal cell-lineage by direct observation. In the section on staining intra-vitam it will be shown that it is possible to trace it with a high degree of accuracy by this method. I will, therefore, add here only some observations made under experimental conditions that confirm the observations, made with vital staining, that this substance is distributed mainly to the first generation of micromeres: Seeing that cleavage takes place in centrifuged eggs always with reference to the original polarity and not to the induced stratification of substances, it often happens that the residual substance of the germinal vesicle, now in the form of the gray cap (Figs. 17 and 22 and Photographs), is located in the lower hemisphere. In such cases it can be seen to stream quite rapidly toward the animal pole during the process of cleavage, so that by the eight-celled stage it is usually found in the upper quartet. If, however, by any chance the third cleavage plane has come in so as to isolate it wholly or in part in the lower quartet it always comes to occupy the uppermost corner of the cells in which it is found (Figs. 41 and 42). This phenomenon may be observed with the greatest ease, because this substance in its massed condition contrasts strongly with the other cell-constituents.

There can, therefore, be no doubt of a strong inherent tendency for this substance to aggregate in the upper hemisphere. Under normal conditions it is originally located in this part of the egg, so that there is every reason to believe, on the basis of these observations alone, that normally it is distributed mainly to the

cells of the first quartet of ectomeres and their descendants. The reader is referred to the section on vital staining for more detailed evidence concerning its ultimate fate.

The distribution of the ectoplasmic spherules, excluding the polar group, corresponds exactly to the distribution of cilia (see Fig. 37). There can, therefore, be no reasonable doubt that they are specially concerned in some way with the production of cilia; this conclusion is practically *demonstrated* by the observations on the differentiation of unsegmented eggs. I would expect, therefore, to find in true prototrochal larvæ of annelids, that the distribution of the homologous substance would be confined to the prototroch, and would thus form a narrower band than in the
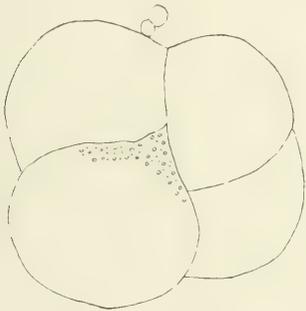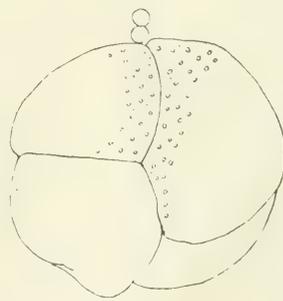

Fig. 41   Fig. 42

Figs. 41 and 42. Two eggs centrifuged before cleavage, now in the eight-celled stage. The stippled areas represent the substance of the gray cap, which tends toward the highest point (*i.e.*, nearest to the animal pole) in the cells to which it is confined. Drawn from the living eggs.

uniformly ciliated larvæ of Chætopterus. The upper division of the ectoplasm in Rhynchelmis has an entirely different fate according to Vejdovsky: like the lower accumulation (my polar ectoplasm) it is restricted to the D quadrant, where it lies near the animal pole; in the four-celled stage both polar accumulations flow into the interior of the egg and surround the nucleus and periplast; subsequently the material appears to be transmitted mainly to the "mesomeres" (first and second somatoblasts).

It would appear, therefore, that the upper division of the ectoplasm has fundamentally different functions in Chætopterus and Rhynchelmis. It is possible, however, that it is really com-

posed of two substances in both forms, and that, in Chætopterus, I have overlooked the part corresponding to the main mass in Rhynchelmis, and that Vejdovsky has overlooked the part in Rhynchelmis corresponding to the main mass in Chætopterus. Seeing that the Rhynchelmis embryo is without locomotor cilia, it would be natural for the substance forming the superficial layer of the ectoderm cells of Chætopterus and associated with the formation of cilia to disappear in its phylogenic history. It is difficult to believe that substances having so similar an original disposition and structure as the upper and lower divisions of the ectoplasm in Chætopterus and Rhynchelmis are entirely different in their morphogenic functions (compare Vejdovsky's Fig. 30, Plate IV, with my Fig. 25).

Undoubtedly it would be possible by a detailed cytological study of each cell in each stage of the cleavage to follow more accurately the fate of the substances with which we have been concerned. A beginning has been made on this problem, but it has proved so difficult, that it seemed desirable to present the facts already determined, rather than delay their publication, and greatly increase the extent of this already too long publication.

The main results concerning the distribution of substances in the normal development are:

(1) The ectoplasmic defect at the animal pole is the place of formation of the apical organ with its flagella.

(2) The polar ectoplasm enters into the cells *1d*, *2d* and *4d*.

(3) The remainder of the ectoplasm forms the superficial layer of the ectoderm cells.

(4) The entoderm cells receive no ectoplasm.

(5) All cells receive some of each kind of endoplasm.

(6) New substances arise from the nuclei and are differentially distributed in some cases at least.

(7) The residual substance of the germinal vesicle is distributed mainly to the first quartet of micromeres.

d.   Intra Vitam Staining

The capacity of living protoplasm to take up stains has been investigated by a large number of workers. Alfred Fischel ('99)

was the first to apply the method to embryological research; his idea was to attempt in this way to differentiate distinct elements of the ovum and if successful to follow them in the embryonic development. "Hinsichtlich des letzteren Punktes galt es mir, im besonderem, als erwünschtes Ziel, vielleicht ermitteln zu können, dass während der Furchung eine Verteilung bestimmter Elemente des Eies auf ganz bestimmte Zellen, also gewissermassen eine Teilauslese der Plasmaarten der Eizelle statthat." Fischel worked with several stains on the ova of a number of different animals, but obtained the best results with neutral red in the development of Echinus microtuberculatus. He found that stained granules appeared in a zone around the spindles and that they had such avidity for the stain, that they would completely decolorize weak solutions and become intensely red. During the resting stage of the nuclei the granules scattered throughout the cell. They were not distributed differentially in the development, but each cell contained them.

Garbowski ('04) is the only other author, so far as I know, who has applied the method to embryological work; his problem was to unite a stained portion of one egg with an unstained portion of another and to follow the subsequent development of the grafted parts. His results, however, do not bear directly on the problem under consideration. My own purpose was precisely the same as Fischel's, namely, to find if it were possible to stain constituents of the egg protoplasm intra vitam and, if so, to follow their history in the subsequent differentiation with special reference to differential distribution of such substances. *Both results were obtained; certain granules take the stain very intensely and are distributed in a very precise fashion to a particular region of the embryo.* Although several stains were used, I shall describe the results from only one, neutral red, which I found, like Fischel and Garbowski, incomparably the best for the purpose.

To obtain the results that I describe one must not use a strong solution, for then the stain becomes diffuse and the eggs do not develop far in it. The best results were obtained from eggs placed immediately after fertilization in a solution of 75 parts seawater plus two and one-half parts of a saturated solution of neutral

red in sea-water. This solution is a very faint rose color, and the
eggs develop in it perfectly normally for more then twenty-four
hours. If there are many eggs in the culture they may entirely
bleach the solution, all the stain being absorbed by certain gran-
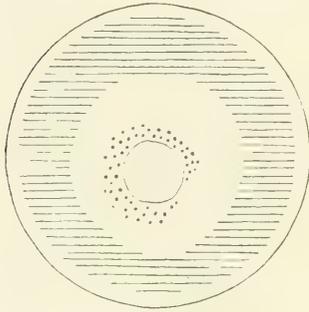
FIG. 43
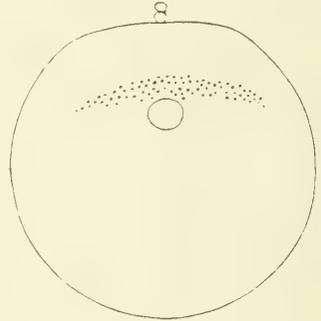
FIG 44
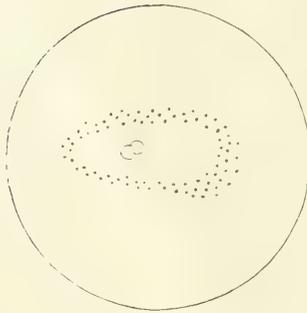
FIG. 45
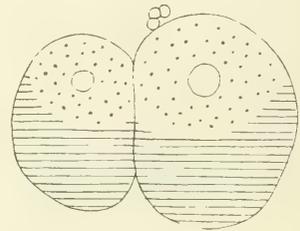
FIG. 46

Figs. 43 to 46. Drawings of living eggs reared in neutral red solution.

Fig. 43. Polar view, stage of first maturation spindle, which is represented by the central circle.
The parallel lines represent the spherular endoplasm. Eggs fertilized at 10.18 A. M.; put in seventy-
five parts sea-water plus two and one-half parts of a saturated solution of neutral red in sea-water at 10.20
A. M.; drawing from the living egg at 10.43 A. M. The dots around the maturation spindle represent
the red-staining granules.

Fig. 44. Later stage of another egg seen from the side; the red-staining granules lie above the
germ-nuclei.

Fig. 45. Stage of first cleavage spindle seen from the animal pole. The two small circles represent
the polar globules. The red granules surround the spindle.

Fig. 46. Two-celled stage. Resting nuclei. Red granules scattered in the upper hemisphere.

ules that become intensely red; such granules retain the stain many hours or permanently, even if the eggs are transferred to pure sea-water. If the stain be made about twice this strength, the eggs develop abnormally after three or four hours; moreover, a diffuse pink color appears in the endoplasm also, but disappears from it very quickly after transfer to pure sea-water, though it remains in the granules that stain in more dilute solutions. Thus the stain is specific for certain granules, and the affinity of these granules for the stain is very intense.

If, then, the stain be of the proper strength one obtains invariably the following results when the eggs are put in immediately after fertilization and are allowed to remain. Up to the time of the formation of the first maturation spindle there is practically no stain in the egg, though by careful examination with an oil immersion lens one can see certain minute, scattered, bright red granules in the ectoplasm. In some cases there appears around the first maturation spindle a complete mantle of bright red granules larger than the ordinary cytomicrosomes and smaller than spherules (Fig. 43). These agree in position with the residual substance of the germinal vesicle. In other cases these granules do not stain until the second maturation spindle is formed or even later.

When the germ-nuclei are formed, these granules form a band above them and later surround them (Fig. 44). When the first cleavage spindle is formed, they form a mantle surrounding it completely (Fig. 45) and are thus divided between the two cells. As the nuclei of the two-celled stage come to rest, the red granules spread out in the upper hemisphere o each beneath the ectoplasmic spherules, *i. e.*, at the periphery of the non-spherular substance (Fig. 46).

As the nuclei elongate for the second division the red granules again accumulate around them and form a mantle to the spindles, and are thus divided between the four cells, in which, as the nuclei come to rest, they again spread out in the upper hemisphere (Fig. 47).

In the preparation for the third cleavage (four cells to eight cells) the red granules are again attracted to the nuclei and spread

out along the periphery of the spindles. But in this case the larger proportion is aggregated around the upper aster, and a relatively small number only around the lower (Fig. 48). Thus the first generation of ectomeres receives by far the larger proportion of the red granules, and this produces a most striking orientation of the egg, the upper quartet being a bright red, and the lower only faintly tinged, *except in the upper left hand corner of each macromere, where the red granules congregate after the cleavage is completed, and thus foretell* their distribution to the second quartet of ectomeres (Fig. 49). The cell *D* furnishes the most striking illustration of this on account of its large size.

I am not sure that all of the red granules remaining in the macromeres are distributed to the cells of the second quartet; but certainly the larger number are, so that very few of the original red granules remain for the third quartet.

In the later cleavage the general result is so clear as to be easily visible with the low powers; the upper half of the segmented egg has an intense red stain, the portion derived from the second quartet very little, and below this there is almost none at all.

This difference continues to increase in intensity during the formation and growth of the trochophore. At about twenty hours the exumbrella of the trochophore (Fig. 51) is brilliant, the red stain is distributed in great blotches that define a broad band interrupted dorsally and leaving only a small area at the apical pole with smaller red spots, in the center of which is the apical tuft of cilia. The subumbrella in comparison to the exumbrella is very lightly stained but the better stained specimens show a few red granules in the ectoderm that appear to define the somatoblastic plate; I am not, however, sure of the distribution of these granules in the subumbrella, which vary greatly in different cultures.

The bright red stain is practically confined to the ectoderm; the entoderm and mesoderm have at most a faint rose tinge. As the trochophore becomes older, the broad prototrochal band becomes relatively narrower, owing no doubt largely to the expansion of the head vesicle in front of it.

If now the cells of the prototroch containing the large masses

of red are examined with the oil immersion lens, it is found that the red mass is situated internal to the nucleus. Five substances may in fact be recognized in each cell: (1) the layer of ectoplasm with its characteristic spherules which are slightly stained; (2 and 3) the nuclear area containing a large vacuole; (4) the bright red mass, analyzable under the 2 mm. lens into a dense group of large red spherules; (5) an aggregation of yellow endo-plasmic spherules (Fig. 52).

Now in a late cleavage stage it will be found that the arrangement of substances is the same with this exception, that the red is in the form of relatively small granules, and the vacuole is not yet formed (Fig. 50). From which it follows, either that the red granules have grown to the size of spherules, or that agglutination and fusion have taken place among them. The origin of spherules from smaller granules is thus demonstrated.

The question now arises, what is the origin of these red-staining granules that have so precise a distribution in the development. One thinks at once of the residual substance of the germinal vesicle with which the aggregation of red granules has the following points in common: (1) the original distribution around the maturation and the first cleavage spindles; (2) the position in late cleavage just central to the nucleus; (3) the size of the granules, intermediate at first between cytomicrosomes and spherules; (4) the distribution to the upper hemisphere.

One is, however, met at once by the difficulty that the amount of these granules in the later development exceeds considerably the original amount of the residual substance of the germinal vesicle. This difficulty may, however, be met by assuming that similar granules arise from other nuclei than the germinal vesicle; there is very definite evidence for this view detailed in the account of the differentiation of unsegmented eggs.

But a more serious difficulty arises; if these red granules are indeed the residual substance of the germinal vesicle, one should find that the gray cap formed from this substance in centrifuged eggs takes the red stain. Now, this is not the case; and to save the hypothesis one can only assume that the denseness of the aggregation induced by the centrifugal force is unfavorable to the

Figs. 47 to 52. Drawings of living eggs reared in neutral red solution.

Fig. 47. Four-celled stage. Resting nuclei. Red granules scattered in upper hemisphere.

Fig. 48. Third cleavage-spindles formed. Majority of red granules accumulated around the upper half of the spindles.

Fig. 49. Eight-celled stage. Resting nuclei. Same egg as Fig. 48, twelve minutes later. Details drawn only in D quadrant. In $1d$ the red granules are uniformly distributed; in $1D$ they occupy the upper left hand corner, or region of the cell $2d$.

Fig. 50. Single cell of about the sixty-four-celled stage. The red granules are now larger and lie just internal to the nucleus.

Fig. 51. Trochophore of twenty-four hours, reared in neutral red solution, the black areas show the distribution of the stain. The largest areas in three irregular rows are in the prototroch. As the larva rotated, the dorsal interruption of the prototroch came repeatedly into view.

Fig. 52. A single prototrochal cell drawn with oil immersion lens in life. The red granules are now very much larger than before. E, Ectoplasm; e, endoplasm; N, nucleus; V, vacuole.
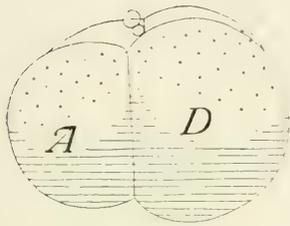
Fig. 47
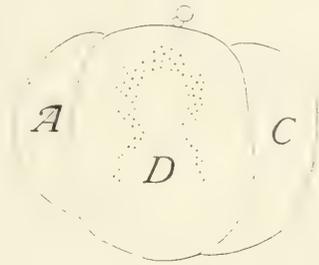
Fig. 48
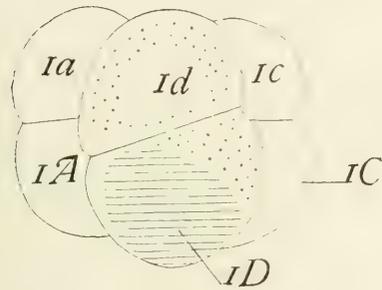
Fig. 49
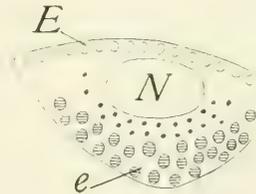
Fig. 50
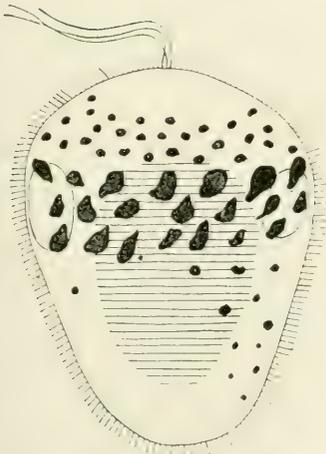
Fig. 51
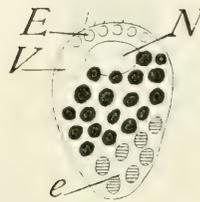
Fig. 52

penetration of the stain. What one does observe is that similar scattered red granules appear on the surface of the clear band, and that these become more abundant in the upper part, and spread over the entire animal hemisphere, whatever be the position of the gray and yellow masses, as in the normal development. Now the clear band always lies against the gray cap, and, as this gradually dissolves and spreads out, it seems probable that the red granules appearing on the clear band are derived from the gray cap. It would thus appear that the residual substance of the germinal vesicle does not consist entirely of erythrophilous granules; but it is probable that the original red granules are derived from this substance.

This is at any rate the conclusion to which I have come. It should be possible to observe this without any uncertainty in the living egg, if the hypothesis is correct; but when I began to realize the significance of the question the season had passed; and I have to rely on the observations made before I realized that the gray cap was the residual substance of the germinal vesicle.

I expect to return to this question another season; but I have little doubt of the correctness of this identification of the red granules distributed to the exumbrella with part of the residual substance of the germinal vesicle and other nuclear derivatives. The resemblance in position, size of granules, and general behavior is too precise to leave much room for doubt.

2. *Differentiation Without Cleavage*

In 1902 I published a paper on Differentiation Without Cleavage in the Egg of the Annelid Chætopterus pergamentaceous, in which I showed that under certain conditions, eggs of Chætopterus might become ciliated, and exhibit other phenomena of differentiation without undergoing any process of cleavage. Similar results have been obtained for other annelid ova by Treadwell ('02) and Scott ('03 and '06). One striking fact noted in Chætopterus was that such ciliated unsegmented ova sometimes exhibited what appeared to be regional homology with the trochophore. But the structure of the ciliated ova was very

variable and such homology could not usually be demonstrated. My general conclusion at that time was that cell division is not necessary for embryonal differentiation of certain kinds; and that the usually disordered and variable character of the forms developing from unsegmented ova indicates that the essential rôle of cleavage in normal development is the localization of processes of different kinds.

However, the conclusion in no way explained how differentiation of specific cell-organs could arise in an unsegmented mass of protoplasm with only one nucleus. The most obvious inference was that the unsegmented ovum already contained substances of specific morphogenic value, that played their rôles whether distributed in cells or not. This idea has been more or less definitely in mind since the original experiments, and I, therefore, took up the subject in Chætopterus again in the summers of 1904 and 1905.

I found that a variety of methods might be employed to bring about differentiation without cleavage:

(1) By exposing the unfertilized eggs for about an hour to the action of potassium chloride in sea-water. (For details of method see Lillie '02.)

(2) By treating fertilized eggs with the same solution but in higher concentration (Lillie '02).

(3) If ova are fertilized after standing two to eight hours in sea-water a variable proportion develop without undergoing segmentation, the proportion being greater when the ova have remained longer without fertilization. Or such eggs might segment at first and the blastomeres fuse together, the subsequent development being without cleavage. The fertilization was often polyspermic in such cases.

On July 28, 1904, a batch of eggs from one female was divided into three parts, which were fertilized after standing three hours, four hours and five hours, respectively, in sea-water. None of these segmented to any considerable extent. The next morning the four-hour lot was swarming with ciliated specimens, all of which had developed without cleavage. On July 30 some eggs were divided into two lots one of which, A, was fertilized after standing in sea-water one and one-half hours and the other B,

. after two and three-quarter hours. The cleavage of lot A was irregular from the start but only about 40 per cent developed without cleavage. In lot B, on the other hand, over 90 per cent failed to segment or the cells fused later on, and most of these eggs differentiated without cleavage. (For details of these experiments see the section on multinucleated, unsegmented eggs.)

(4) Fertilized eggs placed at a temperature of 10–14° C. for about twelve hours remain unsegmented and differentiate when restored to the room temperature, usually without segmentation.

In Experiment 8, 1904, two lots of eggs, A and B, were fertilized at 4.52 and 5 P. M., respectively; each was divided into two lots, A1 and A2, B1 and B2. 8.A1 and 8.B2 were placed at a temperature of about 14° C. about the time of the formation of the first polar body; 8.A2 and 8.B1 were placed at the same time at a temperature of about 16° C. When 8A.1 and 8.B2 were examined the next morning at about 9 A. M. they were unsegmented and they showed almost as sharp a segregation of the residual substance of the germinal vesicle as could be produced with the centrifuge. The preparations show that the maturation spindle remained unchanged in many cases and degenerated in others. In 8.A2 and 8.B1 there were two classes of eggs, viz: young normal larvæ and eggs like 8.A1; clearly the temperature in this case was near the lower margin of the temperature range for cleavage. A large proportion of the unsegmented eggs then underwent differentiation without cleavage at a very rapid rate.

(5) I have some evidence that an abnormally high temperature may produce the same effect (only one experiment not quite conclusive, as differentiation was not followed to a period of formation of cilia).

Careful examination of this mode of development, however, produced, showed, what I had entirely missed before, that the differentiation proceeds by the segregation and differentiation of the substances described in the preceding parts of this paper, but which I had not recognized at the time of my first study.

In describing the phenomena of differentiation without cleavage I shall leave out of account a good many accompanying phenomena which are not directly related to the problem in hand and which,

moreover, were sufficiently noticed in the earlier account (Lillie '02), such as the ameboid movements occurring at various stages of the process, and the more or less abnormal cleavage of a variable proportion of eggs in each experiment.

The unsegmented eggs that undergo differentiation may be divided into two classes according as they are *uninucleated or multinucleated;* the former constitute the type in the KCl cultures and are relatively rare in the fertilized cultures; the latter may owe the multinucleated condition either to polyspermy or to an original cleavage which is subsequently lost by fusion of the blastomeres. The multinucleated unsegmented eggs differentiate nearly as rapidly as normal ova (cilia formed in six or seven hours) whereas the uninucleated ova differentiate more slowly (cilia formed in eight to nine hours).

a.   Uninucleated Ova

The conditions are simpler in the uninucleated ova in some respects and the records more complete; they may, therefore, be considered first.   The process of differentiation depends on segregation of the cytoplasmic materials already described, growth of the nucleus to an enormous size, interaction of nuclear derivatives and cytoplasm, and a final rearrangement and differentiation of substances.   Among the many variable phenomena, these are constant, and may, therefore, be considered as primary.

1.   Early Segregation of Formative Stuffs.—The original segregation of material, whether the eggs be fertilized or not, is like the normal up to the period preceding the first cleavage; the polar globules may or may not be formed (Lillie '02); the polar lobe may appear and is then retracted; but cleavage does not take place. Then the egg-nucleus together with the nonspherular protoplasm begins to sink in toward the center of the egg.   At the same time endoplasmic spherules slowly arise toward the animal pole, as in the normal development, between the nonspherular protoplasm and the ectoplasm (Fig. 53).   The ectoplasm also begins to flow towards the animal pole and is soon aggregated mainly in the upper hemisphere (Figs. 54 and 55); the polar ectoplasm remains at the vegetative pole (Fig. 55).   The original opening in the ectoplasm

at the animal pole may persist and the spherular endoplasm reach the surface here (Figs. 53, 55, 56). The layer of spherular enodplasm is usually considerably thinner on one side, show. ing that there is a bilateral polarization of the substances like the normal. The nucleus has enlarged considerably. This occurs about one and one-half hours from the beginning of the experiment.
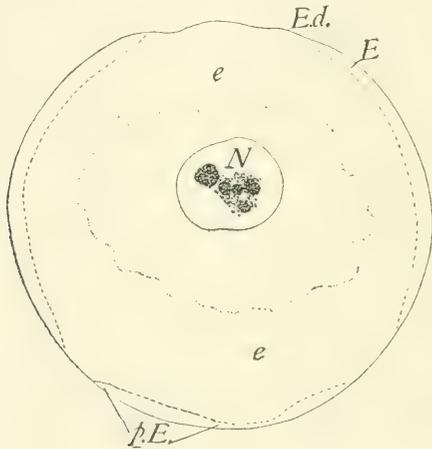
Fig. 53. Section of differentiating uninucleated unsegmented egg. *E*, Ectoplasm; *E.d.*, ecto-plasmic defect; *e.*, spherule bearing endoplasm; *N*, nucleus; *p.E.*, polar ectoplasm. Eggs put in ninety-three parts sea-water plus seven parts 2½ M KCl at 9 A. M., transferred to sea-water at 10 A. M. Preserved 10.39 A. M., one hour and thirty-nine minutes from the beginning of the experiment. The dotted outline represents the boundary of the spherule-bearing endoplasm; the broken line, the ectoplasm.

The ectoplasm now flows more rapidly towards the animal pole and thus forms a relatively narrow polar ring; in some cases the opening disappears and the ectoplasm becomes heaped up in a mass (Fig. 59); as there is relatively little endoplasm in this part of the egg there appear three strata (Figs. 54, 55 and 56). This occurs about two to four hours from the beginning of the experiment. Thus in Experiment 3.1, 1904, I put some unfertilized eggs into ninety-five parts of sea-water plus five parts of 5/2 M KCl at 10.47 A. M., and the eggs were trans-
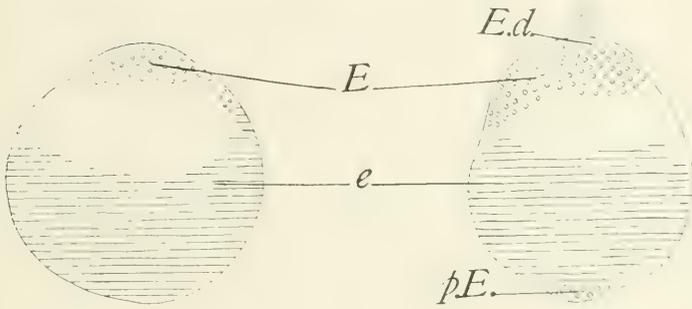
Fig. 54

Fig. 55
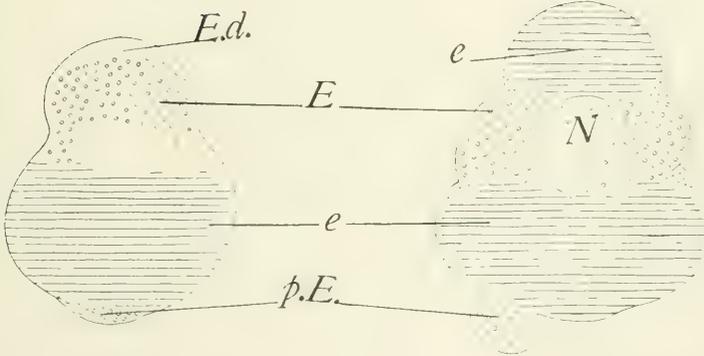
Fig. 56

Fig. 57

Figs. 54 to 58. Stages in the differentiation of uninucleated unsegmented eggs drawn from living material. *E*, Ectoplasm; *e*, endoplasm; *E.d.*, ectoplasmic defect; *p.E.*, polar ectoplasm; *r.s.* remainder of gray cap (residual substance of germinal vesicle).

Fig. 54. Eggs put in ninety-five parts sea-water plus five parts of a two and one-half M KCl solution at 3.15 P. M.   Transferred to sea-water at 5 P. M.   Drawn at 5.15 P. M., two hours from the beginning of the experiment.

Fig. 55. Eggs put in ninety-five parts sea-water plus five parts of a two and one-half M KCl solution at 10.47 A. M.   Transferred to sea-water at 11.52 A. M.   Drawn about 2.45 P. M., four hours from the beginning of the experiment.

Fig. 56. Same history as Fig. 55.

Fig. 57. Eggs put in eighty-five parts sea-water plus fifteen parts of a two and one-half M KCl solution at 11.10 A. M.   Transferred to sea-water at 12 o'clock, noon.   Drawn 4.12 P. M., five hours from the beginning of the experiment.

ferred to normal sea-water at 11.52 A. M., and the conditions shown in Figs. 55 and 56 were found at about 2.30 and 2.45 P. M., respectively. It will be noticed that there is quite a pronounced constriction near the equator between the unlike substances; this often becomes so deep as to produce the impression of a cleavage. The substance of the polar lobe is indicated in both cases, and in one (Fig. 55) an ameboid prominence is formed at the animal pole, owing to extrusion of endoplasm through the ectoplasmic defect occurring there.

In about half an hour to an hour more a very striking change takes place in the majority of the eggs (uninucleated, unsegmented). A mass of yellow spherules that have continued to rise toward the animal pole pushes out through the defect in the ectoplasm and forms a protuberance at the animal pole (Figs. 57, 58, and Photograph L). Thus the ectoplasm becomes a broad band encircling the ovum between the polar masses of yellow endoplasm. (For section of such a stage, see Fig. 60.) The nucleus has now become as large as the original germinal vesicle and it lies in the non-spherular protoplasm beneath the ectoplasmic band. In some cases the ectoplasmic defect at the animal pole becomes covered up (Fig. 59), and then this banded form does not occur; in such cases the ectoplasm simply becomes more massed.
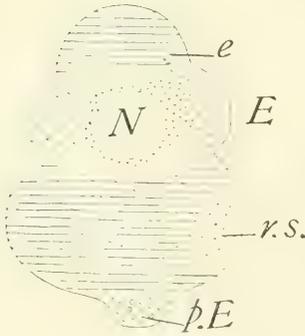
Fig. 58. Eggs taken from female at 9 A. M., centrifuged at 10.45 A. M. Fertilized 10.50 A. M. Drawn about 3.15 P. M., four hours and thirty-five minutes after fertilization.

There are some reasons for thinking that the polar masses of endoplasm are of different composition, thus it will be noticed that the original lower mass (Photograph L) affects the photographic plate less strongly than the upper mass, though to the eye they appeared alike in color.

The mere separation is also evidence for some difference in composition, though it is to be admitted that it does not constitute decisive proof. A third reason is found in the presence in the

lower mass of a small reticular area usually clearly marked at this stage (Fig. 60) and always absent in the upper mass. These facts are mentioned only as indicating that the original (or early induced) diversity of substances in the endoplasm may be greater than one would suppose. The opposed masses in this case correspond roughly to the endoplasm of the first generation of ectomeres (upper mass) and of the other cells (lower mass). The distinction is not between endoplasmic substances a and c, for these occur in each mass.

2. *Concerning the Growth of the Nucleus.*—Nuclear growth is usually accompanied by nuclear division involving the formation and division of a definite number of chromosomes at periodic intervals. Now in the eggs that we are describing the nucleus does not divide, but it grows and undergoes periodic changes that correspond to the periodic changes of dividing nuclei. Thus in any preparation the nuclei of some eggs are composed mainly of nonstaining substances, with only a few scattered stained granules similar to the "nucleoli" cast off at each normal division (Figs. 59 and 60), and other nuclei in ova on the same slide, and thus of precisely the same age and mode of preparation, are dense masses of intertwined chromosomes (Fig. 61). These conditions undoubtedly represent successive phases of nuclear activity, and demonstrate a periodic succession of chromatic and non-chromatic stages. Inasmuch as the number of chromosomes in the older and larger nuclei is much greater than in the younger and smaller, it cannot be doubted that there is a periodic formation and division of chromosomes as in the normal cleavage.

The egg shown in Fig. 61 was killed three hours and seventeen minutes after the beginning of the experiment. After making due allowance for the slower rate of development it would correspond in age to about a normal sixty-four celled stage, which would contain, if unfertilized, $9 \times 64 = 576$ chromosomes. A calculation of the chromosomes in this uninucleated egg gives a roughly corresponding number; the nucleus ran through four sections and there were considerably over 100 chromosomes in each. (All in the one section figured could not be shown in the figure.) The number corresponds so closely to the theoretical requirements,
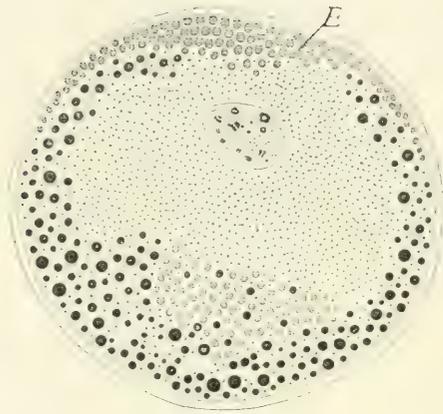
Fig. 59. History same as 53, preserved two hours and nineteen minutes from beginning of experiment. The ectoplasm aggregated in animal hemisphere; ectoplasmic defect covered up; *a* and *c* endoplasms separating out.

that it has the value of an actual demonstration of the view expressed in the last sentence of the preceding paragraph.

The arrangement of the chromosomes in Fig. 61 is suggestive; they are not uniformly distributed, but are arranged in four groups. The number of groups in the entire nucleus was at least double as many. The only explanation of such a grouping that occurs to me is contained in the hypothesis that all descendants of each original chromosome,

of which there were nine, tend to remain together. There is no other evidence for this than I have given. In any event the steady increase in number is a strong support for the individuality hypothesis.

That the nucleus does not divide under such circumstances may be attributed to the absence of centrosomes and spheres, which are usually lacking in the uninucleated unsegmented eggs in the early

Fig. 60. History same as 53, preserved four hours and eighteen minutes from beginning of experiment. Longitudinal section of three-banded form; original animal pole probably to left. Nucleus in achromatic condition. Vacuolated area to right characteristic of eggs in this condition.

stages. The reason for this defect is not very clear, especially in the case of those eggs where the stimulus to development is furnished by normal fertilization. But, even when the stimulus is

supplied by potassium chloride, certain eggs form asters and spin-
dles, so that the potentiality of these structures must be sup-
posed to reside in the egg proto-
plasm.   The series of preparations
upon which I rely for most of
the data concerning the nuclei is
from a potassium chloride series
in which the polar bodies were not
formed;[1] it is thus possible that,
in this case, there is a connection
between the omission of the pro-
cess of formation of the polar
bodies and the absence of asters.
Such an explanation might also
apply to the uninucleated, unseg-
mented eggs found in fertilized

Fig. 61.   History same as 53, preserved three
hours and seventeen minutes from beginning of
experiment.   See text for description.

series; these constitute a relatively
small proportion of all the eggs in such a culture, and in a
normal lot of eggs some fail to fertilize, and do not form
polar bodies; if we supposed that some extract of the sperm
stimulated differentiation in such eggs we would get the above
result.

3.   *Interaction of Nucleus and Cytoplasm.*—After the nucleus
has reached about the size of the germinal vesicle a strong mutual
attraction between the nucleus and the ectoplasm begins to be
apparent, when the nucleus is in the chromatic condition.   This
is shown in one of two ways: Either the ectoplasm is drawn into
the interior of the egg where it forms a mass in contact with the
nucleus, or the chromatic part of the nucleus is drawn out to the
ectoplasm.   It is interesting to note that the former corresponds
to the normal procedure in Clepsine (Whitman '78) and Rhynchel-

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[1] If the polar bodies are not formed, the looped or elongated form of chromosome does not appear
in the uninucleated, unsegmented ova until between one and two hours from the beginning of the
experiment.   Previous to this time one finds only irregular forms similar to the chromosomes of
the maturation spindles.   Thus the chromosomes apparently carry out the maturation divisions
within the egg but without forming daughter nuclei, before the form of chromosome characteristic
of the cleavage divisions appears.
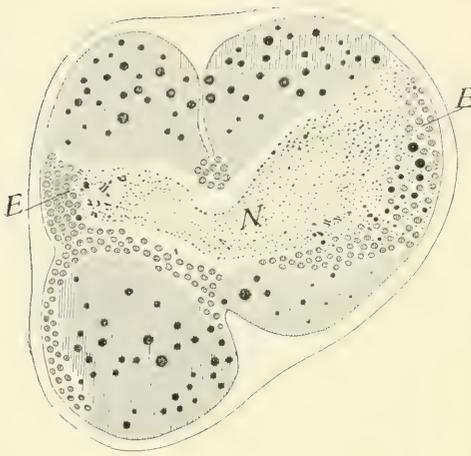
Fig. 62. ˙ Eggs put in ninety-five parts sea-water plus five parts 2½ M KCL at 11.10 A. M., five hours and ten minutes from beginning of experiment. For description see text.

mis (Vejdovsky '88–'92). When the ectoplasm has gathered in the interior it collects on one side on the large nucleus, and a very important change takes place. A large quantity of the chromatin aggregates on the margin of the ectoplasm, and, while the remainder of nucleus returns to the achromatic condition, this portion diffuses in the form of minute particles (microsomes) between the ectoplasmic spherules, which are thus completely interpenetrated by a chromatic network. Eggs in which the ectoplasm has been attracted into the interior apparently do not develop farther.

When the ectoplasm remains external the chromatic part of the nucleus is drawn to it. Fig. 62 illustrates a case in which there were two accumulations of the ectoplasm on opposite sides of the egg; the attraction acting on the nucleus has drawn the latter out into a broad band extending from one mass of ectoplasm to the other. The chromatic portion of the nucleus is next the ectoplasm on each side, leaving an achromatic nuclear band in the center. The chromatin is broken up into small particles which are scattered between the ectoplasmic spherules.

Fig. 63. History same as 62. For description see text.

A single section, such for instance as is shown in Fig. 63, shows the outline of the nucleus still intact; the chromatin in strands with large varicosities is seen stretching off toward the main mass of the ectoplasm, and the farther we trace it from the center of the nucleus the more the chromatin is broken up; radiating lines of granules can be seen extending from larger masses of chromatin in between the ectoplasmic spherules, so that all intermediate stages between masses of chromatin and scattered microsomes may be seen. Many similar sections occur on the same slides. Such granules are very much less abundant between the ectoplasmic spherules in stages preceding this.

The living material furnishes the most conclusive proof of the migration of the chromatin halo of the nucleus. If the living eggs be examined under pressure at this time, one sees a gray halo around each large nucleus (Photograph J and Fig. 58); a little later this may be seen to stretch out toward a surface aggregation of ectoplasm and to flow into it losing its identity in the larger mass (Photograph K).

The intermingling of a large part of the nucleus, distinguishable in the living condition as a gray halo and in sections as a peripheral chromatin layer, is one of the most striking and suggestive phenomena concerned in the differentiation of the uninucleated unsegmented eggs. Concerning the facts there cannot be a particle of doubt. The process can easily be observed in the living condition, and even photographed, as Photographs J and K prove. The preparations are even more conclusive, if possible, for one can study the details more leisurely. Nor is there any doubt, in my opinion, that the chromatin particles become microsomes. The gradation from larger chromatin masses to undoubted microsomes is perfect, and proceeds in an orderly direction from the nucleus outward (Figs. 62 and 63).

The interpretation of this phenomenon must proceed from the assumption that it is the result of a process occurring in the normal development, probably in each generation of cells, but which has been inhibited by the abnormal nuclear conditions, until it breaks loose by sheer force. Its striking character is due to the summation of a number of relatively inconspicuous processes of the nor-

mal development. It, therefore, furnishes to some extent a meas-
ure of the amount of chromatolysis within the same period of the
normal development.

The result necessitates a more careful inquiry into the normal
conditions; at present it does not seem advisable to attempt a
detailed comparison. I may say, however, that the process ap-
pears to me to represent more than the formation of the chromatic
granules that lie between the groups of daughter chromosomes
in the normal anaphase (see description, p 202, and Fig. 25); because
I believe that such granules escape into the cytoplasm periodically
in the uninucleated unsegmented eggs, and do not accumulate
within the nucleus. The matter is still under investigation.

Finally it should be noted that such distribution of chromatin is
not limited to the ectoplasm, but occurs probably in the endoplasm
also in later stages.

4. *Later Distribution of Formative Stuffs.*—That the ectoplasm
undergoes a radical change by this intermixture of chromatin is
proved by two striking phenomena: (1) The mass of chromatin-
charged ectoplasm immediately overflows the remainder of the
substance of the egg, usually, but not always, covering it com-
pletely; (2) the staining reaction of the ectoplasmic spherules
changes radically; whereas previously they could not be stained
with iron hæmatoxylin without overstaining the rest of the egg,
they now take the hæmatoxylin strongly. These two phenomena
will be considered separately.

Overflow (unicellular gastrulation). I observed this process
first in the summer of 1904. The most complete records are in
Experiments 5 and 1 of that season, from which I take the follow-
ing account: Experiment 5 was started at 11.10 A. M. July 28,
1904. Division 1 of this experiment was treated as follows:
The eggs were put into a mixture of ninety-five parts sea-water
plus five parts $2\frac{1}{2}$ M KCl at 11.10 A. M. Part a was transferred
to sea-water at 12.05 P. M. (fifty-five minutes). The designation
of this experiment is thus 5.1.a. At 3.20 P. M. many eggs had reached
the elongated condition shown in Figs. 57 and 58 with two terminal
masses of yellow endoplasm, and a broad equatorial band of ecto-
plasm through which the large nucleus could clearly be seen. At

5 P. M. the ectoplasmic band in many eggs had ruptured on one side transforming it into a saddle-shaped mass, and the two masses of endoplasm met and fused on the opposite side of the egg (Fig. 64). The nuclear area is situated beneath the ectoplasmic accumulation.

It is undoubtedly at this time that the chromatic matter of the nucleus intermingles with the ectoplasm, though I did not observe this in 1904 for the simple reason that the eggs were not examined under pressure. But in 1905 this phenomenon was observed as already described.

FIG. 65   FIG. 64   FIG. 66
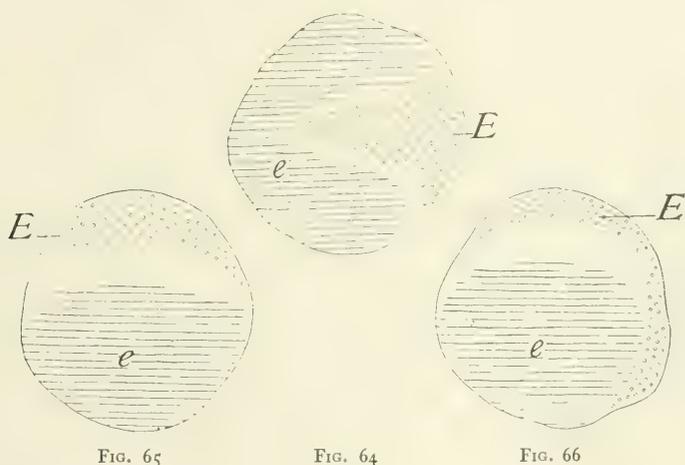
Figs. 64 to 70.   Later differentiation of uninucleated unsegmented eggs from life.

Fig. 64.   Form produced by rupture of ectoplasmic band on one side.   Eggs put in ninety-five parts of sea-water plus five parts 2½ M KCl at 11.10 A. M.   Transferred to sea-water at 12.05 P. M.; drawn at 5 P. M., five hours from beginning of experiment.

Fig. 65.   Slightly later stage than 64.

Fig. 66.   Same egg as sixty-five drawn three minutes later; the ectoplasm has overflowed a large part of the endoplasm.

All stages of the rupture of the ectoplasmic band were observed; the notes read "all stages may be seen; the granular band (ectoplasm) first becomes broader on one side, then the yolk masses (endoplasm) approach and fuse and the band ruptures. Thus the yolk has the characteristic shape shown in the figure."

Then the ectoplasm accumulates in a mass (Fig. 65) and

immediately begins to spread rapidly over the surface of the egg
(Figs. 66 and 67) until there is a complete surface layer of ecto-
plasm. The notes read "This overflow of the gray matter (ecto-
plasm) can easily be watched as it takes place rapidly, and all
stages may be seen at once on a single slide." Two drawings of
the same egg (Figs. 65 and 66) accompanying Experiment 1, 1904,
show that a considerable part of the process of overflow may
take place in three minutes. An uncovered spot, which prob-
ably corresponds to the position of the polar ectoplasm, may persist
for some time, and the egg thus affords a striking picture, as of
epibolic gastrulation in a unicellular mass of protoplasm (Fig. 67).
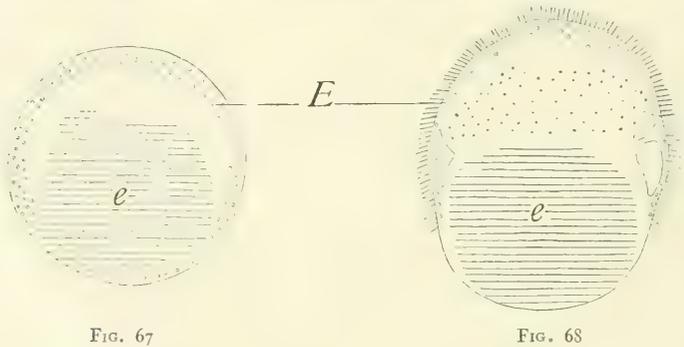
Fig. 67   Fig. 68

Fig 67. Another egg; slightly later stage. Unicellular "gastrula."
Fig. 68. Ciliated uninucleated unsegmented egg drawn about twenty-three hours from beginning
of experiment. The vacuoles are about in the position of the prototroch of the larva.

In many cases the process of overflow is incomplete, or does not
involve the yolk mass at all, thus leading to conditions described
beyond. There is usually more or less endoplasm included in the
overflowing mass; in some cases a very considerable quantity, so
that the layer established by the overflow may be quite thick.
This is a parallel condition to that found in the ectoderm cells
normally, in which, as described on p. 207, only the layer external
to the nucleus is composed of ectoplasm, and the remainder of the
cell of endoplasm.

Change of Staining Reaction. Sections made for the purpose
of studying the process of overgrowth show that it does not begin
until the ectoplasmic spherules have been so modified by the

intermixture of chromatin that their staining reaction to iron hæmatoxylin has changed considerably. Although this approximates their stain to that of some of the endoplasmic spherules, there is not the least difficulty in distinguishing them. The ectoplasmic spherules are smaller, or at least more uniform in size, less spherical, and are massed differently; the substance in which they are embedded likewise appears different from that of the endoplasm, so that the total effect of the ectoplasm even when its spherules stain black is entirely different from that of endoplasm.

It appears as if the ectoplasmic spherules become completely impregnated with chromatin, just before the process of overgrowth takes place; the change in staining reaction is difficult to explain otherwise. The formation of cilia follows very soon after the process of overgrowth; thus it cannot be doubted that the impregnation of the ectoplasmic spherules with chromatin stimulates their latent potencies.

Sections stained in thionin and orange G show that the dispersion of the chromatin is by no means confined to the ectoplasm; but that the endoplasm as well is thoroughly interpenetrated with particles of chromatin, which are beyond all question microsomes. The same thing may also be seen in the iron hæmatoxylin preparations, but it is not shown in so striking a manner as by the thionin and orange stain. The bright orange spherules contrast beautifully with the brilliant blue microsomes clustered around them and in all the interspaces. With thionin and orange staining the ectoplasmic spherules continue to take the orange stain even after the intermixture with chromatin but they take the blue also to a certain extent, so that their general effect is olivaceous.

We have seen that in some cases the nucleus draws the ectoplasm into the interior of the egg, so that it is completely surrounded by the endoplasm. It is probable that this is the reason why many eggs fail to reach the stage of differentiation of cilia. Thus in Experiment 9, 1901, very few of the eggs became ciliated; but the preparations show that, in practically all, the nucleus grew and the ectoplasm segregated in the usual fashion. But the sections also show that the internal migration of the ectoplasm is very characteristic of the series.

5. Formation of Cilia and Other Cell Constituents.—The formation of cilia follows hard on the heels of the ectoplasmic overflow. Some uninucleated eggs begin to revolve eight or nine hours after the beginning of the experiment, and others not until much later. The majority, in the case of the potassium series, never reach this stage at all, probably, as suggested before, owing to failure in the process of overflow.

Typically the entire ectoplasmic surface becomes ciliated, but the cultures show great variety of conditions owing to the following circumstances: (1) A good many eggs break into two or more
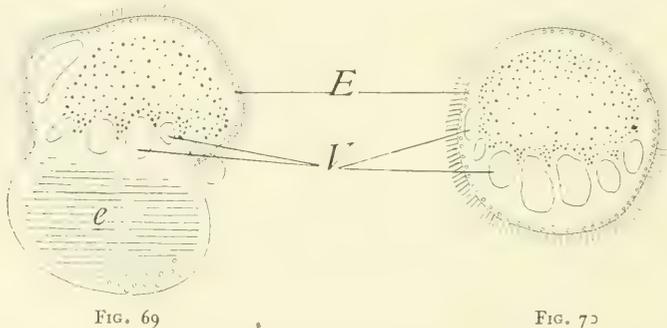
Fig. 69   Fig. 70

Fig. 69. From the same culture as 68; about same age.

Fig. 70. From same culture as 68; about same age. The endoplasm of the egg has been constricted off.

parts owing to excessive development of constrictions between segregated substances (compare Fig. 69). Thus are produced some non-nucleated parts, some parts nucleated and without ectoplasm, other parts nucleated and with relatively little endoplasm. (2) Fusions are very common (see Lillie '02). (3) A larger or smaller part of the endoplasm may remain exposed (compare Figs. 68 and 69).

It is, therefore, not surprising that good examples of clear regional homology with the entire trochophore are rare. Some are, however, found. (The regional homology may be very precise in multinucleated unsegmented eggs; see next section.) The best example of it in uninucleated eggs is shown in Fig. 1 of my 1902 paper. (See also Figs, 68, 69 and 70 of the present

paper.)   The reason for the existence of a regional homology is so clear that discussion is hardly necessary.   Fig. 70 shows a ciliated part corresponding only to the upper halves of Figs. 68 and 69 and resembling only the exumbrella region of the trocho-phore.

In general the following statements may be made concerning the differentiation of the uninucleated eggs.   (1) Organs are never formed, but only such structural elements as may occur in single cells of the trochophore.   (2) Organs may, however, be simulated by the aggregation of the characteristic matter of an organ, for instance in the case of the yellow endo-plasm, which simulates the gut of the trochophore, or the row of large vacu-oles situated near the upper margin of the yellow endoplasm (*e. g.*, Fig. 69) which simulates the row of vacu-oles of the prototroch.   (3) The struc-tural elements appear in the same order of time as in the trochophore. (4) The distribution of structural ele-ments tends to resemble that of the trochophore.   (5) The yellow endo-plasm (yolk ?) is used up, apparently for the maintenance of metabolism,

Fig. 71.   Ciliated unsegmented egg about twenty-eight hours old.   Most of the endoplasm has been consumed. Living.

in the ciliated unsegmented eggs precisely as in the larva (see Fig. 71).   (6) The apical flagellum is never formed.

Inasmuch as a number of conditions were figured and described in my earlier paper (Lillie '02) and because multiplication of examples would be unprofitable, I shall content myself with illustrating in a very few cases the general statements just made.

The first and second statements really require no further elaboration; Figs. 68–71 sufficiently illustrate them.

The order of appearance of the structural elements is: (*a*) cilia; (*b*) intermediate vacuoles; (*c*) ectoplasmic vacuoles.   I was much interested in attempting to discover the mode of origin of the cilia.   From the fact that their distribution, both in the nor-mal larva and unsegmented eggs, is the same as that of the ectoplas-

mic spherules, I was at first inclined to believe that each spherule was the germ of a cilium, and expected, therefore, to find the same number of cilia and spherules. *The number of cilia seems, however, to exceed the number of spherules, and they do not arise directly from the spherules but from the microsomes between or on the surface of the spherules.* This can be seen very clearly by focusing on the surface of an egg that is acquiring cilia with a 2 mm. oil immersion lens. But there is a very intimate relation, and one can convince himself after the rate has slackened that the spherules vibrate in unison with the beat of the cilia.

The intermediate vacuoles appear between the ectoplasm and the endoplasm soon after the formation of cilia. They are practically confined to the original ectoplasmic hemisphere, and are specially well developed usually just above the margin of the endoplasm, thus in a position corresponding to the prototroch. They appear to correspond to similar large vacuoles situated internal to the nucleus in the cells of the exumbrella and particularly of the prototroch in the larva. But, as they are confined by cell-walls, they tend to fuse and thus to produce a fluid-filled space separating ectoplasm and endoplasm (see figures).

The ectoplasmic vacuoles are much smaller and more refringent, and do not appear until about twenty-four hours from the beginning of the experiment (Fig. 71). They are usually massed in a particular region, corresponding to their distribution in the trochophore where they are very much more abundant in the exumbrella. In the ciliated ova of this age, the yellow endoplasm is much reduced in amount, and the eggs as a rule do not live much longer.

(6). It is a curious fact, observed also by Scott in his observations on Amphitrite, that the apical flagella are never found in these ciliated eggs. This indicates a combination of factors in the normal development that is seldom or never realized under the conditions of the experiments. The explanation is probably as follows: The apical flagella arise at the animal pole, and in Chætopterus one can frequently see one polar body at least at their base. This is, however, the place where the endoplasm comes to the surface; *the apical flagella are therefore endoplasmic*

in their origin. In the overflow of the ectoplasm in the unsegmented eggs this exposure of the endoplasm is seldom, or never, preserved. Thus the formation of the apical flagella would be prevented.

The study of the cytological phenomena in sections has been carried only as far as the overflow of the ectoplasm (about eight hours). Up to this time it is possible to follow the history of the original substances of the egg consecutively. I have not studied the later cytological details; the changes that take place in the structure of the cytoplasm are very profound and complex.

b. Multinucleated Unsegmented Eggs

These are characteristic of cultures of fertilized eggs so treated as to inhibit the process of cleavage or to induce fusion of blastomeres. There is a strong tendency towards polyspermy, if eggs are allowed to stand for several hours in sea-water before being fertilized. Such eggs may break up at the first division into a considerable number of blastomeres, and, in many such cases, the blastomeres soon fuse together, and cleavage planes do not again appear. The protoplasm of the egg of Chætopterus is extremely susceptible to external conditions, and one of the most common evidences of this is the fusion of blastomeres.

In Experiment 7.B., 1904, for instance, the eggs were taken from the female at 9 A. M., and were allowed to stand in sea-water until 11.42, when they were fertilized; a series of eggs was preserved and from them entire mounts and sections were prepared. In the entire mounts of eggs preserved twelve minutes after the addition of the sperm it can readily be seen that there are from three to twelve or more sperm nuclei in each egg. These were also seen in the living eggs under pressure. The polar bodies were formed normally in about twenty-five minutes. Figs. 72 to 75 show the form changes of a single egg, from 12.32 to 12.47 P. M. At first this egg became so lobulated that it seemed as though it were dividing into about ten cells at once; then these lobes gradually disappeared and the eggs returned to their spherical condition. At 12.54 the great majority of the eggs had returned to the spher-

ical condition; about 5 per cent appeared like normal two-celled stages; these were probably monospermic.

In Experiment 7.A. some of the same eggs were fertilized at 10.35 A. M., having remained in the sea-water one hour and thirty-five minutes before fertilization. About 60 per cent of these segmented fairly normally, the blastomeres fused together in the remainder "Intermediate conditions were rare or absent." (Quoted from notes.)
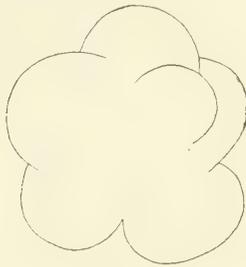
FIG. 72

FIG. 73
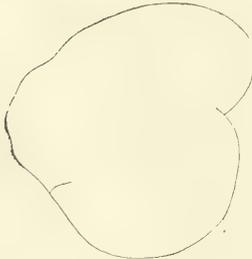
FIG. 74
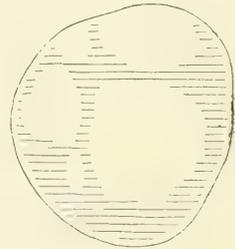
FIG. 75

Figs. 72 to 75. Four views of the same polyspermic egg. The eggs were put in sea-water at 9 A. M. and allowed to stand until 11.42 A. M. when they were fertilized. Fig. 72 was drawn at 12.32 P. M.; Fig. 73 at 12.37; Fig. 74 at 12.44; Fig. 75 at 12.47. At 12.50 the egg was perfectly spherical again and showed no sign of cleavage.

I expect to make a special study of the polyspermy, for which I have the preparations. So in this place I shall consider only a few points. In the polyspermic eggs multipolar spindles arise, and a very uneven distribution of the chromatin results. This is followed by the formation of nuclei of very unequal size, and in the following divisions the mitoses are irregular. Thus there

is an enormous increase of the chromatin, and soon the most of
the nuclei cease to be separate in the resting condition, and form
a vast reticulum situated between the ectoplasm and endoplasm
in the upper half or two-thirds of the egg (Fig. 76). A few
small nuclei may remain separate. In the ensuing periods of
division the individual karyokinetic figures cannot be observed,
and there is a veritable riot of centrosomes, chromosomes, spindle
fibers and astral radiations.
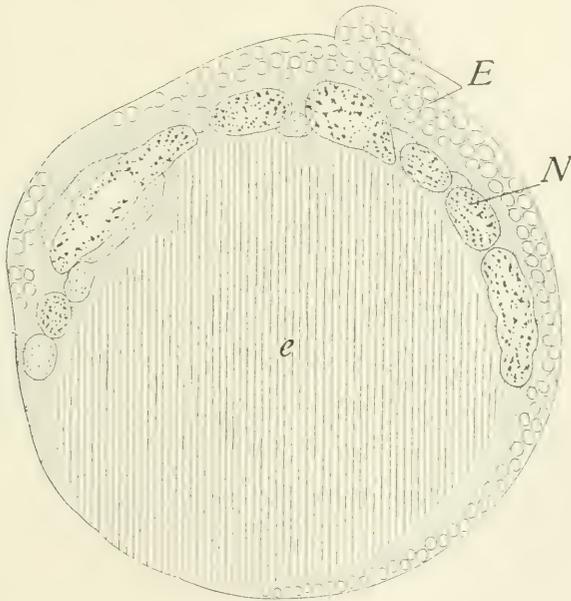
Fig. 76. Optical section of whole mount; multinucleated unsegmented egg, preserved five and
one-half hours after fertilization. The nuclei formed an extensive reticulum; the apparently separate
nuclei of the figure are the optical sections of the broad strands of the nuclear reticulum.

During this period the ectoplasm becomes very thick at the
animal pole, and the endoplasm forms a ball that is attached to
the surface at the vegetative pole by the substance of the polar
lobe (polar ectoplasm) (Figs. 77 and 78).

*Thus it will be seen that the presence of numerous nuclei or of
an extended nuclear reticulum, situated between ectoplasm and endo-
plasm, results in a different arrangement of the ectoplasm and endo-*

plasm from that found in the uninucleated ova where the nucleus is central in position. In the multinucleated ova the polar distribution of substances of the ovum does not differ essentially from the normal. It appears to follow that the curious modes of aggregation of the ectoplasm and the endoplasm in the uninucleated ova are due, in the first place, to the lack of the restraining influence of numerous nuclei, and, in the second place, to the fact that, when nuclear influence is established by the enlargement of the nucleus, it proceeds from a single center in place of many.
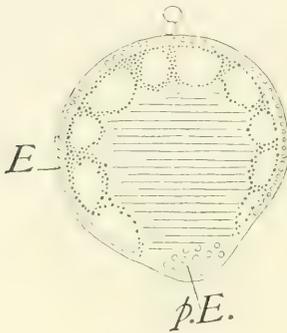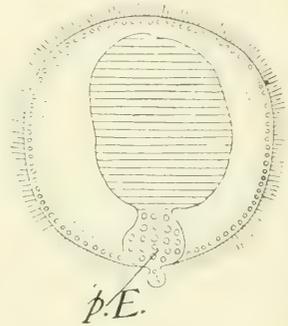
Fig. 77.   Fig. 78.

Fig. 77. Multinucleated unsegmented egg drawn from life four hours and ten minutes after fertilization. The eggs had been allowed to stand in sea-water one hour and fifty minutes before fertilizing.

Fig. 78. Multinucleated unsegmented egg drawn from life eight hours after fertilization. From a culture in which the eggs had been centrifuged one and one-half hours after fertilization.

The maintenance of the normal distribution of ectoplasm and endoplasm in the unsegmented multinucleated ova results in a very perfect homology of the ciliated unsegmented ova with the trochophore. A few examples may illustrate this. Fig. 77 is from a living egg, six hours old. The polar globules show the position of the animal pole; the ectoplasm, endoplasm and nuclei (appearing almost like vacuoles in the living egg) have the positions described for Fig. 76. At the pole of the egg nearly opposite to the polar globules is seen a mass of spherular protoplasm, that corresponds exactly in position and appearance to the mesoblast cells in the normal embryos, which can very readily be seen in the living condition. Thus we can readily homologize ectoplasm,

endoplasm and mesoblast substance with those of the normal larva. Moreover, they have the same positions with relation to one another and to the polarity of the egg as in the normal embryo. The main difference is that the absence of cell-walls permits all of the endoplasm to aggregate in a single mass; whereas in the normal larva it is divided between the cells of the ectoderm, ento- derm and mesoderm, though occupying in each kind of cell a position next the segmentation cavity.

In some cases the substance of the polar lobe is partly separated from the yolk-mass proper (Fig. 78). This affords a fine demon- stration of the existence of such a substance at the lower end of the yolk-mass. The presence of this substance is perfectly characteristic. It is very adhesive, as union by it of two or three ova is very common.

An interesting addition to the information concerning such un- segmented ciliated eggs is given by staining intra vitam with neutral red. It is then found that there is an accumulation of red granules in the upper hemisphere that correspond precisely in position and staining reaction with those of the trochophore (see Part IV, *d*). The only difference is, that in the unseg- mented egg they are more massed, a condition naturally resulting from the absence of cell-walls.

For completeness of demonstration of the principle of germinal localization these differentiated, and yet wholly unsegmented, ova leave little to be desired.

c. Literature and Discussion

Differentiation of entirely unsegmented eggs has been observed in two other genera of polychæta, in Podarke by Treadwell ('02) and in Amphitrite by Scott ('03 and '06). Bullot ('04) failed to observe it in Ophelia, though he looked for it, and he therefore cast some doubt upon its occurrence. I would only point out that the occurrence of segmented ciliated embryos in his cultures by no means demonstrates the absence of the phenomenon of differentiation without segmentation; for Treadwell, Scott and I have all observed the occurrence of ciliated ova both segmented and unsegmented in the same culture-dish. Bullot's figures show

that, in the unfertilized cultures of Ophelia eggs, segmentation was seldom or never, even approximately, normal.

It is clear from the descriptions of these three authors that the occurrence of a certain amount of segmentation is more common under the conditions of the experiment in the forms that they described than it is in Chætopterus, which seems to be peculiarly favorable for the observation of differentiation without any cleavage.

To this list of observations may be added, in all probability, those of Bastian ('05) on a peculiar form of differentiation in Rotifer eggs. Indeed, unless one is willing to adopt Bastian's explanation that particular species of ciliates may arise by direct metamorphosis of rotifer eggs, no other explanation is possible than that he has observed cases of differentiation without cleavage. His peculiar interpretation so colors the description of the observations, that it is difficult to utilize them. But it is probable from the figures that in some cases the eggs form cilia without any cleavage, and that, in other cases, the eggs segment or break into several smaller parts, each of which then differentiates farther without cleavage.

Scott summarizes his results on the differentiation of unfertilized eggs of Amphitrite as follows: "Under the conditions of the experiments certain forms of development occur with or without cleavage and with or without the formation of polar bodies in the unfertilized egg of Amphitrite. Such development takes the form of nuclear divisions, the early differentiation of a layer of ectoplasm, the growth of cilia, the appearance of vacuoles that are found in the fertilized egg of the same age, the development of a brownish pigment, the ameboid movements of the cytoplasm that are connected with cleavage, the ameboid movements at a later stage of development that appear entirely independent of cleavage, and the change in shape of the egg, which in most cases at least is connected with incomplete, arrested or abortive division of cytoplasm. The apical tuft of cilia which is characteristic of trochophores from fertilized eggs is always absent."

Scott justly emphasizes in his theoretical considerations "the intimate relation that exists between cytoplasmic and nuclear

differentiation; the correlation in development between these two factors is very complete where a normal organism results. Inasmuch as the cessation of development is a cumulative process, that is the abnormalities appear in successive transformations of the asters and nucleus, we must look upon the cessation of development as due to incomplete reactions between nucleus and cytoplasm."

Treadwell ('02) observed that ciliated embryos might arise in Podarke without cleavage, and that the differentiation might be carried very far in such cases. One case that he describes is particularly interesting (Fig. 12 of Treadwell); two eggs are fused together and are entirely unsegmented. "Around one portion of the fused mass is a ring of cilia, occupying very much the position, with respect to the cell, of the prototroch in its relation to the trochophore. Not only are the cilia present, but around the embryo, underneath the ciliated band, is an area free from the ordinary pigment of the rest of the cell, but containing granules of a faint yellow color, agreeing in this respect exactly with the prototroch band of the normal trochophore. In this embryo there is, then, not merely a differentiation—without cleavage—of cilia, but of the characteristic protoplasm accompanying these cilia. Or, in other words, we have here in the unsegmented embryo, not merely a differentiation of cilia, but a differentiation of *prototroch cilia.*"

The possibility of a considerable amount of embryonal differentiation without either nuclear or cytoplasmic division may be considered established. This in itself is a fact of considerable importance, for it disposes effectually of all theories of development that make the process of cell-division the primary factor of embryonal differentiation, whether in the form of Weismann's qualitative nuclear division, or of Hertwig's cellular interaction theory. Further, the phenomenon establishes firmly, as I pointed out in 1901, the view that the rôle of cell-division in development is primarily a process of localization. This view is a fundamental part of the doctrine of formative stuffs and is held as such by Wilson, Conklin and others. It may of course be variously elaborated, according to whether it is held, for instance, that cell-

walls are impermeable partitions, as Conklin believes ('05, p. 163), or may permit passage of materials through (*e. g.*, by perforation) as Vejdovsky appears to have observed. The meaning that is given to the term "cell-division" would be important in elaborating the theory; for myself I had meant to include in it only the processes of division of the chromosomes, separation of daughter nuclei and the origin of the cell-wall between the daughter nuclei; movements of the substances within the cell, for instance, would be excluded. It seems to me, however, that the elaboration of a theory concerning the rôle of cell-division in development has to meet with considerable difficulties. I would agree, in general with Conklin, that the localization pattern is something more fundamental and constant than the cleavage pattern; yet the fixity of the latter in all ova of the determinate type of cleavage is a marvelous thing, and cannot be fully explained by any mosaic principle (see General Discussion, p. 254).

V. GENERAL DISCUSSION

Embryological and cytological study has advanced beyond the stage of opinion represented by current theories of development. It is generally agreed that the complex phenomena cannot be reduced to the operation of any single factor, and many possible factors have already been more or less thoroughly discussed, but there is no agreement as to the relative importance of the various accepted factors, nor as to their relations to one another in the various embryonic processes.

1. *Relation of Nucleus and Cytoplasm*

There can be no doubt that the fundamental morphological composition of the protoplasm of the egg of Chætopterus corresponds to the terms of a granule theory and not to those of a filar, reticular or emulsion theory. That a threadlike, reticular or alveolar formation may arise in such protoplasm is unquestionable, but such conditions are secondary ones, due to particular modes of aggregation of the elementary constituents. It is not necessary in adopting a granule theory to go as far as deVries and Altmann

and assume that the granules (pangens of deVries) are the only living elements in the cell, like the bacteria in a zoögloea. The microsomes appear to be the primary living elements of the cytoplasm; I would not venture to assert that they are the only living elements.[1]

Similarly there can be little doubt that the larger granules, the spherules, are products of the microsomes. The origin of some of these from aggregations or growth of microsome-like bodies could be plainly traced by intra vitam staining; moreover, the spherules follow the microsomes in time of origin in the ovogenesis.

Again there can be no doubt that a very large proportion, at least, of the microsomes are of nuclear origin. A great many occur in the residual substance of the germinal vesicle, and, in the history of the uninucleated unsegmented eggs, swarms of microsomes can be seen to proceed from disintegrating chromosomes just prior to the period of differentiation.

Thus my conclusions correspond to certain of the terms of deVries' theory of intracellular pangenesis (1889), provided that we identify the microsomes with deVries' pangens. This theory involves the following assumptions:

1 That the entire living protoplasm is composed of pangens, which constitute the only living element in it. The pangens are invisibly small.

Now the microsomes are not invisibly small, and I regard them only as the primary living elements; not as the sole living elements.

2. There are as many different kinds of pangens as there are independently variable characters, or independent "factors" composing the complex of the characters of the species.

The only cytological evidences that the microsomes are of different kinds are (*a*) that they produce different kinds of spherules, and (*b*) that they proceed from nine different sources in Chætopterus, viz: the nine chromosomes of the egg-nucleus.

3. All the various kinds of pangens of a species occur in the nucleus, and those existing in the cytoplasm come from the nucleus.

---

[1] Indeed a large part of the discussion as to what elements of the cell are living and what are not living seems to me to be purely academic, and likely to remain so, until we possess much more satisfactory knowledge of the mechanics of the vital processes.

This agrees with my opinion concerning the microsomes of Chætopterus.

4. The cytoplasm contains essentially only those kinds of pangens that enter into activity in it. Thus in each variety of cell the immense majority of the pangens remain inactive in the nucleus, and only those leave the nucleus and enter into activity in the cytoplasm that represent the specific cell characters to be expressed.

On the cytological side there is no evidence to correspond to this idea. On the contrary all the chromosomes appear to be active in each cell.

5. Pangens multiply both in the nucleus and also in the cytoplasm.

This is certainly the case as regards microsomes in the nucleus, and possibly also in the cytoplasm.

The theory of intra-cellular pangenesis has anticipated certain observations that may be made concerning the elementary phenomena of development. But it is defective in two important respects: (1) it assumes a degree of original diversity, and certain modes of behavior of the pangens (such as the inactivity of the vast majority in each species of cell) that find no justification in our cytological knowledge, but only in alleged theoretical necessities; (2) it provides no explanation of an essential part of embryonic development, viz: the spatial arrangement of organs and their sequence in time, in short, the unity of the organism by which alone is the possibility of self-sustenance guaranteed. Weismann's theory of germ-plasm proceeds yet farther in unwarranted assumptions as to the original complexity of the germ-plasm, but includes an explanation of the spatial arrangement of organs and their sequence in time, by providing for these factors in the architecture of the germ-plasm.

2. *The Original Diversity of Organization*

All theories postulate a certain original diversity or complexity of organization as the starting point in embryonic development. Now we must inquire what we mean by *original?* For some the fertilized ovum constitutes the starting point; but it is clear, on consideration, that it is relatively far removed from the actual

origin.   Only that can be *original* which is continuous through the series of generations, viz: the germ-plasm in Weismann's terminology, which we identify with the chromosome group.   The entire history of the ovogenesis, as Wilson has repeatedly pointed out, forms part of the embryonic development.   The original diversity of organization is, therefore, contained in the specific chromosome group, which observation has shown to be transmitted from generation to generation.

The next question is as to the degree of original diversity within this chromosome complex.   I would maintain that the estimation of this should rest primarily on the observable facts and not on a mental projection to the lower plane of germ-plasm of the complexity of the higher plane of the adult organization.   The visible diversity of the chromosome complex is usually only quantitative, that is, it consists of a definite number of parts, the individual chromosomes.   In some cases qualitative differences are also observable, for the individual chromosomes may differ in size and behavior (Montgomery '01, Sutton '02, Wilson '05); Boveri ('02) has shown also for the echinids, by some ingenious experiments that, though all the chromosomes are alike morphologically, the individual chromosomes are probably non-equivalent physiologically.   There is, therefore, good reason for believing that there are at least as many different kinds of original substances in the germ-plasm as there are chromosomes.   Considerations as to valency of chromosomes may, perhaps in the future, tend to equate differences in the number of chromosomes in different species; recent observations of McClung ('05) have furnished some arguments along this line.   To maintain that there are no more original germinal substances than there are actual unit chromosomes may, perhaps, be too extreme a position; but it seems to me sounder by far and likely to prove more fruitful as a working hypothesis than the assumption that the germ-plasm is a microcosm of "determinants" of all the characters of the species.

I do not believe that any considerations as to the potencies of the germ-plasm are valid as arguments for the amount of the original diversity, because, if the validity of such arguments be

recognized, there remains no standard but the arbitrary judgment of the individual. Apart from the *a priori* difficulty of accounting for the phenomena of heredity there is no reason for assuming the existence of a large number of original germinal qualities. But, seeing that any species is as distinct from other species in the stage of germ-plasm as in the adult condition, the original germinal qualities, whatever they may be, must bear the stamp of the species.

At present we have no accurate means of estimating the degree or nature of the differences between the chromosomes, but I believe that certain statements may be made about them that follow logically from our present knowledge. In the first place, the differences cannot correspond to the differences between organs or regions, either of the embryo or adult, because the doctrine of the individuality of the chromosomes teaches that each cell receives a descendent of each chromosome. The whole economy of nature forbids us to believe that each cell possesses arm, leg, brain, liver, lung, etc., chromosomes, of which only one class enters into activity in any given tissue, the remainder lying idle. *The fact of the uniform distribution of all chromosomes to all tissues proves conclusively, either that all chromosomes are alike, or that each represents some character of the entire organism. As we have accepted the view that they are originally unlike, we must adopt the second alternative.*

The only observations that we have connecting a particular chromosome with a particular set of characters are those of McClung ('02) and Wilson ('05), according to which the accessory or idiochromosome is a sex-determinant. Now sex is preëminently a character of the entire organism. My hypothesis is, that each chromosome represents some such character or group of characters. It is difficult to imagine what such characters may be; we need a new morphology for their enumeration, and it is to be hoped that this will come from the breeders' experiments; for the only clue that we have to the relation between chromosome-characters and species-characters, consists in the parallelism between the reduction-phenomena in the germ-cells and the Mendelian ratios in inheritance. We might hope, therefore, to get at the nature of chromosome-characters by an enumeration

of the various kinds of characters that are inherited in Mendelian proportions. To attempt this in detail would be too great a task, but they include such characters as color (*e. g.*, inheritance of albinism, or green and yellow endosperm of peas, etc.), stature, pubescence, etc., that are not special characters of particular organs but of the whole organism.

We must be careful, however, to avoid a pitfall here in assuming that there is any *resemblance* between species-characters and chromosome-characters. There is at most only *correspondence* due to genetic connection; and any imaginable degree of knowledge of the unit species-characters would not furnsh a particle of information as to the nature of the original unit germinal characters.

3. *Properties of the Whole (Principle of Unity)*

If the first step in any theory of embryonic development must be certain postulates concerning the original diversity of organization, the second step must be an explanation of the physiological unity of the organism in all stages of its development; for the two main facts concerning any organism are that its parts are diverse, and that it is, nevertheless, a physiological unit. The traditional view, held by many embryologists at the present day, is that the physiological unity arises in the course of embryonic development by the secondary adaptation of originally independent parts to one another. But this explanation has, in my opinion, become untenable, and must be replaced by the view that *there are certain properties of the whole, constituting a principle of unity of organization, that are part of the original inheritance, and thus continuous through the cycles of the generations, and do not arise anew in each* (compare Lillie, '01, p. 275). Weismann places this principle of unity of organization in the architecture of the germ-plasm, but, as I cannot accept his view of vast complexity of the germ-plasm, neither can I accept this principle in the sense of Weismann. My own views agree most nearly with those expressed by Whitman in his paper on the Inadequacy of the Cellular Theory of Development ('93). In this paper

Whitman uses the term organization to express what I have termed above, *properties of the whole or principle of unity*.

If any radical conclusion from the immense amount of investigation of the elementary phenomena of development be justified, this is: that the cells are subordinate to the organism, which produces them, and makes them large or small, of a slow or rapid rate of division, causes them to divide, now in this direction, now in that, and in all respects so disposes them that the latent being comes to full expression.   We see this in the adaptiveness of the process of cleavage of the ovum (Lillie '95, '99; Conklin '96–'97; Meisenhemier '99), in the regeneration of a starving planarian constantly suffering a diminution in the number of its cells while its structure is increasing in complexity (Lillie '00, Schultz '04), in "regulation," and in all cases of morphallaxis (Morgan '00), whether in a protozoan or a metazoan.   The organism is primary, not secondary; it is an individual, not by virtue of the coöperation of countless lesser individualities, but an individual that produces these lesser individualities on which its full expression depends· The persistence of organization is a primary law of embryonic development.

I believe that this conclusion is strongly reinforced by my observations on differentiation without cleavage; for here we see the various substances of the ovum marshalled in order, disposed in a bilateral arrangement and fashioned in the form of a larva; and we see the cilia and other cell-constituents arise in the appropriate locations—and all this without the need of even a single nuclear division.   The question arises whether these phenomena could not be explained by assuming appropriate attractions and repulsions between the elements of the different classes of substances, the spherules and microsomes.   Although such attractions and repulsions undoubtedly exist, and although they appear to me to constitute an important elementary morphogenic factor, yet I find the assumption inadequate to explain the orderly arrangement of the processes collectively.   It seemed at first that the polarity of the ovum might be explained by assuming that the arrangement of endoplasmic substances was produced by mutual attractions and repulsions; but it was found that no alteration of

the arrangement of the endoplasmic substances modified the direction of polarity. Similarly the bilateral polarization of the first cleavage spindle and many aspects of the later cleavages appear to be independent of any chance arrangement of endoplasmic spherules.

Thus there is an apparent inversion in the sequence of embryonic phenomena, by virtue of which those characters that we would expect to appear late, such as the general form and proportions of the embryo, manifest themselves first, and thus lend to the subsequent phenomena an adaptive aspect; as though that which was to be explained preceded the phenomena that could alone account for it. *It is obvious, however, that the adaptiveness of development does not constitute an explanation, but is, on the contrary, itself one of the chief phenomena to be explained.*

The principle of unity transcends all forms of visible diversity hitherto observed; it is a property of the whole distinct from the discernible properties of the parts. Undoubtedly it is capable of further analysis, and it must ultimately be derived from particular relations and properties of material particles. In embryonic development it reveals itself first by axial polarization, second by bilateral polarization and determination of the localization pattern, third by adaptation in cleavage, etc. Analysis of some of these phenomena may some day give a clue to this most mysterious of embryological phenomena.

Morgan ('04) has attempted an "Analysis of the Phenomena of Organic 'Polarity,'" based on the phenomena of regeneration. He concludes that "by means of three assumptions—of totipotence, of heterotropy and of organization-power—we can explain the main features in the result. Each assumption is, moreover, a direct deduction from an experiment or observation." In a footnote he adds, that "the same explanation applies to the development of the egg." By "heterotropy" he means that "the material," though totipotent "is somewhat different at every level, and that this difference corresponds *in kind* to the character of the body at each level." The "organization power," as I understand it, is the same as the "centripetal influence" which, "acting from the surface inward, determines the organization of

the new parts. The action of this centripetal influence is on the new parts as a whole, and determines the relative location of each organ." Later on he suggests that "for want of a better . term, we may provisionally call the property of living material to assume a specific form, the property of *formative organization.*"

It is clear that Morgan here offers an analysis of much more than is generally included under the term polarity; indeed he offers an analysis of regeneration and embryonic development as a whole. It is interesting to note that he defines the factor of "formative organization" so as to be similar to what Whitman calls organization simply, and what I have called influence of the whole. It would be essentially the same idea, if it included heterotropy also, and the latter appears to me to be subordinate to the former principle.

4. *The Mosaic Theory of Development*

The mosaic theory of development of Roux, excepting that inessential part concerning qualitative nuclear analysis, has been strongly supported by the facts of cell-lineage and the recent work of Boveri ('01a), Fischel ('03), Wilson ('04a and b), Conklin ('05a and b), Zeleny ('05), Yatsu ('04) and others. Wilson, Conklin and Fischel especially have shown conclusively that in mollusca, ascidians and ctenophores, the mosaic character of development is based on the principle of germinal localization originally enunciated by His. The work of these authors is too recent and well known to need review here; I only wish to say that I fully accept the results, and am prepared to abide by the theoretical necessities resulting from the assumption that the cleavage mosaic in Chætopterus is as definite a mosaic of potencies as it is in Patella, where each cell of the cleavage-mosaic up to the thirty-two celled stage, or later, differentiates after isolation in substantially the same manner as when forming part of the whole.

But Wilson ('04b) thinks that this result is inconsistent with the conclusion stated by me in 1901, "that the entire organism in every stage of its development exercises a formative influence on all of its parts," although he does not doubt "that this position,

with proper qualifications, is well grounded." He then goes on
to say that "it is clear that the primary localization of formative
stuffs in the unsegmented egg is essentially an act of the "organism
as a whole;" and even though a complete preformation and pre-
localization of specific stuffs for every cell and tissue were assumed
—and I believe with Boveri and Fischel that such an assumption is
not necessary or even probable—we should not escape the neces-
sity for assuming such action of the whole." But in the same
paragraph, while assenting to Whitman's saying that "organiza-
tion precedes cell-formation and regulates it," he takes issue with
him on the ground "that the cytoplasmic aggregation or "organ-
ization" is a progressive or epigenetic process."

As I understand Whitman, "organization" is what I have
called "action of the organism as a whole;" Wilson has either
understood the matter differently, or has forgotten this, when he
identifies "cytoplasmic segregation" and "organization." Organ-
ization, or action of the organism as a whole, is something
that precedes and regulates cytoplasmic localization as much as
it does cell-formation. If this fact were clearly kept in mind I
think that Wilson would find less difference between Whitman's
views (and mine) and his own, than he suspects; for the same
mistaken identification of organization with cytoplasmic localiza-
tion reappears in the succeeding remarks.

But I do not mean to assert that my views are identical with
those of Wilson even when this allowance is made, for he seems
to believe that the action of the organism as a whole ceases when
once the localization pattern is determined, and that thereafter
it is a question of self-differentiation of independently developing
parts with a certain amount of correlative interaction[1] of cells.

[1] It seems necessary to make a special statement of the opinion that I hold concerning the distinc-
tion between "action of the organism as a whole" and the principle of correlative differentiation. By
the former I mean, to use the words of my paper on the organization of the egg of Unio, "that the
entire organism in every stage of development exercises a formative influence on all of its parts."
The principle of correlative differentiation, as I understand it, involves all actions of the *intraorganic
environment*, as I expressed it in my "Experimental Studies on the Development of the Organ in
the Embryo of the Fowl" (1903), that is, "that the rate, degree or mode of differentiation of any
embryonic rudiment is dependent on some part or parts of the same organism external to itself." It
will be seen that, as thus conceived, there is an important difference between the two principles, and
that the principle of correlative differentiation would not include the principle of action of the
organism as a whole without a considerable extension of its usual meaning, which seems to me
undesirable and likely to be confusing.

It appears to me on the contrary, *that the action of the organism as a whole is a continuous process; that physiological unity exists in every stage, and not merely sporadically; no matter to what extent the mosaic principle may apply.* We have to deal with diversity in unity, and unity in the midst of diversity as the two fundamental properties of organisms. The phenomena of regeneration and regulation are incomprehensible on the basis of a *pure* mosaic theory of development. Wilson does not fail to recognize this principle here and there, as in cases of regulation, but it seems to me that he has not given it sufficient weight, and has not kept clearly in mind the distinction between this principle and the derivative condition of germinal localization.

I would like also to give what seems to me the explanation of Wilson's conclusion that "cytoplasmic segregation is a progressive or epigenetic process," based on the results of Boveri, Yatsu, Zeleny and himself. In general it may be said that these authors have found that there is a progressive limitation in the potencies of parts of the egg from the time of the rupture of the germinal vesicle to the eight-celled stage or beyond. Now in Chætopterus there is a visible segregation of substances *already present*, described in Part III, 2, beginning with the breaking of the germinal vesicle, and continuing to the third cleavage at least, by means of which a new germinal topography is produced. Assuming that these substances have limited potencies, as has been demonstrated for similar substances in other eggs, the redistribution of them would inevitably bring about such a result as these authors have described, because the new topography is much more precise than the original one. The rearrangement is an "epigenetic process," if you please; but only the topography is new, not the substances. There is a great deal of evidence in the literature that a similar redistribution is characteristic of the maturation period in most phyla. On the other hand I believe that a true epigenetic process begins with the first cleavage in the production of new substances from the nuclei, and undoubtedly plays a part in the progressive limitation of potency of the blastomeres; but, I believe, only a small part *at first*. (To avoid misunderstanding,

I would state that it is my view that the specific formative stuffs of the later development arise after the process of cleavage has begun.)

Differences in time of origin of formative stuffs no doubt exist in different kinds of eggs, but I do not believe that the differences are so extreme as some would appear to think. Determinateness of cleavage may be a measure of the extent and precision of their localization prior to cleavage, but I think it must be a very *inexact* measure. I fully agree with Whitman that "cell-orientation may enable us to infer organization, but to regard it as a measure of organization is a serious error;" however, one must keep in mind that "organization" precedes and controls "localization," and not confuse the two terms. When this is done I can also agree with Wilson that "a highly differentiated initial cleavage pattern is, therefore, *ipso facto* evidence" (in some degree), "of a high degree of initial cytoplasmic *localization*" (last italics mine).

5. *Concerning Formative Stuffs*

Much has been written of late concerning formative stuffs.[1] (Morphoplasmic stuffs of Wilson, organ-forming substances of various authors.) Unfortunately their actual physical character-istics have been but little examined; indeed, in most cases the so-called organ-forming substances are really germinal areas prob-ably including a variety of substances, and distinguishable only by their localization and by the useful but superficial character of color. As I have attempted to show, the specific character of the substances in Chætopterus is given by the spherules. It is certainly reasonable to expect that formative stuffs in other ova may be differentiated by microchemical methods. Previous observations on this point have been more or less incidental, so that generalization would not be profitable.

According to the theory of formative stuffs developed in the preceding pages, a series of stages characterizes each kind before it reaches its definitive histological condition. First, its origin,

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[1] These "formative stuffs" do not conform to the older conceptions of Sachs, who postulated circulating fluids of formative function. They are supposed to be varieties of protoplasm that probably do not circulate extensively from cell to cell.

in the form of microsomes, from the nucleus; second, the formation of granules of a different order from the microsomes, the spherules; third, the addition of new nuclear derivatives. (The third stage is necessary for there is no final stage of differentiation in non-nucleated parts; in the uninucleated unsegmented eggs the third stage is clearly marked morphologically by the diffusion of chromatin particles among the spherules.) Fourth, definitive histogenesis (*the morphogenic reaction*).

The theory of formative stuffs does away with any "determinant" hypothesis. "Characters" are not due to "unfolding" of the "potencies" of "determinants" but are results of morphogenic reactions between two or more formative stuffs. The "character" need no more be preformed in the reagents (formative stuffs) in the case of a morphogenic than in the case of a chemical reaction. But I do not mean to imply that the morphogenic reaction is a simple chemical reaction, nor that, after it has taken place, the character need appear at once in its definitive form. The morphogenic reaction is probably often of the nature of a response to a stimulus, a phenomenon of irritability, and the definitive "character" resulting may come to expression slowly.

6. *Nuclear Specification*

The logical consequences of the preceding conclusions cannot be avoided: it is clear that the nuclei cannot produce the same kinds of formative stuffs in successive stages of ontogenesis, unless we assume that the organism as a whole manufactures different things out of the same substances at different times and in different places. This appears to be so improbable an assumption that there is no escape from the conclusion that *the nuclei undergo progressive differentiation;* in other words, that each successive ontogenetic stage is preceded by a corresponding nuclear phase.

The possible modes of nuclear specification are: (1) that different chromosomes represent successive ontogenetic phases, and that, therefore, in any given phase the majority are inactive. The objections to this view appear to me to be fatal; for, in the first place, there is not a sufficient number of chromosomes to satisfy

the conditions of the hypothesis; and in the second place, there are grave objections to the hypothesis of inactive chromosomes in any stage of development. This is, however, substantially the view that Wilson presents ('05, p. 292). (2) The second possibility would be that all chromosomes are active in the various stages of ontogenesis, but that only a part of each is active at any given stage. In other words, that each chromosome contains the determinants of all stages, as Weismann supposes, and that these enter into activity successively. This would, however, presuppose an enormous amount of original diversity, an idea that we have already specifically rejected, besides being open to the general objection that the major part of each nucleus would necessarily be supposed to be inactive. (3) Both the foregoing are essentially preformation hypotheses. The third possibility is that the postulated progressive development of nuclei is essentially an epigenetic process.

It appears to me that all the well-established physiological and embryological data point toward this conclusion. Yet, from Nägeli down, nearly every writer on the subject of heredity postulates a degree of original preformation in the idioplasm or nucleus, corresponding to the amount of morphogenic activity supposed to be exercised by it in all stages of the life history. According to Nägeli "every perceptible character is represented in the idioplasm by a rudiment" (Abstammungslehre, 1884, p. 23). According to deVries it is necessary to assume original specific pangens for every heritable property (Intracellular Pangenesis, 1889). Weismann postulates a determinant for every independently varying part. The embryologists have usually not entered into this question; but Wilson has gone so far as to state ('05a) "that the germ consists of two elements, one of which undergoes a development that is essentially epigenetic, while the other represents an original controlling and determining element. The first is represented by the protoplasm of the egg. The second is the nucleus, which, as I have attempted to show, must apparently be conceived as a kind of microcosm or original preformation, consisting of elements which correspond, each for each, to particular parts or characters of the future organism."

According to these writers, therefore, all the characters that are ever to be impressed by the nucleus on the cytoplasm are represented by original preformations in the nucleus. Such a conclusion appears to me to be practically a negation of the evidences of our senses. If such a degree of original diversity is really preformed in the chromosome-complex, it is inconceivable that it should not reveal itself to some one of our senses by variety of behavior or reaction. Moreover, there is not room in the known laws of chemical combination for such diversity of substances within the chromatin of a species as these preformation hypotheses require. It seems to me that all *a priori* considerations should be ruled out of court, unless we are willing to transform biology into a branch of metaphysics dealing with potencies and latencies.

If nuclear specification is to be considered an epigenetic process, the causes thereof may be conceived either to be in the environment of the nucleus, viz: the body of the cell, or to lie within the nucleus itself. In the former case we should have to assume that the locations of nuclei in different parts of the original germinal topography may act as stimuli on the nuclei to differentiate them in various directions. The original cause of cytoplasmic diversity has been traced back to the nucleus, but I do not think that it is necessarily illogical to reverse the order and assume that the cytoplasmic diversity may be a cause of new nuclear diversity. On the contrary it is a widespread biological phenomenon that secretions of the organism react on the organism itself in various ways.

On the other hand I cannot avoid the conclusion that the progressive development of nuclei is to a great extent a process of self-differentiation. There are certain unavoidable corollaries of the argument bearing on the question. If descendants of each original chromosome are transmitted to each cell of the organism, and if each chromosome, therefore, represents some character of the entire organism, as we concluded before, it must follow that each has a series of forms of expression suitable to the successive ontogenetic stages. Thus, if chromosome *x*, for instance, expresses itself by pigmentation in the adult condition, it must have had

some different form of expression in stages prior to the appearance of pigment.   There is, in other words, a correspondence between the mode of expression of a chromosome, and the ontogenetic stage reached by the entire organism.   *There must, therefore, be a progressive evolution of the chromosomes of the same general character in all cells; but this need not exclude local specialization of the nuclei also.*

The differentiation of any particular cell would therefore be the result of an interaction between a specific formative stuff or stuffs, inherited from previous generations of cells, and a new material derived from the nucleus.   Variation in either factor would give a different result.   Thus, for instance, all cells of the embryonic epidermis might be supposed to contain similar stuffs originally; their special lines of differentiation would then be dependent on the nature of the final nuclear stuff.   If, now, we suppose that this may vary with the different external conditions, the line of differentiation would vary with the latter.

To take a specific instance, we may explain Lewis' ('04) interesting discovery that a lens may arise in tadpoles from any part of the embryonic epidermis that is brought into suitable relations to the optic vesicle, by the hypothesis that the stimulus of the optic vesicle causes a different kind of nuclear secretion in the epidermal cells acted on than in others.   In this case the postulated nuclear differentiation would result from the environment. If such a case may be considered typical, we might perhaps generalize by saying that the *progressive evolution* of chromosomes common to all cells is a process of self-differentiation, but that local specifications may result from action of the environment.

According to this conception, therefore, there is an orthogenic and epigenetic progressive development or evolution of the somatic nuclei during the development of the individual, that is common to them all, and in addition local specifications characteristic of particular regions and organs.   The latter are subordinate modifications within the limits set by the ontogenic stage reached in the evolution of the somatic nuclei of the individual.   There would also be involved in the general conception a theory of continuity of the germ-plasm, similar to Weismann's, viz: that the nuclei of the

"Keimbahn" undergo neither evolution nor specification, except such evolution as may be phylogenic in its character.

Without developing the idea any farther in this place I think it will be seen that the view is not inconsistent with the biogenetic law, and that it may be made part of a larger theory of phylogenic development. It seems to me that some such ideas as these result logically from our present cytological knowledge and, indeed they have arisen in my mind in the attempt to interpret current cytological conceptions. They are presented in no dogmatic spirit, but in the hope of stimulating discussion.

7. *Summary of Discussion*

The main points of the discussion may be summarized thus:

(1) The chromosome group of the species contains the total sum of the material transmitted from one generation to another.

(2) The microsomes arise from the chromosomes and constitute the primary cytoplasmic element. They produce the various formative stuffs.

(3) The original diversity, by which I mean the actual degree of heterogeneity of the chromosome group, is probably relatively slight.

(4) There is an original principle of unity, action of the organism as a whole, which expresses itself by axial and bilateral polarization (thus determining the segregation pattern) by adaptation in cleavage, and probably in various other ways, and which is continuous from generation to generation. Only its mode of expression changes and this in accordance with the stage of development of the organism. The unity of the organism does not arise by the secondary process of division of labor.

(5) Apart from the postulated original diversity and the action of the organism as a whole, the entire development is epigenetic.

(6) Each chromosome probably represents in each stage some property of the entire organism.

(7) Each ontogenic stage is preceded by a corresponding nuclear phase; in other words, nuclear evolution is the primary factor in the determination of embryonic stages.

(8) Nuclei probably also undergo local specification as a result of varying intraorganic environment, and possibly also through action of the organism as a whole.

(9) By virtue of the two modes of nuclear evolution and specification, different kinds of formative stuffs arise at successive phases of the ontogenesis and in different parts of the embryo.

(10) The final histogenesis of any cell depends upon interaction of the formative stuffs already present in the cytoplasm with the last formative stuff derived from the nucleus.

(11) The nuclei of the "Keimbahn" undergo neither evolution nor specification except such as may be of a phylogenic character.

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DESCRIPTION OF PHOTOGRAPHS.

The author is greatly indebted to Misses Catharine Foot and E. C. Strobell for the photographs which were taken and printed by them.

All the photographs are from living eggs. The magnification in all is 237 diameters.

All the eggs shown were centrifuged, about 3000 revolutions in one minute, and (with the exception of A and H) then fertilized. *A* to *I* inclusive, show the early stages of such eggs up to the first cleavage, one and one-quarter hours. J, K, L and M were taken from six and one-quarter to seven hours from the time of fertilization.

The first group illustrates the stratification of the egg substances produced by the centrifuge and its relation to polarity, the axis of the egg standing vertical in each case (except in Photograph A, where the axis is not known). The large, dense area seen in each photograph is the massed spherules of the endoplasm, the smaller is the "gray cap" or residual substance of the germinal vesicle. The clear band is seen between them. The photographs show the ectoplasmic layer very distinctly, especially, by contrast, just external to the endoplasmic mass.

Except for the outlines of the polar bodies in B, D, F, G and I, the photographs have been reproduced without retouching.

A. Living egg, unfertilized, shortly after centrifuging. The polar area cannot be seen, hence the relation of the strata to the polarity is not known.

B and C. Two views of one egg with two polar bodies. Centrifuged 8.45 A. M. Fertilized 9.17 A. M. Photographed forty-eight minutes after fertilization. C is a high focus to show the gray cap, B a lower focus to show the polar bodies. The plane of stratification is nearly at right angles to the axis of the egg.

D. Centrifuged 8.45 A. M. Fertilized 9.17 A. M. Photographed 10.13 A. M. There are two polar bodies. The plane of stratification is inclined about 90° to the axis of the egg.

E. History same as D. Photographed 10.20 A. M. There are four polar bodies, a condition observed only once. Plane of stratification inclined about 45° to the axis of the egg.

F. History similar to D. There are two polar bodies. The polar lobe is beginning to form. Plane of stratification inclined about 120° to the axis of the egg.

G. Same egg as shown in F. Photographed five minutes later.

H. Centrifuged 9.08 A. M. Unfertilized. Photographed 10.45 A. M. The gray cap surrounds the maturation spindle and is therefore ring-shaped. During the time that has elapsed since centrifuging the gray cap has spread out considerably.

I. History similar to D. Two-celled stage nearly complete. Polar lobe is nearly at its height. Its substance contrasts strongly with the endoplasm. Most of the gray cap is in the smaller cell and the larger is filled almost entirely with endoplasm.

J. Centrifuged 9.08 A. M. Fertilized immediately. Photographed 3.30 P. M., six hours and twenty minutes after fertilization. This is a uninucleated unsegmented egg showing the large clear nucleus and the chromatin halo surrounding it; distorted and flattened by pressure.

K. History same as J. Photographed 4.10 P. M., seven hours after fertilization. The chromatin halo has risen to the surface where it is mingling with the ectoplasm. Egg distorted by pressure.

L. History same as J. Photographed 3.52 P. M., six hours and forty minutes after fertilization. Egg is flattened by pressure. Three-banded condition. The shadow of the ectoplasmic band may be seen on the surface of the nucleus.

M. History same as J. Photographed 3.45 P. M., six hours and thirty-five minutes after fertilization. Three eggs are shown in one field of the microscope, the one to the left is segmented (abnormal) the central one is multinucleated unsegmented; the egg to the right is uninucleated unsegmented. Shown for comparison of the three main conditions found in such cultures.
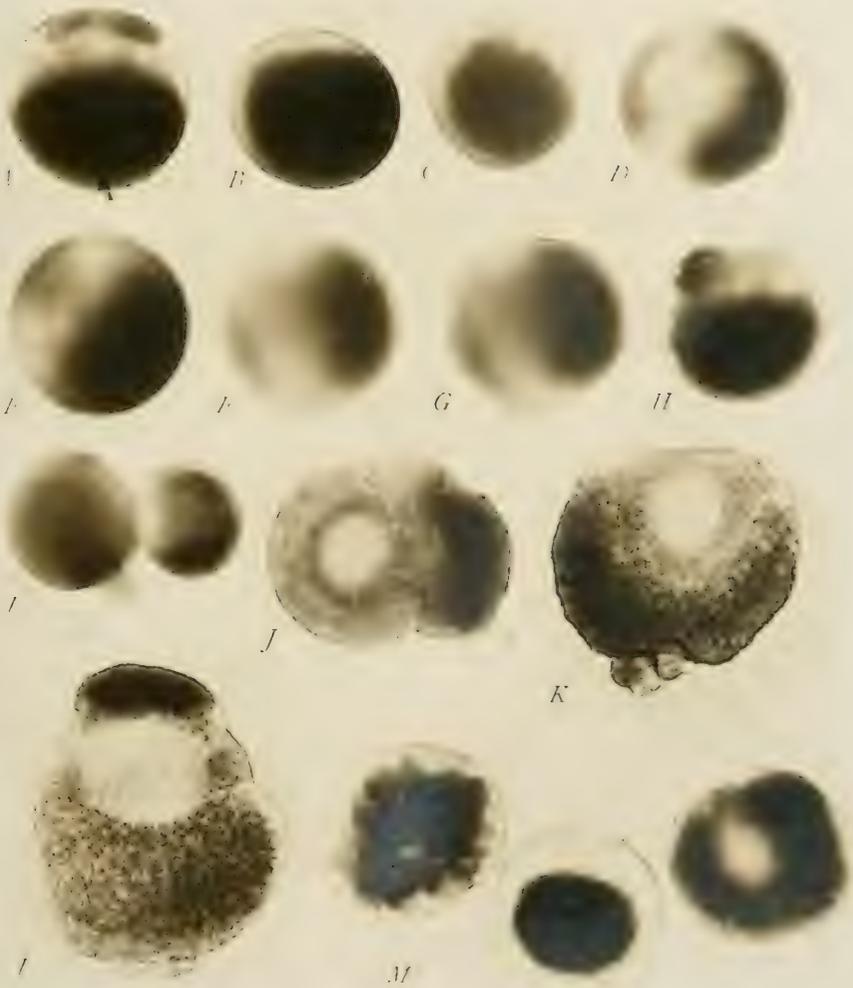
REGENERATION OF GRAFTED PIECES OF PLANARIANS

BY

LILIAN V. MORGAN

WITH SEVENTEEN FIGURES

Experiments in grafting pieces of Planarians were undertaken in order to discover whether regeneration of short pieces grafted on to longer pieces would be influenced by the polarization of the longer piece. It has been found, in Hydra by Peebles[1] and by King,[2] and possibly in Tubularia by Peebles, that long pieces have such an influence on short pieces.

After repeated attempts in various ways to graft two pieces, it was found that in some species of flatworms, a fair proportion of pieces would grow together if held in place between wet pieces of paper. Thick tissue paper was used, tough enough to stand the necessary manipulation, and thin enough to be almost instantly soaked in water, and when wet to cling closely to the pieces of worm. A small piece of wet paper is first laid on a flat surface of paraffin (hardened on the under side of a small dish). As it is very important that the pieces to be grafted should be brought together immediately after they are cut, a second piece of wet paper is held in readiness, and the whole worms are placed with a camel's hair brush on the first paper. The worms are then cut at the level required, and the pieces are turned in the desired relation to one another, and quickly covered by the second piece of paper. If the pieces have not stayed in exactly the right position, they can be shifted to a certain extent by pushing with a dull

[1]Peebles, F., '00. Experiments in Regeneration and in Grafting of Hydrozoa. Archiv f. Entwickelungsmech., Bd. x.

[2]King, H. D., '01. Observations and Experiments on Regeneration in Hydra viridis. Archiv f. Entwickelungsmech., Bd. xiii.

knife on the upper piece of paper. For a moment they will remain in place, but in order to keep them quiet for the length of time necessary for them to grow together, they are held at the sides by the edges of pieces of cover-slip pressed down on the outside paper close to the worms. Two or three fine needles stuck through the paper into the paraffin at the ends of the worms keep them from moving apart. The small dishes are placed in a moist chamber—a large tightly-covered dish with a little water on the bottom—and kept in the dark. It is all important that the grafting pieces should be neither too wet nor too dry. They should be transferred to the paraffin plate with very little water, and the surplus drops should be drained off the pieces of paper after they are soaked. If the worms are too wet they do not stick, and if they are too dry they disintegrate. At the time that the worms are cut, the tips of both tails are cut off in order that the pieces shall be as quiet as possible. The grafting pieces are left in position from eighteen to twenty-four hours. At the end of that time, after the cover-slips and needles have been removed, a little water is added to float the upper paper, which is then very carefully taken off. Since it is difficult to handle very small pieces, the worms were cut anteriorly at the desired levels, and grafted. Only after the pieces had grown together was the one which was to be the short piece in regeneration cut off near the union to make it the required length.

The method was first worked out with Planaria maculata, collected at Van Courtland, N. Y., and at Cold Spring Harbor, L. I. They are apparently the same species but the two forms have some constant and marked differences. Only ten grafts of these worms were successful. The main part of the work was done with Phagocata gracilis from a fresh water pond at Falmouth, Massachusetts.

A. REVERSED GRAFTS PERFECTLY UNITED

The anterior cut surface of a short piece was grafted to the anterior cut surface of a longer piece in order to see whether a reversed head or a normal tail would regenerate at the exposed posterior cut surface of the short piece.

The worms were cut at three different levels: (1) At an anterior level a short distance behind the eyes near the region of the head; (2) at a middle level through the pharyngeal chamber; (3) at a posterior level behind the pharyngeal chamber through the region of the tail. All possible combinations (except two) of these levels were made. In each case, the combinations are designated by the initials of the regions through which the cuts were made, viz: head, middle, and tail regions; the capital letter stands for the longer piece, the small letter for the shorter piece. Thus, "Mh" indicates that the combination is made up of two worms, one cut through the pharyngeal chamber in the middle region of the worm, the other cut behind the eyes through the head region; the two pieces were joined by their anterior cut-surfaces and when grown together, the second worm was cut off posteriorly, leaving only a short piece (h) grafted to a comparatively much longer piece (M) of the other worm. The results of the experiments are noted in the following table:

TABLE I

Regeneration of short pieces of Phagocata gracilis grafted in a reverse direction on to longer pieces

	The Short Component Produced at its Free Posterior Surface			Other Results	Died
	Head	Tail	Closed Knob		
Hh	8	1	5	6	4
Hm	—	10	2	2	—
(Ht)	—	—	—	—	—
Mh	2	—	—	—	—
Mm	2	2	10	1	—
Mt	—	3	8	1	1
(Hh)	—	—	—	—	—
Tm	4	—	—	—	—
Tt	2	1	8	—	—
	18	17	33	10	5
Hh (h long)	—	5	—	1	—

The last column of the table may be left out of account; it simply records the cases which died after being grafted, before they showed any results one way or the other. The next to the last column will be ignored for the present. The first three columns give the cases where the graft was good, the cut surfaces were squarely attached, and a certain proportion of the short pieces regenerated.

It is clear that the exposed cut surface of the short piece is a posterior surface, from which would normally regenerate a tail, if the piece were not attached to the other worm. It has been shown in Planaria maculata[1] that a head is formed at both the anterior and posterior surfaces of very small free pieces, but this I believe has not been found to be the case in Phagocata gracilis; even exceedingly small free pieces of this worm regenerate a head at the anterior end, but invariably a tail at the posterior end. If then from the free posterior surface of a short piece a head develops, the probability is that the short piece is in some way influenced by the longer piece.

The table shows that out of sixty-eight cases, thirty-three (nearly half) closed in, regeneration was prevented, and the combination remained headless. Combinations of this sort are always sluggish as compared with normal worms or with worms with regenerated heads, normal in direction or reversed; they have lived, however, for weeks without showing signs of deterioration. Of the thirty-five which regenerated, about half (eighteen) produced heads, and the other half (seventeen) produced tails. This proportion of heads and tails occurred for the various combinations of the three regions of the worms all taken together, but if each kind of combination is considered by itself the proportions are very different.

Hh. Short pieces from the head region, reversed and grafted to the head region, regenerated in nine cases out of fourteen, and of these nine cases eight produced heads (see Fig. 1*A*, 1*B*), and only one a tail. Varying the size of the shorter piece was tried. In five cases, the shorter piece (h) was left about twice as long

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[1]Morgan, T. H., '04. The Control of Heteromorphosis in Planaria maculata. Archiv f. Entwickelungsmech., Bd. vii.

as the short pieces in all the cases recorded in the first line of the table, but yet much shorter than the long piece (H). In all these cases, a tail and not a head regenerated.

Hm. Ten out of twelve pieces from the middle region, reversed and grafted to the head region, regenerated, but all ten without one exception produced tails.

Mh. Two short, reversed pieces from the head region grafted to the middle region both regenerated and produced heads.

Mm. Middle region on middle region regenerated in only four cases out of fourteen, and of these, half (two) produced heads, and half (two) tails.

Mt. No heads were produced and only three out of eleven pieces regenerated and produced tails.

Tm. Four cases regenerated, and all produced heads.

Tt. Regeneration in only three out of eleven cases; two of the three produced heads, and one a tail.

Of the ten grafts of Planaria, one (an Mh) made a head, probably from the posterior surface of h, but the history was not closely enough followed, to be certain of the origin of the head; another graft (Hh), made a tail at first, but when the new tail had been cut off, near the line of union with the longer piece, a head developed.

It appears from the data for Phagocata that the reversed head region readily produces a head; it occurred in all the cases, but one, when it was grafted to a head region (if the piece h was very short), and in both cases that were tried on the middle region. On account of this uniformity, it did not seem necessary to try Th.

The results from the reversed middle region are much more variable. In the first place, a much larger proportion of these grafts, than of grafts of the head region, did not regenerate at all. Of those that did regenerate, all ten grafted onto the head region produced tails, half of those grafted onto the middle region produced heads and half tails, but all four on the tail region produced heads.

The reversed tail region in far greater proportion than the head or the middle region closed and did not regenerate, but the experiments show that even the tail region, if it does regenerate, may

produce a reversed head; for although of six regenerated pieces (three grafted to the middle, and three to the tail) four produced tails, yet two of those grafted to the tail produced heads.

In these experiments, there is no regularity as to the kind of regeneration of the reversed grafted pieces; but the results show that using each of the three regions of Phagocata gracilis as stock, with some of the other regions as graft, and each as graft, with some of the others as stock, heads may be produced. But some of the possible combinations of the different regions developed only tails. The head region as graft on different stocks, and the tail region as stock with different grafts produced the largest proportion of heads in these experiments. It is possible that when tails are produced, the smaller piece, although very short compared with the longer, may be long enough to be beyond a limit within which a piece may regenerate a reversed head. On looking over the notes to see whether there were data exact enough to throw any light on the point I found in three cases, where the pieces were measured or their length noted and heads were produced that the small piece was shorter than in two cases where the pieces were measured and produced tails. But another piece (which was longer to begin with) closed in, and although it was afterward cut twice until it was very short, it even then produced a tail. The closing-in, in itself, could not have affected the kind of regeneration, for other pieces which closed-in, and were afterward cut, produced heads; these were not actually measured.

B.   IRREGULAR CASES

Having considered the regeneration of a head or a tail at the exposed posterior surface of squarely grafted and perfectly united pieces, the other sorts of regeneration will be examined. The results recorded in the fourth column of the first table are expanded in the first part of a second table.

There are two main classes of examples:

I.   First, when the original cut surfaces did not graft perfectly across the cut ends, surface to surface, but one, or the other, or each of the cut ends, was not covered completely by the other

TABLE II[1]
Phagocata gracilis (Column 4 of Table I)

	I. Head from Exposed Edge of Anterior Surface of			II. Head at Angle of Graft From			Uncertain
	Long piece	Short	Both	Long piece	Short	Both	
Hh	—	—	—	No. 94 (k)	No. 3 (k) (one eye) No. 10 (k)	No. 1 (k) No. 19 (k) No. 95 (k)	—
H long h	—	—	—	—	—	No. 43 (t) (four eyes)	—
Hm	No. 18 (t) No. 89 (k)	—	—	—	—	—	—
Mm	—	No. 90 (k)	—	—	—	—	—
Mt	—	—	—	—	—	—	No. 86

Planaria Maculata

	Long piece	Short	Both	Long piece	Short	Both	Uncertain
Hh	No. 2 (k) No. 3 (k)	—	No. 7 (t) (two heads)	—	—	—	No. 4
Hm	—	—	—	No. 8 (t)	—	—	—
Ht	No. 13 (k)	—	No. 11 (t) (two heads)	—	—	No. 12 (t) (three eyes)	—

[1]The behavior of the exposed posterior surface of the short component is indicated in the table by the small letters "t" and "k;" (t), when a tail was formed; (k), when the piece closed-in, and formed a knob, which did not regenerate in the posterior direction.

surface, but was left in part free. From such a free surface, a head regenerated, and whatever its origin, whether from the short or from the long piece, it gradually assumed (in the examples whose history was followed) the size and position of a head of the large piece.

In Planaria maculata No. 13 Ht (Fig. 2*A*, *B*,) the small grafted piece was from the beginning but a mere fragment at one edge of

Fig. 1.   Phagocata gracilis, combination Hh.   *A*, One day after grafting.   *B*, Eleven days after grafting.   *C* and *D*, Diagrams of the anterior nervous systems of combinations Hh, fourteen days after grafting, drawn from several sections with the camera lucida.   The worm of Diagram *C* had regenerated a reversed head.   *E*, Diagram of the anterior nervous system of a combination Hh, fourteen days after grafting, where h had closed in.   *a*, Line between the large and small components of the grafted combination.   *b*, Line between old and new tissue.

Fig. 2.   Planaria maculata No. 13 Ht.   *A*, After regeneration of the large component had begun. *B*, Eight days later than *A*.

Fig. 3.   Phagocata gracillis No. 89 Hm.   *A*, One day after grafting.   *B*, Twelve days after grafting.   *a*, Exposed anterior surface of the long component H.   *b*, Exposed posterior surface of the short component m.   *x*, Line to which m had been absorbed forty-one days after grafting.

Fig. 4.   Phagocata gracilis No. 18 Hm.   *A*, Five days after grafting.   *B*, Ten days after grafting. *C*, Forty-three days after grafting.   *x*, Outline of the new head sixteen days after grafting.

Fig. 5.   Phagocata gracilis No. 90 Mm.   *a*, Exposed anterior surface of m.

Fig. 6.   Planaria maculata No. 2 Hh.

Fig. 7.   Planaria maculata No. 7 Hh.   *C*, About sixty-eight days later than *A* and *B*.

Fig. 8.   Phagocata gracilis No. 94 Hh.   *A*, Twenty-two days after grafting.   *B*, Diagram of the nervous system of A.
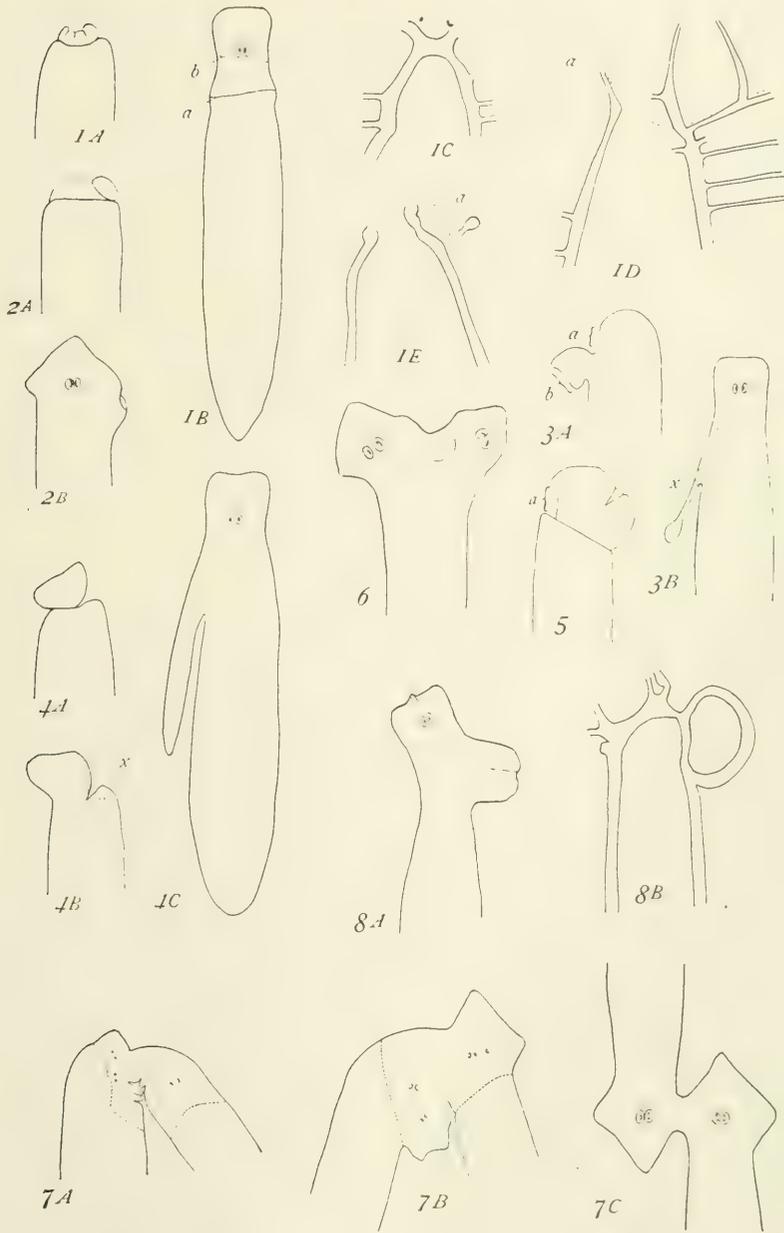
the anterior cut surface of the large piece, and the large piece regenerated freely; the small piece was gradually absorbed, and the combination appeared seventy-one days after grafting like a normal worm.

Phagocata gracilis, No. 89 Hm (Fig. 3*A*, *B*,) was a more interesting case, because the union took place in such a way that part of the anterior cut surface of the long piece was not covered by the small piece and was drawn over to one side. Regeneration took place from the exposed portion, and the new tissue grew much longer on the short side than on the long. A head was thus formed in the normal position of the head of the large piece. The small piece closed in at its exposed posterior surface, and was at first large enough to take part in sticking to the bottom of the dish, but was gradually absorbed, and forty-one days after grafting formed merely a small excrescence on the side of the large worm. It had the same appearance fifty-six days after grafting.

Phagocata No. 18 Hm (Fig. 4*A*, *B*, *C*,) was interesting because of morphallaxis which took place involving both pieces. The small piece when grafted covered about half the anterior surface of the large piece, and was at first pushed before the large piece as it crawled. A narrow head regenerated at the exposed half of the surface of the large piece. The small piece gradually changed its position and turned back at the side of the large piece, developed a tail at its free posterior surface, and crawled with the large piece and in the same direction. The head, in the meantime, became not only as broad as the large piece, from which it originated, but as broad as both components together. The worm died before it became certain whether the smaller tail was going to be absorbed although it seemed to have diminished.

Phagocata No. 90 Mm (Fig. 5) was a case of head growing from the partly exposed anterior surface of the smaller piece. The head assumed the position of the head of the larger piece, and the closed-in posterior part of the small piece formed a slight protrusion from the side of the large worm.

Planarians Nos. 2 and 3 produced heads from part of the exposed anterior surface of the large piece. Their origin was not

followed closely. In one (Fig. 6), there seemed to be two heads from the large piece, the small piece being stuck to the middle of the cut surface between the heads.

In Planaria No. 7 (Fig. 7*A*, *B*, *C*,) the grafted pieces stuck merely by their edges, leaving most of the anterior surfaces of both pieces exposed. Two vigorous worms regenerated, with complete heads and tails, and crawled or attempted to crawl in opposite directions, attached only by the corners of their heads. The eyes at first were very irregular and never became perfectly normal, but were, except one of them, multiple eye-spots in two clusters in each worm. The worms lived for weeks, one of them being sometimes dragged along, while the other, which had a better hold, crawled normally. Usually they crawled side by side, the head of one being doubled up in the angle between the two worms (as seen in Fig. 7*A*, *B*).

Planaria No. 11, Ht, was a two-headed combination, one head and one tail were smaller than the other and seemed to be undergoing absorption. The origin of the regenerated parts was uncertain.

II. The second class of irregular grafts comprises somewhat different phenomena from the first.

In Phagocata No. 94, Hh (Fig. 8*A*), the new head probably came from the large piece, and it may have originated from an exposed edge (as in class I, just considered), but in No. 10 Hh, and No. 3 Hh, the head seems to have originated from the small piece, not from a free surface, but in another way. The union of the two pieces in both cases seemed to be perfect, but the line of union, instead of being straight across the large piece (Figs. 9, 10), was at an acute angle with its long axis. No. 10 died before the head was complete, but a head (Fig. 9) seemed clearly to be developing from the small piece, starting with new tissue that regenerated in the angle between the old tissue of the small piece and the line of union with the large piece, *i. e.*, the head developed from one side of the anterior surface of the small piece, though in contact with the large piece. The head grew in the direction which was nearly anterior in relation to the large piece, and the posterior surface of the small piece closed in, and became a knob

Fig. 9.　Phagocata gracilis No. 10 Hh.　*A*, One day after grafting.　*B*, Ten days after grafting. *C*, Eighteen days after grafting.　*x*, Line where the posterior end of h was cut off a second time.

Fig. 10.　Phagocata gracilis No. 3 Hh.　*A*, One day after grafting.　*B*, Later stage.　*C*, About twenty days after grafting.　*D*, Eighty-three days after grafting.　*E*, Diagram of the anterior nervous system of D.

Fig. 11.　Planaria maculata No. 8 Hm.　*A*, Five days after grafting.　*B*, Seven days after grafting.

Fig. 12.　Phagocata gracilis No. 1 Hh.　*A*, One day after grafting.　*B*, Fifteen days after grafting. *C*, Twenty-nine days after grafting.

Fig. 13.　Phagocata gracilis No. 19 Hh.　*A*, Four days after grafting.　*B*, Twelve days after grafting.　*C*, Forty-seven days after grafting.

Fig. 14.　Phagocata gracilis No. 95 Hh.　*A*, Fourteen days after grafting.　*B*, Diagram of the anterior nervous system of *1 A*.

Fig. 15.　Phagocata gracilis No. 43 H long h.

Fig. 16.　Planaria maculata No. 12 Ht.

Fig. 17.　Phagocata gracilis, dorso-ventral Hh.　*A*, One day after grafting.　*C*, Twenty-nine days after grafting.　*B*, Another specimen, six days after grafting.　*D, E, F*, Diagrams of different positions of a dorso-ventral graft after regeneration.　*I, II*, Worms.　*A, B*, Heads; solid lines represent ventral surfaces; dotted lines, dorsal surfaces.

at the side of the worm. No. 3 (Fig. 10) was somewhat different. The small piece was wedge-shaped, narrowest at the anterior end of the slanting line of union with the large piece. From this narrow part regenerated most of the new tissue from which the head developed; the broad side of the piece lengthened a little at first as though to make a tail, but later closed in and was being absorbed. The head differed from the reversed heads of Table I, in that it did not develop in the middle of the exposed posterior surface, but at the thin edge of one side. At one period a single eye was seen, and also a second pigment spot, which later disappeared, and only one eye remained in the narrow abnormally shaped head.

Planaria No. 8 Hm (Fig. 11*A*, *B*) developed a head at the forward angle of a slanting graft, but its origin was not studied. The small piece made a tail.

In the examples of a single head (next column of Table II) made up in part from both components of the compound worm, the head (where the history was followed) originated from an edge, perhaps slightly exposed of the large piece.

In Phagocata No. 1 Hh (Fig. 12) the point of origin of the head was the anterior edge of a slanting graft. In No. 19 Hh (Fig. 13) new tissue developed from the large piece, especially at the sides of the straight graft. But only one edge grew anteriorly, and the other side of the graft was thrown back. In both No. 19 and No. 1, new tissue from the small piece also regenerated at the line of the graft, and took part in the formation of the head, and one eye came from each component. The posterior edge of the small piece closed. In No. 19, the division between the large and the small piece was always marked by a notch at the forward end of the head; the compound head was always somewhat lateral in position.

The first steps in the regeneration of No. 95 Hh (Fig. 14) were not followed; there resulted from a grafted pair, one worm having an eye from each component, and with a knob from the small piece at the side. A combination of H long h (No. 43, Fig. 15) produced a worm with one head, but with four eyes, and with two tails.

Planaria No. 12 Ht (Fig. 16) produced one head with three eyes, and two tails, and a pharynx developed in the smaller regenerated tail.

Like the perfectly attached unions, the irregular combinations also usually resulted in one way or another in the formation of complete worms derived from parts of two worms. If the point of attachment of the two components is very limited, two nearly complete worms may be regenerated, as Planaria No. 7 (Fig. 7), but usually, even though regeneration of two worms begins, the smaller duplicate parts are later absorbed. If part of an anterior cut surface remains exposed after grafting, the point of regeneration is thereby determined, and the new head grows at that point; it may be from the larger component, Pl. No. 13 (Fig. 2), Ph. No. 89 (Fig. 3), Ph. No. 18 (Fig. 4), or it may be from the smaller Ph. No. 90 (Fig. 5).

If a cut anterior surface is not exposed, new tissue may still grow at the line of the graft, and, one edge growing forward, form a head, at first lateral, then more and more anterior in relation to the larger component. Such a head may be derived from one component, No. 94 (Fig. 8), No. 3 (Fig. 10), No. 10 (Fig. 9), or from both, No. 1 (Fig. 12), No. 19 (Fig. 13), No. 95 (Fig. 14).

In all these cases, whether the head be derived from a free surface, or from the growing edge of the line of the graft, the posterior part of the smaller component (whether it closes in or whether it regenerates a tail) is almost always gradually absorbed, leaving one complete worm whose body and tail are derived from the larger component, and whose head is derived from the larger component or from both, or even from the smaller only. In the last case, No. 90 (Fig. 5), No. 3 (Fig. 10), No. 10 (Fig. 9), the tail is absorbed of that same component from which is derived the head of the final combination. In the combination worm, however the head may arise, the larger body persists as the body of the worm. Part of a second head may also regenerate, but it is usually abnormal and in a crowded position [No. 43 (Fig. 15), Pl. No. 12 (Fig. 16)] and perhaps degenerates. One of two eyes degenerated in No. 3 (Fig. 10). A clear case of morphallaxis in the formation of the final unit is seen in No. 18 (Fig. 4), where the

head (derived from the large piece) at first occupied but half the width of the large piece, the small piece regenerated a tail, which grew back to a posterior position before it was absorbed, and the head widened until it extended across the width of both components. All the observed cases of a head being formed at one edge of the line of graft without a free surface are of the combination Hh.

C.　DORSO-VENTRAL GRAFTS

Another set of experiments was carried out to see what sort of regeneration would take place if two worms were so attached by their anterior cut surfaces that the dorsal surface of one worm was continuous with the ventral surface of the other, *i. e.*, one worm was turned over on its back before grafting together the anterior ends. After many unsuccessful attempts to get the pieces to stick together, six grafted pairs were obtained from pieces cut anteriorly in the head region (dorso-ventral Hh), and four combinations of tail regions (dorso-ventral Tt). In one Hh, part of an anterior surface remained exposed, and two normal worms regenerated and pulled apart. After about a week, all the other five Hh combinations without exception, had regenerated in one and the same way. From both flat surfaces at the line of union of the two pieces a head regenerated; at the same time the small piece had regenerated a tail at the free posterior cut surface (a normal product), and the result of such a combination is that a larger worm crawls about carrying attached to it, posterior to the eyes, another smaller worm at first shorter than itself. In other words, two worms appear to be attached to each other by the "napes" of their necks, the larger one carrying the smaller one curled up above it (Fig. 17 *A, B, C*). As the smaller one grows, it also tries to attach itself and sometimes the combination is so twisted that both worms crawl at once. The two cases of dorso-ventral graft obtained in Planaria maculata regenerated in the same way. The dorso-ventral grafts of Phagocata of the combination Tt behaved like those of the combination Hh in two cases where they could readily be observed; in the other two, two heads showed, but the pieces were very small and not carefully made out.

The preceding description of the regeneration of dorso-ventral combinations does not fully represent the state of the case; for, when the worms are of different colors and the grafted combination is more carefully examined, it is seen that the head of the crawling worm has its dorsal surface pigmented like the dorsal surface of the worm carried above it. In other words, the head is a composite of ventral material regenerated from one worm, and dorsal material regenerated from the other worm, and both heads belong in part to both worms. The relations of the heads to the old parts are shown in a diagram (Fig. 17*D*) of a side view of the combination; the dorsal surfaces are represented by dotted lines, the ventral by continuous lines. If worm *I* is loosened from its crawling surface, the heads may be thrown in opposite directions (Fig. 17*E*), and now the worms appear to be attached by their throats, and worm *I* appears to possess head *B* instead of head *A*. If allowed to return to the position of least resistance, head *A* will again be thrown forward from the larger worm *I*, and the ventral surfaces of worm *I* and head *A* will be continuous in the crawling position on the bottom of the dish. Diagram F, representing a position midway between *D* and *E*, will show most clearly how the composite heads have grown from the upper and lower edges of the graft.

In dorso-ventral grafts, there is no chance for the nerves of the two worms to unite, and this may be a factor in the production of two heads at the graft, and hence of no regeneration of a head at the free posterior cut surface of the small piece. From this point of view, the results of the experiments fall directly in line with the results of the plain grafts of Tables I and II.

The relations of the nervous system have not been worked out in all cases, but many combinations were sectioned and showed results of interest. As far as the nerves have been studied, the general rule holds that whenever a head is developed, its nerves are connected with the longitudinal nerve-trunks of one or both components of the graft, and if there is a single head involving the smaller component, the longitudinal nerves of the small component are connected with those of the larger component; the single head may be a reversed head on the small piece, or a laterally

compound head, the derivation of the head being judged by the position of the eyes. If a reversed head is developed at the posterior surface of the small piece, it is found in section that the longitudinal nerves of small and large components meet squarely end to end and thus the nerves are perfectly continuous, and in the new head, sometimes (Fig. 1C) apparently normal "brain" and intercerebral commissure are developed; sometimes the commissure is very small. If a tail is formed again, the longitudinal nerves of the two pieces unite end to end, but there is no brain or commissure. In one specimen, the longitudinal nerves in the new tail are connected at intervals by smaller nerves as they are throughout a normal worm.

In a case of what was supposed to be reversed regeneration (Fig. 1D), the anterior nerves are in the small component, and with a large connecting nerve are shunted off to one side connected with only one longitudinal nerve of the large component; the left nerve of the small component is continuous with the right nerve of the large component; the connecting nerve leaves the longitudinal nerve at the level where the normal commissure should connect the two longitudinal nerves of the large piece; but exactly through this region passes the line of the graft, and the normal commissure is but slightly developed. This worm was thought to be among those which showed externally perfect head regeneration, but it may have been confused in embedding with closed-in pieces since the eyes have not been found in the sections. The chief interest lies in the one-sided connection of the nerves of the larger and smaller components.

In grafts in which the small piece closed in, and in which no regeneration occurred, there was found no brain or commissure. In the examples that were sectioned, the longitudinal nerves ended abruptly, sometimes with a few nerve branches in place of the commissure, and in one case, a third large nerve in the knob that remained of the small component (Fig. 1E) was connected by a few nerves with one of the longitudinal nerves.

The union of the longitudinal nerves of the two components seems to take place at the time that the pieces grow together; the nerve-trunks are continuous in surface view when the grafted

pieces are first released from the papers (eighteen to twenty-four hours after being placed together), and in sections of a graft then killed, the nerves are found united. The line of union of the two pieces of worm can sometimes be seen in sections of worms killed some time after the pieces grew together because of the accumulation at the line of union of parenchyme nuclei.

In Phagocata No. 94 (Fig. 8*B*) the nerve of the closed-in knob of the small piece has formed a closed ring with branches. The ring is united with the longitudinal nerve of the side of the large component on which the knob is attached.

In No. 95 (Fig. 14*B*), where one eye has developed in each component of the graft, the longitudinal nerves of the large component are connected by a commissure, the eye of the large component is innervated by nerves from the brain connected with one longitudinal nerve; from the commissure a branch passes to the nerve ring in the knob derived from the small component, and anteriorly from this ring a large branch nerve is developed from which the eye, derived from the small component, is innervated. Again this branch and the brain in the other side of the large component are connected by nerves but slightly developed in the normal position of a commissure.

In No. 3 (Fig. 10*E*), where there is but one eye which is derived from the small component of the graft, the longitudinal nerves of the large component are connected by a commissure and are also connected anteriorly by the nerve of the smaller component of the graft, forming with the commissure a complete ring. Two anterior nerves, which also are connected together arise, one from the longitudinal nerve of the large component, the other from the nerve ring of the small component, and from this a nerve extends to the one eye.

The fact that regeneration at line of graft a slanting occurred in cases of the combination Hh only has not been explained. It might be supposed that the material of the head region most easily forms a head, the heads being here produced even when the usual outlet for regeneration, *i. e.*, a free surface, is blocked by the graft. In line with this view would be the case of No. 3, where the reversed head grew, not in the middle of the piece, but at the thin

edge of the piece, that is the point where the tissue was nearest to the normal place for head-regeneration. The question as to whether (as in Tubularia)[1] a head regenerates sooner or more readily at different levels from behind forward, has not been studied. It is shown by the results given in Table I, that regeneration of a head even in a reverse direction may take place at any level of the worm, and the condition of the nervous system in two of the class of cases under discussion suggests that a nerve ending freely is a factor in regeneration. In neither of the worms sectioned (Figs. 8 and 14) do the longitudinal nerves of the two components meet squarely as in the worms with reversed heads (Fig. 1*C*). In the cases where the line of graft slants, the free anterior end of a longitudinal nerve trunk may then, as in dorso-ventral grafts, be a factor in determining the position of the new head, although there is no exposed cut surface of either worm.

It would be of interest to know the condition of the nerves in a case like Planaria No. 2 as compared with Phagocata No. 19, and Phagocata No. 58. In Planaria No. 2 (Fig. 6), it seems that two heads have regenerated from the anterior surface of the large component of the graft, one head each side of the small closed piece. In Phagocata No. 19 (Fig. 13), new tissue grew from the large component at both sides of the line of graft, but a head developed on one side only, and was later composed in part of the smaller component of the graft. In Phagocata No. 58, the small piece closed in, the anterior surface of the larger component regenerated on both sides of the small piece, and the new tissue completely surrounded it, but a head never developed. The worm was watched for ninety-six days.

Reviewing the results as far as worked out, it appears that in all cases of graft, the eye and its nerve are developed from the same component of the grafted worm. With two worms cut off anteriorly there are four exposed anterior nerve ends. When these, by the conditions of the graft, cannot unite, two perfect

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[1]Morgan, T. H., and Stevens, N. M. '04. Experiments on Polarity in Tubularia. Journ. of Exp. Zöol., vol. i.

heads are formed (dorso-ventral grafts and a very one-sided graft like Planaria No. 7, Fig. 7). When they can unite end to end (perfect plain grafts), they do so, and regeneration of the anterior ends of the nerves is excluded; if a head is then developed it is not the anterior, but the posterior, exposed ends of the nerves that come to innervate the eyes, and may even form normal brain and commissure, or may regenerate in a way less like a normal anterior nervous system. In irregular grafts, various ways of combining the nerves of the small component with those of the large lead to different arrangements of the nerves of the two sides. When the small component closes in, its nerves may end freely posteriorly, as in square grafts (Fig. 1E), or may form a ring attached in various ways to the nerves of the large component. In No. 94 (Fig. 8) the ring is shunted in with one longitudinal nerve of the large component. In No. 3 (Fig. 10) it is joined with the commissure and with one longitudinal nerve and innervates the one eye which is derived from the small component. In No. 95 (Fig. 14) the ring is connected by a large nerve to the commissure, sends a large branch to the eye derived from the small component, and the branch is joined by a few nerves with the anterior nerve of the other side of the large component.

The relations between the kind of regeneration (as seen in surface views) and the condition of the nervous system is what would be expected, though apparently perfect regeneration of a head and eyes may be accompanied (as in Leptoplana littoralis)[1] by imperfect regeneration of cerebral commissure and brain.

Some points in regard to the closing-in of the exposed posterior surface of the small component may be further examined. Closing-in of the posterior end of the small component never occurred where there was regeneration of two heads in the anterior tissue of the components, either complete heads [dorso-ventral grafts (Fig. 17) and Table II, column 3, Planaria No. 7 (Fig. 7) and No. 11], or partially complete [Table II, Phagocata No. 43 (Fig. 15), Planaria No. 12 (Fig. 16)]. It always occurred (except

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[1] Morgan, L. V. Incomplete Anterior Regeneration in the Absence of the Brain in Leptoplana littoralis. Biol. Bull. vol. ix.

in two cases), Table II, Phagocata No. 18, (Fig. 4) and Planaria
No. 8 (Fig. 11), where a single head originated in some way from
the anterior end of one or both components (Figs. 2, 3, 5, 8, 9,
10, 12, 13, 14). It often occurred in plain square grafts (Table I),
but never when the shorter component was not very short (Table I,
H long h). Closing-in of the anterior surface of various kinds
of Planarians, when the worms are simply cut across without
grafting, is a very common phenomenon; it has often been
observed and is frequently noted in the literature on regenera-
tion in different species and genera of Planarians.[1] Closing-in
of posterior cut surfaces of ungrafted worms, so far as I know,
has not been observed.

It might then appear that when the posterior surface of the small
component closes in (Table I, column 3; Table II, columns 1, 2,
4, 5 and 6, except Phagocata No. 18 and the compound-headed
worms Phagocata No. 43 and Planaria No. 12), it acts like an
anterior cut surface of an ungrafted worm. If this were always
true, it would follow that when the anterior surface of the small
component takes part in the formation of the head, and at the
same time, the posterior surface closes in, both surfaces of the
small component act like anterior surfaces of a cut worm. On
looking closely at the facts, it will be seen that the closing-in of
the posterior surface of the small component never occurred
when all of the anterior surface of the small component took part
in the formation of the head (dorso-ventral grafts and column 3
of Table II); in those cases a tail was formed from the short com-
ponent posteriorly. The cases where head-formation occurred
anteriorly, and at the same time closing-in occurred posteriorly
in the small component are cases of slanting grafts and of all other
grafts where part only of the anterior surface of the short com-
ponent contributed to the new head (Table II, columns 2, 5 and 6,

[1]Morgan, T. H., '98. Experimental Studies of the Regeneration of Planaria maculata. Archiv
f. Entwickelungsmech. d. Organismen, Bd. viii. Lillie, F. R., '01. Notes on Regeneration and Regu-
lation in Planarians. Am. Journ. of Physiol, vol. vi. Schultz, E., '02. Aus dem Gebiete der Regenera-
tion. II. Ueber die Regeneration bei Turbellarien. Zeitschr. f. wiss. Zool., Bd. lxxii. Morgan, T. H.,
'04. Notes on Regeneration. Biol. Bull., vol. vi. Child, C. M., '05. Studies on Regulation. IV, V
and VI, Journ. of Exp. Zoöl., vol. i. Morgan, L. V., *loc. cit.*

the origin of the parts was not followed in Phagocata No. 43 and Planaria No. 12). The condition of the nervous system in slanting grafts indicates that there is a difference between the closing-in of these posterior surfaces and the closing-in of the posterior surfaces of reversed square grafts without regeneration at the line of graft.

The longitudinal nerve trunks of the short component in the slanting grafts (Figs. 8, 10 and 14) did not join end to end with those of the long component, but formed a closed ring joined in some other way with the longitudinal nerves of the long component. In contrast to this the posterior ends of the longitudinal nerves of closed-in pieces of square grafts without regeneration at the line of graft ended freely. It may then be true that the closing-in of reversed square grafts is like the closing-in of the anterior cut ends of ungrafted worms, while the behavior of the nerves may be a factor in the closing-in of the posterior ends of the small pieces in slanting grafts where part of the anterior surface contributes to the new head. The hypothesis that the closing-in of reversed square grafts without regeneration is a phenomenon of an anterior surface or of one acting like an anterior surface leaves unexplained the case of a graft where the short piece at first closed in, but after it was twice cut off produced a tail; as the shortness of the piece seems to be a factor in the production of reversed heads, it is unexplained why the piece when longer acted like an anterior surface, when shorter made a tail.

SUMMARY

1. Short pieces of Phagocata gracilis, cut from different parts of the worm, and grafted by the anterior surface to the anterior surface of long pieces from different parts of another worm, sometimes produce heads, sometimes produce tails and sometimes close in at the exposed posterior surface.

2. The numbers of the combinations cut from different regions of the worms, which either failed to regenerate, or produced heads, or produced tails, are fully summarized in Table I, page 271, and on page 272. The number of grafts that was studied of any one

combination was comparatively small, and with large numbers the proportions in the results might be different.

Longer pieces from the head region of a worm, reversed and grafted on the head region of another worm, regenerated tails at the exposed posterior surface of the short component.

3. When (because of imperfect grafting or for some other cause) regeneration occurs at some point other than the exposed posterior surface of the small component, various results follow. Examples of Phagocata and Planaria are fully summarized in Table II, page 274, and on page 275.

4. If the conditions of the graft are such that part of an anterior surface is exposed (even if only a very small fraction of the whole surface) a head is regenerated from the exposed part of that surface. A head may regenerate from each component of the graft (Fig. 7), or only from the large component (Figs. 2, 3, 4, 6), or from the short component (Fig. 5), according to which surfaces are partly exposed.

5. A single head formed in this way (no matter from which component or in what position it originates) gradually grows to the size and position of a head of the larger component, the smaller duplicate parts of the compound worm are absorbed, and one complete compound worm results.

6. In a few cases where both long and short components were cut through the head regions of the worms, a head regenerated at one edge of the line of graft, although there was apparently no exposed (anterior) surface (Figs. 8–14).

7. In the last cases (paragraph 6) except one (Fig. 13, and one where the original line of graft is not recorded, Fig. 8) the line of graft was not straight across the worms, but at an acute angle with the long axis, and the head regenerated at the forward angle of the graft. The heads were derived from one component (Figs. 8 and 10), or from both (Figs. 12–14).

8. Where the nervous system of these grafts was studied, it was found that the longitudinal nerves of the two components did not squarely unite, and probably anterior ends of the longitudinal nerves remained free.

9. These heads like those of paragraph 4 acquired by degrees

the size and approximate position of a head of the large component, and the small knobs or tails at the exposed end of the small components showed (where their history was followed) that they were being absorbed.

10.   Two compound worms with extra eyes were observed (Figs. 15, 16) but their history was not recorded.

11.   Another kind of combination, "dorso-ventral" grafts, tried with one combination of Planaria and with two combinations of different regions of Phagocata, produced uniform results (except in one case of imperfect graft). In the grafted combination, the ventral surface of one worm was continuous with the dorsal surface of the other. All the examples produced double worms, the two heads being at the line of graft, on opposite sides of the combination, and each head being derived in part from each component of the graft (Fig. 17).

12.   The heads in these cases regenerated at the normal place for head regeneration, but not from a free cut surface. The cut anterior ends of the longitudinal nerve trunks must, however, have ended freely.

13.   The exposed posterior surface of the small component in each case of dorso-ventral graft regenerated a tail, and never closed in, and the small component became in a short time a more or less nearly full-sized worm, attached to the other worm.

14.   In single-headed compound worms where the nervous system was studied, it was found that the longitudinal nerve trunks of the two components are connected in one way or another and the anterior nervous system and the innervation of the eyes are derived from the two components according to which part of the new head arise from each.

15.   In square grafts, the anterior ends of the longitudinal nerves of the two components unite end to end. The posterior ends of the longitudinal nerves of the short component may form commissure and brain, if a reversed head regenerates, but they may end freely if the posterior end of the short component closes in.

16.   In slanting grafts, which regenerate a head at the line of graft, the longitudinal nerves of the short component form a ring

connected in one way or another with the longitudinal nerves of the large component.

17. The nervous system of double worms, like that of Fig. 7, and of dorso-ventral grafts (Fig. 17) has not been studied, but the conditions of the graft are evidently such that the longitudinal nerve trunks of the two components could not unite, and in accordance with this is the fact that double worms (not one compound worm) are formed.

New York, January, 1906.

EXPERIMENTS ON THE BEHAVIOR OF TUBICOLOUS ANNELIDS

BY

CHAS. W. HARGITT

WITH THREE FIGURES

During the past summer it came in my way to collect numerous colonies of the serpulid annelid, Hydroides dianthus, which is very abundant in the waters about Woods Hole. Finding them to be well adapted to aquarium life I kept colonies under observations upon my laboratory table during almost the entire summer, and made such experiments and observations on their reactions and various forms of behavior as suggested themselves from time to time.

In connection with the observations upon Hydroides, at the suggestion of Dr. J. P. Moore, I also included species of Potamilla and Sabella, though they were very much less numerous than the former species. I am under obligations to Dr. Moore for identifying the several species.

As is well known, many of these annelids are remarkably sensitive to the slightest disturbances of various sorts, such as vibrations, the intervention of shadows, etc. In connection with interesting observations concerning the habits of various tubicolous worms Dalyell,[1] remarked concerning Amphitrite bombyx that "it is impatient of light," withdrawing into its tube instantly upon the interception of the light.

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[1] The Powers of the Creator Revealed. London, 1853. Quoted from *Andrews' Jour Morph.*, vol. v, p. 287.

Claparède,[1] has also called attention to a similar feature in Branchiomma köllikeri, stating that "it is very sensitive to changes in the amount of illumination, for a slight movement of the hand at a distance of a meter from the aquarium, causes all the animals to withdraw into their tubes as soon as the shadow falls upon them. Yet Sabellas, having no eyes, remained immobile and unaffected."

It will be seen from some of the following observations that the reference to Sabella is more or less incorrect, since our species of Sabella, at least, are quite well provided with eyes, and are also subject to the same stimuli as are others, differing only in degree.
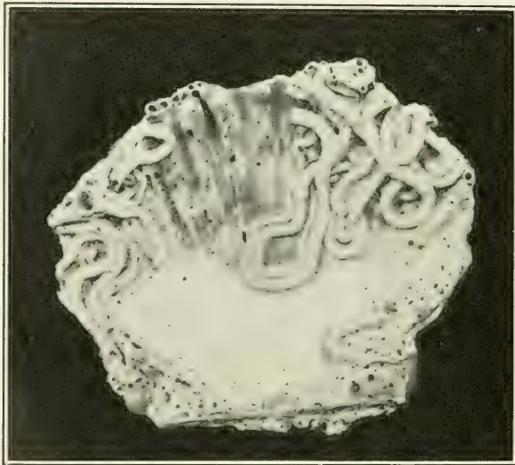
Fig. 1. Several individual tubes of Hydroides dianthus, showing general aspects when growing freely upon shells or similar substratum. (Somewhat less than natural size.)

Similar observations have been made also by Darwin[2] and others upon earthworms, the significance of which is probably of the same general character as the former. Still later observations upon species of Tubicolidæ have been made by Andrews, Loeb, Nagel, and others, which will be considered in detail in a later connection.

[1] Annelides Chetopodes du Golfes de Naples. 1868. Quoted from Andrews Jour Morph., vol. v, p. 287.
[2] The Formation of Vegetable Mould through the Actions of Worms. 1881.

My observations extended to the following named species: Hydroides dianthus, Potamilla oculifera, and Sabella microphthalmia, chiefly the first. These were available in considerable numbers, and collected from various shells about the docks of the United States Fish Commission, from shells, various bivalves, Venus, Pecten, etc., from rocks dredged from depths varying from two or three fathoms to fifteen to twenty in Vineyard Sound and Buzzards Bay. The other species were obtained in part from among colonies of Cynthia collected from the docks, and in part among colonies of Hydroides. Their numbers were smaller than those of Hydroides and the observations correspondingly
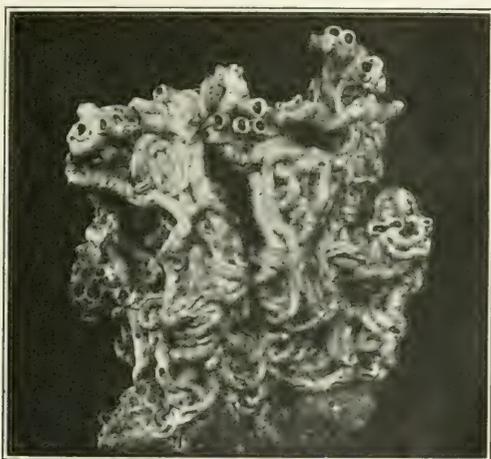
Fig. 2. Colony of Hydroides dianthus growing in complex mass from flat rock base. The various aspects of the mouth of the tubes may be easily distinguished, showing vertical, lateral and downward relations referred to in the paper. (Somewhat less than natural size.)

less extended. As will be observed in a later connection the limitations of experiments on species of Potamilla and Sabella were due in part to their comparative indifference to the various tests applied.

EXPERIMENTS ON HYDROIDES DIANTHUS

The general character of these annelids is so well known that no particular account is necessary. The photographs of several typi-

cal conditions will show quite enough to make clear the habitat and modes of growth. Colonies growing upon shells are seldom large, while those growing upon rocks are frequently quite large, often including from thirty to fifty, or even more, distinct specimens, each inhabiting its own tube, but forming inextricable masses variously intertwined, and among which are usually various other annelids, corals, hydroids, etc., the whole comprising a most interesting ecological community, as well as a most beautiful display of richly varied form and color, rarely surpassed among the almost infinite variety of marine life. It may be noted in passing that most of this richness and variety of coloration is to be found in the
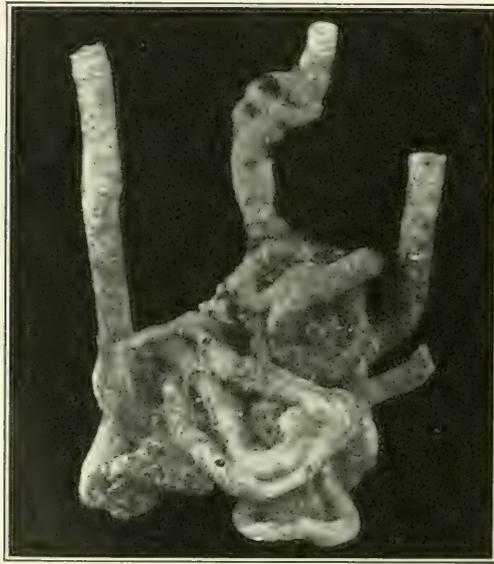
Fig. 3. Colony of Protula intestinum, from Bay of Naples. The serpentine aspects of the tubes referred to in the paper are easily recognized. The coiled tubes to be seen upon the central tube of the colony is particularly interesting as clearly indicating the indifference of the creature to the influence of gravity. (Somewhat less than the natural size.)

annelids themselves, a fact which has been long known and commented upon, but little understood. This feature will be further considered in connection with the several accounts in which it may be involved.

Though I had often observed the general sensitiveness of these creatures to sudden intervention of shadows of various sorts, my attention was particularly attracted to the matter in the present instance by the observation that shadows of even the slightest degree, such as those produced by a strip of white paper, or even a glass rod, seemed quite as effective a stimulus as those produced by the hand or other opaque screen. And this was the more noticeable in that the specimens were before a north window, and thus in diffused light.

Having determined upon a series of observations and experiments, the colonies were arranged in several aquaria, certain of which were placed upon a table before a window facing south, the others upon a table facing northwest, the latter also in such a position that a sixteen candle-power incandescent lamp was available for certain experiments at night.

First Series

The first series of experiments was made to determine exactly the character of the stimulus to which the reactions of the worms were due; that is, whether they were the result of differences of the intensity of light, or whether to the suddenness of the stimulus, or the apparently negative effects of shadow stimulus. Loeb ('93) had pointed out, in a simple experiment made by opening and closing the shutter of a window before which specimens of Hydroides uncinata were living in an aquarium, that "only the decrease in the intensity of light acts as a stimulus upon the animals."

This experiment I repeated many times and under variously modified conditions. The general fact that only the decrease of light, or in other words the shadow, is effective was abundantly confirmed. Drawing the shade before the window, interposing the hand or a sheet of paper, always sufficed to insure the prompt retraction of the animals. Now by continuing the presence of the shadow, the worms extended themselves quite as before. When thus extended the curtain, or other intervening object, may be removed, allowing the increase of light to its former intensity.

This was variously done, in the case of the curtain, vertically; in the case of the hand, screen or cardboard, etc., by either lateral, downward, or vertical withdrawal; but in every case the added increment of light produced no effect whatsoever upon the behavior of the animals.

The experiment was further varied by employing artificial light, an incandescent electric light of sixteen candle-power, hung just over the table upon which the aquarium was placed. Allowing the specimens to become fully extended, which was quite as common by night as by day, in darkness as in light, the light was suddenly flashed directly in their faces, so to speak, but in no case was there evidence of any specific reaction. On the other hand, the sudden extinction of the light almost always gave the same response as the shadow, as could be seen by immediately turning on the light again.

Again the experiment was made in bright sunlight. Allowing the specimens to fully expand under an appropriate screen they were then subjected to the sudden increment of full sunlight but without the slightest reaction. This was varied by covering the aquarium with a dark box; leaving them in total darkness until fully expanded, then quickly removing the box and allowing the full sunlight to fall directly upon them gave the same negative result as in the former.

There can be no doubt, therefore, that the reaction is not due to simply a difference of light-intensity alone. For whether in diffused or direct sunlight, whether in natural or artificial light, the response is to the shadow, sudden diminution of light, a purely negative condition. But it may well be doubted whether this can be properly designated as simply negative phototropism or heliotropism. The phenomena are much too complex to be explained by any single factor. Details on this point will be taken up in a later connection, however.

The experiments were varied by interposing the screens at a distance of from a very few inches, perhaps two or three, to five, ten, fifteen, twenty, etc., up to forty or fifty, but without materially affecting the results. In the diffused light before a north window the shadows from the greater distances were not always equally

effective, but before a south window when the light was bright, even though diffused, the results were as certain and effective as at the shorter distances. No attempt was made to determine the limits beyond which the shadows might become too indefinite to act as a stimulus.

Furthermore, the experiments were still varied by varying the *time* or *rate* of the shadow movements. It was found that ordinarily little difference could be detected as to the reactions under a swift and a slow motion as ordinarily made by the movement of the hand. But if pains were taken to insure a very rapid movement, as by propelling an object before the aquarium by mechanical means, such as a spring, it was possible to pass the shadow of a small object before the specimens without producing sufficient effect to act as a stimulus. Likewise it was possible to interpose a screen by such very slow degrees as to avoid any direct reaction on the part of the specimens.

Another experiment was tried to test the effects of a constantly recurring, or rhythmic shadow. This was effected by arranging a pendulum so adjustable as to secure a rhythm of from about a quarter of a second to a full second. With the full second movement there was more or less constant reaction with each passing shadow. With the half second movement it was found that, after the first few beats, a considerable portion of the worms failed to respond at all; and with the quarter second beats almost all the colonies became indifferent to the presence of the passing shadows.

These results are extremely interesting as indicating the possible relations of the reactions to protective or adaptive ends. May it not be possible that in these rhythmic shadows we have a simulation of the more or less rhythmic shadows resulting from the ripples or wave action, which are phenomena of more or less constancy and of course affecting in much the same way specimens living in the shallower waters?

In connection with this matter of rhythmic shadows it was observed that where experiments were repeated with any considerable frequency specimens sooner or later became somewhat irresponsive, often failing entirely to react to any of the usual tests.

This I am inclined to regard as the result of fatigue. But it may naturally be asked if this might not be equally well explained as the result of a condition similar to that induced by the rhythm just considered?

That it is a matter of fatigue, or a closely related phenomenon, rather than the latter, seems to me strongly indicated by the fact that it results from any form of shadow stimulus which may be applied, such as a vertical or lateral; slow or rapid; made by the hand, by a small rod or broad screen; in short, by any or all of the various tests applied successively or in any order whatsoever. These tests applied singly and at irregular intervals rarely failed to produce the appropriate reaction. It was only when repeated at such rapidly recurring intervals as to leave insufficient time for readjustment that the animals gradually became irregular and indefinite in their behavior. At almost every point the results simulated so intimately the fatigue phenomena of higher organisms as to leave a very strong impression of its similarity, or even identity, differing, if at all, chiefly in degree rather than in character.

Second Series

The second series of experiments was directed to a determination of the exact localized areas involved in the various reactions. The first step toward a solution was the observation that when the crown of gill filaments was directed away from the source of light, as would be the case when the opening of the tube, and consequently the direction of the head was turned from the light, it frequently happened that the worm failed to respond to the usual shadow stimulus. This was variously repeated and with cumulative evidence to the effect that the outer, or under surface of the gills was distinctly less sensitive to these stimuli than the inner surface. This is only what might naturally be expected, since it is the inner surface which in expansion sustains direct relations to sources of contact or approach from the surrounding medium, while on the other hand the outer surface in the unfolding and expansion of the plumose filaments would be least exposed.

Furthermore, it was found by close observation that the terminal portions of the filaments were likewise more sensitive than the basal portions, which is again what might naturally be anticipated. If one watched carefully the behavior of a specimen following a retraction of the gills it would be seen that the following protrusion was a very cautious process, so to speak. The tips of the gills would be gradually extended, barely protruding beyond the margin of the tube, and in this position the creature would wait for a time, the filaments in the meantime actively vibrating, as if searching for any source of danger. Next they would be protruded somewhat farther, with another pause, and finally the entire crown would be protruded and gradually expanded. The same process was frequently observed, but in reverse order, in a contraction following a very slight stimulus.

Following an unusually vigorous stimulus, as a very dense shadow or a mechanical disturbance, the worms would frequently remain for some time deeply withdrawn, and in again expanding would do so with unusual caution.

This was most strikingly demonstrated in the following experiment of further attempting to locate the sensitive areas by a process of graduated excision of the gill filaments. This was attempted by a quick snip with scissors as the specimens were quietly expanded near the surface of the water. The operation was found, however, to be an exceedingly difficult one, owing to the lightning-like rapidity of the contraction of the worm at the slightest disturbance. I succeeded in several cases in clipping off about one fourth of the terminal portion of the filaments, and found in every case that there followed an appreciable loss in the acuteness of the sensory reaction, though as will be seen in the later discussion of "mixed stimuli," it is not beyond doubt that this apparent loss was due to the interposition just here of another stimulus, that resulting from the mutilation, which for the time being superseded that of the light. This is further suggested in the fact that immediately following such an excision the creature seemed to go through various maneuvers, rubbing the filaments over each other in the most curious fashion, protruding and withdrawing them in very unusual ways. And it was further ob-

served that within perhaps a dozen hours the worm behaved in quite a normal fashion, and seemed to have regained much of its former sensitiveness, though its responses were less definite or exact than before.

Attempts to excise further portions of the same gills upon a following day showed the operation to be a much more difficult matter than in the first instance. This was not only due to the same extremely rapid reaction mentioned before, but also to the fact that the creature had apparently acquired a degree of caution in protruding far beyond the tube, and had likewise apparently become much more easily disturbed than before. This became even more marked upon a third or fourth attempt. It was as if it had acquired an experience which served much as in higher animals, and will have a peculiar significance in some of the later discussions.

I finally succeeded in excising entirely the gills down to the base of the palps in the case of several specimens by substantially the same process, except that it was necessary, in order to secure this result to place the scissors about a quarter of an inch below the orifice of the tube, and then to cut off that much of the tube, and in the process "catch" the crown of the retreating worm. In a few other cases I forced the worms out of the tubes by thrusting a bristle into the smaller portion of the tube, posterior to the worm, and then gradually irritating it till it emerged entirely, or so far as to allow careful excision of the entire gill area, after which the worm was allowed to readjust itself in the tube.

This last expedient was found, however, to give less satisfactory results than the former, as a rule, since it often happened that by the process of forcing the worm from the tube there was great liability of injuring it sufficiently to seriously interfere with the subsequent experiments, or to so modify the reactions as to give inconclusive results.

As a result of these several experiments it was clearly demonstrated that the sensory centers are within the gill areas, and chiefly the more distal portions, though with the basal third of the gills intact the creature still retains more or less of sensory activity. But with the entire gill area removed close to the palps, the sensory

power is wholly lost for the time being. It must be stated incidentally that the capacity of regeneration is well developed in these worms, and new tissue begins to make its appearance within about two days, and with this regeneration there is recovered in a more than proportional measure, the sensory function. The entire gill is regenerated in about two weeks, or in some cases perhaps less.

It will be evident, I think, from these several experiments, that the sensory area is quite definitely circumscribed in this species, and it may also be stated in this connection, that it is equally so in the other species included within the present account. But it remains to consider the further question, whether there be any special sensory organ, answering the structural or functional purposes of an eye? In reply it may be said that such organs, the so-called eyes, have long been known in many of these worms, and that they have been generally regarded as having a visual function. The citation from Claparède in an earlier section of this paper is one of many which might be given in support of the view.

On the other hand it is likewise equally well known that there are not lacking many species, not only among worms, but cœlenterates, molluscs, etc., which are totally devoid of anything of the sort and are nevertheless, quite as sensitive as species having an abundant supply of these "eyes." Such is the case, for example, with species of Hydroides. So far as known the gills are wholly devoid of anything like the "eyes" of Potamilla or Sabella; and yet the latter are, so far as my observations go, much less sensitive than the former.

Andrews several years ago made a critical study of the eyes of annelids, comparing them with similar organs of molluscs. Commenting on points of the comparisons, he remarks, "In both cases the animals respond very quickly to slight sudden changes in the intensity of illumination, bivalves seeking safety by retreat within the hard shell, the annelid withdrawing into firm tubes." But he continues, "The great number and position of these organs suggests doubts as to their usefulness as eyes, the same that have been made to the like organs of Arca" (op. *cit.*, p. 287.)

This author found no structure which was in any degree comparable to the "eyes" in the other species investigated, including Potamilla and Sabella. Yet he found that "the seat of sensation is also in the branchiæ; when these are cut off more and more, the animal still reacts till nothing but the bases of the branchial stems remain." This last point differs slightly from my own observations as just given above, though it would seem to be rather a difference of degree than of kind.

It may be mentioned in passing that Dr. A. L. Treadwell, who has carefully studied the histology of the branchiæ of Hydroides, assured me that he was not able to distinguish anything comparable with the eye-like organs found in other species of annelids.

It seems fairly certain, therefore, that we have in these annelids a condition of highly sensory activity, more or less definitely localized, yet without any specialized organs, as sensory centers, or media of photic coördination. Whether it shall be found upon further investigation that there exists in these annelids certain specialized cells, similar to those found by Langdon[1] in the earthworm, which may be regarded as having a similar function must be left for the future to determine, though the probability of such sensory cells may be confidently anticipated.

Third Series

A third series of experiments was conducted in relation to the effects of colored light upon the reactions of these worms.

The general influences of various parts of the spectrum on organic processes are too well known to call for special details. It will be sufficient to refer to the observations of Lubbock,[2] Graber,[3] and Engelmann,[4] as examples of many who have given attention to the matter. Others will be cited in connection with particular phases to be considered later.

My experiments with colored light were made with dark ruby glass such as is commonly used in photographic dark rooms, and

[1]Langdon, F. E.: Jour. Morph., vol. xi, 1895, p. 193, etc.

[2]Lubbock: Ants, Bees and Wasps, 1882, p. 186, *et seq.*

[3]Graber, V.: Grundlinien des Helligkeits- u. Farbensinnes der Tiere, 1884.

[4]Engelmann, Th. W.: Ueber Licht- u. Farbenperception niederster Organismen, Arch f. d. gesam. Phyisol., Bd. xxix, 1882.

with deep blue cobalt glass. No attempt was made to determine whether the glass was approximately monochromatic. However, for the purposes involved, it answered fairly well.

The first experiment consisted in the very simple process of interposing a plate of colored glass between the aquarium and window, with no attempt to protect other portions of the aquarium from the diffused light of the room. As might have been anticipated, under the circumstances no definite results were distinguishable.

The next experiment was made by enclosing the entire aquarium under a dark box, one end of which was fitted with a colored glass. The specimens were left here for about a half hour before observations were attempted, and then no differences could be distinguished. After another half hour further tests were made, but with no appreciable differences as compared with those under natural light. In this instance the red glass was used. The experiment was varied by interposing blue glass, and again the aquarium was left for about the same time, and no differences being apparent, the apparatus was left for another hour before testing, and again with negative results. Finally it was left over the entire night and tested about 8 A. M. the next day, but again with negative results. As will be seen by the later experiments the apparent failures were due to the fact that immediate attention was not given to the matter of tests and observations.

At 8.15 the aquarium was placed under the red glass and testing immediately, it was observed that there were no responses to any of the former tests, even perfectly opaque screens like a thick pasteboard, gave only negative results. That is, reactions were wholly inhibited. After an hour, further tests showed that specimens were quite recovered and reacted to any of the usual tests quite promptly and energetically. Repeating the tests at intervals during another hour they gave the same positive reaction which had been shown under normal conditions. Removing the red screen entirely, in order to repeat the tests under blue glass, it was found that the entire colony was strangely irresponsive; none of the usual tests making any impression whatever upon them. Repeating the tests in every way possible failed

to secure any reaction for at least two minutes, when it was noted that the specimens were apparently recovering from a sort of dazed condition, and within five minutes all were reacting quite as promptly as under normal conditions.

The specimens were left for about forty minutes under natural conditions and were then placed under the red light again and carefully observed, and with the same results as in the last experiment except that it was found that after the first effects of inhibition due to the red light, they were found to gradually recover sensitiveness in from five to ten minutes. Testing as before at intervals of from five to ten minutes during half an hour they reacted quite as under normal conditions. Then again the red screen was removed and the animals carefully watched and tested as before with the same results, namely, that immediately following the removal of the screen they were found to be quite indifferent to any of the usual tests but that in from two to five minutes they had quite recovered and reacted as under normal conditions. By actual count the reactions were as follows: At the end of one half minute none; end of one minute one specimen; end of one and one-half minutes four specimens; end of two minutes ten specimens; end of two and one-half minutes all but five of the colony of twenty-five specimens. These did not fully recover normal activity until some six minutes had elapsed.

At two o'clock they were placed under the blue screen and carefully watched as before. There was at first the same inhibition of reaction as in the former case. At the end of two minutes the first indication of recovery of sensory activity was noticed. Repeated at intervals of one minute it was found that in four minutes apparently the entire colony became normally responsive. Tests made at intervals of about five minutes for nearly half an hour seemed to show that there was a higher average of response than under the red light. At 2.25 the screen was carefully removed and tests showed specimens to be acutely sensitive, responding to the slightest shadow.

The experiment was repeated, after adding a new and fresh colony to the aquarium, and with almost identical results.

Again at four o'clock the same experiment was repeated, and with the same results, the creatures losing for about five minutes any sensory discrimination, but quickly recovering it with apparently greater acuteness than under natural light. This was likewise the case at the conclusion of the experiment at six o'clock, when the screen was removed, every specimen reacting with great promptness and vigor.

These experiments were repeated again and again, under varying conditions of light, temperature, etc., but with so large a measure of constancy as to preclude the possibility of the operation of merely accidental or incidental causes.

As has been noted in an earlier section, the coloration of these creatures is very variable, ranging from orange-red on through yellow, dull brown, blue and purple of all degrees of combination to almost pure in some cases. In connection with the experiments on colored light occurred the query, whether there might be any possible relations between the various colors of the worms and the exposure to varying intensities of light? While their arrangement in the colonies gave no apparent support to an affirmative probability, still it seemed well to carefully test the individual reactions of the more conspicuously colored specimens. This was accordingly done in repeated instances but with entirely negative results. Whether in natural light or under the influences of artificial and colored light; whether among a group of bright orange-colored specimens, or in deep purple colored specimens, not the slightest individual difference could be distinguished. And the same was equally true whether the experiments were made with colonies just taken from the natural habitat or those long in the aquarium. It may be assumed therefore that these rather remarkable colors have little or no adaptive relations to light, any more than to selective protection. It seems most remarkable that in a single colony of these worms numbering perhaps thirty or more specimens, and probably arising from a single brood or generation, all this range of color variation should be found, and that without any apparent significance in relation to their habits or life history, yet such seems to be the case here as in many other equally well known instances among this and other phyla or classes.

In this connection may be mentioned an illustration of observations cited by the writer in an earlier paper,[1] namely, the decline of color brilliance under the artificial conditions of the aquarium. In Hydroides this was quite marked, as was also the case with Protula. The colors in which this was first noticeable were the reds, orange and yellow, though it was not lacking in the bluish tints. These changes were less evident in Potamilla and Sabella, the colors of which are less striking.

It might be inferred from what has gone before that these color changes would have little effect upon the sensory responses of the specimens, and such was the case. While as already shown, any evident decline in vigor was likely to involve a corresponding decline in sensory qualities, but there was nothing to indicate that there was any necessary relation between these phenomena and that of decline of color.

EXPERIMENTS ON OTHER ANNELIDS

Reference has been made repeatedly to Potamilla and Sabella, but no special account has been given of definite experiments made upon them. As already stated, the numbers of these specimens obtained were comparatively few, and the experiments much less extensive than upon Hydroides. But one species of each was found, namely, Potamilla oculifera, and Sabella microphthalmia. In size and habit they are very similar. Their tubes are not calcareous and rigid as with Hydroides, they are much smaller in size, with a habitat among sponges, ascidians, etc. Their general behavior is similar to that of Hydroides, but very much less striking. The experiments made upon them were the same as have been already described for the other species. In various experiments with light of varying intensities—to electric and colored light, to touch, etc., they gave no reaction which was not much more clear and convincing in the former. To colored light their behavior was almost uniformly negative, as was also the case with that of the electric lamp. Furthermore, their reaction time was much more sluggish and uncertain than in the case of Hydroides. Their

[1] Science, vol. xix, 132, 1904.

reaction to shadow stimuli, while similar to that of Hydroides, was as before very much less striking. It was frequently the case that only after some two or three more or less dense shadows had been cast over them that a reaction followed, and then comparatively slow, and so to speak, deliberate-like. Tactile stimuli, and excision of portions of the gills induced, as in Hydroides, apparent caution and more promptness of reaction.

Concerning the behavior of Protula intestimum a brief reference has already been made in another section. It may be worth while to cite a few additional observations which serve to confirm and accentuate those of the other species. Protula is a large tubicolous annelid, quite common in the Bay of Naples which like Hydroides secretes dense calcareous tubes curiously coiled in serpentine aspects, as shown in Fig. 3. These tubes are often 180 mm. or more in length and from 5 to 8 mm. in diameter at the opening. The color is usually of a more or less uniformly bright orange red, and a colony of these creatures fully expanded forms a most brilliant picture.

Their reactions to the various stimuli already mentioned are very similar to those of Hydroides, though somewhat more erratic. Thus it frequently happens that while at one time responses to shadows are very prompt and decisive, at another time they may be apparently quite indifferent. Perhaps on the following day this may all be changed and the colony acutely sensitive to the slightest variation in the intensity of light. Furthermore, they seem less adapted to continuous aquarium conditions than do species of Hydroides, a lowering of vital tone being more or less evident after about a fortnight. This is also associated with a decline in the color tone of a more marked degree than is the case with the former species. And with these changes the reactions to various stimuli, especially light, become very uncertain or even entirely lacking.

GENERAL DISCUSSION

The foregoing experiments and observations bring before us certain clearly defined facts which call for further consideration and explanation. This may be facilitated, perhaps, by a com-

parison with similar facts from other sources, some of which have already been referred to. Patten,[1] whose somewhat extended observations and study of the eyes of molluscs is well known, has offered suggestive comments concerning their reactions to varying intensity of light. For example, he has observed that in Arca, whose sensory organs are very numerous and highly complex, the reaction is much less marked than in species of Avicula in which the slightest shadows, such as that of a pencil, causes instant response. He makes a similar comparison between Cardium and Pecten, the former of which is extremely sensitive to varying light intensity but only slightly so in reference to mechanical disturbances like a jar of the aquarium or the action of waves; while the latter is highly sensitive to mechanical stimuli but much less so to shadows, though in this case again the sensory organs are both numerous and complex.

Hence he concludes that "We are led to suspect the presence of some other factor which must, when known, account for the apparent agreement in functional powers between two organs so widely different in structure."

Concerning Pecten, Patten also observed the phenomenon of fatigue, to which reference has been made previously, finding that specimens experimented upon frequently become erratic in responses, "so that finally, even quite deep and sudden shadows may produce only restless or uneasy movements, or perhaps no effects at all" (*ibid.*, p. 614).

Darwin's observations concerning the reactions of earthworms to light are too well known to call for more than passing notice, except to point out that this critical observer expressed no hesitation in ascribing to these creatures definite light perceptions, nor did he hesitate to suggest the coöperation and coördination of other factors in bringing about the varying results obtained in his experiments. For example, he observed that, under the conditions of feeding, mating, etc., or where they were otherwise preoccupied, they either failed entirely to react to the light stimulus, or did so in very different degrees of promptness or directness. He even

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[1] Mitt. Zoöl. Sta. Neapel, Bd. vi, p. 608, *et seq.*

suggests that at times their behavior indicated changes of nervous states, "as if their attention were aroused or as if surprise were felt."[1]

Under the later development of the theory of tropisms, and its extension to the phenomena of animal behavior, its dominance has relegated the earlier views to the limbo of discarded anthropomorphisms so called. Without essaying any review of the *pros* and *cons* of this problem, it may be said that already a reaction has taken place and frankness compels a reconsideration of some of these discarded and discredited views. Such a review has already been made by Jennings[2] so far as it relates to the lower organisms, and his conclusions must, it seems to me, be equally true for many if not most higher animals as well.

Among those who have given especial attention to the behavior of annelids, the most important are Loeb,[3] and Nagel,[4] and to a less extent Rádl.[5]

As is well known, Loeb maintains without hesitation that the behavior of these annelids in their relations to light is governed by the same laws as is that of plants. Indeed, concerning the general orientation of these creatures he contends that they are governed by two fundamental influences, namely, gravity and light, and in support of this view describes various experiments, and gives illustrations as to various details.

Concerning the influences of gravity as a factor in the case of the species I am unable to find any adequate confirmation of Loeb's views. An examination of the several photographs presented herewith will, it seems to me, show that neither in the case of single individuals nor in that of colonies is there any such uniformity as to position or relation as would indicate the predominant influence of any single factor or force. Where single specimens are found growing either upon a shell, or upon a solid and fixed

[1]The Formation of Vegetable Mould. London, 1881.

[2]Contributions to the Study of the Behavior of Lower Organisms. Washington, 1904.

[3]Der Heliotropismus der Tiere und seine Ueberlinstimmung mit dem Heliotropismus der Pflanzen. Würzburg, 1890.

[4]Der Lichtsinn augenloser Tiere. Jena, 1896.

[5]Untersuchungen über den Phototropismus der Tiere. Leipzig, 1903.

support, as a rock, the general position is more or less prone, with a varying serpentine aspect, as shown in Fig. 1. In certain cases the mouth of the tube would be found in one direction, and in others quite otherwise. Sometimes the head projected upward, often downward. This is better shown in some measure in the case of colonies. While here the general tendency of the tubes is vertical in growth, many are found diverging laterally, and in not a few cases directly downward, as may be seen in Fig. 2. In this figure the open mouths of several tubes may be observed near the top. This colony is attached to a flat stone, whose center of gravity would serve to maintain a constant position, hence the variously coiled tubes are evidence of a correspondingly shifting behavior on the part of the worms during their growth. This is perhaps still better seen in Fig. 3, a colony of Protula, several of which I studied at Naples. While here as in the former figure, the general aspect of the tubes is toward the vertical, still their lower serpentine coils show the varying conditions of orientation at different periods of growth. But if further evidence be needed it is found in the tube coiled about the larger central one the mouth of which opens almost directly downward. But furthermore, colonies kept in the aquaria for nearly two months, during which time the tubes had grown almost half an inch, showed not the slightest evidence of any response to gravity. They did, however, show unmistakable evidence of adjustment to conditions favorable for respiration, and for the capture of food; and these I regard as of far more importance in determining orientation than either gravity or light.

Zeleny,[1] who has reared the larvæ of these serpulids in connection with his investigations upon their regeneration and regulation, has incidentally recorded brief, but very interesting observations concerning the behavior of the young worms in relation to "gravity, light and food, but was able to find no general rule, although some groups seem to be arranged with respect either to maximum food-obtaining ability, or with respect to a lateral stimulus of unknown character."

[1] Biol. Bull., vol. viii, p. 309.

While not sufficiently extended to warrant definite conclusions, these observations go to corroborate my own as to the importance of adjustments in reference to respiration and obtaining food.

Concerning the reactions to light Loeb[1] holds very decided views, and has taken Nagel sharply to task concerning a suggestion that the behavior of such creatures as responded to the stimulus of shadows might be due to something akin to a sense of apprehension as to the approach of an enemy or other threatening danger. In opposition to this he undertakes to show the necessity for the existence of some organ of perception similar to a cerebrum in order to enable the creature to possess any instinct, or sense of self-preservation, such as Nagel's suggestion implies. Loeb suggests, on the other hand, that the effect of light may tend to induce a muscular expansion or stretching so that the worm emerges from the tube. If now, a sudden diminution of light follows, as in the case of a shadow, an opposite muscular reaction is set up, and consequently the worm rapidly withdraws into the tube, and the sudden retraction of the worm is only the expression of the rapid extension of the contraction wave of the worm's musculature.

Of the relations of light to many of the phenomena of life there is not the slightest doubt. That in many cases these relations may be so intimate as to warrant designating them by such terms as phototropism, heliotropism, etc. But that there is any such correlations involved in the aspects of behavior under review as to warrant regarding them in the light of cause and effect seems not only doubtful, but wholly inadequate. As Ràdl has remarked, "Diese Erklärung Loebes ist vielleicht ebenso einfach wie unrichtig" (*op. cit.*, p. 78).

In the present case there are many difficulties in the way of the rigid application of any theory of tropisms. In the first place the widely differing degrees of responsiveness among closely related species of slightly differing habitat is difficult to explain by this means. Again, the varying behavior under slightly differing

[1]Archiv f. d. gesam. Physiol., Bd. lxvi, 1897, p. 461.

conditions, as for instance, where specimens have been rendered wary under attempts to excise the gills, or where a given specimen has been placed for a time so near the surface of the water that it has been compelled to extend itself in an unusual manner to bathe its gills at all, under which circumstances it often fails entirely to react to the ordinary stimulus. In this case we have apparently what Jennings has designated as "physiological states" namely, conditions in which the internal changes involved in respiration perhaps, have served to render the animal indifferent for the time to stimuli which under normal conditions are very constant and effective in their action, or perhaps as suggested above, complex or *mixed stimuli.*

·That light of itself is not a determining factor in impelling the worms to emerge from the tubes is evident in that this attitude occurs in darkness as well as in light, by night as well as by day.

Furthermore, it must be recalled in this connection that the particular stimulus involved in these observations, as previously pointed out, is not light at all directly, but the lack of light, or the shadow. The response is, therefore, induced by a negative stimulus, if such an apparent paradox be tolerable in relation to phenomena of behavior. Of course, it is not overlooked that Loeb has designated these and similar reactions as due to "negative heliotropism." At the same time it is not clear that in the present case we are dealing with phenomena at all comparable with those associated with negative heliotropism as ordinarily understood. For as already observed, the phenomena are not in themselves negative. They are not dependent upon any given degree of light, or rather darkness, but to the suddenness of the change.

Rawitz[1] long ago called attention to exactly this peculiarity in describing the reactions of species of acalephs. Referring to the suggestion of Drost concerning the effect of shadows as stimuli, he says "Ein Reiz kann immer nur von etwas positivem, also in diesem Falle vom Lichte, ausgeübt werden, niemals aber von

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[1]Der Mantelrand der Acalephen. Jenaische Zeit. f. Naturwiss, Bd. xxii, xxiv, xxvii.

einer Negation. Und Schatten ist eine Negation, die des Lichtes namlich,'' etc.

But it will be asked, if we are not to regard these reactions as due to some form of light stimulus, whether it be positive or negative, nor perhaps even to photokinesis, how then shall they be interpreted? For myself they seem much better explained as activities concerned with protective adaptation than by any merely mechanical or mechanico-chemical adjustments. If the tubes in which these creatures have their homes have been acquired as protective adaptations, and if it be granted that they, in common with other annelids, have some sensory capacities, including light perceptions, then it would not seem a far call to interpret the phenomena in question as sensory reflexes which have become instinctive through multiplied generations of struggle with various predatory enemies, whose approach cast fateful "shadows before.''

It only remains to consider, briefly, the reactions involving the influence of colored light. In a general way the principal features concerned under this head have long been known. Experiments, have shown that violet rays have a definitely higher phototropic quality than have those of lower refrangibility, such as orange and red.

Nagel[1] has called attention to the same facts in relation to molluscs, though he enters into few details, and frankly admits the desirability of additional observations. He believes that all the colors of the spectrum, except red, have a measure of stimulus for such organisms as show, like his mussels, sensory reactions to the presence of light.

Loeb[2] has also made similar observations, and concludes that "since the heliotropic phenomena appear only weakly or not at all behind dark red glass, while they occur just as in diffuse daylight behind dark-blue glass, the few red rays which penetrate the dark-blue glass cannot be responsible for the heliotropic phenomena which take place so energetically behind this screen but can be due only to the activity of the more refrangible rays.''

[1]Der Lichtsinn augenloser Tiere, Jena, 1896, p. 53.
[2]Physiological Studies, vol. i, p. 18.

A comparison of these views with the facts cited in connection with my observations will show that in several particulars there are obvious differences. In the first place, my observations show that, though there is an inhibitory quality in the dark-red rays when first brought into relation with the worms, it is only temporary. Within some ten minutes, or after time has allowed the specimens to become adjusted in their sensory activities to the new conditions, they become almost if not quite as responsive to the shadows as under normal light. And, moreover, the same thing happens under the influence of the dark-blue rays. Here there is a brief period of inhibition, as in the case of the red rays though the adjustment is more rapid. Usually within five minutes the animals have become accustomed to the new light and behave quite as under normal light. I have pointed out in the earlier connection that it was not certain that the red glass with which my experiments was made was monochromatic, but since it was sufficiently so to render the light passing through it nonactinic, it may be assumed that it was sufficiently so to prevent the passage of the essentially blue or violet rays, at any rate in sufficient measure to act as a stimulus. It seems not improbable that the apparent discrepancy concerning the effects of the red rays may be due to the fact that a longer time is required in adjustment to these rays than to the blue.

In the next place, and here I have seen no similar records, the most striking difference appears in the fact, previously mentioned, that following the removal of the red screen there was a most remarkable inhibition of all sensory responses under the effects of normal light. For several minutes, not more than five, the animals behaved in the most striking manner, much as if asleep, and recovered sensory activity in much the same way as if awakening. Nothing of this sort was apparent when suddenly brought from under the influences of the blue rays. Here if anything their sensory activities seemed even more than normally alert.

However, while this behavior seems more or less peculiar and singular, I am inclined to believe its explanation may be found along the same line of influence which has already been cited, namely, the inhibitory effects of the colored light when first applied.

Under both the blue and red there was a period of inhibition for some time varying in the two cases, as pointed out above, but most effective in the case of the red. Recovery of sensory activity under these changed conditions involved, of course, some physiological change in the sensory apparatus in response to the changed conditions. Now in the sudden emergence from the low refrangibility of the red rays which had involved the preceding adjustment there was involved likewise another physiological change but in the reverse order.

SUMMARY

1. Under the varying degrees of light intensity furnished by the sixteen candle-power incandescent lamp, the diffuse light from north and south windows, and direct sunlight, the results of all experiments involving increased intensity of light were uniformly negative. On the other hand, experiments involving a sudden decrease of light intensity gave results as uniformly positive. However, the behavior does not seem to be essentially comparable with that usually designated as negative heliotropism.

2. Experiments continued without interruption for some time gave rise to behavior analogous to that of fatigue.

3. Various experiments involving the direction of light contact, the excision of branchiæ, etc., showed that the sensory areas are located in the branchial filaments, chiefly the inner and terminal portions.

4. When the animals are brought under the influence of red and blue light sensory activities are for a time inhibited. This is more marked under the red than the blue. On the other hand, when brought suddenly from the colored light into normal white light there is apparently an intensified sensory acuteness due to the blue light, while the effects of the red seem to have been just the opposite, namely, to positively inhibit sensory response for a period of from two to five minutes.

5. Species of Potamilla and Sabella behave in essentially the same manner as do those of Hydroides, though with less acuteness, promptness and certainty. This is somewhat remarkable since

these species are abundantly supplied with "eyes," which are entirely lacking in Hydroides.

6. Species of Protula likewise behave in essentially the same manner as do the others, though apparently somewhat more erratic and uncertain in their reactions.

7. The experiments tend to discredit the theory of tropisms, since no single factor, such as light or gravity, furnishes an adequate explanation.

8. The experiments strongly suggest the presence in the gill filaments of these creatures of sensory cells and nerve endings through which are coördinated by means of nervous centers the various aspects of behavior toward protective or physiological ends.

INHERITANCE OF DICHROMATISM IN LINA AND GASTROIDEA

BY

ISABEL McCRACKEN

In a series of experiments with the dichromatic species of beetle, Lina lapponica, as described in a previous paper,[1] it was determined by breeding through a series of four generations that the behavior in heredity of the alternate characters of the species "spotted-brown" and "black" follows, in general, Mendelian behavior.

This was evidenced by the fact that the broods from a first cross between spotted individuals and black individuals are either wholly spotted or the broods are made up of individuals, some of which (and the majority) are spotted, and some are black. In other words, it appeared that "spotted-brown" was either completely or partially dominant, depending upon unknown causes.

That black is a recessive character was evidenced by the fact that it frequently did not appear in a first cross between S and B, in which case it was extracted in the next generation and thereafter bred true.

The proportions between alternate characters from hybrids in Lina showed no parallelism with typical Mendelian proportions, however, and called for further experimentation.

EXPERIMENTS WITH LINA

I have during the past season (1905), sought to determine the exact proportion of dominant to recessive in successive generations bred each generation from hybrid dominant parents. The individuals used as parents in each generation were spotted individuals, from broods composed of both spotted and black individuals, that had

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[1] Inheritance of Dichromatism in Lina lapponica, Journal of Experimental Zoölogy, vol. ii, pp. 119-136.

proven their hybrid character by producing black as well as spotted offspring.

Diagram I shows character of matings and color character of offspring throughout four generations from hybrid parents. S standing alone signifies a brood in which S was completely dominant, S B standing side by side signifies a brood consisting of both spotted and black individuals, the spotted being present in larger numbers (or partially dominant).

```
S x S                          S x S
|___|                          |___|
                                 |
S and S B                      S and S B


    S and S B                              S B
    |_____|

    S and S B                          S B
    |_____|

        S and S B
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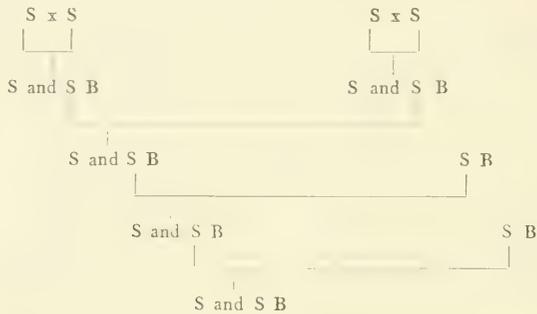
Diagram I. Showing pedigree through four generations from hybrid S.

In the past year's experiments, as in the previous ones, the hybrid S parents of the first generation produced two sorts of broods, broods in which S was completely dominant and broods in which S was partially dominant. In 1904, by breeding consecutively from completely dominant S broods, pure S broods were obtained in the third generation. Breeding in 1905 consecutively from hybrid S parents chosen from broods in which S dominated partially, in each generation both sorts of broods were obtained, that is, completely dominant S broods and partially dominant S broods. In the partially dominant S broods the proportion of dominant to recessive gradually increased through the fifth generation when hibernation began.

It may be noted here that last year (1905) there occurred in broods both from dominant and recessive parents, an occasional wholly melanic individual, thorax as well as wing covers being totally black. Such individuals were utilized for a study of "sport-variation" in heredity.

Table I gives a summary of the data in each generation from S x S parents, including total number of broods, number of broods in which S dominated completely, number of broods in which S dominated partially (designated as "mixed broods"), total number of melanic or sport individuals, total number of individuals and proportion of S to B in partially dominant S broods.

TABLE I

	Total No. of Broods.	Total No. Completely Dominant S Broods.	Total No. of Mixed Broods.	Total No. of Melanic Sports.	Total No. of Individuals.	Proportion of S : B in Mixed Broods.
First generation	118	13	105	10	3442	3.8 : 1
Second generation	45	27	18	1	1050	6.1 : 1
Third generation	142	121	21	1	4736	8.7 : 1
Fourth generation..........	19	16	3	0	549	26 : 1

The increasing proportion of spotted individuals produced in successive generations shows a progressive dominance of the S character. That B might eventually be excluded from the partial dominant S line seems highly probable, but conclusive evidence cannot be obtained on this point until success attends hibernation.

It seems, however, that we may consider S as dominant in a greater sense than that denoted by Mendelian terminology. It is progressively dominant in a way not accounted for by Mendelian law.

EXPERIMENTS WITH GASTROIDEA

The relation of dominant to recessive in alternative characters of the kind studied is more clearly brought out in the following series of experiments with Gastroidea dissimilis.

Gastroidea dissimilis is a dichromatic species belonging to the same family of beetles as Lina lapponica, the Chrysomelidæ.

When an adult of this species first issues from its pupal case, the wing covers are soft and yellow. During the process of harden-

ing all individuals become at first a smoky brown and later black. From this black condition the individual color-development proceeds gradually in one of two directions. In one series of individuals there is a gradual deepening of the pigment to a shiny permanent deep blue-black condition. In the other series there is a passing from the black condition to a shiny permanent bright green condition. The color is fixed in each series within two or three hours of issuing.

We find, therefore, in Gastroidea, in its color-development, the same condition found in Lina, that is, a primary condition through which all individuals pass (and in which one series of individuals remains), and a secondary condition into which one series of individuals only passes.

The two colors, black and green, are therefore represented in the species, no intermediates having been observed during the course of the experiment involving the handling of many thousands of individuals (about 26,000). The beetle is small, about 5 mm. long, feeds on dock or rhubarb and breeds from late in February until early in September, producing normally five or six generations in a year. Under laboratory conditions the breeding season was prolonged through December and seven generations were reared from a lot collected March 16, 1905.

The experiment began with two hundred adults, one hundred black, one hundred green. These had been mating out of doors, blacks and greens promiscuously, and ovipositing had begun. It was comparatively certain that there were no pure bred individuals in the collection, since presumably crossmating had been going on ever since dichromatism had been established in the species.

The first eggs were obtained March 16. These hatched March 24 and issued as adults April 23. At this season, therefore, mature adults were obtained in less than forty days from the egg. Females oviposited five or six days after maturing, each female ovipositing ten to fifteen egg masses during the following few weeks with from twenty to thirty eggs in a mass, frequently running as high as fifty eggs in the mass. With this material as a nucleus, I sought to determine whether there was a behavior of

the color-alternatives in heredity corresponding to that of Lina lapponica. My plan was to breed successively from completely dominant hybrids, if such were found to be present, to breed successively from partially dominant hybrids, and, finally, from recessives that had from one to several pair of dominant ancestors.

In the following tables results in total are tabulated for seven generations of laboratory reared lots.

Table II gives the data of the first generation reared from outdoor collected adults. Individuals used as parents for these data were confined in the laboratory with mates of similar color. Since it is altogether possible that some or all of the females had mated with individuals of alternate color before laboratory isolation, the results indicated give little more than known parentage for second generation data.

TABLE II

First Generation

Color Character of Matings.	Number of Green Broods.	Number of Black Broods.	Number of Mixed Broods.	Total No. of Individuals.	Proportion of B : G in Mixed Broods.
G × G	33	0	6*	580	1 B in each of six G broods.
B × B	0	2	39	1207	1.2 : 1

*One B individual in each six broods.

The single black individuals occurring in otherwise green broods of G x G are possibly due to earlier matings of the female parents. The data from the B x B matings seemed to point to B as the dominant type, the actual results being presumably somewhat modified by uncontrolled matings previous to collection.

In succeeding generations the same method of mating was followed as with Lina lapponica in 1904, that is, females of one brood were confined in a breeding-jar with males of another and *vice versa*.

For second generation data five categories of matings were established as follows:

A–G $\times$ G, each parent from broods of B $\times$ B parentage that had produced mixed broods.
B–G $\times$ G, each parent from broods of G $\times$ G parentage that had produced broods of green only.
C–B $\times$ B, each parent from a mixed brood of B parentage.
D–B $\times$ B, each parent from broods of B $\times$ B parentage that had produced broods of black only.
E–G $\times$ B or B $\times$ G, the G parent from a pure G brood, the B parent from a completely dominant
           B brood.

Table III gives a summary of the data of these matings.

TABLE III

Second Generation

Mating Category.	Grand-parents.	Parents.	No. Green Broods.	No. Black Broods.	No. of Mixed Broods	Total No. of Individuals.	Proportion of B : G in Mixed Broods.
A	B $\times$ B	G $\times$ G (m^1)	16	0	0	337	
B	G $\times$ G	G $\times$ G	4	0	0	98	
C	B $\times$ B	B $\times$ B (m^1)	0	5	25	740	1.2 : 1
D	B $\times$ B	B $\times$ B (cd^2)	0	1	0	22	But 1 brood reared.
E	G $\times$ G B $\times$ B	G♀ $\times$ B♂ or B♂ $\times$ G♀	0	1	15	384	3.6 : 1

    m^1 in parenthesis means that the individuals mated were from mixed broods.
    cd^2 in parenthesis means that the individuals mated were from broods in which B was completely dominant.

This data shows that the recessive greens (G) breed true under either condition A or condition B. The dominant blacks (B) produce, under condition C, either broods in which B dominates completely or mixed broods in which B dominates partially. Since but a single brood was reared under condition D, the data are insufficient. B $\times$ G produces either completely dominant B broods or mixed broods.

For third generation data five categories of matings were established as indicated by Diagrams 2, 3, 4, 5 and 6.

Diagram 2. Mating category A.

First matings............ B x B   B x B   B x B   B x B

First gen..... .... B-BG   B-BG   B-BG   B-BG

Second gen.............. G   G

Third gen.. . ... G
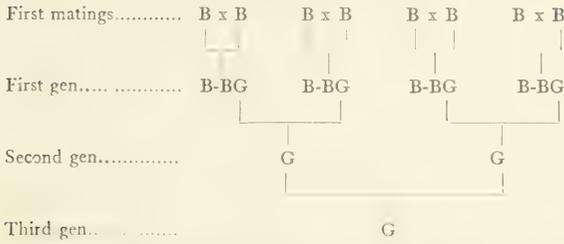
Diagram 3. Mating category B.

First matings... .... B x B   B x B   B x B   B x B

First gen.................. B-BG   B-BG   B-BG   B-B-G

Second gen............... B-BG   B-BG

G
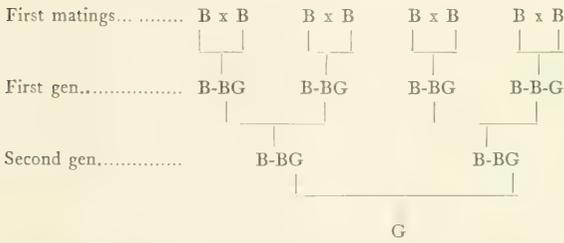
Diagram 4. Mating category C.

First matings. .... B x B   B x B   B x B   B x B

First gen.................. B-BG   B-BG   B-BG   B-BG

Second gen..... .... B-BG   B-BG

B-BG
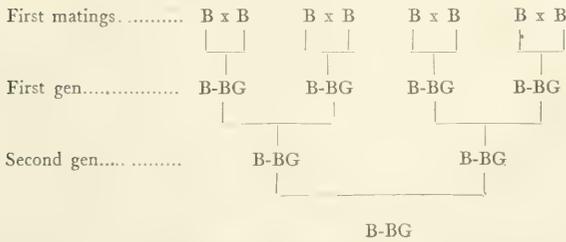
Diagram 5. Mating category D.

First matings............ B x B   B x B   B x B   B x B

First gen.................. B-BG   B-BG   B-BG   B-BG

Second gen............ .... B-BG   B-BG

B-BG

Diagram 6. Mating category E.

First matings............ B x B   G x G   B x B   G x G

First gen.................. B   G   B   G

Second gen.............. B   B

Third gen................. BG
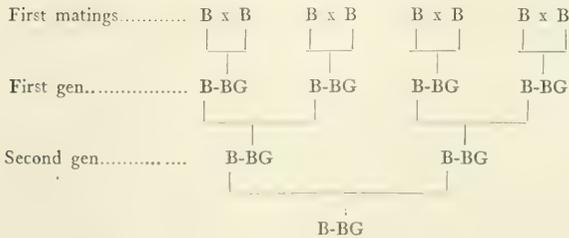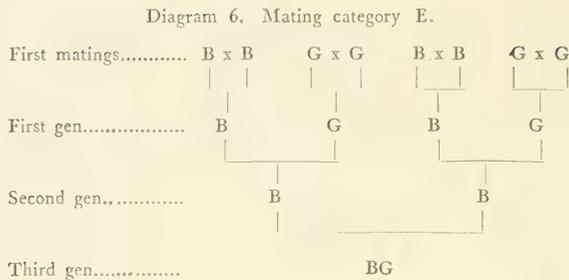
Inspection of these diagrams shows that the original black parents (B x B of the first matings, except in Diagram 6) may be considered hybrids inasmuch as they produce both black and green individuals.

Table IV gives a summary of the results of third generation matings.

TABLE IV

Third Generation

Ma-ting Cate-gory.	Great-grand-parents.	Grand-parents.	Parents.	No. Green Broods.	No. Black Broods.	No. Mixed Broods.	Total No. of Indi-viduals.	Proportion of B : A in Mixed Broods.
A	B × B	G × G	G × G	29	0	0	733	
B	B × B	B × B (m)	G × G	27	0	1*	653	
C	B × B	B × B (m)	B × B (m)	0	8	19	688	2.76 : 1
D	B × B	B × B (cd)	B × B (cd)	0	18	3	518	4.2 : 1
E	B × B \ G × G ∫	B × G	B × B (cd)	0	0	15	420	2 : 1

*One B individual in a brood of nineteen, otherwise G individuals.

Comparing categories A and B, we find, with the single exception of one individual, that broods of green individuals only are produced by G × G matings, whether there are one (Category B) or two (Category A) generations of green parentage.

Comparing Categories C and D, we find that under each of these conditions, that is, whether the B parents are chosen from broods in which B dominates completely, or from mixed broods,

two kinds of broods result, broods in which "black" dominates completely, and mixed broods. The comparative number of broods in which "black" dominates completely is, however, much greater in Category D than in Category C, and the comparative number of black individuals in mixed broods is much greater in Category D than in Category C. This shows a decided difference in the influence of the black character in these two categories.

In the fourth generation, ten categories of matings were established as indicated in Table V. This table gives a summary of the results of these matings.

TABLE V

Fourth Generation

Mating Category.	G-great-grand-parents.	Great-grand-parents.	Grand-parents.	Parents.	No. G'n Broods	No. Black Broods	No. of M'd Broods	Total No. of Indi'd.	Pro'n of B:G in Mixed Broods.
A	B × B	G × G (m)	G × G	G × G	17	0	0	457	
B	B × B	B × B (m)	G × G (m)	G × G	17	0	0	404	
C	B × B	B × B (m)	B × B (m)	G × G (m)	6	0	0	69	
D	B × B / G × G	B × G	B × B (m)	G × G (m)	8	0	0	322	
E	B × B	B × B (cd)	B × B (m)	G × G (m)	4	0	0	73	
F	B × B	B × B (m)	B × B (m)	B × B	0	5	8	250	5.5 : 1
G	B × B	B × B (cd)	B × B (cd)	B × B	0	9	0	199	
H	B × B / G × G	B × G	B × B (cd)	B × B (m)	0	0	3	52	3.8 : 1
I	B × B / G × G	B × G	B × B (m)	B × B (m)	0	2	8	293	5 : 1
J	B × B / G × G	B× B(cd) / G × G	B × B(cd) / G × G	B × G	0	2	1	76	4 : 1

Comparing Category D of Table IV with Category G of Table V we find, as in Lina lapponica, that by selecting individuals in which there is complete dominance, the recessive character is eventually eliminated. In other words, the fourth generation from hybrid parents, along the line of complete dominance, breeds true to the dominant character, no recessives appearing in the offspring.

Comparing Categories A, B, C, D and E in Table V we find G × G breeding true in the fourth generation whether the immediate parents only are green or there has been a lineage of green for one, two or three generations.

In the fifth generation, seven categories of matings were established as indicated in Table VI. This table embodies a summary of these matings.

<div align="center">TABLE VI</div>

<div align="center">*Fifth Generation*</div>

Mating Category.	GGG-[1] grand-parents.	GG-grand-parents.	Great-grand-parents.	Grand-parents.	Parents.	No. G'n Bd's.	No. Bl'k Bd's.	No. Mix. Bd's.	Total No. of Individ's.	Prop. of B : G in Mixed Broods.
A	B × B	G × G	G × G	G × G	G × G	32	0	0	1529	
B	B × B	B × B	G × G	G × G	G × G	24	0	0	679	
C	B × B	B × B	B × B	G × G	G × G	5	0	0	120	
D	B × B	B × B	B × B	B × B	G × G	14	0	0	370	
D	B × B	B × B	B × B	B × B	G × G	14	0	0	370	
E	B × B	B × B (m)	B × B (m)	B × B (m)	B × B	0	35	8	1336	8 : 5 : 1
F	B × B	B × B (cd)	B × B (cd)	B × B (cd)	B × B	0	15	0	397	
G	B × B	B× B(cd) ⎫ G × G ⎭	B× B(cd) ⎫ G × G ⎭	B× B(cd) ⎫ G×G ⎭	G × B	0	2	6	242	6 : 1

[1]The letter G in this connection indicates "great."

Data in Table VI shows G × G continuing to breed true in Categories A, B, C and D, that is, whether there has been a line of green parentage for four generations or the immediate parents only are green. Comparing Category F of Table V with Category E of Table VI we find an increasing proportion of broods in which black is wholly dominant and an increasing proportion of black individuals in the broods in which black is but partially dominant. In Category F, Table VI (compare Category G, Table V) black in the completely dominant line continues to breed · true.

In the sixth generation six categories of matings were established as indicated in Table VII. This table embodies a summary of the results of these matings.

TABLE VII

Sixth Generation

Mating Category.	GGGG-grand-parents.	GGG-grand-parents.	GGreat-grand-parents.	Great-grand-parents.	Grand-parents.	Parents.	No. of Green Broods.	No. of Black Broods.	No. of Mixed Broods.	Total No. Individuals.	Prop'n B : G in Mixed Broods.
A	B × B	B × B	B × B	G × G	G × G	G × G	35	0	1 B in br. of 30	1034	
B	B × B	G × G	G × G	G × G	G ♀ × B ♂ / B ♂ × G ♀	G × G	9	0	1 B in br. of 28 / 1 B in br. of 25	283	
C	B × B	B × B (cd)	B × B (cd)	B × B (cd)	B × B (cd)	B × B	0	18	0	475	28 : 1
D	B × B	B × B (cd)	B × B (cd)	B × B (cd)	B × B (cd)	B × B	0	5	6	349	16 : 1
E	B × B	B × B (m)	B × B (m)	B × B (m)	B × B (m)	B × B	0	8	6	419	
F	B · B	G × G	G × G	G × G	G × G	B × G	0	8	17	815	3 : 1

The data in Categories A and B show single black individuals in each of three otherwise green broods out of a total of forty-four broods, and a total of 1317 individuals of G × G parents. Unless this can be attributed to a mishap due to accident in the handling of breeding jars (a possibility where hundreds of breeding-jars are being daily cleaned and cared for) this is a return of latent B in normally recessive G. It will be noticed that such an exception to the general behavior in G × G matings was also recorded in Table IV (third generation).

Comparing data in Category F, Table VI, with data in Category D, Table VII, we find a discrepancy in the behavior of what was apparently pure B, that is likewise unaccounted for unless by accident or reversion. Table VII, Category D, records the presence of a recessive G (possibly a *latent* G in Castle's terminology)[1] in each of six broods from pure B parentage.

Otherwise the behavior of G and B in the sixth generation is consistent with their behavior in preceding generations in similar categories.

In the seventh generation, nine categories of matings were established, as indicated in Table VIII.

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[1]Castle, W. E., 1905: Heredity of Coat-color in Guinea pigs and Rabbits.   Carnegie Inst. Pub., No. 23.

TABLE VIII

Seventh Generation

Mating Cate-gory.	GGGGG-grand-parents.	GGGG-grand-parents.	GGG-grand-parents.	GG-grand-parents.	Great-grand-parents.	Grand-parents.	Parents.	No. of Green Broods.	No. of Black Broods.	No. of Mixed Broods.	Total No. of Individ.	Prop'n on B : G Mixed Broods.
A	B × B (m)	B × B (m)	G × G	G × G	G × G	G × G	G × G	71	0	0	2013	
B	B × B	B × B (m)	B × B (m)	G × G	G × G	G × G	G × G	9	0	0	223	
C	B × B	B × B	B × B	B × B	B × B	G × B	G × G	37	37	0	944	
D	B × B	G × G	G × G	G × G	C × B	G × B	B × B	0	0	49	1638	3.4 : 1
E	B × B	B × B	G × B	B × B	B × B(cd)	B × B (m)	G × G	34	0	0	976	
F	B × B	B × B (m)	B × B(m)	B × B(m)	B × B(cd)	B × B(cd)	B × B	0	10	0	333	
		B × B (m)	B × B(m)		B × B(m)	B × B(cd)						
G	B × B	B × B	B × B	B × B	G × B	B × B (m)	B × B	0	22	41	1678	4 : 1
H	G × G	G × G	G × G	G × G	B × B (cd)	B × B(cd)	B × B	0	21	0	559	
I	B × B	B × B (cd)	B × B (cd)	B × B	B × B (cd)	B × B(cd)	B × B	0	34	0	976	

Categories A, B, C and E show consistent behavior of recessives.

Category F shows the possibility of complete domination of B even from a line of partially dominant ancestry.

The proportions in Categories D and G remain consistent with proportions in corresponding categories of previous tables.

Table IX embodies a comparison of broods of seven generations reared successively from B × B in which the parents were partial dominants, that is, black individuals from mixed broods containing a larger number of black than green individuals. This table shows the proportion of B : G (of dominant to recessive) in successive generations, and the rate of increase from generation to generation of the dominant B.

This data shows a progressive dominance of B, which either reduces G to a latent in the seventh generation or wholly eliminates it.

TABLE IX

	Total No. of Broods.	Total No. of Individs.	Total No. Broods B Completely Dominant.	Total No. Broods B Partially Dominant.	Prop'n of B : G in Mixed Broods.
First generation (Table II)..............	41	1207	2	39	1 . 2 : 1
Second generation (Table III G)	30	740	5	25	1 . 12 : 1
Third generation (Table IV C)	27	868	8	19	2 . 76 : 1
Fourth generation (Table V F)	13	250	5	8	5 . 5 : 1
Fifth generation (Table VI E)	43	1336	35	8	8 . 5 : 1
Sixth generation (Table VII E)	14	419	8	6	16 : 1
Seventh generation (Table VIII F)[1]	10	333	10	0	All B

[1]Not absolutely, but practically, comparable with categories of other generations with which it is compared.

COMPARISON OF RESULTS IN LINA AND GASTROIDEA

The fact that in one species (Gastroidea) black is the dominant character, while in the other species (Lina) black is the recessive

character, shows that there is nothing in the character as such that makes for its actual behavior.

That green is the final color in the color-development of the individual in Gastroidea has already been pointed out. In the paper previously referred to, it was pointed out that black is the final color in the color-development of individuals in Lina lapponica. We have, therefore, a similarity of behavior in the establishment of the definitive color in the two-color series in each species. That is, we have in each species a primary color-condition through which all individuals pass and in which one series remain ("black". in Gastroidea, "spotted" in Lina). We have in each species a secondary color-condition into which some of the individuals pass and there remain ("green" in Gastroidea and "black" in Lina). It has been shown that it is the final or secondary color-condition that becomes the recessive character in each species.

If it could be shown that the primary or first condition into which each species enters represents the older or ancestral condition, we would then have in Lina and Gastroidea a condition found by Castle in *pigmented* versus *albino* and *long* versus *short-coated* guinea pigs; that is, a dominance of the ancestral or older character.

SUMMARY OF RESULTS

1. In Gastroidea dissimilis "black" is dominant, "green" is recessive. In Lina lapponica "black" is recessive and "spotted-brown" is dominant, though not typically so. In each species the dominant color is that appearing first in the color-development of the maturing adult, and the recessive color is that appearing last in the color development.

2. In each species the recessive character breeds true at once (with the possibility of the recurrence of the latent dominant). The dominant character breeds true in the third or fourth generation from a hybrid through the completely dominant line (with the possibility of a recurrence of the latent recessive).

3. In each species there is progressive or accumulative dominance from generation to generation through the partially dominant line.

In conclusion it is evident that under the conditions of the breeding experiments with Lina lapponica and Gastroidea dissimilis, the heredity of the alternate characters in dichromatic species differs materially from typical Mendelian heredity of alternate characters. In the latter, the relation between dominant and recessive characters is a perfectly stable one, assuming the definite numerical proportion of 3 : 1. In the former, there is apparently an actual *prepotency* of the dominant character that in the long run effectually eliminates or reduces the recessive character to a latent one.

LOCOMOTION OF AMŒBÆ AND ALLIED FORMS

BY

ORIS P. DELLINGER

WITH TWO PLATES AND TWENTY-NINE FIGURES IN THE TEXT

Few subjects are more fundamental or have been more assiduously studied than the movements of the Amœba. The earlier writers maintained that the explanation of these movements was to be found in the contractility of protoplasm. M. Schultz, Brücke, DeBary, Kühne, Haeckel and others held this view. They did not fully analyze their theories, however, although Brücke did postulate an internal contractile framework to account for the streaming of protoplasm.

Early in the discussion, objections were raised to the contractile theory. Wallich ('63) called attention to the fact that the currents of protoplasm in Amœbæ did not begin at the posterior end or in the interior but that they commenced at the point of advance and extended backward. Bütschli, Hofmeister, Nägeli and others made similar observations and on that account relinquished the contraction theory. All these observations were made by looking down on the Amœba from above, while if it is viewed from the side, the difficulties can be readily explained.

BERTHOLD'S THEORY

Many other theories followed, none of which need be mentioned until Berthold's. In the year 1886, he advanced the opinion that the protoplasm of Amœbæ behaves much the same as a drop of fluid which is spreading on a solid surface. If a drop of inorganic fluid is caused to adhere more strongly at one side than the other, it will roll toward the more adherent side. He believed this movement to be exactly what we have in Amœbæ. Jennings ('04, p. 208) says: "By a proper arrangement of the conditions

almost every detail of amœboid locomotion may be clearly imitated''
(by a drop of fluid).   Berthold further compares the movements
to a drop of water fleeing from a rod wet with ether; but, as
Bütschli points out, the character of the movements here is entirely
different from those of a drop adhering to a solid surface.
Bütschli, in discussing Berthold's view, says (p. 190): "I consider
it as incorrect to suppose that Amœbæ really adhere to the solid
substratum.   I do not entirely dispute the fact that local adhesions
at the hinder end, or occasionally also in the pseudopodia during
their retraction, may come under observation.   On the other
hand, I consider it certain that an extensive adhesion is absent.''
Berthold was obliged to offer some other explanation to account
for the protrusion of a free pseudopod, as in that case there is no
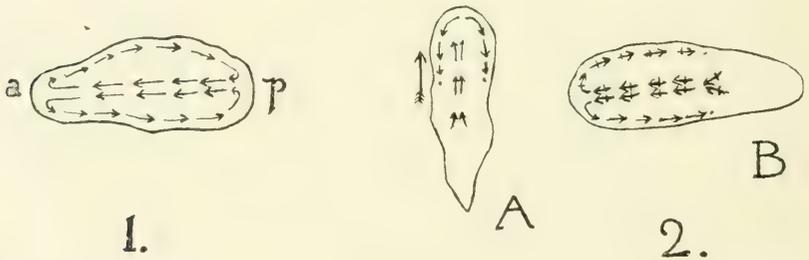

Fig. 1.   Diagram of the currents in a drop of clove oil in which the surface tension is lessened
on one side.   The drop elongates and moves in the direction of *a*.   (After Jennings.)

Fig. 2.   Diagram of the currents in a progressing A. limax.   A, top view; B, side view.   (After
Rhumbler from Jennings.)

surface to which to adhere.   He explained it by a contraction
theory.   We shall see later that neither form, currents, nor
adhesion at the point of advance, required by Berthold's theory
are present.

SURFACE TENSION THEORY OF BÜTSCHLI AND RHUMBLER

Bütschli ('92) and Rhumbler ('98) followed with the surface
tension theory.   According to these two authors, and also many
others, the Amœba is a drop of complex fluid which moves about
as a result of local changes in surface tension.   The currents in
such a drop are forward in the central axis and backward along
the surface (Fig. 1).   Fig. 2 will show the same currents in the

Amœba, according to Rhumbler. It is evident that this theory would explain the putting out of free pseudopods, but, as Bütschli points out, it would not account for the very fine pseudopods of actinosphærium-like forms. Unfortunately for their theory the necessary currents are not present. Even Bütschli calls attention to the fact that he could not always observe them. Neither is the Amœba the shape of a drop of fluid acting under changing surface tension and, what is more to the point, there are definite points of attachment. As Jennings points out, this theory would not explain many phenomena of the free pseudopods.

MOVEMENTS OF AMŒBA AS DESCRIBED BY JENNINGS

Great credit is due Jennings ('04) for the careful experiments and observations by which he throws doubt on the surface tension theory of Rhumbler and Bütschli, and brings forward his contraction theory. For this reason a careful review of his work will be taken up before going on to my own observations and experiments.

Jennings bases his view on the following:

(1) Experiments and observations to determine what movements take place.

(2) Observations as to the shape of advancing Amœbæ.

(3) Belief that the anterior edge is closely applied to the substratum.

(4) Observations which seem to indicate contractility of the ectosarc.

His experiment consisted in mixing soot with water in which Amœba verrucosa was moving. Particles of the soot became attached to the surface of the Amœba and by watching these, he could distinguish what currents were present. He sums up his results as follows:

"In an advancing Amœba substance flows forward on the upper surface, rolls over at the anterior edge, coming in contact with the substratum, then remains quiet until the body of the Amœba has passed over it. It then moves upward at the posterior end, and forward again on the upper surface, continuing in rotation as long as the Amœba continues to progress. The motion of the upper surface is congruent with that of the endosarc, the two forming a single stream" (Fig. 3).

According to his description, Jennings derives the shape of an advancing Amœba from two observations.   These are as follows :

"In an Amœba that was creeping on the lower surface of a cover glass, I was able to define with some accuracy the parts that were attached and those that were not.   A small flagellate was moving briskly about between the Amœba and the cover glass but its excursions were limited by a visible line running parallel with the anterior edge of the Amœba and extending at the sides back to about one-third the animal's length from the rear.   The zone between this and the margin was pressed close to the glass, and was evidently attached to it.   The more pointed posterior end was held quite away from the glass, leaving a broad passage-way through which the flagellate finally escaped.

"The results of this observation were confirmed by another.   An Amœba verrucosa in full career was suddenly turned on one lateral edge by a strong current from a rotifer, and its upper edge coming in contact with the cover glass, it remained in that position some time without change of form.   It could be seen that the under surface was concave, the edges very thin and flat, while the posterior portion was thick and arched" (p. 145).
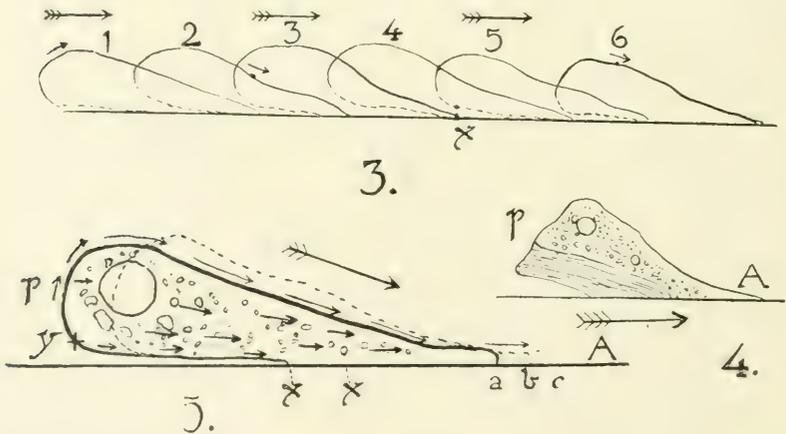
Fig. 3.   Diagram of the movements of a particle attached to the surface of A. verrucosa in side view.   As the Amœba moves forward from position *1*, the particle moves forward to the positions shown in *2, 3, 4, 5* and *6*.   (After Jennings.)

Fig. 4.   Jennings' conception of the shape of an advancing A. verrucosa, seen from the side.   The anterior edge is thin and applied closely to the substratum while the posterior end is raised high in the water.   The Amœba is attached only by the anterior third to the substratum.   (After Jennings.)

Fig. 5.   Shape of advancing Amœbæ in general.   (After Jennings.)

From these observations Jennings concludes that the form of the advancing Amœbæ verrucosa is that given in Fig. 4, and of Amœbæ in general, that of Fig. 5, in which we see the thin anterior edge applied closely to the substratum while the thick posterior end is raised high above it. On the same page he gives a diagram of an advancing anterior edge and on the preceding page, the following observation and conclusion:

"A certain feature of the advance of the anterior edge seems of much significance. Each wave seems to arise just behind the previous anterior boundary line and overlaps it, leaving it buried. This line often remains visible for a short time after the new wave has been formed. The new wave rolls over the preceding one in such a way that its original upper surface becomes applied to the substratum. This is demonstrated by the rolling under of small objects on the upper surface of the advancing wave. A diagram of the movement at the anterior edge is given in Fig. 45. The movements can be imitated roughly by making a cylinder of cloth, laying it flat on a plane surface, and pulling forward the anterior edge in a series of waves. The entire cylinder then rolls forward just as the Amœba does.

"The essential features of the movement seem to be; (1) the advance of the wave from the upper surface at the anterior edge; (2) the pull exercised by the wave on the the remainder of the upper surface of the body, bringing it forward. Most of the other phenomena follow as consequences of these two. The flowing forward of the granules of the endosarc seems to demand no special explanation, since a fluid containing granules within a rolling sac must necessarily flow forward as the sac rolls. By the movement forward of the anterior end a space is left free; by the rolling forward of the posterior end the fluid is piled up and pressed upon and must flow forward into the empty space in front. Possibly there may be other causes at work in producing the endosarcal currents, but such currents would be produced without other cause in a sac moving as Amœba does."

From the above it will be seen that Jennings' conception of an Amœba is an elastic sac filled with a fluid. If we give the sac contractility, which from observations (p.147) he has a perfect right to do, we have the Amœba from which he derives his theory of the movements.

Further discussion of his paper will follow my own observations and experiments, but I may add that, although I can confirm some of his observations, I think, had he studied good side views of his specimens, he would have reached far different conclusions.

AMŒBÆ AND DIFFLUGIA STUDIED FROM ABOVE AND FROM THE SIDE

In connection with a comparative study on the cilium, it was necessary for me, as many students of contractile protoplasm have done, to begin with the pseudopod of the Amœba. Before I had proceded far, it became evident that I must repeat Jennings' ('04) observations; the more so because a study of prepared slides of the pseudopod led me to doubt his conclusions as to the structure of Amœbæ.

I did not confine myself to Amœbæ but gave considerable attention to Difflugia, Actinosphaerium and Euglena. Difflugia is practically an Amœba in a shell, and has pseudopods that move in a particularly definite way. Apparently little attention has been paid to this form. Jennings ('04) and Penard ('90) refer to it, the latter calling attention to the activity of its pseudopods.

It was necessary to answer the following questions before I could proceed:

1. How does an Amœba form a pseudopod?
2. What evidence have we for a contractile substance in Amœba and how is this substance distributed?
3. How does an Amœba attach itself?
4. What determines its form while in motion?
5. What causes Amœbæ to advance in a certain direction?

My first observations were made in the usual way by watching the forms from above. I used Difflugia acuminata, D. spiralis, Amœba proteus, A. limax, A. verrucosa, A. radiosa and several small forms of Amœba which I did not determine. Amœba proteus was especially well suited to my purpose, as it was large enough to be easily seen with the unaided eye.

Difflugia

Difflugia acuminata extends a slender pseudopod to its full length free in the water. That it is free is evident from its motions. The pseudopod then becomes attached at its tip (Fig. 6) and contracts, drawing the mouth of the shell up to the point of attachment. A new pseudopod appears *pari passu*, generally

near the base of the first (Fig. 6), and increases in length as the first is contracted. The new pseudopod, which is fully extended when the mouth of the shell reaches the point of attachment, swings into the line of advance and becomes attached. The cycle is then repeated. Figs. 6 and 8 show the position of the
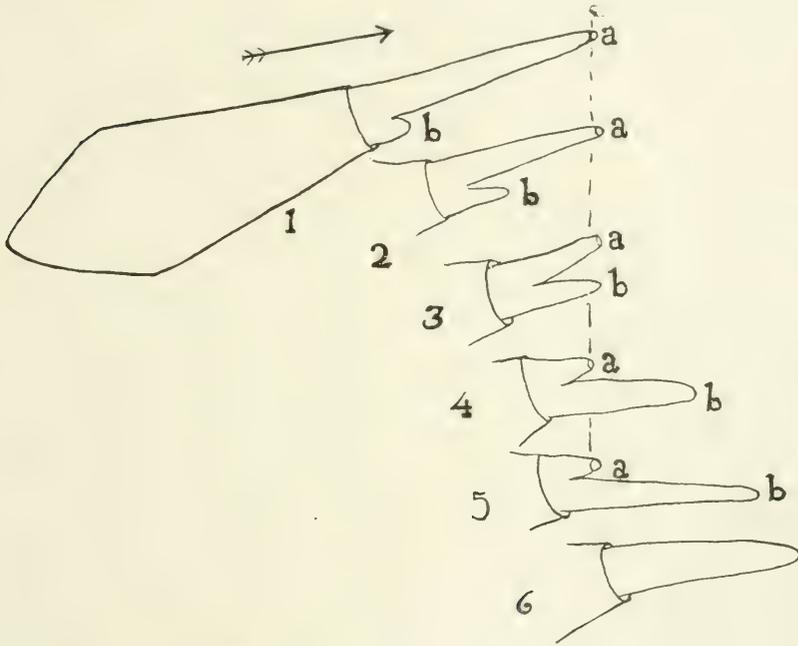

Fig. 6. Diagram of Difflugia acuminata showing changes from one pseudopod to another. *a, a, a, a, a* show points of attachment; *b, b, b, b, b,* the new pseudopod at different stages of its formation. (The diagram was made with camera lucida by drawing the paper at right angles to the line of advance.)

mouth and pseudopod at different stages in the change from one pseudopod to another. Fig. 7 shows four successive positions of the shell at the time when a pseudopod becomes attached, and also the lines of advance of the shell and of the tips of new pseudopods as they form and are carried forward. That the pseudopods while free take no part in drawing the shell forward is evident from the figure.

Often Difflugia acuminata apparently moves with a single pseudopod, in which case the new pseudopod is extended directly

over the tip of the old.   I obtained evidence to prove this from a specimen in which the second pseudopod appeared at the side instead of over the end of the first (Fig. 9).   It is evident that the
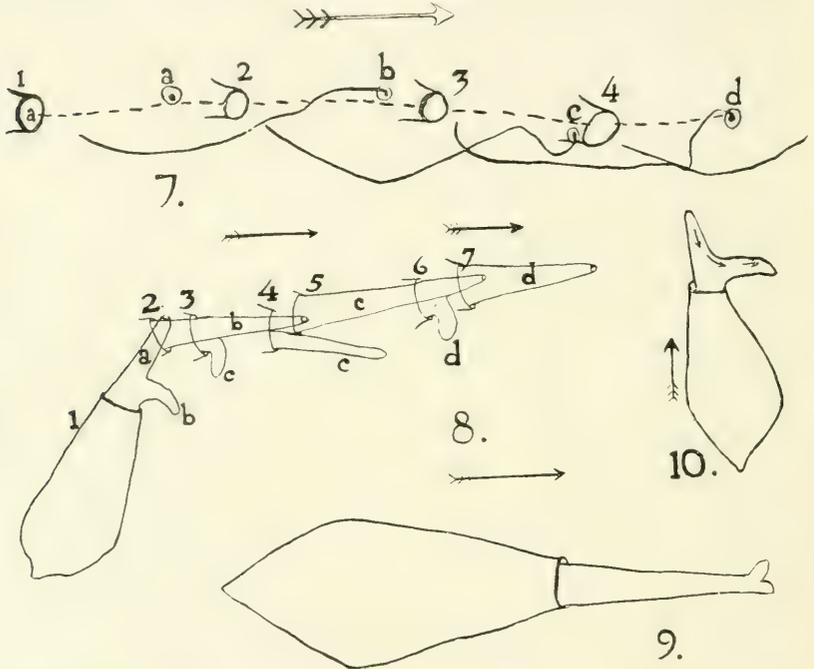

Fig. 7.   Diagram showing position of mouth at time the pseudopod was attached and the lines of advance of mouth and of forming pseudopods.   *1,2,3* and *4* show positions of mouth when attachments *a,b,c* and *d* are made.   Dotted line indicates advance of the mouth and the solid lines follow the tips of the forming pseudopods.   (Camera lucida.)

Fig. 8.   Diagram of D. acuminata as it advances.   At position *1,a* is attached; at *2,b* is attached and *c* is appearing; at *4*, the mouth of the shell is well up to the point of attachment of *b* and *c* is long; at *5*, *c* is attached; at *6*, *c* has contracted and *d* is appearing; at *7*, *d* has become attached.   (Camera lucida.)

Fig. 9.   D. acuminata with new pseudopod appearing near the tip instead of near the base.

Fig. 10.   Diagram of currents in advancing D. acuminata.   The substance of the contracting pseudopod flows over into the new pseudopod.

last two methods of locomotion are but variations of the first. The movements were sometimes complicated by combining these methods.

The particles in the contracting pseudopod flowed around into the new pseudopod (Fig. 10), giving the exact picture of the flow

of particles in retracting and protruding pseudopods of Amœbæ. Another point of resemblance was the shrunken and wrinkled appearance of the pseudopod as it contracted.

Difflugia spiralis carries its shell when in motion much as a snail does, and thus the mouth and base of the pseudopods are not visible unless viewed from the side. Yet watching it from above one sees the end of a pseudopod suddenly appear, wave about for a time, and then become attached. The shell then moves over it, at the same time another pseudopod appears and the above is repeated.

Amœbæ—Absence of Rolling Movement in all Except Amœba Verrucosa

My first experiment with the Amœba was the one Jennings performed with lampblack. I tried at first Amœba proteus, A. Limax and some smaller Amœbæ which I did not determine. In these forms I never obtained his results, although I repeated the experiment many times. The attached particles would merely oscillate back and forth a little but never revolved as he describes (Fig. 11). The position of the particles on the Amœbæ made no difference. I watched particles attached to the anterior end, to the posterior end, and to the middle, but never found any revolution.

I next experimented with Amœba verrucosa. The particles became attached much more readily in this form and I found the currents exactly as Jennings describes them. Diatoms, granules and particles of all kinds passed to the anterior end, over the edge, then stopped until the Amœba passed over them, when they were picked up and the above repeated (Fig. 3).

There may be rotation in other species but I am convinced that it is not common. Evidence for this will appear later in the paper.

I next gave my attention to the direction of the currents of protoplasm. By watching the granules it is easy to tell just what the currents are. In general the granules flow forward rapidly in the middle and spread out in all directions at the

anterior end. Some of the granules at the border stop while those in the center flow on. These may remain quiet for some time and then enter the current, or some may not enter the current until the body of the Amœba is almost past, when they enter at the posterior end. The large granules of the endosarc oscillate back
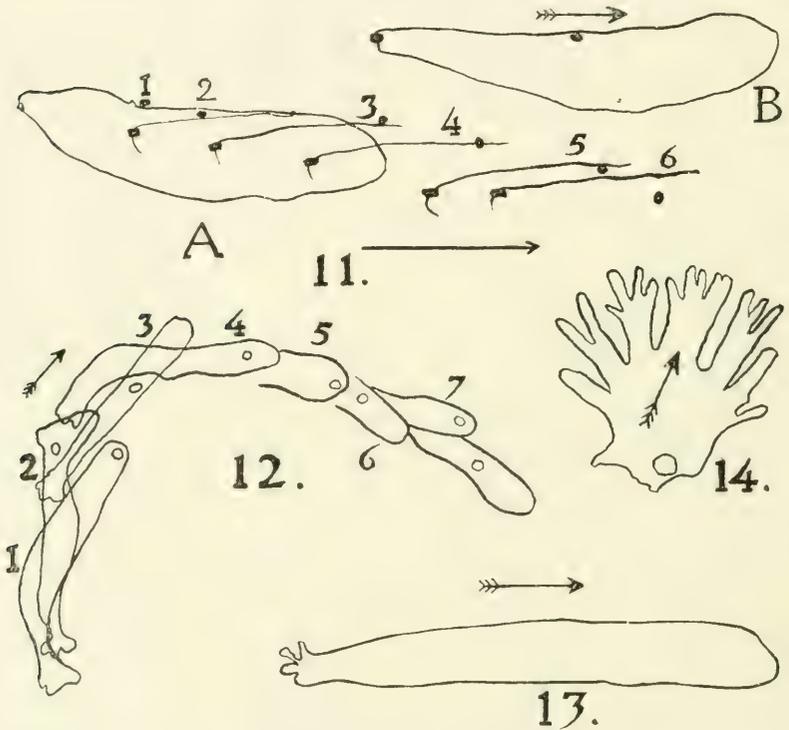
Fig. 11. *A*, diagram showing position of the particles of lampblack on an advancing Amœba at different stages of the advance. *B*, the same Amœba twenty minutes later. During all that time they had oscillated back and forth but had kept nearly the same position. (Camera lucida drawing.)

Fig. 12. Diagram showing movements of a large particle in the endosarc during different stages of the advance. (Camera lucida drawing.)

Fig. 13. Diagram of A. proteus moving in a clear field.

Fig. 14. Diagram of A. proteus moving in the presence of small algæ on which it is feeding.

and forth but keep the same relative position (Fig. 12). The currents at the anterior end are like those in a pseudopod formed free in the water. The granules do not flow forward in straight lines but zigzag along as if flowing around obstacles. In

Amœba verrucosa there are definite streams and channels which anastomose, and the flow reminds one of the flow of corpuscles in a frog's web. Diatoms flow forward with the other granules if lengthwise with the currents. If lying across the channels they appear to be caught and move with the Amœba itself.

In Amœba verrucosa there is also a peculiar jerking of the particles at the posterior end at regular intervals, as if at this point there were regular flows forward. I was unable to explain this while studying them from above but a side view revealed the cause.

The anterior end of Amœba verrucosa is very regular in contour and advances evenly in all directions. Here again it is an exception, for in all other species studied the anterior end advances by a series of lunges, which are never directly forward but alternate from side to side of the direct line of advance. When there are many pseudopods put out in front, as is often the case, the one through which the Amœba advances alternates from side to side. This phenomenon probably accounts for the absence of rolling of the ectosarc in these forms.

The various shapes of advancing Amœbæ find an explanation in the reactions of the animals to food. If Amœbæ are traveling in a clear field or moving from one mass of debris to another, they move by long loops (Pl. II, Figs. 7, 8, 9) or maintain a long, slender form and are attached at several points (Fig. 13 and Pl. II, Fig. 10). When moving and feeding on algæ they reach out many pseudopods and then present a palmate form (Fig. 14, and Pl. II, Fig. 13).

Other observations made were:

1. The shape and wrinkled appearance of the posterior end indicate that the substance is contracting in this region.

2. The particles in Amœbæ that are rolled along retain their relative position and do not flow as in an advancing Amœba. This observation is easily made by rolling an Amœba about with a rod. This gives good evidence for two things: First, that the Amœba is not an elastic sac filled with a fluid, which if rolled along would produce the endosarcal currents (Jennings, quoted above). The flow of the particles in the endosarc must be due to something else. Second, that the endosarc has a definite struc-

ture that holds the particles firmly, however much the Amœba is rolled about.

There is no question as to the methods of movement in Difflugia. A slender pseudopod reaches out, attaches at the tip, and contracts, drawing the shell forward. A new pseudopod is formed by an expulsion of the substance from the contracting pseudopod. The new pseudopod is full length when the mouth of the shell reaches the point of attachment of the old and swings in to the line of advance and attaches. The above cycle is then repeated.

The similarity of the two forms suggests that Amœbæ move by the same general method. The movements of an unidentified Amœba furnishes some support for this view. This form extends a slender pseudopod free in the water, attaches it by the tip and draws the rest of the body up to this point. It then establishes a point of attachment close behind the first, and freeing this, the above is repeated. My observations before obtaining a side view indicated that Amœba proteus moves in the same way.

STUDY OF AMŒBÆ AND DIFFLUGIA FROM THE SIDE

The discussions of Amœbæ for the past fifty years call to mind the story of the two knights and the shield. If a single observation of an Amœba accidently turned on its side by a passing rotifer has given ground for a theory of the shape, attachment, and movements of Amœbæ, it becomes clearly necessary to devise some means by which Amœbæ and similar forms can be studied in side view. Such a view will give points of attachment and support, and upon these depends an understanding of the strains and contractions which produce the movements both for advance and for the formation of pseudopods. So far as I can find, no mention of the study of Amœba in this way is to be found in the literature.

The apparatus devised is very simple. One edge of an ordinary slide is ground square and polished. Long cover slips are cemented to this with the edges extending beyond the polished surface so as to form a narrow trough (Fig. 15). With the microscope

brought to a horizontal position, specimens pipetted into the trough move along the edge of the slide and are easily observed in side view.[1]

Difflugia

Difflugia spiralis moves exactly like D. acuminata (Fig. 16); that is, it extends a pseudopod free in the water, attaches it near the tip (*1*, Fig. 16) and draws the shell forward. As the shell is drawn forward a new pseudopod appears (*2*, Fig. 16), grows in length as the first is shortened (*3*, Fig. 16), is full length when the mouth of the shell reaches the point of attachment, and is then
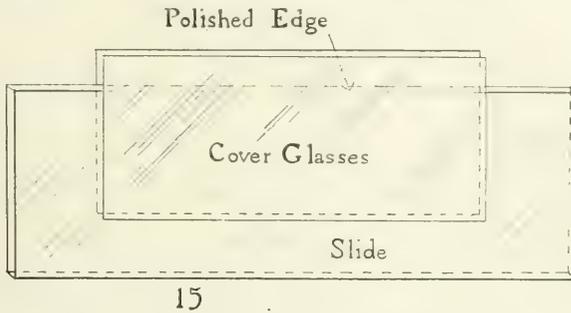
Fig. 15. Diagram of apparatus designed to study Protozoa in side view.

swung into the line of advance and attached. The attachment of the pseudopod in both cases is near the tip and is definite.

When Difflugia moves with one apparent pseudopod, as described above, the new pseudopod is put out as a direct continuation of the old. It is always short and the points of attachment are closer together than when moving with two pseudopods (Fig. 17).

Difflugia spiralis often creeps on a vertical surface. At such times the points of attachment can be easily demonstrated by jarring the table. The unattached parts vibrate while those attached move with the glass. This same form often creeps on the ceiling (Fig. 18), and then the movements are the same as when on the floor.

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[1] The cover slips were cemented with varnish and baked in an oven while closely pressed to the slide. Only a trace of cement was used and great care exercised to prevent any of it reaching the polished surface.

Amœbæ

Advancing Amœba verrucosa is attached at the anterior and posterior ends and is free everywhere else (Fig. 19). The anterior end is extended along the substratum but is apparently free
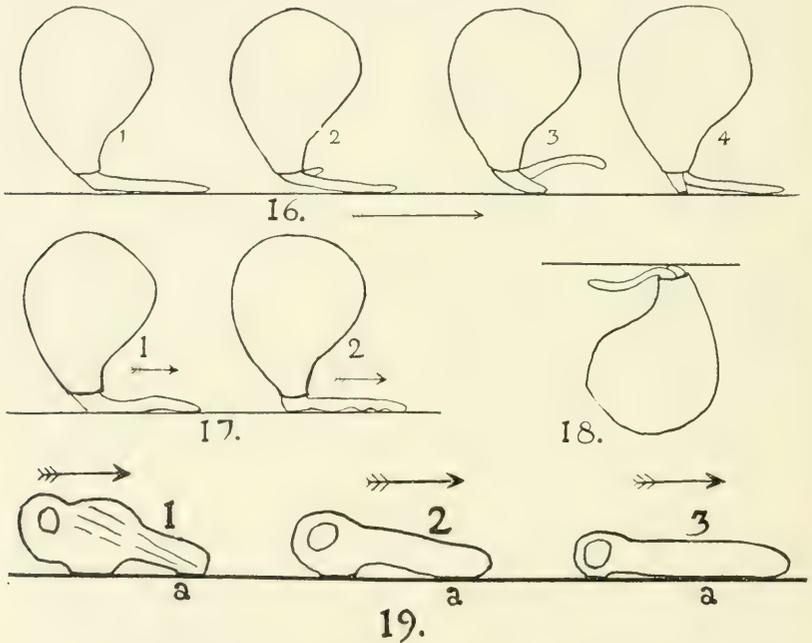
Fig. 16. Diagram of D. spiralis changing from one pseudopod to another. At position *1*, the first pseudopod is attached; at *2*, a new pseudopod is appearing; at *3*, the mouth of the shell is well up to the point of attachment of the first and the new pseudopod is reaching over to attach; at *4*, the new pseudopod is attached.

Fig. 17. D. spiralis moving with one pseudopod.

Fig. 18. D. spiralis creeping on the ceiling.

Fig. 19. Side view of advancing Amœba verrucosa showing the various shapes it assumes at different stages of advance. At position *1*, a new attachment has just been formed at the posterior end and it is quite thick. At position *3*, the Amœba is just ready to form a new attachment at the posterior end. It is seen that the anterior end is not thin, and although put out along the substratum is not attached except at definite points.

from it. It is thick and blunt (Fig. 19, and Pl. I, Figs. 1, 2, 3 and 4). There is a sudden flow of the substance forward when the attachment at the posterior end is released and the Amœba in this region becomes much thicker (*1*, Fig. 19, and Plate I, Fig. 1).

This accounts for the jerking motion noted when observed from above. The flow of the substance forward is due to the pull from the attachment at the anterior end and contraction of the posterior end. As the substance flows forward the posterior end becomes thinner and is drawn up to the point of attachment (*2, 3,* Fig. 19). A new attachment is then formed at the anterior end and the one at the posterior end is released. The strain due to the pull from in front causes the sudden flow forward. The old attachment at the anterior end becomes in part the new attachment at the posterior end, but when the sudden flow forward occurs, the attached surface is extended back much farther.

It is difficult to understand how Jennings arrived at the conclusion that the anterior end is thin. On the other hand the observations from which he derives the shape, and on which he bases his view as to the thickness of the posterior end and point of attachment, are easily explained. If the Amœba is seen in the stage of its movement shown in Fig. 19, the posterior end is thick but the anterior end is not thin, neither is it the only point of attachment. The escape of the flagellate, which he describes might be explained by the change of attachment at the posterior end, or by the Amœba being jarred loose (Pl. I, Fig. 4).

Admitting the shape as he gives it, it does not seem to me that he explains the endosarcal currents. It is true they would occur in a sac rolling on the floor, but unfortunately for his explanation the sac he observed was hanging from the ceiling, in which case the particles should fall down toward the posterior end instead of flowing forward as he finds them. If Fig. 4 is inverted we have the Amœba as he saw it.

All other forms of Amœbæ advance much more like Difflugia. They extend the anterior end free in the water and attach it at or near the tip and then contract. At the same time the posterior end is contracting and the substance thus pushed and pulled forward goes to form the new anterior end. This continues as long as the Amœba advances (Figs. 20, 21, 22 and 23, also Pl. I, Figs. 6, 7 and 8). Often the anterior end is pushed along the substratum but no attachments form except at definite points.

In other cases the anterior end is lifted free and then curves

down to the substratum and attaches, forming a long loop (Pl. II, Figs. 1 to 10). The posterior end is then released and the substance flows over to the anterior end. At the same time another anterior end is extended. Amœba proteus frequently uses this method (Fig. 24) and a small undetermined form appears to move
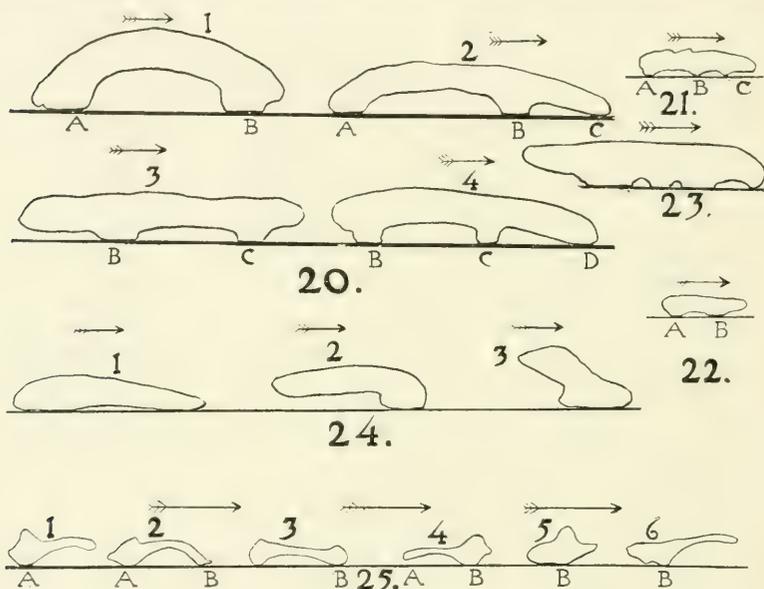
Fig. 20. A. proteus, side view showing shape and points of attachment and the method generally used by this form in advancing. At position *1*, the Amœba is attached at *a* and *b* and forms a long loop; at position *2*, a new anterior end has been extended; the points of attachment at *a* and *b* are well defined and the substance is drawn up into posts; at position *3*, attachment *a* has been released and a new attachment formed at *c*; a new anterior end has been pushed out at *4* and brought to the substratum and attached. The Amœba continues this as long as it advances.

Figs. 21 and 22. Side views of small undetermined Amœba showing points of attachment.

Fig. 23. Side view of A. limax showing points of attachment.

Fig. 24. Side view of A. proteus showing how it moves by long loops.

Fig. 25. Side view of the small Amœba referred to above, showing how it loops along in changing from one point of attachment *a*, to another *b*. At *2*, attachment *b* is just forming; at *4*, attachment *a* is released.

in this way altogether (Fig. 25). The points of attachment are always well defined and never extensive (Fig. 20, and Pl. I, Figs. 6, 7 and 8). Sometimes they are far apart, the Amœba forming a long loop, or they may be close together (Fig. 26).

The substance is often drawn up into well defined posts. The attachments are formed quickly and at no time was there any evidence that a viscid substance is present. An Amœba was jarred loose from the slide by striking the table when the parts of the body that had been in contact were easily seen. The surface at these points was perfectly flat and looked as if it had been planed off.

The granules at the anterior end move outward in all directions but never backward. At the posterior end the granules flow
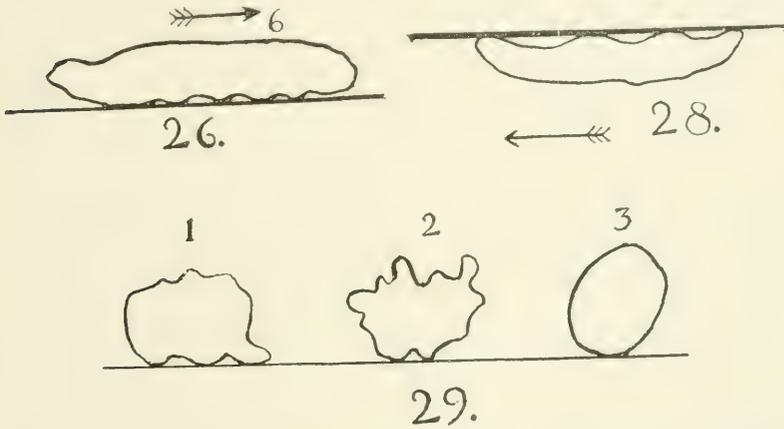
Fig. 26. A. proteus, side view, showing points of attachment close together. The anterior end is just reaching out to form a new attachment.

Fig. 28. Side view A. proteus creeping on the ceiling.

Fig. 29. A. proteus at rest showing points of contact. The Amœba does not seem to touch with an extensive contact.

forward in a steady, even stream. The currents are really the same as seen from above, the upper and lower surface then becoming the lateral edges. Particles attached to the surface oscillate but are never carried around the Amœba.

An Amœba proteus covered with carmine granules was advancing in the usual way. The particles moved along with the Amœba, keeping the same relative position. Two particles almost opposite each other on the upper and lower surface of the advancing anterior end kept the same distance from the tip as it advanced. The one on top never rolled over and passed to the under side.

Particles attached to the tip would sometimes pass to the upper surface. At no time did particles move along the upper surface to the anterior end and then pass underneath. On the other hand, particles on the under surface flowed along with particles on the upper surface. I am convinced that in species studied there was no flowing of the upper surface over the anterior edge to the under side.

Amœbæ often creep on a vertical surface or, by inverting the cell, on the ceiling, and at such times the shape is the same as when creeping on the floor (Fig. 28). A large Amœba proteus creeping on the ceiling was jarred loose and tried to attach itself again. It had partly succeeded when it was loosened at the new attachment. In rounding up it then went through the stages shown in Fig. 27. The entire process took about fifteen minutes. If the
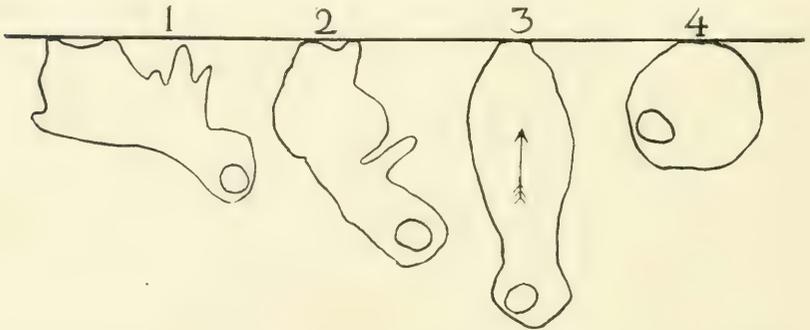
Fig. 27. Side view of A. proteus that was jarred loose from the ceiling at all but two points of attachment, which were near the anterior end. Shapes it assumed in rounding up. It hung in position *3* for ten minutes during which time all the large granules in the endosarc kept the same position.

Amœba is a sac filled with a fluid in which the endosarcal particles are suspended it seems that these particles would settle to the bottom. This was not the case. Since particles do not flow as they would in a sac, we must assume a more definite structure in the endosarc. Whatever structure is assumed must account for the behavior of the endosarcal particles while the Amœba is moving and at rest. In other words, the endosarc must be so constituted as to allow the particles to flow at one time and to hold them securely at another.

Other observations were:

1. Amœbæ at rest do not touch with an extensive contact (Fig. 29).

2. Amœbæ often change form without advancing. They do this in the presence of Paramœcia and appear to be fishing for them.

3. Amœbæ when rolled about, or when starting to move, after a rest, extend pseudopods in all directions. Difflugia when turned on its back will do the same. In either case as soon as a pseudopod becomes attached the animal moves off in the direction of the point attached.

CONCLUSIONS

What answer to the questions asked at the beginning of this inquiry are to be found in the above observations?

1. Advancing Amœbæ at no time approximate the shape of a drop of fluid adhering more closely at one side than the other. Neither do they behave like a drop acting under changing surface tension. I think we are warranted in saying also that they are not sacs of contractile ectosarc rolling about. Such an assumption would not explain the phenomena in moving Amœbæ as we have found them from a side view.

2. The definiteness with which Difflugia extends a pseudopod, swings it about, brings it into the line of advance and attaches it; the manner in which the shell is drawn up to this point of attachment; and the creeping about over a vertical surface and the ceiling, indicate that in this form there is a contractile substance.

3. In advancing Amœbæ the movements are essentially those of Difflugia. Often they are exactly the same. The anterior end is extended free in the water and attached. There is then a contraction of the substance back of this point and a flow of the substance toward the anterior end results. The movements of Amœbæ are due to the presence of a contractile substance.

From observation we are warranted in assuming that such a substance is present.

4. The granules of the endosarc do not flow as if they were suspended in a fluid in a contractile sac. On the contrary they are held in definite positions under conditions that would indicate that the endosarc has definite structure. A coarse reticulum of contractile substance distributed through the endosarc would account for the phenomena as we have observed them.

I wish to acknowledge my indebtedness to Dr. C. F. Hodge, under whose direction the research was made, for the many suggestions and the encouragement given me. Also to Mrs. A. Forrest Dellinger for help in preparing the drawings and plates.

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DESCRIPTION OF PLATES

Plates I and II are photographs of living Amœba taken with B & L $\frac{2}{3}$ objective and No. 1 eyepiece All but Nos. 10 and 13 in Plate II are of the same Amœba.

PLATE I

Fig. 1. Amœba verrucosa, side view. Just after attachment is formed at the posterior end. The Amœba is thick at the posterior end and has a definite point of attachment in that region. The anterior end is also attached and the lines observed from above are seen to run to the point of attachment at the anterior end.

Figs. 2 and 4. A. verrucosa, side view. In Fig. 4 the Amœba is just ready to form a new attachment at the posterior end. It is noticed that the anterior end is not thin but is thick.

Fig. 3. A. verrucosa side view. Was creeping on the ceiling and was jarred loose except at the anterior end. The anterior end is rounded and is not thin.

Fig. 5. A. verrucosa, top view.

Fig. 6. A. proteus, side view. The Amœba is attached at a and b and the anterior end is being advanced to form a new attachment. Note the definiteness of the points of attachment.

Fig. 7. A. proteus, side view. The Amœba is attached at a and b. The substance at b is drawn up into a well-defined post. The anterior end is coming to the substratum to form a new attachment. It moved from c to d while the plate was being exposed. A new pseudopod is appearing at e.

Fig. 8. The same Amœba a few seconds later. e has grown in length. Attachment at a is releasing and another has been formed at f.

PLATE II

Figs. 1 to 6. A. proteus, side view. Changing from one attachment to another. At position 1, the anterior end has reached over to attach. At 2, the attachment at the posterior end is released. At $3,4$ and 5 the substance is flowing over to the anterior end. At 6, the substance is well over, and the Amœba is putting out a new pseudopod at the anterior end.

Figs. 7, 8 and 9. Amœba proteus, side view. Moving by long loops. The Amœba has just brought the anterior end to the substratum but has not attached it. It slides along and is not attached until the position in Fig. 9 is reached. The substance then flowed over to the anterior end as in Figs. 1 to 6. Note the smallness of the pseudopods at the posterior end on which the Amœba is resting.

Fig. 10. A. proteus, top view. Showing form taken when moving in a clear field. The Amœba in this photograph is attached at the anterior and posterior ends and probably forms a loop.

Figs. 11 and 12. A. proteus, side view. Upon small pseudopods, which is often the case. A new anterior end is being extended in 11 and is brought to the substratum and attached in 12.

Fig. 3. A. proteus, top view. Moving in the presence of algæ, with numerous pseudopods at the anterior end.
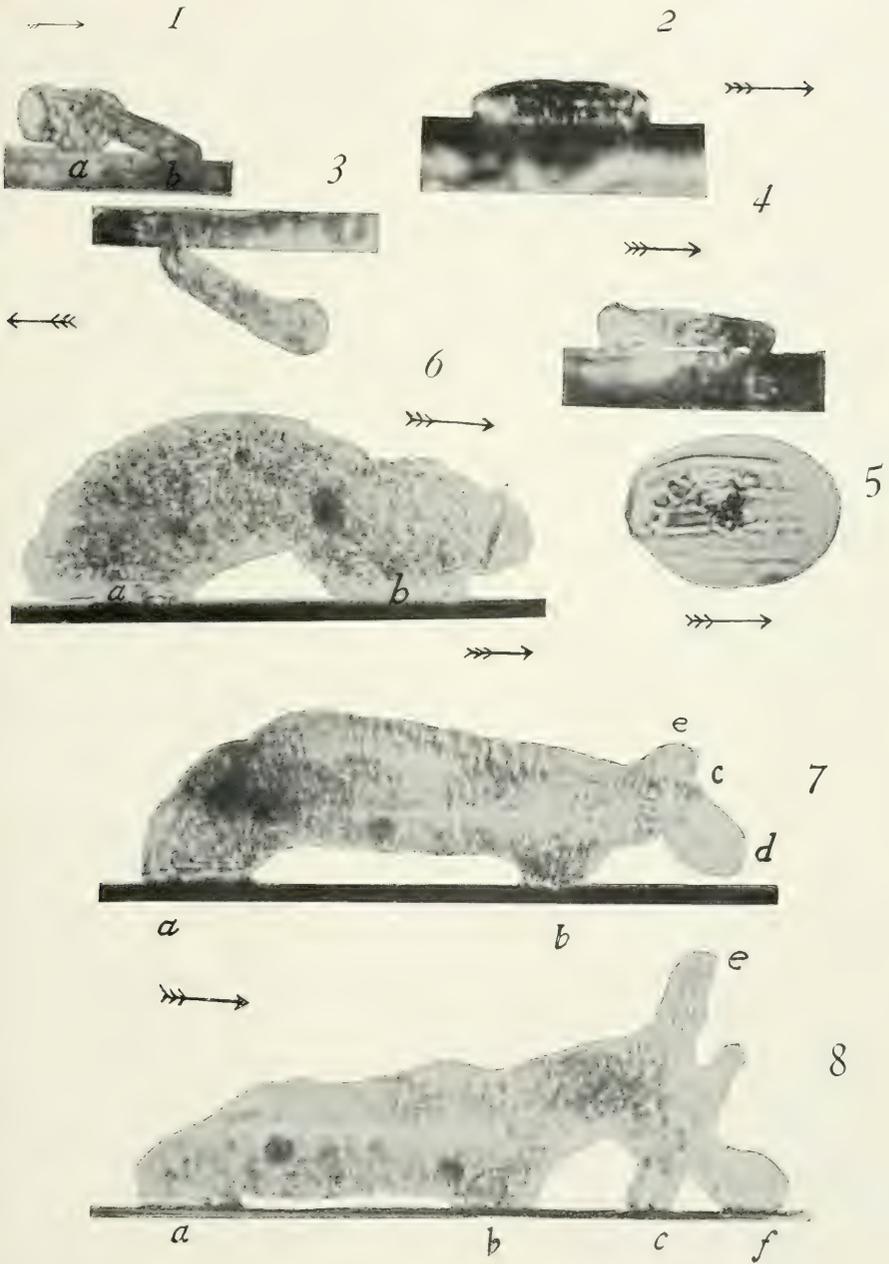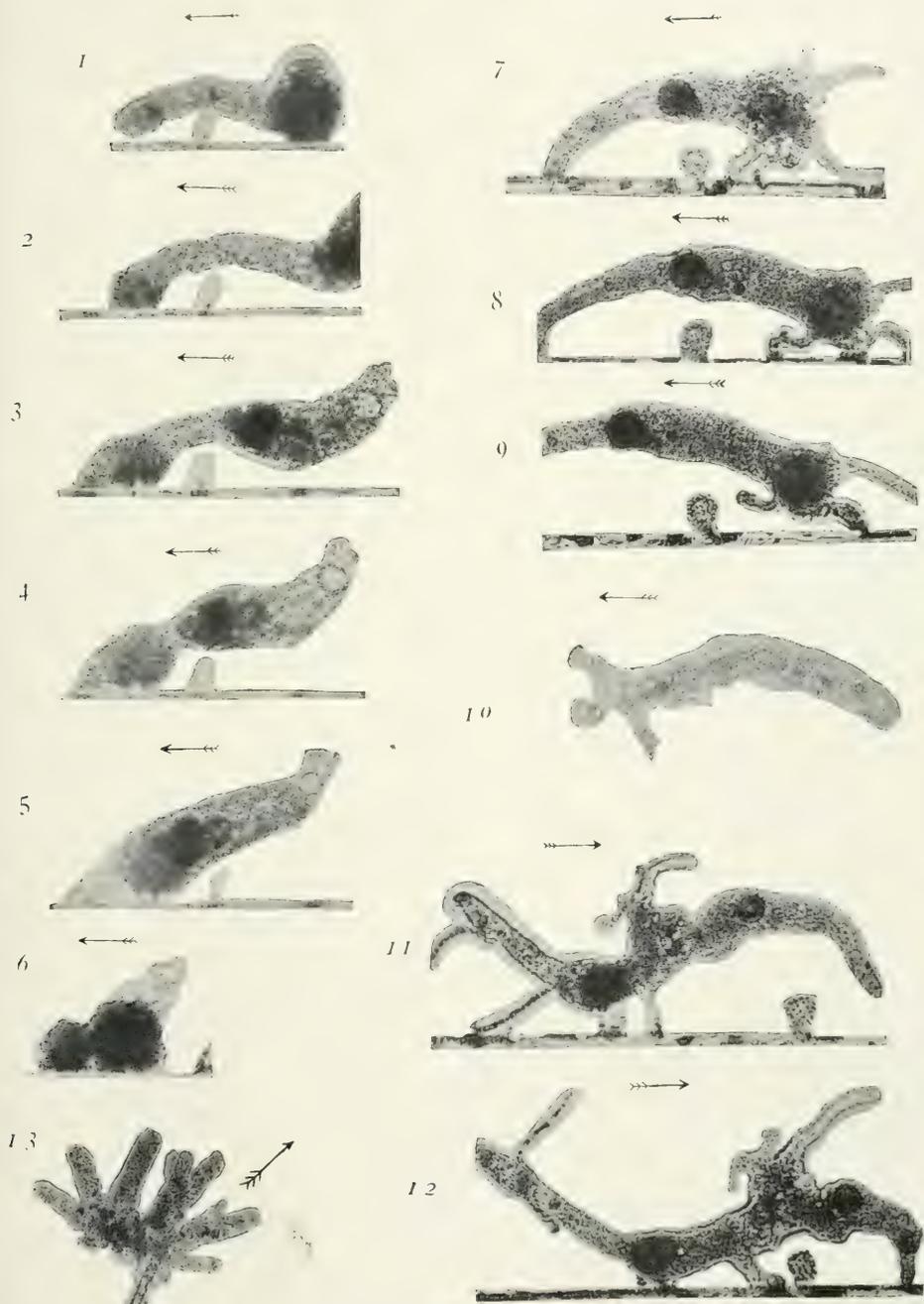
LIGHT REACTIONS IN LOWER ORGANISMS

I. STENTOR CŒRULEUS

BY

S. O. MAST

Professor of Biological Science at Hope College, Holland, Mich., U. S. A.

WITH SIX FIGURES

I. INTRODUCTION

The light reactions of Stentor cœruleus have been described
in detail by Jennings ('04a, p. 29) in a paper, entitled "Re-
actions to Light in Ciliates and Flagellates." His work shows
conclusively that such reactions do not take place in accordance
with the tropism theory as set forth by Loeb, Verworn, or Holt
and Lee, but that the animals orient by means of "motor
reactions," *i. e.*, by turning toward a structurally defined side
(aboral), when stimulated, and then proceeding on a new path
at an angle with the old one. My experimental results, while

differing in a few minor details from those of Jennings, lead to the same general conclusions. Our methods, however, have been quite different. Jennings laid particular stress on the detailed movements of the individuals, while I directed most careful attention to the regulation of the stimulus. Moreover, he dealt with some phases of the subject more extensively than I did, while I dealt with others more extensively than he did. My experiments were, however, entirely completed and much of this paper was written before the work of Jennings was published. The facts that, working entirely independently of each other on the same organism and with different methods, we have obtained results which lead to the same general conclusion and that this conclusion is not in agreement with the commonly accepted tropism theories, have led me to prepare this paper for publication even at the risk of repeating some matters already published by others.

I wish, before continuing further, to express my appreciation of many valuable suggestions by Dr. G. H. Parker, under whose direction the work was pursued, and my indebtedness to Prof. E. L. Mark, not only for granting exceptional facilities for carrying on the work but also for his encouraging interest. I am also under obligation to Dr. H. S. Jennings for helpful criticism.

2. MATERIAL AND APPARATUS

The animals used in the following experiments were obtained by letting aquatic plants collected in a pond known to contain Stentor, decay in battery jars nearly filled with water. Stentors begin to multiply rapidly after Paramecium disappears or while it is decreasing. If, however, the jars contain more than about one-tenth as much vegetable matter as water, fermentation takes place to such an extent that the Stentors are apparently all killed, for while in such cultures there are usually numerous Paramecia, Stentors never appear, or if they do, there are very few of them. The cultures should be kept in diffused daylight or total darkness at a temperature of about 22° C. If conditions are favorable, Stentors usually appear about ten days after

the cultures are put up, and then multiply very rapidly, frequently becoming so numerous that the walls of the jars to which they attach themselves, especially near the surface of the water, become densely covered with them in the course of a few days; but they soon decrease in numbers again and frequently disappear altogether within a week. It was found that if a few stalks of hay were added to cultures about to decrease in numbers they could be kept in good condition much longer than otherwise. Frequently it happens that after the Stentors seem to have completely disappeared in a given culture they reappear and multiply very rapidly. The disappearance and reappearance of Stentors in a culture is due largely, as Peters ('02) has shown, to chemical changes brought about by the decaying vegetable matter. While working on the following experiments, several cultures were constantly kept on hand. This insured an abundance of good material at all times.

Stentors, as is well known, are usually found most abundantly on the shady side of the vessel in which they are kept. If put into a dish containing water and placed in front of a window, they swim about for a time seemingly at random, but soon orient and then swim from the source of light, apparently as nearly parallel with the light rays as possible. How and why do they orient? Do they move parallel with the rays of light, and if so, why? What is the minimum light intensity which causes orientation? Is this a constant?

Answering these questions involves quantitative as well as qualitative work. In order to make quantitative work worthy of the name, it is, as Jennings ('04b, p. 507) has well stated, at least necessary to know what we are trying to measure, *i. e.*, we must understand the reactions of the organism with which we are working in detail, and know the precise value of the stimulus as it reaches the organism; and to know this we must understand the physical and chemical properties of the stimulus. The results and conclusions of the following experiments present a striking illustration of the importance of the latter. Strasburger ('78) found that certain swarm spores were positive in large vessels and negative in hanging drops in the same light intensity.

Rothert ('03) obtained similar results, and both offered quite extensive theoretical explanations for the cause of this; but neither got at the truth of the matter because they did not recognize the effect of the curvature of the surface of the hanging drop on the light as it entered the water. Chmielevsky ('04) proved conclusively by theoretical considerations and by means of photographs that the intensity of a hanging drop of liquid is frequently greatest in parts of the drop farthest from the source of light, so that it is more than probable that the organisms in hanging drops in the experiments of Strasburger and of Rothert reacted to light precisely as they did in the vessels.

In the following experiments particular stress was laid on the study and regulation of the light used as a stimulus. The work was carried on in a large basement dark-room in which the temperature varied but little. This room was supplied with gas, carbon filament incandescent lamps of various candle-power, a carbon arc lamp, and six-glower and single-glower Nernst lamps. Moreover, the room was so situated that sunlight, direct and diffused, could also be made use of. The Nernst single-glower lamp was found to be the most satisfactory source of light for all experiments, both quantitative and qualitative, providing the intensity required was not great. Such a glower consists of a single, small, straight rod composed of oxide of zircon. The rods are about 1 mm. in diameter and vary from 1 cm. to 2.5 cm. in length, thus producing when heated a compact source of light. They are heated in air by an electric current and need not be protected by a globe, so that much reflection and refraction are avoided. The glowers were generally not used in connection with the regular Nernst lamp, but were mounted in front of an opening in a box the inside of which was painted dead black (Fig. 1, d, c), to prevent reflection from the background. The box used was nearly cubical in form, each side being about 35 cm. long. The end containing the opening was made of a heavy asbestos pad to avoid danger from fire, since the temperature of the glower becomes very high. In case one glower did not produce light of sufficient intensity, three were so grouped that a cross-section would form a small equilateral triangle.

When these were heated they appeared much like a single glower somewhat enlarged. We have, then, in the Nernst glower, arranged as described above, a light the direction of the rays of which can be pretty accurately controlled, certainly more nearly so than the direction of rays from carbon incandescent or arc lamps. But the light intensity of the Nernst glower, while it probably varies less than that of the carbon arc, certainly varies more than that of the carbon incandescent. It varies with the voltage, the age and composition of the glower, the temperature of the room and especially with currents of air. Most of these factors were, however, under control. The light intensity with known voltage and temperature was frequently measured; the voltage was recorded from time to time during the progress of nearly all quantitative experiments by means of a meter in the circuit; the temperature of the room was nearly constant and drafts of air were avoided as much as possible. But as a matter of fact the variation under the most favorable conditions was so great that delicate quantitative results must be accepted with caution. The variation in light intensity, and its relation to the variation in voltage are indicated in the Appendix (p. 394).

A modification of a piece of apparatus devised by Sabine and Yerkes (Yerkes, '03) was used extensively in the following experiments and found very serviceable. By means of it a field of light can be produced which either is uniform in intensity throughout or gradually increases in intensity from one end to the other. The rays producing the field are all practically perpendicular to the plane of the field and there is little diffusion. The defects in Oltmanns'[1] method of producing light of graded

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[1]Oltmanns ('92) produced in an aquarium light gradually increasing in intensity from one end t the other, by placing a hollow prism filled with a mixture of India ink and glycerine-gelatine between the source of light and the aquarium. The India-ink mixture of course absorbed only a little light at the thin end of the prism, but gradually more toward the thicker end. Oltmanns and others assumed that the light rays in the aquarium under such conditions were parallel with each other and perpendicular to the side through which they entered. That this is not true, is clear from a theoretical as well as from a practical standpoint. The India-ink mixture contains numerous solid particles of carbon in suspension, which, together with particles in suspension in the water in the aquarium, unquestionably diffuse the light more or less.

intensity, which have been pointed out by Strasburger ('78, p. 588), Miss Towle ('00), and others, seem to ˙ be largely if not entirely avoided in this method. The piece of apparatus used in these experiments will be referred to as the light-grader. Its construction will be readily understood from the accompanying diagrams (Figs. 1, 2).

The walls of the apparatus are all light-proof and dead black inside, so as to prevent reflection. The outline of a cross-section at any point is square. The upper portion of the front wall of the vertical part of the apparatus is hung on hinges forming a door. From the bottom of this door is hung a loose vertical curtain, which can be so opened that observations can be made without admitting light. The glower is parallel with the minor axis of the lens. It is mounted in front of a small opening in a light-proof box, painted inside dead black, which thus forms a non-reflecting background. The glower and stage are at the conjugate focal points of the lens, and, therefore, at equal distances (50 cm.) from it. The plano-convex cylindrical lens used is 25 cm. long, 10 cm. wide and has a radius of curvature of 12.5 cm.

A cylindrical lens will not form a single definite image of an object, but rather a series of images, since by means of it light is focused only in reference to one plane. If, then, the object, e. g., a Nernst glower, is placed at one of the conjugate focal points so that the distance from the lens to the glower is equal to that from the lens to the image, and the glower is so arranged that it is pependicular to the axis of the lens, the image will not consist of a narrow band of light as large as a glower, which would be true if the segment of a sphere were used as the lens, but it will consist of a comparatively large field of light, the length of which is proportional to the functional length of the lens, while the width is equal to the length of the glower, regardless of the functional width of the lens (see Fig. 2). But since the amount of light which passes through the lens is directly proportional to the functional width of the lens and the width of the field is constant, it is clear that the intensity of light in the field, if we disregard the amount of light absorbed by the lens, must also be
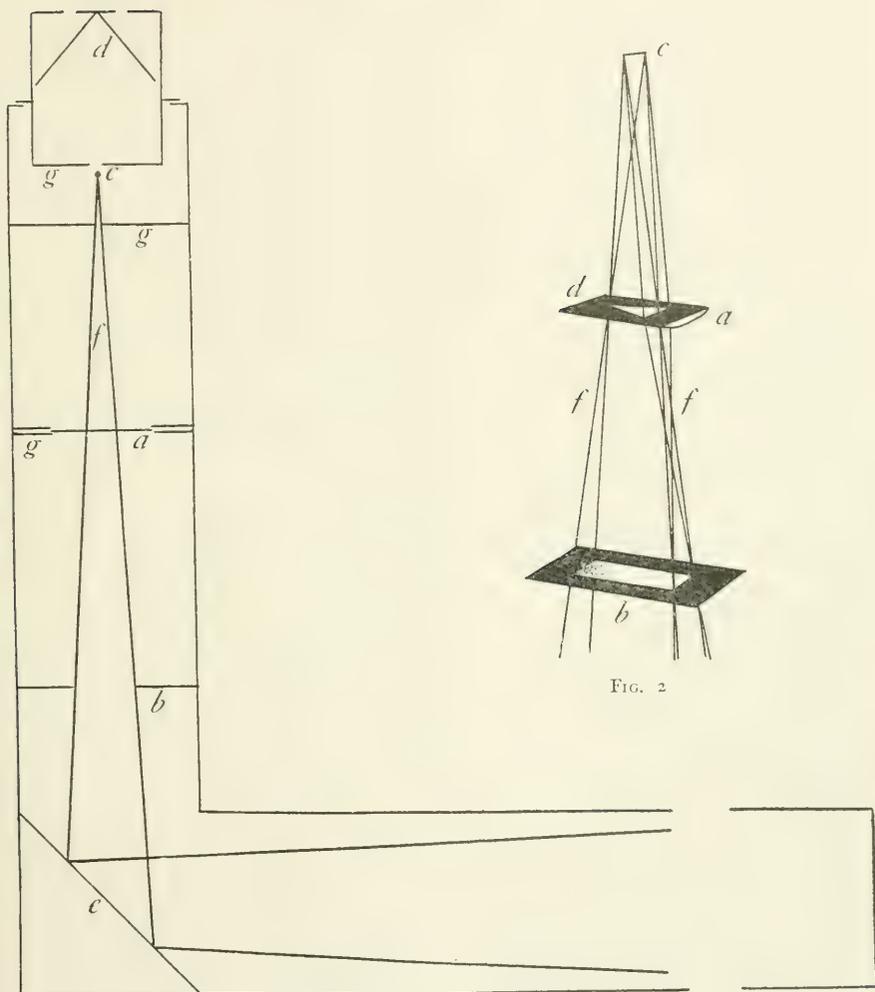
Fig. 1. A vertical section of the light-grader. The lens *a*, which is a segment of a cylinder, has its longitudinal axis lying in the plane of the section; *b*, stage; *c*, Nernst glower; *d*, non-reflecting background; *e*, mirror; *f*, light rays; *g*, opaque screens.

Fig. 2. Stereographic view of light, lens and image. *a*, Lens; *b*, field of light graded in intensity and produced by the image of the glower; *c*, Nernst glower; *d*, opaque screen containing a triangular opening and lying on the upper flat surface of the lens; *f*, light rays.

theoretically proportional to its functional width. Direct measurements of the light intensity with different functional widths of the lens proved this to be true within the limits of error. If, then, the lens be covered with an opaque screen containing a triangular opening, the base of which is parallel with the minor axis of the lens as represented in Fig. 2, there will result a rectangular field of light in which the intensity gradually diminishes from the end produced by light which passes through the base of the triangular opening to the opposite end, where theoretically it fades into darkness. Practically, however, it was found to be impossible to cut the apex of the triangular opening so as to prevent an apparent line at the end of least intensity. Since the light intensity of the field is proportional to the functional width of the lens, it is evident that the rate of diminution in intensity depends upon the ratio of the altitude of the triangular opening to the length of its base, *i. e.*, decreasing the altitude or increasing the base causes an increase in the rate of diminution and vice versa. The facts that a field of light, either uniform in intensity or graded in intensity, can be produced by merely changing the form of the opening in the screen over the lens, and that the intensity can be changed by altering the width of the opening, and readily calculated for any width if it is known for a given width, make this a very desirable piece of apparatus for quantitative as well as qualitative work.

3. OBSERVATIONS

A. Moving Stentors

a. In Light Uniform in Intensity

In studying the reactions of Stentors to light-rays perpendicular to the plane of the field, animals were put on the stage of the light-grader in a shallow aquarium[1] containing about 3 mm. of water taken from the culture jar and

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[1] The aquarium used was made by enclosing a space of desired size on a clear piece of plate glass with a ridge of paraffin attached to the glass with balsam.

carefully filtered. A field of light of the desired size and intensity, produced by regulating the opening in the screen, was thrown into the middle of the aquarium. The shallowness of the water restricted the movements of the animals almost entirely to the plane of the field and thus prevented orientation. . If, under these conditions, a uniform field of light about 16 candle-meters in intensity is thrown into the aquarium and a considerable number of Stentors introduced and evenly scattered, it will be noticed in the course of a few seconds that nearly all of the Stentors have left the light area (*a*), as represented in Fig. 3. These animals, as stated above, can not orient toward the source of light; how, then, do they get out of the light area and how do they keep out?
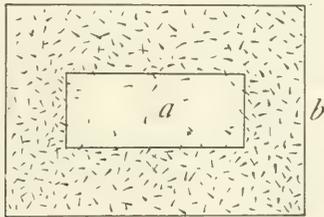
Fig. 3. Distribution of Stentors as seen in the light-grader 30 seconds after a field of light 16 candle-meters in intensity throughout was thrown into the middle of the aquarium containing water 2 mm. deep. *a*, Field of light, natural size; *b*, shaded area of aquarium. In this experiment the animals could move only in directions perpendicular to the rays of light.

After being stirred up, as they are when transported from the culture jar to the aquarium, they move rapidly, so that those in the light area soon swim out of it, but without any noticeable change in their course as they pass from light into darkness. The animals in that case get out of the light area by mere random movements. But when animals in the dark happen in their random movements to come in contact with the light area, they suddenly stop, swing their anterior end towards one side, and then continue on a new path which forms a definite angle with the old one. The new course may take them back into the dark area at once, but if it does not, as frequently happens, they soon stop, turn again towards one side and begin again on a new path.

This reaction may be repeated several times before the animal succeeds in getting out of the light area; indeed, Stentors were frequently seen to repeat this reaction until they finally got out of the light area at the side opposite the one at which they entered. Thus we see that the animals are kept out of the light area by means of what Jennings has called the motor reaction.

It is evident from the preceding description that the light rays in this experiment are practically perpendicular to the plane of the field, and that the movements of the animals are almost entirely restricted to this plane, so that the rays strike them perpendicular to their longitudinal axis; therefore the direction of the direct rays certainly can not affect their movements. But it may be argued that there is sufficient light reflected from other individuals and from particles of various other substance in the water to affect the direction of movement of Stentors and that they leave the light area because of this reflected light, to which they orient. That this is not true is clearly shown by the fact that Stentors are found swimming at random in the shaded regions close to the edge of the light area, as represented in Fig. 3. The shaded area contains practically as much reflected light as the light area, so that if the re- flected light affected the direction of movement at all, we should expect it to affect that of the Stentors in the shaded area as well as that of those in the light. But if this were true, we should expect the animals in the shaded regions to be less numerous near the edge of the light area than elsewhere; we should also expect to find them oriented toward the light area, but this is not the case. The animals are as numerous in this region as they are farther from the light area and are here found swimming at random. That the motor reaction is not due to the temperature is evident from the fact that Paramecium, which is more sensi- tive to heat than Stentor (Jennings, '04a, p. 31), does not respond when it passes into the light area. Thus we must conclude that the animals do not leave the light area by orienting to reflected rays, but that they are carried out of this area by random move- ments caused by the stimulation of the light, and that they are

kept out by means of a motor reaction induced by an increase in light intensity as they attempt to enter.

If the light is not very intense many of the animals, after having given the motor reaction several times without succeeding in getting out of the field, become quiet or continue on in a straight course, no longer responding with the motor reaction. Such animals have apparently become acclimated. Some animals give the motor reaction apparently as soon as their anterior end touches the field of light, others do not respond until they are entirely within the field of light, while still others pass into the field three or four millimeters before reacting, and if the light is not extremely intense, there are always some which apparently are not affected by the light at all. These responses show clearly that there is great variability in the sensitiveness to light among animals from the same culture, and this is true even under the most favorable circumstances. The variability, together with the fact that Stentors readily become acclimated to light, makes quantitative work exceedingly difficult.

The fact that the motor reaction is given by some animals as soon as their anterior ends reach the light area, shows that stimulation of this end is sufficient to induce a reaction, but it does not show that the posterior end is not sensitive, nor does it show that the posterior end is less sensitive than the anterior. This question will be dealt with later.

In the experiment described above, Stentors were frequently seen to enter the field of light so that their paths form rather acute angles with the edge of the field. Animals thus entering the field, in responding with the motor reaction, often turned toward the center of the field, and in so doing were of course carried farther into the light area; whereas, had they turned toward the edge they would immediately have been carried back into the dark area again. This indicates, as Jennings ('04a, pp. 33-35) very clearly showed by direct observation, that they always turn toward a structurally defined side regardless of the region stimulated. That this is really what takes place, can be demonstrated as follows: Water to the depth of about one centimeter is put into the aquarium in the light-grader so that

the Stentors can readily swim in all directions. If, now, by means of a mirror held beneath the aquarium, a field of light about 20 candle-meters in intensity is suddenly flashed on animals swimming at random in the dark, nearly every animal, no matter in which direction it is moving,—from the source of light or toward it,—stops almost instantly, turns, and then starts on a new path, *i.e.*, responds with the motor reaction. If the animals are numerous, they may be seen literally to turn in every direction at the same instant. If reaction takes place in accordance with the tropism theory as defined by Loeb, Verworn, or Holt and Lee, we should of course expect all animals not oriented to turn from the source of light, but we should not expect those which happen to be already oriented when the light is flashed on them to turn at all. This, however, is not the case. As stated above, practically all turn; some of those moving at right angles to the rays turn toward the source of light and others from it; and of those already oriented, some turn to the right, others to the left. This is a most striking and convincing experiment. It strongly supports the direct observations of Jennings by which he demonstrated that Stentor always turns toward a structurally defined side and shows clearly that there is no apparent relation between the direction of the rays and the direction of turning.

When Stentors which are oriented to a given light respond with the motor reaction to an increase in intensity of the light, they are for the time being thrown out of orientation, but by repeated response to the more intense light they soon become oriented again. If, however, the light intensity is increased very much (125± candle-meters), they may again respond after having become oriented. As a matter of observation, under such conditions the responses in many individuals are repeated in such rapid succession that the animals appear to be turning about a pivot at their posterior end.

Jennings ('04a, pp. 49-50) writes: "When a large number of Euglenæ are swimming toward the source of light, if the illumination is suddenly decreased in any way, they give the typical motor reaction described in my previous paper as a response to

other classes of stimuli (Jennings, '00, p. 235). That is, they turn at once toward the dorsal side.'' Euglenæ are positive, so that when they are swimming toward the source of light they are oriented; when, under such conditions, they respond to a decrease in light intensity, they are, as was found to be true in case of Stentor, thrown out of orientation. These reactions seem decidedly fatal to any theory which assumes that animals remain oriented because when in this position similar surfaces on opposite sides of the body, or locomotor appendages on such surfaces, are equally stimulated.

We have thus far shown that when Stentors pass from a dark into a light region they respond with the motor reaction. Will they respond likewise when they pass from a region of one intensity to that of another?

Two adjoining regions of different light intensity can readily be produced by reflecting the field of light in the light-grader so that the reflected field overlaps the field produced by direct light. Where the two fields overlap, the light will of course be more intense than elsewhere, although it is exceedingly difficult to see any line of demarcation between these regions of different light intensity; but when Stentors swimming in the regions of lower intensity happen to strike this plane they respond with the motor reaction much as if they had come in contact with a glass wall. If, however, they strike the plane while swimming from the more intensely illuminated region, they pass on apparently unaffected. It is decidedly interesting as well as instructive to watch the results when these animals reach the practically invisible plane between the regions of different light intensity; if moving in one direction, without any response whatever; if moving in the opposite direction, with a marked reaction.

b. In Light Graded in Intensity

Holt and Lee ('01, pp. 471-475) found that if Stentors are put into an elongated aquarium in which the light becomes gradually more intense from one end to the other, they collect at the dark

end of the aquarium. These investigators produced such light conditions by means of Oltmanns' prism, as follows: "Parallel rays of light fell horizontally at right angles to the long axis of a narrow trough destined to receive the organisms. Between the trough and the incoming light there intervened a prismatic screen. Thin at one end, it there let almost all the light through; but becoming gradually thicker it gradually diminished the intensity of the rays till at the opposite end the screen was opaque and intercepted all the light." "If numbers of blue Stentors are put into a trough that is moderately illuminated under the conditions described above, the animals at first swim away from the light until they encounter the farther wall of the trough. They then swim backward a little distance and start off in a new direction, as Jennings has described for other species of Infusoria, some toward the light end of the trough and some toward the dark end. Soon they strike the wall again, and again start off in one direction or the other, and this series of movements is repeated many times. The preponderance of movement is toward the dark end, and in time by far the majority are found there swimming about." Holt and Lee explain the cause of the preponderance of movement toward the dark end by applying Verworn's hypothesis. The animals swimming from the side of the aquarium nearest the source of light toward the opposite side are, they say, so deflected toward the dark end, because the side of the animals facing the light end of the aquarium is more strongly illuminated than the opposite side. This is due in part to the decrease in light intensity from one end of the aquarium to the other, but more especially to the fact that the side facing the more highly illuminated end of the aquarium receives considerable light which is reflected from the water and sides of the trough at the well lighted end. The fact that an appreciable quantity of light is thus reflected is sufficiently attested by Strasburger ('78) and Miss Towle ('00).

Thus it is clear that Holt and Lee in their explanation of the cause of the "preponderance of movement toward the dark end" make use largely of two factors, the swimming from the source of light and the light reflection from the more highly illuminated

end of the aquarium. Can these factors be eliminated and, if so, will the animals still collect at the darker end of the aquarium? If, with these factors eliminated, they still collect at the darker end, it is clear that the hypothesis of Verworn, as applied by Holt and Lee, will not explain the cause of such a collection.

A field consisting of rays practically perpendicular to the plane of the field and graded in intensity can readily be produced in the light-grader by placing a screen containing a triangular opening over the lens, as described above (pp. 364-366). If, now, a considerable number of Stentors are put on to the stage in the light-grader in an aquarium containing thoroughly filtered water about two millimeters in depth, and such a field of light is thrown
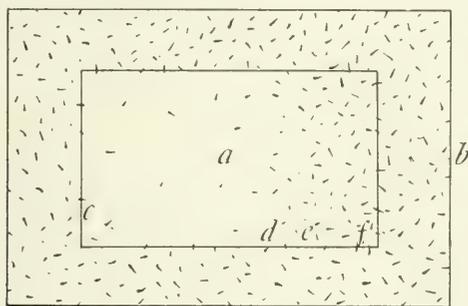
Fig. 4. Distribution of Stentors as seen in the light-grader one minute after a field of light graded in intensity was thrown into the middle of the aquarium containing water 2 mm. deep. The shallowness of the water forces the animals to move at right angles to the light rays. *a*, Field of light; *b*, shaded area; the light intensity at *c* is 16 candle-meters; at *d*, 4 candle-meters and at *e*, 1.6 candlemeters. The intensity at *f* was so low that it could barely be distinguished from that in the shaded area.

into the aquarium perpendicular to the bottom, it is found that in a short time nearly all the animals have left the more intensely illuminated regions of the field, while there are as many in the region of lower intensity as there are in the shaded area. This result is represented in Fig. 4.

If the light area is made as large as the aquarium, the animals collect at the darker end. In this experiment there are a good many animals in the light area when the light is first turned on and each one will cause diffusion of light, *i. e.*, will be a secondary source of light, reflecting rays parallel with the plane of the field.

Those at the most highly illuminated end of the field will reflect
more light than those at the darker end. May not, then, the
collection of the Stentors at the darker end be due to orientation
to these reflected rays, as has been suggested by some who believe
ray-direction to be the primal cause of orientation? An argu-
ment has already been presented in this paper (p. 368) which
seems to indicate very clearly that the reflected light would not
under the above conditions affect the direction of movement
of the animals. But to test this more in detail and to learn
precisely how the animals react in collecting at the darker end
of the aquarium, a square field of light 2.5 cm. on a side was
thrown into the middle of an aquarium on the stage in the light-
grader. The field of light was 125± candle-meters at one end
and 0+ candle-meters at the other. The aquarium contained
water about two millimeters deep, taken from the culture jar
and carefully filtered. Special precautions were taken to pre-
vent diffusion of light by dust particles on the lens, on the water,
or on the mirror used to reflect the light after it had passed
through the aquarium into a dark chamber, where it was absorbed
(see Fig. 1, p. 365). One Stentor at a time was taken with a fine
pipette from a dish kept in the dark, then carefully dropped
into the center of the field, and its reactions studied. In this
way the effect of light reflected from numerous animals was of
course eliminated. The movements of the animals, practically
restricted to the plane of the field by the shallowness of the water
in the aquarium, could be distinctly seen with the naked eye, but
of course their structure could not be made out. As soon as an
animal was released in the center of the field it responded with
the motor reaction several times in rapid succession, moving
but a short distance between successive responses, until it appar-
ently became acclimated to the intensity of light to which it
was subjected. If at the end of this period it happened to be
headed so that in moving forward it did not pass from regions
of lower to regions of higher light intensity, it usually continued
making nearly a straight path until it got out of the light area.
If, however, it happened to be oriented so that its movements
carried it into regions of higher light intensity, it continued only

a short distance before again responding with the motor reaction, and such response was repeated at short intervals, until the animal happened to become so directed that when it moved forward it no longer passed from regions of lower to regions of higher intensity. It thus continued on a straight course out of the field, there being no longer any stimulation to induce the
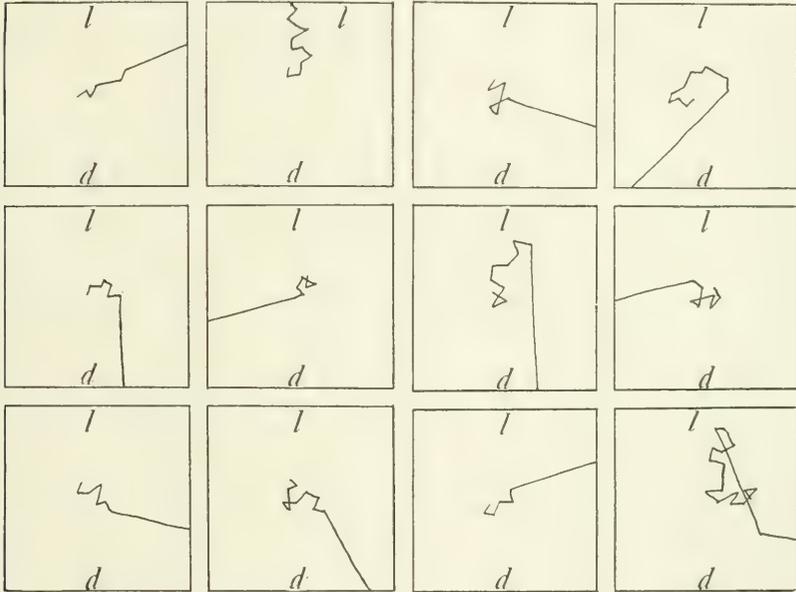
Fig. 5. Each square represents the field of light at its actual size. The rays of light strike the plane of the field practically perpendicularly. The higher light intensity at the side *l* was 125± candle-meters, and that at the opposite side, *d*, 0 + candle-meters. The lines within the square represent, approximately, the paths of twelve Stentors taken at random from a dish in the dark and dropped one at a time as nearly into the center of the field as possible. The angles in the paths indicate points where the animals gave the motor reaction.

motor reaction. It will be seen from this description that Stentors in light graded in intensity, moving perpendicular to the direction of the rays, do not orient and then continue in a definite direction. They may continue moving in any direction excepting in such as would carry them from regions of lower to regions of higher light intensity. This fact will become more evident by referring to the accompanying figures (Fig. 5), which were

made by tracing the paths of the animals on a sheet of paper as
they proceeded on their course.   With a little practice it was
possible to watch the animals and draw at the same time, so
that while these tracings do not represent the paths in detail very
accurately, they at least represent the general course taken by
the animals.

It will be seen from these figures that only one of the twelve
animals left the light area at the most highly illuminated end,
and that this one continued responding with the motor reaction
at short intervals until it got out of the area.   Evidently it did
not happen to become headed in such a direction that movement
after a reaction would no longer carry it into regions of higher
light intensity.   It will also be seen that there is absolutely
no indication of orientation with reference to degrees of light
intensity, *i. e.*, the animals move continuously in any direction
except such as would carry them rapidly into regions of higher
light intensity.   Subsequently 64 additional animals were put
into the graded field one at a time.   Of these, four left the field
at the end of highest light intensity, 26 at the end of lowest
intensity, and 34 at the two sides.

We have in the above experiments eliminated the two prin-
cipal factors (p. 372) upon which Holt and Lee based their
explanation of "preponderance of movement toward the dark
end," and have found that such movement still takes place.
Evidently, then, their explanation will not hold.   Jennings
('04a, p. 48), after stating this explanation and describing the
motor reaction of Stentor due to light stimulations, continues
with the following paragraph:

"There is evidently nothing in this account [referring to the
observations of Holt and Lee] which is inconsistent with the
method of light reaction which I have described.   On the
contrary, the reason why the organisms finally swim toward the
dark end and gather there becomes much more evident when
the reaction method that I have described is taken into con-
sideration.   Let us suppose that the Stentors, after striking
the back of the trough, turn in equal numbers toward D [dark
end of trough] and toward L [light end of trough].   In those

swimming toward D the anterior end is directed away from the source of strongest light (due to reflection from the lighted end of the dish L), and the animals are passing into a region of less intense light. There is thus nothing to cause the 'motor reaction,' with its accompanying change in the direction of movement. In the Stentors swimming toward L, on the other hand, the strongest light falls on the anterior end, and the organisms are passing into a region of more intense light. Either of these factors taken separately may, as we have seen, cause the motor reaction (the turning toward the right aboral side), thus changing the direction in which the Stentors swim. The animals which start to swim toward L will therefore soon be turned, and only when the direction of movement is toward D will there be no cause for further change."

In the experiments described above, we have substantiated Jennings' ideas as set forth in this paragraph; we have shown that the preponderance of movement toward the darker end is due to the motor reaction induced by an increase in light intensity, which consequently prevents continuous movement toward the more highly illuminated end of the aquarium.

It should, however, be kept in mind that these experiments were completed before Jennings' paper was published, and therefore were in no way influenced by his explanation.

B. Attached Stentors

In cultures many of the Stentors are usually found attached by the posterior end to solid objects of various kinds near the surface of the water. The relative number of attached and free individuals, however, depends upon the condition of the culture. In a given culture under certain conditions nearly all the animals may be free, whereas, in the same culture perhaps only a few days later, nearly all may be attached. From casual observations, it was thought that the number of free animals depends upon the rate of reproduction. In some cultures it was difficult to study the reactions of free-swimming animals, because they attached themselves again almost immediately after being de-

tached. On the other hand it was difficult in some cultures to study reactions of fixed Stentors because the least disturbance seemed to cause them to separate from their support.

Attached Stentors exhibit great variability in sensitiveness to light. For while the reactions are marked in some cultures when they are subjected to a sudden change—*e. g.*, either to an intensity of 120 candle-meters or to rather weak diffused daylight—in others only slight reactions are induced by sudden exposure either to strong direct sunlight or to the light from a carbon arc of 250 candle-power at a distance of 25 cm. (4000 candle-meters). In testing quite a number of cultures at different times, from December to the following August, I did not find any in which there was no response to sudden exposure to direct sunlight; but in many cultures there were individuals that did not appear to be affected at all, and, as stated above, there were some cultures in which the response was not definite. It is, therefore, not surprising that Jennings was unable to get any light reactions from attached Stentors in the cultures that he studied. He says ('04a, p. 32): "Such individuals do not react at all to light. When light is thrown on them they remain in the positions in which they are found at the beginning, neither contracting nor in any way changing their position. No matter whether the light is weak or strong, and without regard to the direction from which it comes, fixed Stentors give no reaction and show no orientation with reference to light. The contact reaction apparently inhibits the light reaction completely."

This, as we have seen, is true only under certain conditions, but the interference of contact reactions probably has something to do with the variation, which is much greater in attached than in free swimming animals. Contact reactions probably also tend to keep the animals in such a position that their longitudinal axis is perpendicular to the surface to which they are attached, for they are usually found in this position even when attached to vertical surfaces or to the upper surface of solids, and when attached to such surfaces they must maintain their position against gravitation.

The reactions in detail are as follows: If Stentors in favorable

condition are put into a dimly lighted aquarium containing water a few millimeters deep, they soon become attached to the bottom, take a position such that their longitudinal axis is approximately vertical and soon become quiet. If, now, the light is slightly increased, they begin to swing about their point of attachment, presumably turning toward their aboral sides. If, however, the light is suddenly increased considerably, they at once contract, but in the course of a few moments they expand again and then slowly swing as they do when the light intensity is only slightly increased; after this they soon become quiet or break loose. These reactions are entirely independent of the direction of the light rays. They are precisely the same in animals hanging from the surface film or from a cover glass floating on it, whether illuminated from above, below or from the side. There is, however, some evidence that the same reaction is induced by light of lower intensity when thrown on the anterior end than when thrown on the posterior, indicating that the anterior end is more sensitive than the posterior (p. 389.)

Attached Stentors illuminated from the side do not orient, as might be expected at first thought. Experiments with reference to this point were repeated many times with animals in various conditions and with light varying in intensity from very strong direct sunlight striking the animals at various angles, to that which would barely induce a reaction; but in no case was there any definite indication of orientation. Assuming the tropism theories to be correct, we should, of course, expect the animals to orient, *i. e.*, to turn until symmetrical portions are equally stimulated and then stop, and we should not expect any reaction in animals already oriented when subjected to light stimulation, unless the stimulations were intense enough to cause contraction. But, as stated above, such is not the fact; when slightly stimulated, the Stentors swing about their point of attachment regardless of the direction of the rays, and if illuminated from the side they do not stop swinging when the anterior end becomes directed away from the source of light. Evidently, then, the reactions of attached Stentors are not in accordance with the tropism theories. Are they in harmony with orientation by means of motor reactions?

The anterior end of Stentor, as will be shown later (p. 389), is more sensitive to light than any other portion of the surface of the body, so that when it is turned from the source of light the stimulation on the animal as a whole is weaker than when it is turned in any other direction. It is this which keeps free-swimming animals oriented after they have once attained such a position by means of the motor reaction (Jennings, '04a, p. 45). Why, then, do not attached Stentors remain oriented, when, in swinging about their point of attachment, they happen to reach a position in which their anterior ends are directed from the source of light? We have shown that free-swimming animals which are already oriented respond with the motor reaction if the light intensity is suddenly increased and thus are thrown out of orientation, and that if animals hanging from a surface film are suddenly illuminated from above they begin to swing about their points of attachment; so that the mere fact that the anterior end is directed from the source of light is not sufficient to prevent the motor reaction, *i. e.*, turning toward a structurally defined side. We have also seen that attached Stentors are readily acclimated to light and, as this process of acclimation proceeds, we should expect to find a stage at which the turning of the anterior end from the source of light would cause enough reduction in stimulation to prevent further swinging, *i. e.*, to inhibit the motor reaction, and this is probably true. We find attached Stentors when not stimulated arranged so that the longitudinal axis is perpendicular to the surface to which they are attached. With these animals acclimation has proceeded so far that there is no longer sufficient stimulation when the anterior end is directed from the source of light to induce the motor reaction. We should, therefore, expect them to maintain a position perpendicular to the surface to which they are attached.

If the light intensity, then, is great enough to cause a sufficient stimulation when the anterior end is turned from the source of light, attached Stentors respond with the motor reaction which prevents orientation; but if it is not intense enough to induce the motor reaction under these conditions, the tendency to take a position perpendicular to the surface on which they are attached

again prevents orientation, so that the fact that attached Stentors do not orient is precisely what we should expect if the animals orient by means of motor reactions.

In closing this section of the work, let me state clearly that, while I have found absolutely no evidence of tropic responses in any of my experiments with Stentor, I do not wish to be understood as intimating that difference in intensity on opposite sides of these organisms, or even that the direction in which the light rays pass through them, may not affect the direction of their motion. I shall, however, state again, as I have stated several times in the preceding pages, that the reactions of Stentors are not in accord with the tropism theory as defined by either Loeb, Verworn, or Holt and Lee.

C. *Threshold for Light Stimuli*

a. With the Animal's Side Illuminated

Since the threshold for light stimuli varies greatly with different individuals and in the same individual under different conditions, it is evident that the mere determination of it without correlating the physiological conditions of the animals with the individual variation in the threshold can be of little value. We have, however, one problem that can be attacked without such correlation. I have assumed in some of the preceding discussions, that the anterior end of Stentor is more sensitive to light stimuli than any other portion of the surface. Now, this assumption can be tested if the threshold can be ascertained when animals under any given conditions are illuminated on the side, and again when, under the same conditions, they are illuminated on the anterior end. To obtain the threshold for animals with the side illuminated, they were put on the stage of the light-grader in an aquarium containing water only about two millimeters deep. Then the intensity of a small field of light thrown into the middle of the aquarium was reduced until the animals when passing from darkness to light no longer responded with the motor reaction.

The movements of the animals were practically restricted to the plane of the bottom, so that when the animals passed into the field of light their sides only were fully illuminated.

Three methods were used to ascertain the lowest intensity to which animals, under the above conditions, react.

First Method.—A field of light of uniform intensity throughout was thrown into the aquarium and the intensity reduced by decreasing the functional width of the lens. The reactions of the animals as they passed into the light area were then studied. While theoretically this method, as well as those which follow, should yield accurate quantitative results, practically the results must be considered as at best only rather gross approximations, for in the first place it was impossible to prevent fluctuations in the light intensity; in the second place Stentors are very readily acclimated to light of low intensity; in the third place it was difficult to see at times if a response was given or not, owing to the low intensity to which many responded; and, finally, the individual variation was so marked that without statistical methods the point of lowest intensity to which a definite number responded could be only roughly determined. The threshold also varies greatly in different cultures and in the same culture under different conditions. For example, a culture was tested March 3, at 3 o'clock, and definite reactions to a change in intensity of 1.6 candle-meters were obtained, although only a small proportion of the whole number reacted. The same Stentors were tested again after having been in the light-grader 90 minutes in darkness undisturbed, and then only an occasional animal responded to a change in intensity of 4.8 candle-meters.

It was found that the movements of the animals could be followed best by studying their shadows cast on a piece of white paper held about 20 cm. below the aquarium. The paper, if unglazed, diffuses the light, and therefore reflects it very nearly equally over both the dark and the light area, so that the intensity difference is not appreciably affected.

A large number of Stentors from different cultures were tested for their threshold to light stimuli, as described above, on the following days: February 20, 21, 22 and 23; March 4, 10 and 16;

also August 1, 2, 3 and 4. In all the cultures experimented with animals were found that gave unquestionable reactions when they passed from darkness into a light intensity of 1.5 candle-meters and in some culture when they passed into an intensity of 1.2 candle-meters. But no definite reactions were obtained under any conditions from animals which passed from darkness into light of an intensity lower than 1.2 candle-meters.

Second Method.—In this method, as in the one described above, the field of light in the aquarium was uniformly illuminated, but in place of studying the reactions directly, the animals were left undisturbed for a given time (15 to 60 minutes) after the light was turned on, and then their distribution ascertained by counting those in the field of light and those in dark areas equal in size to that of the field of light. The results of thirty such experiments were recorded. There were, however, a considerable number more in which the results were so evident that the distribution was not studied by actual count and no record was made of them. These tests were performed on the following days: February 20, 21, 22 and 23, and on March 3 and 4, the same days on which experiments described under "First Method" were performed, and the animals used were taken from the same cultures in both cases. The light intensity of the field in these tests varied from less than 1.2 to 3.2 candle-meters. In five tests it was 3.2 candle-meters; in four of these tests there were decidedly fewer Stentors in the field at the close of the test than in an equal area outside; in the other one the number was about equal in the two areas, but these animals had been used in the preceding experiment and were therefore probably acclimated to the light. In twelve of the tests, the light intensity was 1.6 candle-meters; in eight of these there were definitely fewer animals in the light area than elsewhere; in the remaining four the distribution was about uniform throughout the aquarium. In eight tests the intensity was 1.2 candle-meters; in four of these there were fewer Stentors in the light area than elsewhere, but the difference was not very marked, while in the other four it was questionable which contained the greater number. In the five remaining tests the light intensity was slightly less than 1.2 candle-meters; in all but one

of these, the light did not effect the distribution and in this one the outcome was questionable. These results agree very well with those obtained by the first method, in which, as here, it was found that the least change in intensity which would produce any re-action under most favorable conditions was 1.2 candle-meters. The tabulated results of these experiments, as well as of those that follow, will be found in the Appendix (pp. 394-399).

Third Method.—A field of light graded in intensity from one end to the other was produced by means of a screen containing a triangular opening placed over the cylindrical lens. The point of least light intensity along the side of the field to which the animals responded when passing into the light was then ascertained, either directly by observing the movements of the animals, or in-directly by studying the effect of the light on the distribution of the animals after having been exposed to it for a given length of time. The intensity of any area in the field could readily be calculated by measuring the functional width of the lens as determined by the opening in the screen at the point through which the light passed to produce the given area. A large number of experiments on animals from various cultures and under various conditions were carried out in accordance with this method, the result being that the threshold was found to vary from 1.2 to 4 candle-meters. In no instance was there any definite evidence of reaction to a change in light intensity of less than 1.2 candle-meters.

In all of these experiments individuals were met with which were apparently not affected in the least in passing from darkness into a light intensity as high as 125 candle-meters. The threshold of the most sensitive animals also varied considerably in different cultures, but I think we may safely conclude that the threshold for Stentors from the cultures tested under the most favorable conditions was not less than 1.2 candle-meters when the sides of the animals were illuminated.

b. With the Animal's Anterior End Illuminated

Let us now consider the threshold of Stentors from the same cultures under similar conditions, but with the anterior end illumi-

nated in place of one side. Stentors are approximately conical in form, the posterior end being somewhat pointed while the anterior end is an almost flat surface approximately perpendicular to the longitudinal axis of the animal. If, then, the light rays are perpendicular to the longitudinal axis, they must be parallel with the anterior end, and this end therefore will not be exposed fully to light, but the posterior end being pointed will be practically as highly illuminated as if it were turned toward the source of light. In the experiments discussed above, the light rays were practically perpendicular to the longitudinal axis of the animals, therefore the only portion of the body not fully exposed to light in these experiments was the anterior end. If, however, the movements of the Stentors in an aquarium are not restricted in relation to the source of light, it is evident that sooner or later, in random swimming, the anterior end, as well as the sides and posterior end, may be turned toward the source of light and thus fully exposed. If, now, the animals leave that part of the aquarium nearest the light, it may be due to motor reactions induced by stimulation of any part of the surface. But if the anterior end of the animal is more sensitive than any other portion of its surface and the light intensity is gradually reduced, it is clear that a condition of illumination will be reached in which the motor reaction will be induced only when the anterior end is turned toward the source of light. If, then, the threshold is found to be higher when the movements of the animals are restricted to a plane perpendicular to the light rays than when they are not thus restricted, we may conclude that the anterior end is more sensitive than any other portion of the surface. The following experiments prove this to be true.

In order to ascertain the threshold when there was no restriction of movements, the Stentors were put into an elongated aquarium, one end of which faced a Nernst glower that was on a level with the bottom of the aquarium. The aquarium was then moved from the source of light until a place was reached at which the animals no longer left the end nearest the light. Theoretically this method seems very simple, but practically it is quite otherwise; as in the case of the experiments described above, only

rather gross approximations can be looked for.   We have here
to deal with the fluctuations in the intensity of the source of
light, with reflection of light from the ends and sides of the
aquarium, with absorption of light by the water in the aquarium,
and with the difficulty of determining, without statistical methods,
the lowest intensity to which the animals respond, a difficult prob-
lem because of the marked individual variation of the animals
and the ease with which they become acclimated.

These difficulties were controlled as far as possible in the fol-
lowing way.   The variation in light intensity in the Nernst
glower is due largely to variations in voltage.   Having ascertained
the intensity for a given voltage, the intensity for any other
voltage can be approximately calculated, so that by means of
a volt-meter in the circuit it was possible to ascertain the
approximate light intensity at any given time.   Four per cent of
the light which reaches the end of the aquarium nearest the
source is reflected and again four per cent of that which passes
through the opposite end; moreover, there is considerable re-
flection from the sides of the aquarium and from the surface of
the water.   Owing to such reflection and to the absorption of
light, the variation in intensity in an aquarium illuminated from
the end by light, even from a single point becomes exceedingly
complicated.   With such a complicated field it is of course utterly
impossible to do quantitative work worthy of the name.   For use
in the following experiments an aquarium was constructed in
accordance with the plan shown in Fig. 6.   The construction
will be readily understood by referring to the figure.

In these experiments the light was placed on a level with the
bottom of the aquarium, so that there was no reflection from this
surface.   The space between the walls was filled with water to
prevent reflection from the sides.   The velvet between the end
walls absorbed most of the light which passed through the
aquarium and consequently prevented most of the reflection from
the end farthest from the glower.   Reflection from the surface of the
water was prevented by cutting off the light which would other-
wise reach it by means of a screen in front of the aquarium.   The
Nernst glower was entirely surrounded by screens, only one of

which contained an opening. Through this, light escaped producing a field just large enough to cover the front end of the aquarium, and since the whole apparatus was in a dark room no other light reached the aquarium. The heat rays were cut out by passing the light through 7 cm. of distilled water[1] in a vessel with parallel sides.
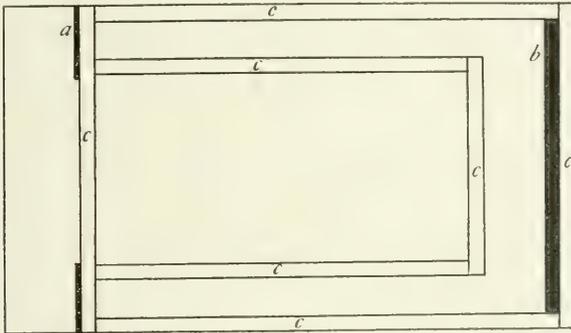
Fig. 6. Plan of aquarium, natural size. *a*, Screen; *b*, piece of black velvet; *c*, vertical walls 2.5 cm. high. This aquarium was made by gluing glass slides together with balsam. It was contructed for the purpose of eliminating as far as possible the effect of reflected light (see text).

The aquarium was filled to a depth of five millimeters with water taken from the culture jar and thoroughly filtered, so as to eliminate absorption as much as possible. Then, after properly

[1]To those who still use alum solutions for absorbing rays, the following statements by Nichols ('93, p. 15) may be of interest:

"Melloni (Thermochrose, p. 165), using an Argand lamp with a glass chimney as a source, found that a layer of alum solution 9.21 mm. thick transmitted 12 per cent of the total incident radiation and that distilled water transmitted only 11 per cent."

"Shelford Bidwell (Nature, Vol. xliv, p. 565), using a paraffin lamp as a light source and the thermopile, obtained the following results:

Solutions	Diathermacy
Empty cell	1000
Water, distilled...	199
Tap water	200
Alum, saturated solution.....................................	204

"Neither potassium alum nor ammonium alum in solution alters the diathermacy of distilled water in the region studied," *i. e.*, wave lengths 0.776 to 1.41μ.

Nichols and Coblentz ('03, p. 272) state that water transmits 67 per cent at wave lengths 1μ and becomes completely opaque only when wave lengths 1.8 μ is reached. The upper limit of wave length in red is usually considered to be 0.76 μ.

arranging the aquarium with reference to the light, quite a number of Stentors taken directly from the culture jar were introduced and evenly scattered. The animals were then left undisturbed for some time, 10 to 60 minutes, before their distribution was studied. It was soon found that 10 minutes was sufficient time to produce an effect, if the light intensity was not below the threshold. If, on studying their distribution, it was found that there were definitely fewer within an area, about five millimeters wide at the end of the aquarium nearest the light than elsewhere, and that those in the remaining area were still practically equally scattered, the intensity at this end was considered the threshold. It will be noticed that owing (1) to reflection from the end, (2) to absorption, (3) to the difficulty in deciding whether a change in distribution has occurred, and (4) to possible acclimation, the threshold as read would tend to be slightly too high. Sometimes, if the threshold was not found at the first trial, as was usually the case, the animals were thoroughly stirred up and a second trial made with the same animals, but it was soon found that the threshold of such animals was higher than that of animals fresh from the culture jar, even if the light on the first exposure was not above the threshold in intensity, so that in nearly all experiments fresh Stentors were introduced for each trial.

In all, 62 exposures were made in accordance with the method just described (see Appendix, pp. 397-399). These experiments were performed, on February 25, 26, 29; March 1, 2, 3, 8, 11, 13, 15, 16, 17, and August 1, 2, 3 and 4. The Stentors used were taken from the same cultures from which those used in ascertaining the threshold in the light-grader had been taken. Many of the experiments in the two series were performed on the same days, or nearly so, and in the same room, so that the conditions in both were practically the same with the exception of illumination. But, as already stated, the threshold in the light-grader was found to vary from 1.2 to 4.8 candle-meters, whereas in these experiments it was found to vary from 0.235 to 0.646 candle-meters. This contrast is brought out more strikingly in the following tests: During the period from August 1 to 4, inclusive, Stentors fresh from the culture jar were repeatedly tested under various conditions

in the light-grader and no definite reactions were obtained to intensities less than 1.78 candle-meters, while animals from the same culture jar tested at different times during the same period in the elongated aquarium illuminated from the end, reacted definitely to an intensity as low as 0.287 candle-meters.

It will be remembered that in the first of these series all portions of the surface of the animals excepting the anterior end were subjected to illumination, while in the second series the entire surface, including the anterior end, was exposed to light. The difference in the threshold in the two series must, then, be due to the exposure of the anterior end in the second series; and since the threshold is lower in this series than in the first, we can safely conclude that with reference to stimulation by light, the anterior end is the more sensitive portion of the surface of the animal.

The difference in sensitiveness between the anterior end and other portions of the surface of Stentor was further tested in the following experiments. In these experiments the light was placed on a level with the animals, which were hanging from a cover glass floating on the surface film in a small aquarium. A mirror was fastened above the aquarium and another below it, each at such an angle that the light reflected from above fell on the posterior end, and that from below, on the anterior end of the animal. Then, by properly screening the light and using the mirrors independently, the threshold was obtained when the animals were illuminated on either end. While the results thus obtained indicate a threshold of lower intensity when the anterior end is illuminated than when the posterior end is illuminated, I cannot consider these indications conclusive, since, on account of the extreme individual variations and the high intensity of light required, it was very difficult indeed to locate the threshold.

Jennings ('04a) demonstrated by direct observation that ciliary action in Stentor, Paramecium, Oxytricha and other organisms produces currents which carry liquid from some distance ahead of the animals to the oral groove. Now, if the stimulating agent can be carried with the liquid, "The result is a stimulation on the oral side of the body, not elsewhere." In giving the motor reaction, these organisms always turn toward the aboral surface,

i. e., away from the side stimulated, whenever the stimulating agent is such that it can be carried in the current, *i. e.*, chemical or thermal. Roesle ('02) showed that the peristome region is more sensitive to mechanical stimuli than any other portion of the surface of the body, and it is also probably true that the same region is more sensitive to chemical and thermal stimuli. If so, it is clear that when animals are put into a chemical solution which acts as a stimulus, or into a liquid at a temperature above the threshold, the oral side is more strongly stimulated than the aboral, and we should expect precisely what is found to be true, *i. e.*, the animals under these conditions respond with the motor reaction and in so doing turn the anterior end away from the oral side, which is more strongly stimulated than the aboral.

The effect of a stimulating agent on a given surface depends upon the sensitiveness of the surface and upon its degree of exposure. Mechanical and radiant-energy stimuli are produced by agents which are in general not affected by currents and thus can be applied to any desired portion of the surface. It is, however, found that the animals always turn toward the aboral side regardless of the surface to which these agents are applied. Can these facts be explained?

In Euglena the eye spot is in all probability more sensitive to light than any other portion of the body. It is located in the dorsal lip near the surface of the body. These organisms are positive to light of moderate intensity. They swim with their anterior end facing the light, and in giving the motor reaction they turn toward the dorsal lip, in which the eyespot is situated, *i. e.*, toward the region most sensitive and consequently most strongly stimulated, just as Stentor, Paramecium and Oxytricha, all of which are negative, turn from the side most strongly stimulated in case of thermal or chemical stimuli. But it will be recalled that here the location of stimulation is not so much due to variation in sensitiveness as to variation in exposure of different regions of the surface, owing to currents. We have demonstrated that the anterior end of Stentor is more sensitive to light than any other portion of the surface. Roesle showed, as stated above, that the peristomal region in certain Infusoria is more sensitive

to mechanical stimuli than any other surface area. Is it not probable, then, that this region in Stentor is also more sensitive to light than any other? If this is true, then in stimulating the anterior end with light the oral region will be more strongly affected than any other, and, in turning from the oral side when the motor reaction is given, the animals turn from the part most strongly stimulated precisely as in response to thermal and chemical stimuli. This would likewise be true if they were stimulated by light striking any other part of the surface of the body than the anterior end, for these animals are translucent, so that if they were illuminated from the side, for example, light could reach the oral region by passing through the animal. As a matter of fact, we have as yet no experimental results with regard to light reactions in Stentor which cannot be explained by assuming a given structure in the region of the oral groove to be the only portion of the body which is sensitive to light.

In giving the motor reaction to all light, chemical, or thermal, stimulations, Stentor probably turns always from the side most strongly stimulated, but apparently not so with regard to mechanical stimulations, for in such stimulations the motor reaction can be induced by touching any part of the surface. Does this mean that a certain physiological change induces a given definite reaction, which has become fixed in the race, possibly by "survival of the fittest," and that all local stimulations are effective only in so far as they tend to cause general physiological changes? Or are the local stimulations produced by some agents (chemicals, change in temperature, light) still in a measure local signs which induce reactions in harmony with them, as well as cause a definite physiological change, while in those produced by other agents (mechanical) the local sign is lost and the response is induced because of a given physiological state? Or may not the effect of stimulating mechanically any point on the surface be transmitted to the region of the peristome and there produce changes greater than were produced at the point actually touched and thus call forth a local sign which may regulate the reaction?

Whatever the final answer to these questions may be, the facts thus far established by experiment seem to indicate that the motor

reaction, _i. e._, the turning toward a structurally defined side, originated in response to stimuli which produced local signs.

4. SUMMARY

1. Stentors free to swim in all directions orient and swim from the source of light.

2. They orient by means of motor reactions, _i. e._, by turning toward a structurally defined side and then proceeding on a new path which forms an angle with the old one. If a single reaction does not result in orientation, it is repeated until the anterior end of the animal happens to become directed from the source of light.

3. The motor reaction is induced by a sudden increase in light intensity regardless of the relation between the direction of the rays and the direction of movement of the animals at the time the intensity is increased.

4. If a source of light to which Stentors are oriented is increased in intensity, the animals respond with the motor reaction and are thus thrown out of orientation, but by repeating the motor reaction they soon become oriented again.

5. The anterior end of Stentor is more sensitive to light than any other part of the surface of the body. The minimum threshold in animals stimulated by rays perpendicular to the longitudinal axis is 1.2 candle-meters, but in those stimulated by light striking the anterior end it is only 0.25 candle-meter.

6. The threshold varies greatly in individuals under the same conditions, and in the same individuals under different conditions. Stentors readily become acclimated to light, but much more readily under some conditions than under others.

7. Stentors once oriented remain oriented, if the light intensity is not too high, because they are least sensitive to light when the rays strike the posterior end.

8. Attached Stentors respond to increase in light intensity by contracting or by swinging about their point of attachment. If the increase is great and sudden, they contract; if it is not very great nor sudden, they swing about their point of attachment.

These reactions are independent of any relation between the position of the animals and the direction of the light rays.

9. Attached Stentors do not orient, for if the light intensity is above the threshold when the anterior end is turned from the source of light, the stimulation induces the motor reaction, which prevents orientation; and if it is below the threshold, the tendency to take a position such that their longitudinal axis is perpendicular to the surface on which they are attached, again prevents it.

10. The variation in the threshold to light stimuli in attached Stentors is much greater than in free-swimming ones. In some physiological conditions they respond definitely to a light intensity of 1.20 candle-meters, whereas in others they respond very indefinitely when exposed to an intensity of 4000 candle-meters.

11. The threshold in attached Stentors is probably lower when light strikes the anterior end than when it strikes the posterior end.

12. The light reactions of Stentor, both free-swimming and fixed, cannot be explained by the application of the tropism theory as defined by either Loeb, Verworn, or Holt and Lee.

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6. APPENDIX

TABLE I

Light Intensity, 222-Volt Nernst Glower

Date	Photometer to Glower in cm.	Photometer to Candle in cm.	Candle per Hour in Grains	Voltage	Standard Candle-power
Feb. 25	200	52.78	?	?	19—
Feb. 26	200	52.25	?	?	19±
Feb. 26	200	54.1	?	211±	18±
Feb. 27	201	63.89	165.72	212—	13.62
Mar. 17	200	56.5	154.3	204-209	16.15

TABLE II

Light Intensity, 110-Volt Nernst Glower

Date	Photometer to Glower in cm.	Photometer to Candle in cm.	Candle per Hour in Grains	Voltage	Standard Candle-Power
July 15	200	47.8	138.87	108.5-110	20.27
July.15	200	44.89	154.3	110-111	24.42
July.27	200	47.8	154.3	110±	22.48
Aug. 1	200	44.17	157.38	111.5-113	26.88
Aug. 3	200	48.32	163.56	109.5-111	23.35
Aug. 27	200	45.2	140.96	110-111.5	23.00

It will be noticed that the light intensity of the 110-volt glower is higher than that of the 222-volt glower. This is due to the comparatively low voltage. Later, in comparing the intensity of the 110-volt glower with that of the 222-volt glower directly, with a voltage of 110± and 222±, respectively, it was found that the candle power of the latter was 1.62 times as great as that of the former.

TABLE III

Light Intensity at Stage in Light-grader, 222-Volt Nernst Glower.

Date	Photometer to Candle in cm.	Photometer at Stage	Candle per Hour in Grains	Voltage	Functional width of Lens	Standard Candle-meters
Mar. ?	106.78	"	161.86	206–208?	0.75mm.	1.193
Mar. ?	71.6	"	161.40	206–208?	1.5 mm.	2.52
Aug. 2	60.37	"	157.39	221–223	1.5 mm.	3.57
Aug. 27	42.41	"	138.87	224–225	3.0 mm.	6.5+

The light intensities given in the tables were obtained by taking 30 successive readings at intervals of one minute. The distance from the photometer to the candle is in every case an average of 30 readings. A Lummer-Brodhun photometer was used.

TABLE IV

Threshold for Stentors with the Side Illuminated

First Method (see p. 382)

Date	Voltage	Reaction in Light-grader as Stentors pass from Darkness into Light of the following Intensities in Candle-meters			
		1.2	1.2	1.6	3.2
Feb. 20	?	no	no	?	marked
Feb. 21	?	no	no	definite, but not in all	
Feb. 22	?	no	?	large majority	
Feb. 23	?	no	no	about 1 in 5	
Mar. 4	208±	no	?	definite	
Mar. 10	205–207	no	about 1 in 10	many	
Mar. 16	209–210	no	?	a few	
Mar. 16	206–208	no	?	definite, but only a few[1]	

[1]After these Stentors had been in the aquarium in the light-grader 1.5 hours, no reactions were found to lower intensity than 4.8 candle-meters.

August 1 to 4, voltage 220–222. During this period Stentors fresh from culture jars were tested several times under various conditions and no definite reactions were seen to intensities lower than 1.78 candle-meters.

TABLE V

Threshold for Stentors with the Side Illuminated

SECOND METHOD (see p. 383)

Date	Time of Exposure in Minutes	Light Intensity in Candlemeters	No. of Stentors in Field of Light	In Equal Field to Right	In Equal Field to Left
Feb. 20	48	3.2		definitely+	definitely+
Feb. 20	33	3.2	4	16	18
Feb. 20	70	3.2	6	12	16
Feb. 21	63	1.6	7	13	12
Feb. 22	?	1.6	3	8	9
Feb. 22	56	1.6	8	15	16
Feb. 22	53	1.2	11	15	9
Feb. 22	38	1.2	10	16	15
Feb. 23	90	1.6	9	9+	9+
Feb. 23	60	1.6	12	18	24
Mar. 3	65	1.6	19	30	
Mar. 3	65	1.2	22	27	
Mar. 3	65	1.2–	32	31	
Mar. 3	90	1.6–	17	30	
Mar. 3	90	1.2	14	23	
Mar. 3	90	1.2–	28	32	
Mar. 3	65	1.6	30	24	
Mar. 3	65	1.2	29	32	
Mar. 3	65	1.2–	44	44	
Mar. 3	60	1.6	24	18	
Mar. 3	60	1.2	27	32	
Mar. 3	60	1.2–	22	32	
Mar. 4	120	1.6	9	24	
Mar. 4	120	1.2	16	16	
Mar. 4	120	1.2–	24	16	

The temperature varied from 19° C. to 24° C. in these experiments, but this change in temperature produced no apparent effect on the reactions.

TABLE VI

Threshold for Stentors, the Anterior End Illuminated (see p. 384)

Date	Time of Exposure in Minutes	Tempera-ture in Degrees C.	Voltage	Condition of Stentors	Distance from Light in cm.	Reactions	Intensity in Candle-meters
Feb. 25	60	?	?	used for some time	588	none	
Feb. 25	70	?	?	fresh	588	marked	
Feb. 26	48	?	?	used for some time	588	none	
Feb. 26	30	?	?	fresh	588	marked	
Feb. 26	95	?	?	same again	650	quite definite	
Feb. 26	100	?	?	same again	806	none	0.26
Feb. 26	45	?	?	fresh	806	none	
Feb. 27	30	17.5	211	fresh	806	none	
Feb. 27	50	18	211	same again	806	none	
Feb. 27	30	18	210	fresh	723	none	
Feb. 27	50	18	207	same again	723	none	
Feb. 27	60	18	212	fresh	670	none	
Feb. 27	30	21	211	same again	670	slight	
Feb. 27	45	21	113	same again	670	marked	
Feb. 27	25	21	210	fresh	975	very few within 5 mm. from light end of aquarium	
Feb. 27	20	22	211	same again	900	none	0.209
Feb. 29	26	22	210	fresh	900	none	
Feb. 29	14	22	210	same again	900	none	
Feb. 29	15	22	210	same again	806	none	0.26
Feb. 29	20	22	211	fresh	806	marked	
Feb. 29	53	21	212	same again	825	none	
Feb. 29	20	21	210	fresh	825	none	0.24
Feb. 29	60	21	211	same again	800	very few within 3 mm. of light end	0.265±
Feb. 29	55	21	210	fresh	800	definite	0.265±
Feb. 29	60	21	212	same again	800	definite	0.265±
Feb. 29	65	22	212	fresh	836	none	
Mar. 1	30	22	210	fresh	836	marked; many at dark end	
Mar. 1	20	18	209	same again	836	marked; many at dark end	
Mar. 1	60	?	208	same again	836	none	

TABLE VI—*Continued*

Date	Time of Exposure in Minutes	Tempera- ture in Degrees C.	Voltage	Condition of Stentors	Distance from Light in cm.	Reactions	Intensity in Candle- meters
Mar. 1	135	22	212	same again	800	none	0.265
Mar. 1	20	21	?	fresh	800	very few near light end	
Mar. 1	60	20	212	same again	835	few near light end	
Mar. 2	40	20	212	fresh	835	many at dark end; marked	0.235
Mar. 2	60	21	213	same again	850	none	
Mar. 2	60	22	211	fresh	850	marked	
Mar. 2	25	?	210	fresh	900	very few within 1 cm. of light end	0.209
Mar. 2	15	22		used before	900	none	very low; light filter- ed through thin paper
Mar. 3	30	20		fresh	900	none	ditto
Mar. 8	10	?	209	same again	825	?	
Mar. 8	50	19	211	same again	750	?	
Mar. 8	65	?	207	fresh	750	very many near dark end	
Mar. 8	20	?	?	used before	800	quite definite	
Mar. 8	35	19	208	used before	900	?	0.209
Mar. 8	17	21	208	used before	800	marked; few near light end	0.265
Mar. 11	40	?	209	fresh	900	none	0.209
Mar. 11	20	21	212	used before	800	quite definite	0.265
Mar. 13	20	20	206	fresh	800	definite, but not very marked	0.265
Mar. 15	35	20	208	fresh	800	marked	0.265
Mar. 15	45	21	211	fresh	800	none	
Mar. 16	75	21	210	fresh	600	marked	0.446
Mar. 16	40	22	209	fresh	600	marked	
Mar. 17	90	?	?	fresh	500	?	
Mar. 17	38	?	?	fresh	300	very marked	
Mar. 17	45	21	213	used before	500	marked	0.646
Mar. 17	35	?	?	used before	500	?	0.646
Mar. 17	17	?	212	fresh	500	very definite	0.646
Mar. 17	20	?	213	used before	500	none	0.646
Mar. 17	60	22	208	fresh	500	few within 1 cm. of light end	0.646
Mar. 17	50	?	?	used before	500	definite	0.646

TABLE VI—*Continued*

Date	Time of Exposure in Midutes	Tempera-ture in Degrees C.	Voltage	Condition of Stentors	Distance from Light in cm.	Reactions	Intensity in Candle-meters
Aug. 1	?	?	111–113	fresh	800	very few within 1 cm. of light end	0.365
Aug. 4	?	?	110–111	fresh	800	definite	0.365
Aug. 4	?	?	110–112	fresh	900	slight, but still very evident.	0.287

A 222-volt Nernst glower was used in all the experiments in this series excepting the last three, in which a 110-volt glower was used. For intensity of light, see table on intensity, p. 395.

THE INFLUENCE OF LIGHT AND HEAT ON THE MOVEMENT OF THE MELANOPHORE PIGMENT, ESPECIALLY IN LIZARDS

BY

G. H. PARKER

With Three Figures

It is now generally recognized that, of the various factors concerned in the integumentary color changes in lizards, none is so important as the migration of the pigment granules in the large pigment cells of the derma, the melanophores, erythrophores, etc. These cells are situated as a rule in the deeper part of the derma, and their bodies are either embedded in the more or less opaque guanine layer or lie proximal to this layer. Their processes extend distally between the guanine particles, and, as finely divided branches, form a rich arborization on the proximal face of the epidermis. When these distal processes are filled with pigment granules from the bodies of the cells, they form a dark covering on the distal face of the guanine, and the skin in consequence is dark-colored. When, however, the pigment migrates from the distal processes proximally into the bodies of the cells, the guanine layer becomes exposed to the light, and, since this layer is made of reflecting particles, the skin under these circumstances has a lightish appearance. Thus a *distal* migration of the pigment results in a *dark-colored* skin, a *proximal* migration in a *light-colored* one.

The migration of the pigment is influenced both by internal and external factors. Not only do the emotional states and other nervous conditions of the lizard make themselves evident

in the color changes of its skin, but external factors, such as heat and light also induce these changes. It has been pointed out recently by Parker and Starratt ('04, p. 464) that, in all lizards on which they could get records, a *high* temperature is accompanied by a *proximal* migration of the pigment, whereby a light-colored skin is produced, and a *low* temperature by a *distal* movement, thus giving rise to a dark coloration. Light on the other hand seemed to affect different lizards differently. Thus in Chamæleon and Anolis light induces a distal migration of pigment, while in Stellio, according to Filippi ('66), and in Varanus and Uromastix, according to Thilenius ('97), light calls forth a proximal migration. After having studied Anolis and become familiar, in this lizard, with one in which light produced a distal migration of pigment, I was naturally desirous of examining a species in which the reverse took place. Unfortunately the lizards studied by Filippi and by Thilenius were all old-world species and hence were not readily accessible to me in a living condition. I was, therefore, obliged to seek for a representative of this type of color change among more available forms. Hoffmann ('90, p. 1353) states on the authority of Wiedersheim, that the horned toad, Phrynosoma orbiculare, on cool overcast days is dark colored, and that it changes to silvery gray when sunlight falls upon it. Wiedersheim believed that this change was caused by a difference of temperature and not by illumination, but as no proof of this view was given, Keller ('95, p.132) was free to assert that these changes were due in his opinion to light. In that case Phrynosoma would show agreement with Stellio, Varanus and Uromastix, in that a strong light would induce a proximal migration of the pigment and a weak light, or none, a distal movement. Since Phrynosoma is abundant in the southern and western parts of the United States and since its color changes gave promise of being the reverse of those in Anolis, so far as their relation to light was concerned, I decided to make it an object of study.

Preliminary trials made at my suggestion by Mr. A. S. Pearse showed that horned toads obtained from dealers were not usually in a very satisfactory condition for work of this kind, and further that the color changes, though present in Phrynosoma, were not

of a very pronounced type. The subject was, therefore, abandoned till through the kindness of Miss S. R. Armington I came into the possession of a large vigorous specimen of Phrynosoma blainvillei Gray, from San Diego, California. This specimen had been well fed, had recently shed its skin, and showed in a clear and unmistakable way a series of color changes that had been only faintly indicated in other specimens. The results recorded in this paper refer in the main to the reactions of this one specimen, though they have been checked by observations on other horned toads in the laboratory. I am under obligation to Miss Armington for the privilege of working with this animal.
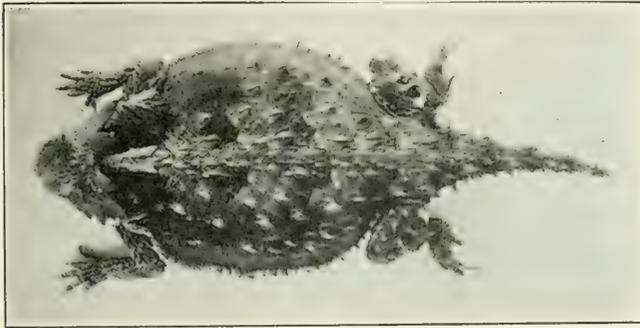
Fig. 1. Dorsal view of Phrynosoma blainvillei, showing the dark coloration due to exposure to daylight. $\times \frac{2}{3}$.

Since both light and heat influence the migration of the pigment in the melanophores of lizards, it was obvious that in experimenting on Phrynosoma, both factors were to be kept in mind. In a room at 19° C., after an exposure for several hours to bright but diffuse daylight, the horned toad was deep brownish, mottled with black and white (Fig. 1). The head was brownish gray. Down the middle of the back from the head to the tail ran a broad brownish streak, which was much lighter on the neck than elsewhere. Right and left of this lighter portion and covering most of the neck were two large patches almost black in hue. The trunk was marked by four chestnut-brown transverse bands

alternating with darker bands. The most anterior of the brown bands was immediately behind the neck and crossed the trunk from foreleg to foreleg. The most posterior one was on the pelvis. The tail was also marked by transverse bands, five dark ones alternating with four brown ones. On the lateral edges of the trunk the color was chestnut-brown, and the claw-like scales of the edge, numbering about twenty to a side, had dark, almost black bases with white edges and tips (Fig. 2). The legs were banded transversely dark brown and black. The ventral side of the animal was light-colored, the trunk being yellowish with numerous gray splotches.

After the horned toad had been kept in the dark at 19° C. for several hours, it became distinctly lighter excepting on the ventral surface, which remained unchanged. The head assumed a light yellowish-gray tint, the brownish dorsal line and the brownish transverse bands on the trunk and the tail also became lighter, as did the chestnut-brown edges of the body. But the most marked change was in the lateral claw-like scales; these lost their dark bases and became yellowish white (Fig. 3).

Since it was not always easy to follow the color changes in the complicated pattern of this animal, I selected for further study the lateral claw-like scales, in which these changes went on in a clear and conspicuous way. Each scale is somewhat triangular in form with its claw-like apex turned posteriorly. As already stated, these scales in bright diffuse daylight at 19° C. are deep gray or even black except on their free edges and tips, which are yellowish white (Fig.2). After an hour or so in the dark at 19° C. the whole scale became yellowish white (Fig. 3). These scales have a well developed guanine layer and a deep-seated layer of melanophores whose pigment when in the distal branches produces the dark central areas and when withdrawn leaves the whole scale whitish. In some of the experiments the dark central areas did not disappear entirely even after the animal had been in the dark for some time, but they remained visible as faintly grayish or greenish spots. On examining these spots under a hand lens they were found to contain scattered, minute, dark points, evidently particles of pigment which had not yet been

carried proximally far enough to be hidden by the guanine layer and which thus gave rise to the grayish or greenish tint.
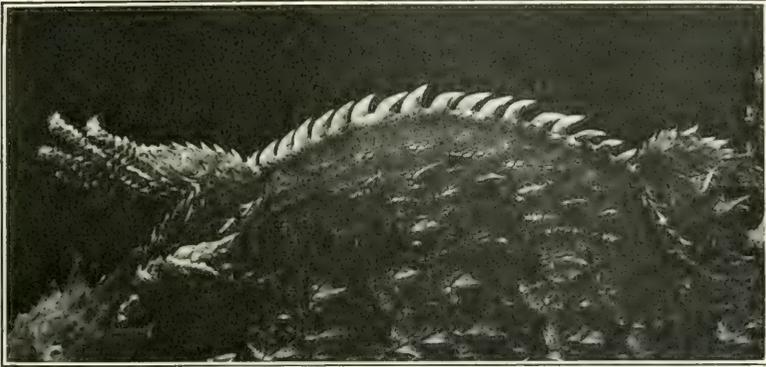

Fig. 2
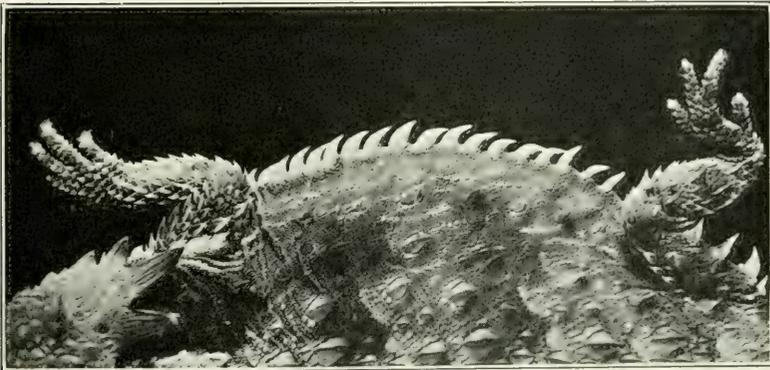
Fig. 3

Figs. 2 and 3. Dorsal views of the right side of Phrynosoma blainvillei, showing (Fig. 2) the dark coloration due to exposure to very bright daylight and (Fig. 3) the light coloration due to retention in the dark. The negatives from which these figures were made were taken from the same animal—Fig. 3 immediately after the animal had been taken from the dark, and Fig. 2 after exposure for an hour and a half to bright daylight. The illumination for photographing was diffuse daylight and the exposure in each instance was 7 seconds. The two plates were then developed for the same length of time in the same fluid. In Fig. 3 the very faint bands and patches on the lateral claw-like scales are shadows. These also occur in Fig. 2; otherwise the dark color on these scales in Fig. 2 is due to the melanophore pigment. The effect of this pigment can be most fully appreciated by comparing the last three lateral scales in the two figures. The magnification is only slightly over natural size.

The complete proximal migration of the pigment is carried out at a somewhat different rate from the distal migration. At 19° C. the distal migration was accomplished in about fifteen minutes and the proximal one in a little over half an hour. Thus in Phrynosoma, as in Anolis (Carlton, '03), the distal migration takes less time than the proximal one.

I next tried the effect of temperature changes on the migration of the pigment. At 15° C. in bright diffuse daylight, the lateral scales had very pronounced dark centers, much as at 19° C. under similar illumination. These dark centers were partly but not completely lost when the animal was kept in the dark at 15° C. As these centers usually disappeared completely at 19° C. in the dark, it is clear that a low temperature favors a distal position for the pigment.

At 32° C. the animal when in the dark was light-colored and the lateral scales showed no traces of dark centers. Their tint was a clear ivory white. When the lizard was transferred to the light at this temperature, it became rapidly darker, the lateral scales always showing gray centers, which sometimes became almost black, but not so much so as at 19° C. Hence a high temperature favors a proximal position for the pigment.

Similar results, both as respects light and temperature, were obtained from two other horned toads in the laboratory, but these animals were much less satisfactory for experimental purposes because of the conditions of their skin. In both the epidermis was filled with fine particles of dirt that rendered the pigment changes visible only with difficulty, nor were these two lizards as quick in response as the first one was, a fact that may have been due to their lack of proper food and surroundings.

From these observations it is evident that both heat and light influence the pigment migration in the skin of Phrynosoma blainvillei, a bright light and a low temperature calling forth a relatively rapid distal migration of the pigment and the absence of light and a high temperature inducing a proximal migration. Bright light is antagonistic to high temperature and no light to low temperature. Since the animals were dark-colored in a bright light at a high temperature and light-colored in the dark

at a low temperature, it follows that between 15° C. and 32° C. light or its absence is a more effective stimulus than heat or cold and in the main controls the resulting color. I believe, therefore, that the dark coloration noticed by Wiedersheim (Hoffmann '90, p.1353) in Phrynosoma orbiculare on sunless, cool days was probably a light reaction, though the low temperature doubtless favored it. It seems to me improbable, however, that Keller ('95, p.132) is correct in assuming the reaction to be due exclusively to light. The blanching of the animal, which is stated by Wiedersheim to occur when sunlight falls upon it, is not so readily explained. My three specimens of Phrynosoma remained quite dark in full sunlight even after several hours of exposure.[1] Had they turned light, I should have suspected that it was due to heat, but in all my experiments with these animals the effect of heat has invariably been found to be subordinate to that of light and the animal has always been dark-colored in daylight. How we are to explain Wiedersheim's observation, I am at a loss to say, but it is not impossible that Phrynosoma orbiculare differs from P. blainvillei in its relation to light and heat, and that like Anolis it may react to a high temperature by withdrawing its pigment even when illuminated. In such a case the blanching in sunlight would be a temperature effect and not due to light. That such a condition is not improbable may be inferred from the observations of de Grijs ('99, p.51) on Phrynosoma cornutum. In this species the individuals are said to be dark-colored in an unheated cage and light-colored in a warm one, even though illuminated.

If the light color assumed by some species of Phrynosoma in bright sunlight is not due to a reversal of the ordinary light reactions, such as are seen in Chamæleon and Anolis, but to a temperature reaction, may it not be that the blanching of the other supposedly exceptional cases, such as Stellio, Uromastix and Varanus, is also to be explained in this way? Unfortunately there is not sufficient evidence at hand to settle this question, but

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[1]In this respect my results agree with the observations of Gadow ('o1, pp. 510, 521), who states that Agama stellio when basking and geckos when in broiling hot sunlight are almost black.

in two of the best known cases, Uromastix and Varanus, this interpretation is not impossible.

Uromastix, according to Thilenius ('97, p.536), is dark colored at night, but whitens after the morning sunlight has been on it for an hour or more, and remains so till evening, when it again becomes dark. No conclusive evidence is given as to whether this reaction is due to heat or to light, but if, as I suspect, temperature is the controlling factor, these changes are precisely what should be expected, and the fact that Uromastix has been observed by de Grijs ('99, p. 51) to become light-colored in a warm cage favors this view.

The relations of Varanus to heat and light have been stated by Thilenius ('97, p. 536) more fully than those of Uromastix. Varanus is said to be dark-colored in the shade at a temperature of 45° C. to 50° C., but to blanch in the morning sunlight before the thermometer had reached 30° C. Such an instance might seem to be a conclusive case of reversed light action, but in my opinion the dark color in this instance is due, as in Phrynosoma blainvillei, to the action of the diffuse daylight (shade) irrespective of the relatively high temperature. The blanching in sunlight, however, notwithstanding the statement as to temperature made by Thilenius, I believe to have been due to heat. It is true that Thilenius states that the temperature of the morning sunlight had not yet risen to 30° C. when the change took place, but it seems to me hardly possible that such could have been the case. Even the early morning sunlight usually has much more heat in it than this. Possibly Thilenius took the temperature with an ordinary mercury-bulb thermometer, and it may be that the reflection from such a bulb would be sufficient to allow so low a reading to be recorded, but with a black-bulb thermometer I feel confident that a higher temperature would have been found, and such a bulb would have imitated much more nearly the conditions in the dark absorbing skin of the lizard than the reflecting surface of a glass-and-mercury thermometer would. I, therefore, believe it probable that the blanching of the skin of Varanus in sunlight is a temperature reaction, such as de Grijs has observed in other lizards, and not a reversed light reaction.

If these conclusions are correct, the reactions to light and to heat of the melanophores and other like cells in the skins of lizards can be stated very simply, as follows: Light causes a distal migration of the pigment granules of these cells; its absence a proximal one. A high temperature causes a proximal migration of the pigment; a low temperature a distal one. So far as I am aware, there are no real exceptions to these rules. Light causes a distal migration of pigment and its absence a proximal one in Chamæleon (Brücke, '52; Keller, '95, etc.), Anolis (Carlton, '03; Parker and Starratt '04), and Phrynosoma blainvillei; and I know of no lizard of which the reverse is true. Heat causes a proximal migration of pigment and cold a distal one in Chamæleon (Brücke, '52; Keller '95); Eumeces schneideri, Tarentola annularis, Uromastix, Sceloporus undulatus, Crotaphytus collaris, Phrynosoma cornutum, Amphibolurus barbatus, Agama mossambica, A. stellio, A. inermis and Cachryx defensor (de Grijs, '99, pp. 51-54), and Anolis (Parker and Starratt, '04). Here, too, I know of no exceptions. At unusually high temperatures, such as obtain in the full sunlight of torrid deserts, the light reactions of the melanophores of certain species of lizards are apparently subordinate to their temperature reactions, and hence these forms may appear light-colored in full sunlight. Thus Amphibolurus barbatus, according to de Grijs ('99, p. 54), darkens in the early morning sunlight but changes to light gray as the heat of midday comes on, and Anolis (Parker and Starratt, '04) becomes green, *i. e.*, its melanophore pigment migrates proximally, at 40° C. to 45° C. irrespective of illumination. The same is probably true, for reasons already given, of Stellio (Filippi, '66) Phrynosoma orbiculare (Hoffmann, '90), Varanus and Uromastix (Thilenius, '97) Calotes emma (Gadow, '01, p. 519), and most desert-inhabiting agamids and iguanids (de Grijs, '99, p. 54). Thus, in my opinion, the apparently exceptional cases of light reactions in melanophores are really instances of normal temperature reactions and do not show any reversal of the regular processes. I believe, therefore, that the simple rules already stated as to the relation of heat and light to the pigment migration in the melanophores of lizards will hold for all those lacertilians in which such changes occur.

Not only do the melanophores of lizards show uniformity in the migration of their pigment under the influence of light, but the rule that holds in these cases appears to be of much wider application. It is well known that in the eyes of most vertebrates the pigment of the retinal pigment-cells migrates back and forth under varying illumination, and here, as in the melanophores, light causes a distal migration and its absence a proximal one. The same has been shown to be true by Hess ('05, p. 421) for the retinal pigment in the eyes of cephalopods. The compound eyes of many arthropods show similar changes. According to Exner ('91) and others, the dark pigment of these eyes is often divided into two layers, one distal and the other proximal. The proximal layer consists of dark pigment granules within the retinular cells and these granules migrate back and forth under changes of illumination. The distal migration is made in the light and the proximal one in the dark exactly as with the melanophores. Not only is there agreement in the direction of the migration but the relative rates in the few cases known show a certain similarity. Thus, in the retinular cells in Palæmonetes (Parker, '97), the distal migration is more quickly accomplished than the proximal one, a condition parallel with that seen in the migration of the pigment in the melanophores of Anolis (Carlton, '03; Parker and Starratt, '04) and of Phrynosoma.

In all these instances the direction of the migration is in strict relation to the source of light and is not determined by such obvious structures of the cell as the nucleus. Thus in the melanophores of the lizards, in the retinal pigment cells of the vertebrates, and in the retinular cells of certain crustaceans, Gammarus (Parker, '99), for instance, the distal migration of pigment is in a direction away from the nucleus, while in the retinular cells of most crustaceans and insects (Exner, '91; Parker '97) it is toward that organ. Thus the position of the nucleus seems in no way to influence the direction taken by the migrating pigment.

As the pigment particles of the melanophores are within cell limits and, when illuminated, move toward the source of light,

the phenomenon may be described as a form of intracellular phototropism positive in character. It is, however, highly improbable that the pigment granules take any active part in this operation; in my opinion they are simply transported by the protoplasm, which in some instances certainly receives directly from the light the stimulus to motion. That the pigment particles are not necessarily concerned in the motion is seen in the retinular cells of Gammarus, whose distal pigment, which is most exposed to light, fails to migrate, whereas that which is somewhat more proximal in the same cell performs the characteristic migration (Parker, '99).

It is probable that the forms of pigment migration already discussed are more or less adaptive in their character. The dark color of the lizard's skin in moderate illumination at a moderate temperature insures, possibly, among other things, a certain degree of warmth which would be superfluous, if not dangerous, at a higher temperature, and in consequence the skin becomes light-colored in hot sunlight. The movement of the retinal pigment in both vertebrates and arthropods is well calculated to protect the receptive organs of their retinas from overstimulation by light and to improve the sharpness of their retinal images. Thus these pigment changes are not without adaptive character. Admitting such to be true, it might be supposed that if a case arose in which a reversed migration of pigment would be of service to the organism, such a form of migration would be evolved and a set of pigment cells in which the pigment granules under illumination would migrate away from the source of light instead of toward it would be produced. In this connection it is interesting to observe that such a reversed movement of pigment does occur in the distal pigment cells of the compound eyes of many arthropods, but that where this has been studied with fullness, as in Palæmonetes, it has been shown that these cells, though they contain the same kind of pigment as the proximal cells do, have a pigment change based upon a wholly different principle; the cell as a whole migrates distally and proximally and the pigment granules within show no intracellular rearrangement (Parker, '97). Hence it seems probable that the melanophores, retinal

pigment cells, and other like structures in which dark pigment granules exhibit migratory movements, are restricted as to these possibilities, and that in light they always transport their pigment toward the source and never in the reverse direction.

Whether heat is as uniform in this particular as light seems to be, cannot be stated with certainty. Parker and Starratt ('04) have pointed out the uniformity of its effects on the melanophores of the vertebrate skin and it is possible that this uniformity also extends to the retinal pigment, for Herzog ('05), following up certain observations made by Kühne ('79, p. 334), has shown that in the frog for a range of temperatures between 18° C. and 0° C. the retinal pigment migrates distally with a decreasing temperature and proximally with an increasing one, as the pigment of the integumentary melanophores does, but in ranges above 18° C., according to Herzog, the reverse takes place. However, the relation of the retinal pigment migration to temperature has been so little studied that further investigation will be necessary before safe general statements can be formulated.

SUMMARY

1. Phrynosoma blainvillei can change the color of its skin from a light yellowish gray with dark bands and spots to a dark chestnut brown mottled with black.

2. The light coloration is produced by the proximal migration of the pigment granules out of the processes of the melanophore and other like cells into their bodies, thus exposing to the light the reflecting guanine layer.

3. The dark coloration is produced by the distal migration of these pigment granules from the cell bodies into their processes, whereby the guanine layer becomes covered and cut off from the light.

4. In Phrynosoma blainvillei the proximal migration is favored by heat and the absence of light, and the distal one by cold and light.

5. In Phrynosoma blainvillei between 15° C. and 32° C. light or its absence is more effective as a stimulus to color change than heat or cold.

6. The blanching of certain lizards in strong sunlight, which has been supposed to be evidence of a reversed light reaction, is probably not a light reaction at all, but a temperature reaction normal in direction.

7. As in the retinular cells of Palæmonetes, the distal migration of the melanophore pigment of lizards is more quickly accomplished than the proximal migration.

8. It is probable that in all melanophores in which there is a migration of pigment, light or a low temperature will induce a migration toward the source of illumination and the absence of light or a high temperature a migration in the reverse direction.

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SOME REACTIONS OF CATERPILLARS AND MOTHS

BY

ALFRED G. MAYER AND CAROLINE G. SOULE

It is believed by many naturalists that the larva of Danais plexippus is warningly colored, for it is conspicuously ringed in black, white and yellow; often displays itself openly upon the leaves of its food plant, and it is not known to be subject to the attacks of birds or other vertebrates. The larva normally feeds only upon species of milkweed (Asclepias), although in captivity it may sometimes be induced to feed upon the leaves of the carrot. Each individual larva commonly spends its entire life upon a single plant. It usually lives upon or near the upper half of the plant, and only rarely does it descend to a point below the middle of the plant.

In fact, were the larva to crawl down the stem and escape from the plant it might possibly starve before finding another Asclepias. We have frequently placed larvæ upon the lower part of the stem of a milkweed (Asclepias) and in all but one instance they crawled up the stem and remained for days at or near the top of the plant feeding upon the leaves. In one case, however, the larva crawled upward but moved away from the milkweed upon some blades of grass which touched it.

It must be borne in mind, however, that the caterpillar of D. plexippus is sometimes found upon very small or immature milkweed plants; too small to provide them with sufficient food upon which to mature. Under these conditions it would seem that they must either find other milkweeds or starve. If this be the case their success in finding other milkweeds must be facilitated by the fact that these plants usually grow in clusters, but we have no evidence leading us to conclude that the larva of D. plexippus has the ability to direct its course to a milkweed rather than to any other plant.

The larvæ of Argynnis, Catocala, and some other lepidoptera feed only at night, and crawl a considerable distance away from their food, and remain hidden during the day, and these caterpillars must in some way be guided back to their food.

That the larva of D. plexippus may also have this ability, seems probable from the fact that if the caterpillar be repeatedly disturbed it finally curls up and drops off the milkweed.

Our experiments show that under normal conditions the caterpillar of D. plexippus is constrained to remain upon the milkweed in obedience to two reactions which the creature displays with almost machine-like regularity. It is negatively geotactic, and positively phototactic. If the larva be placed head downward, in darkness, upon a vertically suspended string, it will usually turn at once, and crawl upward. If now the string be reversed so as to cause the larva to be again head downward, it again reverses its direction and crawls upward. This may be repeated time after time with the same result. Often when the string is reversed the larva continues to crawl downward for a short distance, but it is restless in so doing, and shows a tendency to frequently stretch its anterior segments outward at right angles to the body, and to sweep widely to and fro with its head. This is sure to result soon in the head being bent completely back upon the body, and in consequence the larva crawls upward. In crawling upward the creature is more at ease and rarely stretches its head horizontally outward. Thus in its travels the larva is almost certain to go farther in an upward than in a downward direction. This is especially true if the larva be in ordinary daylight but it also takes place in the dark. Larvæ were placed head downward upon a vertical string which was placed in an absolutely dark chamber, and at the end of three minutes the string was observed. If the larva had turned and crawled upward the string was reversed, and observed again at the end of another three minutes. Thirty-seven experiments upon three larvæ gave the following results. The larvæ, at the expiration of three minutes, had turned and crawled up to a point above their original position in twenty-seven cases. In four cases the larvæ continued downward, and were found head downward and still descending, while in six other cases the larvæ

had ascended above the original position, but had again turned and begun to descend. It is apparent that the larva shows a tendency to crawl upward against the force of gravity, and this reaction alone would tend to retain it at or near the top of its food plant. No difference is apparent in the reactions of well-fed or hungry larvæ, excepting that the latter are more active.

In another series of experiments a milkweed was planted in a flower-pot and suspended upside down. Larvæ placed upon the young leaves, at what was now the lowest part of the plant, crawled upward, and sooner or later they went up the stem of the plant and crawled off upon the flower-pot. One larva crawled up the entire length of the plant (nineteen inches) and off upon the flower-pot in four minutes and twenty-seven seconds. Upon being again replaced upon the young leaves, it again crawled off the plant in about six and one-half minutes. The plant was then reversed and placed in its normal upright position. The same larva was then placed at the base of the stem, and promptly crawled upward to the top of the plant where it remained feeding for fifty-three hours, when it was removed for further experiments. Frequent repetitions of this experiment showed that the larvæ do not long remain upon a plant which has been turned upside down.

We may conclude that the larva has no inherent tendency to seek out the young leaves, and that its remaining near the top of the plant it not a matter of "judgment" or conscious preference, but is due to negative geotaxis, and may be a wholly unconscious reaction. It is worthy of note that the tendency to turn and crawl upward only affects the larva when in motion. It will often remain *at rest* head downward for hours at a time, but upon *beginning to move* is almost certain soon to turn upward.

But, another influence contributes to cause the caterpillar to remain at or near the top of the plant. This is due to the fact that the creature is positively phototactic, and is particularly sensitive to the ultra-violet rays. If the larva be placed in the middle of a horizontally placed opaque tube, one end of which is stopped with a cover impervious to light, while the other end is open to admit diffused daylight, the larva always turns and crawls toward the open, lighted, end of the tube. Experiments were made upon

six larvæ under the above conditions, using a pasteboard tube eleven inches long and one and one-half inches in caliber, and they crawled fifty-one times to the open end of the tube and never to the dark closed end. The larvæ were pushed back into the center of the tube after each trial and the experiment so arranged that the head-end of the creature faced the dark end of the tube but invariably the larva turned and crawled toward the daylight. This positive heliotropism in caterpillars is well known, and Loeb ('05, p. 42) states that it was shown by all of those upon which he experimented even by the larvæ of the willow-borer which live in the stems of willows where they are not exposed to light. When this experiment with the horizontal tube is tried, substituting the light of a 36-candle power kerosene lamp, four feet distant, for daylight, a very different reaction takes place, for under these conditions the larvæ crawled to the light end of the tube twenty-eight times, and to the dark end twenty-one times. The light of a kerosene lamp is deficient in ultra-violet rays, and it seemed probable that the larvæ might be sensitive to these rays and hence practically indifferent to the light of kerosene, while they always crawled toward diffused daylight. In order to test this the diffused daylight was obliged to pass through a layer of bisulphide of carbon one and one-half inches thick before entering the light end of the tube. Experiments were made upon four larvæ under these conditions. One of them crawled five times to the light end, and eight times to the dark end of the tube; another larva went twenty-one times to the light and one time to the dark end, another went once to the light and ten times to the dark end, and the fourth larva went three times to the light end and never to the dark end of the tube.

Thus in forty-nine trials the caterpillars went nineteen times to the dark end of the tube, and thirty times to the end which admitted diffused daylight deprived of its ultra-violet rays. Now, if ordinary diffused daylight be admitted at one end of the tube the larvæ *always* crawl toward it, and are also much more active than when under the influence of the daylight deprived of the ultra-violet rays. We see, then, that they are attracted chiefly if not wholly by the ultra-violet rays and are but little if at all influenced by the rays that constitute, to us, the visible spectrum.

Even hungry larvæ in diffused daylight deprived of the ultra-violet rays move with extreme slowness, and often remain for hours at a time motionless; and apparently unstimulated. They act similarly in the dark. In ordinary diffused daylight, however, they are very active, and rarely remain quiescent more than a few minutes at a time. Intense sunlight renders the larva very restless but it becomes less active when in the shade. When hungry the larvæ are much more active than when well fed.

At night when its negative geotropism is apparently the only factor tending to maintain the caterpillar near the top of the plant, the sluggishness of its movements in the darkness must be a safe-guard, preventing its moving to any considerable distance.

The negative geotropism of the caterpillar is, however, more potent than the influence of the diffused daylight of an ordinary room, as is shown by the following experiment. If we place the larva in the middle of a vertical pasteboard tube having the upper end closed with a cap so as to prevent the entrance of light, while the lower end remains open admitting the diffused daylight of the room, the larvæ show a greater tendency to go upward into the darkness than downward toward the light. Thus two larvæ, placed in such a tube, went fifteen times to the dark (upper) end of the tube, and eight times to the light (lower) end, although they were invariably placed head downward, nearer the lower than the upper end, and facing the light at the beginning of each trial.

It will be remembered that Loeb showed that the starving larvæ of Porthesia chrysorrhœa are positively phototactic, while they become practically indifferent to the influence of light when well fed. Larvæ of D. plexippus are, however, always positively phototactic to moderate daylight, whether well fed or starving. Lubbock (Avebury) ('83) showed that ants are always sensitive to the ultra-violet rays, reacting strongly against them, and Loeb found that nocturnal moths are attracted mainly if not wholly by the more refrangible rays of the spectrum.

In 1903 Parker published a beautiful series of observations, showing that when the mourning-cloak butterfly, Vanessa antiopa, alights in sunlight it comes to rest with its head turned away

from the sun, and with its body in line with the rays of light. On cloudy days or in shaded places this reaction is not displayed, and even in brilliant sunshine it is overruled by the chemotropic response to food. This negative phototropism is seen only in intense sunlight, and then only after the butterfly has been upon the wing; *i. e.*, after a certain state of metabolism has been established. The butterfly will creep or fly toward a source of moderate light, such as a lamp in a darkened room, or a window admitting light; and it is even attracted toward intense sunlight until it has begun to fly about, when it becomes negatively phototropic. When the eyes are blackened no reaction to light occurs, and the butterfly flies upward.

It appears that positive phototaxis and negative geotaxis are all that are required to maintain the larva of D. plexippus upon the young leaves of its food plant, and to practically prevent its wandering away from the plant itself. The larva certainly displays no "judgment" in finding its most nutritious food, and probably its reactions are almost if not quite unconscious, for they are displayed with almost machine-like regularity at every recurrence of the stimulus, however as Jennings ('04) has shown, animals are not machines for their method of behavior is often that of trial and error and internal as well as external factors modify their behavior.

Another criterion of consciousness is the presence of associative memory, but all of our attempts to detect the presence of such memory have failed.

A large number of experiments were made upon larvæ which will eat only certain definite kinds of leaves, and will starve to death without taking a single bite of other sorts of leaves. These larvæ can be induced to eat sparingly of previously uneatable leaves, however, if the sap of their proper food plant be pressed into the previously distasteful leaves. For example, the larva of D. plexippus can be induced to eat sumach (*Rhus*) leaves if the latter be thickly covered with the milky juice of Asclepias. This only occurs, however, when the larva has become very hungry.

Conversely, milkweed leaves can be rendered uneatable by covering them thickly with the milky juice of sumach. The larva appears not to be guided by any sense of color in seeking its food,

for it will eat Asclepias leaves which have been painted over with aniline red.

We find that any caterpillar can be induced to bite at or devour any foreign substance, if one proceed as follows: Allow the larva to begin eating its proper food plant, then slide up in front of it a distasteful leaf, sheet of paper, piece of tinfoil, etc. The larva will then take a few bites out of the foreign substance, but will soon withdraw its head, often snapping its mandibles and thrashing from side to side. Very soon, however, it recommences to devour its proper food in a normal manner. Under these conditions if the foreign substance or distasteful leaf be presented to the larva at intervals of not more than one and one-half minutes, about the same number of bites are taken at each presentation. The instinctive mechanism of eating once set in motion continues as if it possessed momentum. This may be taken to show that the larva does not retain the memory of its disagreeable[1] experience for an interval of a minute and a half. If, however, we make the interval about thirty seconds, the larva will take fewer and fewer bites of the distasteful object and will soon refuse it altogether, and stop eating whenever it is presented.

The following table gives the results of observations of the above sort upon larvæ of D. plexippus; the intervals of presentation of the distasteful leaf being as nearly as possible one and one-half minutes apart. The table shows the number of bites taken by the larvæ at successive presentations of a leaf of the Virginia Creeper, Ampelopsis.

An inspection of the table shows that the successive reactions in any single series of experiments are very irregular, but that these irregularities tend to disappear when we take the sum of the corresponding reactions in a large number of experiments. For example, in the column headed "Summation of series showing at least nine reactions," we see that the numbers are relatively more nearly equal each to each than are those of any one of the

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[1] The words "disagreeable," "agreeable," "distasteful," etc., are used in default of better expressions. We must not forget that the larva may be quite unconscious, and may act by reflexes, which may be very complex and controlled by varying internal and external conditions, thus being capable of much modifiability, although possibly unconscious.

Table I

Showing the Number of Bites taken by Larvæ of D. plexippus at each Presentation of a Leaf of Ampelopsis, the Ampelopsis being Presented at Intervals of 1½ Minutes

No. of Trials	Larva No. I Oct. 8, 1901	Larva No. I Oct. 7, 1901	Larva No. I Oct. 6, 1901	Larva No. I Oct. 5, 1901	Larva No. I Oct. 2, 1901	Larva No. II Oct. 2, 1901	Larva No. II Oct. 5, 1901	Larva No. II Oct. 9, 1901	Larva No. II Oct. 6, 1901	Larva No. II Oct. 7, 19.1	Larva No. III Oct. 2, 19:1	Larva No. I Oct. 4, 19 1	Larva No. II Oct. 4, 1901	Larva No. IV Sept. 7, 1902	Larva No. V Sept. 12, 1902	Larva No. V Sept. 13, 1902	Larva No. V Sept. 14, 1902	Larva No. V Sept. 15, 1902	Larva No. V Sept. 16, 1902	Larva No. VI Sept. 26, 1902	Summation of all Series of at least 9 Reactions
1	13	8	2	0	8	3	10	8	8	6	3	13	0	3	6	0	1	6	1	3	94
2	2	16	2	0	7	6	0	10	0	16	3	2	8	2	4	4	17	17	8	3	117
3	6	6	4	0	4	0	11	14	25	14	7	1	10	4	4	4	12	41	8	8	169
4	1	5	3	0	6	0	17	26	0	2	3	0	6	0	7	0	21	20	7	7	105
5	6	11	2	0	9	7	2	6	24	14	2	2	5	1	3	0	9	25	8	10	140
6	6	0	2	0	8	5	2	19	29	18	0	0	14	2	6	0	10	13	0	15	130
7	0	4	6	0	2	8	18	30	0	8	2	2	8	0	0	1	3	0	6	19	87
8	1	3	3	0	9	5	28		8	3	0	2	12	1	0	0	6	29	15	14	142
9	2	7	2	3	4	6	53		13	9	2	5	16	0	0	0	4	11	0	9	144
10	0	3	1	1			23		10	6			13				6	16	13	5	
11	2	0		0						7							1	12	2	13	
12	4	10								4							0		10	9	
13	3	0								4							5		0	5	
14	9									0							7		17	0	
15	3									2							5		5		
16	0									19							4		9		
17	3									3							11		11		
18	3									4							23		11		
19	4									11											
20	0									0											
21*	6									10											

*From the twenty-first to the fortieth trial, inclusive, this series gave the following for the number of bites at each trial :

6, 0, 2, 5, 7, 1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 2, 0, 4, 0.
6, 0, 2, 5, 5, 7, 1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 2, 0, 4, 0.

component series. The fact comes to light that there is no sensible falling off of the reaction, the number of bites taken at the ninth reaction being fully equal to those taken at the first. It can hardly be affirmed that the larvæ must take a certain definite number of bites from a foreign leaf before being able to "recognize" or react against its "injurious" or "distasteful" nature, for when larvæ are placed in a chamber surrounded with these same leaves they will starve to death before taking even a single bite. The instinct to eat must first be initiated by the presence of food proper to the larva, but once the mechanism of this instinct is called into play, it continues as if by its own momentum. The phenomenon might, therefore, be termed the "momentum of reaction." As the value of this momentum remains the same, and does not decrease with successive reactions it probably represents an unconscious reflex on the part of the larva. The presence of its proper food plant causes the eating reaction to come into play, and this reaction continues for a constant and apparently unmodified time against a regularly recurring succession of counteracting stimuli.

Larvæ do, however, become accustomed to certain stimuli, and if such a stimulus be repeated, it finally ceases to produce any reaction. If one blows a current of air upon a moving larva, or raps sharply upon the surface on which it is crawling, the creature contracts and remains motionless for some time. If this stimulus be repeated every time the larva begins to move, it finally loses all effect. For example, a single blow of the breath sufficed to halt a caterpillar of D. plexippus every time it started for four successive times; at the fifth start, however, two blows were necessary to cause it to cease moving; then, however, one blow sufficed until the nineteenth attempt to start, when eleven blows were necessary to stop it. Then one blow sufficed until the forty-seventh attempt at start when five blows were necessary; on the forty-eighth attempt to start, one blow still sufficed to stop it; but when it started for the forty-ninth time, one hundred blows had no effect upon it whatsoever.

Usually, however, the results are not so irregular; for example, one blow sufficed to stop a caterpillar in thirty-seven attempts to start, but at its thirty-eighth attempt one hundred blows had no effect and it moved steadily onward.

When the larva of D. plexippus has become insensitive to blows of the breath, it still reacts to mechanical shocks, stopping when the twig upon which it crawls is sharply struck, mechanical shocks can, therefore, be distinguished from other mechanical or chemical stimuli.

Davenport ('97), Loeb ('00), Massart ('01) and Jennings ('02, '04) have studied this final failure to react to a repeated stimulus. The studies of Jennings upon various fixed Infusoria are especially instructive, and he concludes that the phenomenon is not necessarily due to fatigue either of the muscles or of the sensory or perceptive power, but as Davenport has expressed it, "When an organism has been stimulated by contact for some time it becomes changed so that it no longer responds as it did at first." In other words, internal factors come to modify its behavior. We must bear in mind that in some cases this failure to respond to repeated stimuli may be due to fatigue as has been shown to be the case in the rejection of pieces of filter paper by the tentacles of Metridium (see L. F. Allabach ('05); *Biol. Bulletin*, vol. x, p. 35).

In another series of experiments designed to test the presence or absence of associative memory, larvæ of Pyrrharctia isabella were placed in a wooden box sixteen inches long, five inches wide and four inches deep, which was divided into two compartments by means of a central wooden partition. This partition was pierced by a small opening. The chamber on one side of the partition contained moist earth and growing food plants while the chamber on the other side was barren. The larvæ were placed in the barren chamber and after wandering about they found their way through the opening into the food chamber. Then after being allowed to eat for a few minutes they were thrust back through the opening into the center of the barren chamber. Apparently, however, they failed to learn the direct path to the food, but always wandered about upon successive trials. For example, one larva was placed in the barren chamber twenty-one times; during the first ten times it found the food in the average time of 50.8 minutes. The average of the next ten times was, however, 101 minutes and the final trial consumed 434 minutes. Another larva which was placed thirty-eight times in the barren chamber appeared to do

better for it made an average of 88.8 minutes for the first ten, 66.1 minutes for the second ten, 52.8 for the third ten, and 129.2 minutes on the last eight trials. Another larva made twenty-five trials giving an average of 29.3 minutes on the first ten, 17.3 minutes on the second ten, and 18.8 minutes on the last five. Another larva made the following averages in forty-two trials. For the first ten 58.7 minutes, second ten 58.1, third ten 39.1, fourth ten 32.2. On the whole it appears that the larvæ do not learn, even after repeated trials, to shorten their paths to the food.

It was evident that the larvæ are attracted by the presence of the food, however, for when the food chamber contained earth with no plants the larvæ entered it at much more protracted intervals than when food was present. For example, when the food-chamber contained growing plants, a larva entered it forty times, the average time for each trial being forty-seven minutes. When the plants were removed leaving only the moist earth the larva crawled into the chamber less frequently, the average time in nine trials being 228 minutes, and when both earth and plants were removed it made an average during seven trials of 417 minutes.

It will be remembered that Yerkes ('02) and Yerkes and Huggins ('03) found that the green crab and the crawfish are able to learn simple labyrinth habits.

A study was made of the instinct to spin the cocoon, in order to, if possible, reduce it to a set of reactions to definite stimuli. The experiments were carried out upon Samia cynthia and Callosamia promethea. In both of these moths, however, the larvæ accommodate themselves to very varied conditions. For example, we have seen several cocoons of S. cynthia which were in all respects similar to normal cocoons excepting that they were much larger than the average. These large cocoons, however, contained each a single large inner chamber in which there were two pupæ. Apparently two larvæ had started to spin side by side at about the same time, using one and the same leaf and had mutually accommodated themselves to the conditions so as to contribute each its share toward the spinning of a single cocoon large enough to accommodate both of them. If all leaves be removed, the larvæ of C. promethea and S. cynthia will still spin cocoons even upon a perfectly flat surface.

These larvæ normally start the cocoon by covering the leaf-stalk with a layer of silk. They then spin down the middle of the upper side of the leaf and draw the edges of the leaf together, forming a tube. After spinning an outer case of moderately loose silk, the inner case of densely spun silk is constructed. This inner case is ellipsoidal, and its innermost surface smooth and polished. The silk at its upper end is, however, loose and the strands are longitudinal so as to allow for the exit of the moth. The lower end of the inner case is densely woven and its innermost surface polished. The cocoon hangs vertically downward and the larva always pupates with its head turned upward. We find that this upward turning of the head of the larva is in response to gravity, for if the cocoon be reversed in the dark immediately after the outer envelope is completed, the larvæ often become reversed and still pupate head upward, but facing what would under normal conditions be the lower end of the cocoon. Miss Caroline G. Soule first tried this experiment in 1900 with twenty-eight larvæ of S. cynthia, reversing the cocoons of nineteen immediately after the formation of the outer envelope, and of nine a few hours after it had been completed. In all of the cocoons which were turned upside down immediately after the formation of the outer envelope, the larvæ were reversed in their cocoons and pupated head upward. In thirteen of these cocoons the silk of the inner chamber was loose at both ends. In one cocoon the larva had bitten a hole in the lower end when it became the uppermost, and had spun the other solid. Five cocoons had no exit arranged, and their inner capsules were solid at both ends. In all of the nine cocoons which were turned upside down, a few hours after the completion of the outer envelope the larvæ pupated head downward, and the cocoons were normal in all respects.

In 1901–'02, a similar experiment was tried upon twenty-seven cocoons of C. promethea. Twenty-one of these were not affected; the larvæ pupating head downward. In six other cocoons the larvæ pupated head upward, thus being reversed in reference to the cocoon. In one of these cocoons the inner case was loosely spun at both ends, while in the five others it was spun as in the normal cocoon and thus the head of the pupa faced the densely

woven end of the inner capsule which apparently allowed of no ready exit. We tried enclosing larvæ in capsules of glass and of cotton network resembling the inner capsules of the cocoon in shape but the larvæ still spun an inner capsule of silk in a normal manner. We then opened a normal cocoon and after removing the pupa placed a spinning larva within it. The larva spun a new inner capsule which was nowhere attached to the cocoon within which it was made. Evidently the instinct to spin the cocoon may be adjusted to widely different environmental conditions, some of which are never met with in nature. It cannot be reduced to a simple reaction of thigmotaxis. That is to say, the larva does not cease to spin merely because it feels itself pressed upon by the confined space of the cocoon, for even if it be placed with a cocoon already spun, it re-spins it. The larva does, however, orient itself in reference to gravity and normally pupates head upward.

Darwin ('71) supposed that the peculiar and often brilliant coloration of male Lepidoptera had been brought about as the result of long continued selection upon the part of the females.

Studies were carried out upon the mating instinct in order to determine whether the females exercised any selection in the choice of their mates.

In 1900 Mayer studied the mating instinct in Callosamia promethea in which it appeared that the female exercised no choice in the matter. It seemed desirable to repeat these experiments upon a large scale and with this in view more than 1500 cocoons of C. promethea were collected during the winter of 1091–'02. Large numbers being gathered by our friends Messrs. Clarence Riker and William F. Patterson. The cocoons were hung under trees and the moths allowed to fly about unconfined. It is important in such experiments to maintain the conditions as close to those of nature as possible for confinement often produces remarkable alterations in behavior. For example, larvæ which feed only during the night, in a state of nature, will often eat during the entire day in captivity. About six hundred males emerged from the cocoons and the wings of about one-half of them were painted with scarlet or green ink while the others were allowed to remain normal in color. It was evident that the males whose wings were

scarlet or green succeeded fully as well in their attempts to mate as did the normal males.   More than three hundred attempts to mate were observed.   Seventy per cent were successful and thirty per cent gave rise to no visible resistance on the part of the female although no mating occurred.   In one instance only was a normal male resisted successfully by the female, while in another case the male succeeded in mating although the female made some show of resistance.   Two of the green colored males were successfully resisted by two females, although even a slight showing of resistance on the part of the female was too rare and exceptional to be of any moment in selection.   Therefore, the peculiar black coloration of the male appears not to have been caused by sexual selection on the part of the female, or at any rate the female promethea moths of the present time show no dislike for abnormal coloration in the male.

In Porthetria dispar the male is brown and the female white, and it is possible that the peculiar coloration of the male may have been developed under the influence of sexual selection on the part of the female.   The experiments of A. H. Kirkland ('96) upon the mating habits of P. dispar, show that in common with C. promethea the males are attracted solely by the odor of the female.   In C. promethea, however, Mayer found that old females are more attractive than young, whereas the reverse is the case with P. dispar.   Moreover, the severed wings or scales of the females of C. promethea do not attract the male while those of the female of P. dispar are highly attractive.

Experiments were made to determine whether the females of P. dispar exercised selection against maimed or abnormally colored males.   The wings of an equal number of males and females were clipped off, and the success achieved by the wingless males in their attempts to mate was much less than that of the perfect males, as will appear from the following table which records the results of one hundred attempts to mate.

TABLE II.

Showing the Reactions of Females of P. dispar whose Sight is Normal

	Mated without Resistance	Male Resisted Unsuccessfully by the Female	Male Resisted with Success by the Female	Female Attacked by Male without Resistance on Her Part but without Mating	Per cent of Mating without Resistance by the Female	Per cent of Resistance on the Part of the Female
Perfect ♂ + perfect ♀	19	3	7	17	65.5	34.5
Wingless ♂ + perfect ♀	4	2	11	5	23.5	76.5
Perfect ♂ + wingless ♀	10	1	3	4	71.4	28.6
Wingless ♂ + wingless ♀	6	0	6	2	50	50

The above table shows that the perfect males were much less apt to meet with resistance from the females than were the wingless males. Some further experiments were made to discover whether the selection against the wingless males was determined by sight. The females were blinded by painting their eyes with a thick layer of quick-drying asphaltum. The results are shown in the following table:

TABLE III.

Showing the Reactions of Blind Females of P. dispar

	Mated without Resistance	Male Resisted Unsuccessfully by the Female	Male Resisted with Success by the Female	Female Attacked by Male without Resistance on Her Part, but without Mating	Per cent of Mating without Resistance by the Female	Per cent of Resistance on the Part of the Female
Perfect ♂ + perfect ♀ —	10	2	2	15	71.5	28.5
Wingless ♂ + perfect ♀	7		2	4	77.7	22.3
Perfect ♂ + wingless ♀ —	1		1.	6	50	50
Wingless ♂ + wingless ♀	4		1	4	80	20

It appears from the above tables that when the female is blinded the wingless males succeed fully as well as do those which are normal. Selection on the part of the female is therefore conditional upon the possession of sight.

Another series of experiments was carried out in which the females were normal in all respects while the wings of the males were painted scarlet or bright green. This abnormal coloration, however, failed to affect the results, and the colored males succeeded quite as well as did normal males. Apparently the female selects against wingless males but not against those showing abnormal colors.

These experiments upon P. dispar would have been made more extensive had it not been that they simply confirm the excellent and very extensive experiments of Crampton upon Saturnidæ. Crampton shows that normal males are more apt to mate with normal than with abnormal females, and also that abnormal males mate less readily than normal males. Moreover, abnormal females lay fewer eggs. In a word the actual basis of elimination is *correlation*, not sexual selection exercised by the female in respect to color.

The mating instinct in the males of C. promethea and P. dispar is a phenomenon of chemotaxis. Sexual selection on the ground of color alone does not affect it, and there is no associative memory connected with it. We frequently placed a normal male among females, and after mating had taken place, the antennæ of the male was covered with flour paste. Under these conditions the male never again mated, but often did so immediately when the flour paste was dissolved with water. It appears, therefore, that the mating instinct can only be called forth through the sense of smell, and not through associative memory.

The males fly toward the females against the wind. Frequently we have observed a male flying up against the wind until he passed by the side of and beyond the female. Under these conditions he would often remain poised on his wings, and the wind would drift him back until he came to leeward of the female, when a few vigorous strokes of his wings would bring him more or less toward her again. In other words, the male pursued the method of trial and error so ably shown by Jennings ('04) to be prevalent in the animal kingdom.

Miss Soule found that if the wind be allowed to blow from a female toward a male Saturnid moth both of the same species, the

male may be induced to mate with a female of another species confined in a cage with him, thus demonstrating that the mating instinct is called forth through chemotropism.

SUMMARY

1. The caterpillar of the milkweed butterfly, D. plexippus is positively heliotropic to the ultra-violet rays, but almost if not quite unresponsive to the rays which to us constitute the visible spectrum. This caterpillar is also negatively geotropic. These two reactions serve to maintain it near the upper part of its food plant, and to lessen the risk of its wandering down the stem and starving before being able to find another milkweed.

2. The caterpillar has no inherent perception of the proper form or color of its food but is guided by a chemical sense. Once the eating reaction be set into play it tends to continue so that the larva may then be induced to eat substances which it would never have commenced to eat in the first instance. This tendency to continue its activity in the face of a non-stimulus, we have called the *momentum* of its reaction.

3. After a larva D. plexippus has commenced to eat a milkweed leaf, we may cause it to bite at leaves which it would not normally eat. If this "distasteful" leaf be presented to the feeding larva at intervals of one and one-half minutes, the caterpillar takes about the same number of bites each time, showing that it is not influenced after one and one-half minutes by the events of its past experience. If, however, the distasteful leaf be presented at intervals of about thirty seconds the larva takes fewer and fewer bites at each successive presentation and soon refuses it altogether, and ceases to eat the "distasteful" leaf when it is presented.

4. No associative memory of more than one and one-half minutes' duration can be demonstrated in larvæ of lepidoptera.

5. A constantly repeated stimulus loses its effect, and this may be due not to fatigue, but to internal changes which express themselves in modified behavior.

6. The caterpillar of Samia cynthia and Callosamia promethea are negatively geotropic when about to pupate, and always pupate head upward. If the cocoon be turned upside down after the outer case has been spun they will often pupate head upward, thus facing what in nature would have been the lower, closed part of the cocoon.

7. The mating instinct of Porthetria dispar is, on the part of the male, a reaction of chemotaxis, but the normal females display a decided selection against wingless males, although not against abnormally colored males. When the female is blinded she does not select against wingless males.

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MODIFIABILITY IN BEHAVIOR

II. FACTORS DETERMINING DIRECTION AND CHARACTER OF MOVEMENT IN THE EARTHWORM

BY

H. S. JENNINGS

Johns Hopkins University Baltimore, Md., U. S. A.

When an organism moves in a certain direction, what are the various factors by which this direction is determined ? This is the question with which the present paper deals, using the earthworm as a type. The problem, as will appear, is a complex one, even for so low an organism.[1]

According to certain theories of tropisms, much held up to recent times, the direction of movement in any lower organism is always determined in a precise and simple way by the impact of external agents. For a brief statement of this view we may quote the words of Davenport and Perkins ('97) in the introductory paragraph of a paper on the directed reactions of the slug:

"It is almost an axiom in modern zoölogy, that whenever an organism, or any mass of protoplasm whatsoever, migrates in a definite direction, it does so because it is guided from without by the direction of impact of an irritant."

Just how the external irritant acts in guiding the organism has likewise been formulated in a simple way. The formulation most commonly accepted has been that which represents the result as due to the local action of the stimulating agent on the motor organs

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[1] The author had in hand a more extensive piece of work on the behavior of the earthworm, when the excellent paper of Harper ('05) appeared. This made a statement of many of my results superfluous. But while the work of Harper went farther in certain directions than my own, I have brought out certain facts, and especially have dealt with certain general relations which Harper did not take up. It seems worth while, therefore, to publish a summary of my results, bringing out the general relations in which I was chiefly interested.

of the region on which the stimulus impinges, as set forth especially by Loeb and by Verworn. Davenport again has made a convenient summary of this theory, in direct application to the earthworm, in a discussion of the way in which this animal reacts to light:

"Represent the worm by an arrow whose head indicates the head end (Fig. 1, *A*). Let solar rays *SS* fall upon it horizontally and perpendicularly to its axis. Then the impinging ray strikes it laterally, or, in other words, it is illuminated on one side and not on the other. Since, now, the protoplasm of both sides is attuned to an
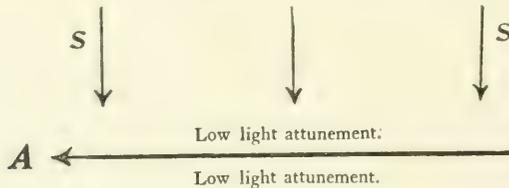
Fig. 1. Diagram to explain a tropism in a muscular organism, such as the earthworm. (After Davenport.)

equal intensity of light, that which is the less illuminated is nearer its optimum intensity. Its protoplasm is in a phototonic condition. That which is strongly illuminated has lost its phototonic condition. Only the darkened muscles, then, are capable of normal contraction; the brightly illuminated ones are relaxed. Under these conditions the organism curves toward the darker side; and since its head region is the most sensitive, response begins there. Owing to a continuance of the causes, the organism will continue to turn from the light until both sides are equally illuminated, *i. e.*, until it is in the light ray. Subsequent locomotion will carry the organism in a straight line, since the muscles of the two sides now act similarly. Thus orientation of the organism is effected. The same explanation, which is modified from one of Loeb ('93, p. 86), will account, *mutatis mutandis*, for positive phototaxis" (Davenport, '97, p. 209).[1]

Both this axiomatic view that the direction of movement is always precisely determined by external agents, and the theory that this determination is due to direct local action of the agent in question, have begun to give way before a careful examination of the

[1]In making the above quotations, there is no intention of representing Davenport as a sponsor or defender of the views set forth. In his valuable general works, Davenport has summed up in an unsurpassed way the general views prevailing at the time, and our quotations are given merely as such summaries.

facts as to just what organisms do. A thorough acquaintance with
the behavior of Paramecium, or of any other typical free-moving
lower animal, renders the above quoted axiom quite untenable.
If the animal is placed in such a position that the external condi-
tions are uniform in all directions, so that there is nothing in them
to guide it, it does not remain quiet. Paramecium when placed
in such a situation simply goes straight ahead, and the same is
true of most other lower animals. In other words, the differentia-
tions of the animal's body are sufficient to determine the direction
of locomotion, provided there is nothing else to do so, and often
this factor prevails even when there are external factors which
work decidedly against it. An animal often goes in a certain
direction merely because there is nothing to prevent its forward
movement. The animal is a going machine, and need not be
prodded up by a present outside stimulus in order to move; it
carries within itself both the impulse to move, and factors deter-
mining the direction and character of the movement. The move-
ment of such a creature as Paramecium is indeed defined in many
ways, even in a uniform environment.

If such relations are evident in examining even so low an animal
as the infusorian, it will not be surprising if we find them to hold
in even higher degree of so complex an animal as the earthworm.

Of course external agents frequently do determine the direction
of movement. But thorough study shows that their action is
rarely of the simple and direct character set forth in the summariz-
ing statement above quoted; it is usually, on the contrary, very
indirect and complex. For the earthworm, this was perhaps first
made evident by the work of Miss Smith ('02). This work showed
that the directive action of light, heat, chemicals, etc., on the earth-
worm Allolobophora is far from being of the precise, unequivocal
character that the local action theory would require. She found
that in place of orienting itself by bending steadily to one side under
the unilateral action of a stimulus, the earthworm frequently
alternated movements in various directions, till finally one direction
was selected for further progress. This direction was by no means
usually one of precise orientation. This result was confirmed by
Holmes ('05), who finds that "random movements" play a large

part in determining the direction of locomotion in the earthworm, as well as in various other lower organisms, under the action of light and other stimuli. Holmes' observations inclined him even to doubt that the earthworm has any localizing power in the negative reactions to light, in the sense that it is anywise more likely to turn first directly away from the lighted side.

In essential agreement with such results are the statistics of Parker and Arkin ('01), who found that in reacting to one-sided illumination the earthworm usually (in 65.5 per cent of all cases) merely starts ahead, and that when it turns, it may turn either toward or away from the source of light, the number of turns away from the light being only 26 per cent greater than the number of turns toward the light. The large overplus of movements that are not as required by the theory of direct local action of course require explanation. Harper ('05) showed clearly that under strong light the earthworm Perichæta may turn directly away from the light and he brought out some of the factors on which depend the other movements, that are not determined in direction by the impact of the light rays. It is a further inquiry into the determining factors of movements that do not depend directly on the localization of the stimulus, that concerns us in the present paper.

In the paper of Holmes ('05) the movements that are not in relation with the direction of the source of stimulation are characterized simply as "random." But, of course, the movements are random only with relation to the external stimulus considered; it is evident that they must be determined in some way. It cannot be admitted in a scientific treatment of the matter that any movements are random in the sense of undetermined. If we leave the matter here, calling the movements *merely* random, we leave our treatment open to the charge of inexactness, and of the use of nonscientific concepts; the movements are left without a determining cause. Why does the animal turn sometimes to the right, sometimes to the left, sometimes go straight ahead, or perform other activities, even though the external conditions remain the same?

We are forced then to take up the fundamental question, What factors determine the direction of movement of the given organism at a given moment? This question the local action theory of

tropisms tried to settle in an axiomatic, *a priori* way, taking into consideration only external factors. What is required is that the question shall be taken up as a problem for objective investigation, attempting by analytical experimentation to differentiate the various factors involved, both internal and external. The paper of Harper made incidentally a first advance in this direction; the present paper endeavors to deal with the problem more explicitly, and to advance the analysis farther.

As will appear, the problem is really an extraordinarily complex one, instead of being of the simple character assumed by the local action theory, so that the present essay can give only a general outline of the various classes of factors involved, taking into consideration situations where the external conditions are made as simple as possible.

EXPERIMENTS

The common earthworm, Lumbricus terrestris L. (L. herculeus Sav.), was used in the present experiments. The method of work was the simplest, not to say crudest, possible. The earthworm was placed on a large sheet of wet filter paper in a dimly lighted room, pains being taken to keep it moist; then the simplest possible stimuli, precisely localized, were applied to it. The stimulus chiefly used was a touch with the tip of a finely pointed glass rod. Precisely localized chemical and thermal stimuli were also used but essentially the same result were obtained with all. It is evident that in these experiments (as in those of other recent authors) the animals are under most unnatural conditions, and for certain purposes this would of course be a serious disadvantage. For our present object, however, this is not the case, since these circumstances must be taken into consideration as well as all others, in a general investigation of the factors that determine the direction of movement.

When a localized stimulus is applied to one side of the earthworm, does it respond by a simple contraction or relaxation of the muscles of the side of the region affected, thus turning the head from or toward the stimulated side?

Analysis of the results of a large number of experiments shows

that when a local stimulus is applied to one side of the anterior part of the earthworm—say to the sixth metamere—any one of the following varied methods of action may result:

1. There may be merely a slight swelling of the region stimulated.

2. The worm may turn the anterior end away from the side stimulated.

3. It may turn the head toward the side stimulated.

4. It may creep backward.

5. It may creep forward.

6. It may creep first backward, then forward.

7. The head may be merely retracted.

8. The animal may make a sudden right-about-face, interchanging the position of anterior and posterior ends, in the way to be described later.

9. The anterior fourth of the body of the worm may be raised in the air and waved wildly about.

From this list it is clear that the reaction must depend on many factors, since we can distinguish at least nine different methods of behavior which may follow on the stimulus. Of course the different actions performed in different cases are not matters of chance but are determined in some way. What are the determining factors?

So far as I have been able to determine these factors by analytical experimentation, we can distinguish at least the following groups:

I. External Factors

1. Intensity of the Stimulation. This is, of course, universally recognized as a determining factor in behavior. Other conditions remaining the same, in the earthworm a weak stimulus may induce a mere swelling of the body at the region stimulated (this varying in extent in different cases); a stronger stimulus may cause a slight turning of the head away from (or sometimes toward) the point stimulated; a still stronger one a retraction of the head, without turning; a very strong one an immediate rapid crawling backward. In connection with variations in the other conditions an almost

infinite variety of combinations of reactions may be produced by varying the intensity of the stimulus.

2. Localization of the Stimulus. The different effects of differently localized stimuli are of course universally recognized; this is indeed one of the corner stones of the orthodox tropism theory. Certain of the relations of reaction to localization of the stimulus in the earthworm are of interest.

Stimulation first of one side, then of the other, may induce in the two cases movement in opposite directions. But this is by no means always the result; the direction of turning depends, as we shall see later, on a multiplicity of factors. Other conditions remaining the same, a stimulus of a certain intensity on the right side of one of the anterior twenty metameres usually causes a turn to the left; the same stimulus on the left causes a turn to the right. It is thus clear that there exists the possibility of an immediate relation of the direction of the movement to the side stimulated, as Harper showed. This is the factor which the local action theory of tropisms takes into consideration, though there seems to be no evidence that the turning is due merely to direct local action. But in any case it is only one factor out of many. In the region back of about the anterior third of the body, this factor plays little or no part, as we shall immediately see.

Comparing stimuli applied to anterior and posterior parts of the body, very different results are reached. A rather strong stimulus on the dorsal surface of the anterior third of the body (to just behind the clitellum) usually causes the animal to creep backward. This backward movement is usually soon followed by a forward one. A similar stimulus on the posterior half of the body usually causes the worm to creep rapidly forward. Between these two regions there is an area comprising five to ten metameres in which a strong dorsal stimulus causes an immediate stretching of the entire worm, the anterior region starting forward, the posterior region backward. The anterior movement soon prevails, as a rule, so that the worm crawls forward. The final result then is in all these cases a movement forward; this is preceded in the case of anterior stimulation by a movement backward, in intermediate stimulation by a movement in both directions, while

only in the case of posterior stimulation does the forward movement take place directly. Under other conditions, to be mentioned presently, the worm may crawl steadily backward. Further, which of these three results we get in a given case depends largely on the intensity of the stimulus and on other factors not yet considered, as well as upon the localization of the stimulus.

Lateral stimulation on the anterior one-fourth or one-third of the body may, as we have already seen, cause a turning away from the side stimulated. Often, however, it causes merely a backward movement; sometimes a forward one (for the conditions on which these depend, see later). But *lateral stimulation of the posterior two-thirds of the body never causes turning away from the side stimulated*, so far as I have observed. In this region a strong lateral stimulus causes merely a rapid movement forward. The adaptive relation of these facts to the movements of the animal under natural conditions is evident. An attack in the anterior region is best escaped by moving backward; in the posterior region by moving forward. Forward movement is more rapid than backward movement, so a stimulus at the middle of the body, or even a little in front of it, is more quickly escaped by a forward than by a backward movement. Lateral attacks in the anterior region may be escaped through a sidewise turn, but lateral stimulation in the posterior region will be much more quickly avoided by a rush forward. These adaptive relations of course do not show us why the earthworm moves as it does, but their existence is interesting, and it is possible that in the long run they may have something to do with determining what movements shall occur.

The fact that strong stimuli on the lateral surface of the posterior half of the body do not tend to cause turning to one side is one that needs to be kept in mind in practical experimentation. Thus, Parker and Arkin ('01) found that light directed against the side of the anterior end of the worm causes turning away, but directed against the posterior part it does not cause the turning. This, of course, does not in itself indicate that the posterior half is less sensitive to light than the anterior half (though this may be probable on other grounds), for even if the light acted most powerfully on the posterior half of the animal, it would not cause a

bending, but merely a movement forward. The argument for less sensitiveness in the posterior half from the results of Parker and Arkin rests on the assumption of the correctness of the local action theory of tropisms for light reactions; this assumption can hardly be said to have shown itself correct.

A very powerful stimulus, constituting a severe injury, usually causes the part anterior to the injury to crawl rapidly straight ahead, while the part behind the injury squirms about violently. Close examination of this "squirming" shows that it is exactly the movement that is required, in the natural conditions under which the worm lives, for urging the body forward with great rapidity. The worm is, of course, usually in a narrow burrow. Under the circumstances the "squirming" throws first one side of the body, then the other, against the sides of the burrow, while at the same time the setæ are extended and moved in such a way as to exercise a powerful leverage against the sides. In this way, by repeated successive impulses from each side, the worm shoots forward through its burrow, tending thus to escape from the injurious agent. The anterior end is not turned about from side to side, but is held more nearly straight, so that it does not interfere with the movement induced by the powerful impulses coming from behind. At the same time it aids the flight by creeping as rapidly forward as possible.

In a well known paper Norman ('97) described the fact that when the strong stimulus is a cut, actually dividing the worm into two halves, the parts anterior and posterior to the cut behave respectively in the two ways above described, and brought this into relation with the question of the existence of pain in the lower animals. It was argued that, since only the part behind the cut shows the squirming reaction, while the part in front does not, it cannot be held that there is any indication of pain in the worm, since it must be supposed that the anterior region is at least as sensitive as the posterior one. But since the behavior of both anterior and posterior pieces is of precisely the character that would under natural conditions most assist the worm to escape from the stimulating agent, it may be questioned whether the difference between the two has any bearing on the probable exist-

ence of pain. There would seem to be no more and no less reason for supposing the strong lateral movements which urge the animal forward in the "squirming" to be accompanied by pain, than the rapid forward creeping of the anterior part.

The remaining more important determining factors in the movements of the earthworm must be classified as

II.   *Internal Factors*

These are extremely varied, and a complete classification is impossible at present.   Some of the chief ones are the following:

3. The reaction to a given stimulus depends partly on what the animal has done, and on its position, just before receiving the stimulus.

This factor shows itself as follows: The earthworm makes with its anterior end side to side movements in creeping, turning first to the right, then to the left.   If now we stimulate it just after it has turned its head far to the right, it at once as a rule jerks its head to the left.   This occurs whether the stimulus is applied to the right side, to the left side, or to the dorsal surface, so that the direction of turning becomes independent of the localization of the stimulus.   If the animal is lying bent to the right, it is most likely to bend to the left when stimulated, without regard to the precise point of stimulation.[1]

4. The reaction to a given stimulus depends partly on a general tendency of the animal to move in a certain way, namely, forward rather than backward.   This is seen in the different results of localized stimuli on the posterior and anterior parts of the body. In the former case the animal moves away from the source of stimulus (forward), and continues to do so.   In the latter case it first moves away from the source of stimulation (backward), then

[1]In describing movements that depend upon such a multiplicity of factors as do those of the earthworm, it is difficult to treat of any one factor separately without making the effects of that factor appear more absolute and independent of the presence of others than the experimental results show to be the case.   Thus, while *as a rule* the results are as given above, different results may be reached when other factors than those considered in this paragraph become the determining ones.   This remark applies to the discussion of each of the factors taken up separately in the text.

this movement is as a rule supplanted by movement toward the source of stimulation (forward).

5. The reaction to a given stimulus depends partly on the direction in which the animal is crawling at the moment when it is stimulated. In a quiet worm, posterior stimulation causes movement forward, anterior stimulation movement backward. The same results are usually observed when the animal is very quietly crawling forward or backward. But at times, when creeping forward it is found that stimuli on any part of the body—even at the anterior tip—merely cause the worm to hasten the forward movement. In the same way, a worm that is creeping backward may persist in this movement, and even hasten it, in spite of repeated strong stimulation at the posterior end. The physiological state seems to have taken a sort of set, causing the worm to obstinately persist in following the direction in which it has started. Sometimes, when the worm is in a similar condition, a stimulus at the advancing end of the animal (anterior or posterior) causes a cessation of the movement for a few seconds, then the worm starts again in the direction it was pursuing. Stimulation causes it to suspend operations for a time, but it is not to be easily turned aside from the course it is following, and it soon resumes this course.

6. The reaction to a given stimulus depends partly on previous stimuli received. I have not by any means worked out in detail all the manifestations of this principle; they are very numerous. A worm which has not received marked stimuli for some time, and is at complete rest, may not react at all to a slight stimulation. Two or three similar stimulations, however, rouse it up, and now it reacts readily. After more stimuli, or more intense ones, its reaction to any given stimulus changes in character and increases in intensity. We can distinguish from this point of view a series of different physiological states, each manifesting itself by a different reaction. A partial list of these, with the characteristic behavior, is as follows:

(*a*) The state of rest, in which the worm does not react readily to slight stimuli, such as a touch with the tip of a glass rod.

(*b*) A state of moderate activity, in which a touch at the anterior end causes movement backward; at the posterior end movement

forward, while lateral stimuli (in the anterior region) cause turning away from the side stimulated.

(*c*) A state of excitement, after repeated stimuli, in which the animal persists in the direction of movement once begun, merely stopping for a few seconds when stimulated at the end which is advancing.

(*d*) A state of greater excitement, in which stimuli merely cause the animal to hasten its movements in the direction in which it has started, without regard to the localization of the stimulus.

(*e*) A state of still greater excitement, after long-continued and intense stimulation. Now the worm responds to a stimulus at the anterior end, that would in a resting worm cause only a comparatively slight reaction, by a rapid "right-about-face." The body is suddenly doubled at its middle, so that the anterior and posterior halves become parallel, with the two ends pointing in the same direction, then the posterior half is quickly whipped about, so that the whole worm is again straight, but is facing the opposite direction from that in which it was pointed before the reaction. This peculiar reaction takes place with such rapidity that one can distinguish the way in which it occurs only after many repetitions. After this right-about-face the worm usually crawls rapidly in the new direction.

In the natural condition, within the burrow, this reaction would, of course, instantaneously direct the animal downward, if attacked as it creeps toward the surface.

(*f*) A state of still more intense excitement, after repeated strong stimulation that is of such a character as to actually injure the tissues. The worm now responds to a repetition of the stimulus (and often when the new stimulus is only slight) by lifting the anterior fourth of the body into a vertical position, and waving it about in a frantic manner. This behavior is usually alternated with the right-about-face reactions, and with persistent rapid crawling forward or backward. The spectator is involuntarily inclined to feel that the animal is tormented, and that continuation of the experiment is cruelty; this may, of course, be due only to the peculiar constitution of the spectator, and not to that of the worm.

It is beyond question that many other physiological states could

be distinguished, each with its characteristic method of action. I have considered only those appearing under relatively simple conditions of stimulation, and such as can be formulated in a more or less definite way. Many other variations in reaction that may easily be observed I have not taken up above, because recounting them would add merely a heap of details, the interrelations of which are not clear. I have further omitted all physiological states resulting from varied states of metabolism, and have not attempted to study possible lasting modifications of physiological state ("habits," etc.), but have merely dealt with the movements from moment to moment.

Yet with even this limitation of the field, it is clear that the cause for movement in a given direction, or of a certain character, is in the earthworm not simple, but excessively complex. The present external stimulus is only one of the numerous variable factors involved. The movement at a given time demonstrably depends, not alone on present external conditions, but also on former external conditions, former actions of the organisms, and present internal physiological conditions that are determined in many different ways. The direction of movement of one of these organisms cannot be represented as a simple function of the direction of impact of some external force, but is the complex resultant of many different factors.

GENERAL

Cause of Change of Reaction Under Uniform External Stimulation.

In the present and in previous papers I have shown that even while the external conditions remain the same, the reaction of the organism changes. Such a statement appears to be looked upon by some as leaving the movements without determining factors, and as therefore leading to vitalism and mysticism. But such an idea results only from a failure to realize what animals are. They are not static structures, but are bundles of processes, and it is this that gives us a key to the understanding of changes in behavior even under uniform external conditions. In every animal

processes of the most varied character are occurring: the taking of oxygen and other substances, digestion, assimilation, dissimilation, secretion, excretion, usually some form of circulation, etc. Whenever we think of an animal—an earthworm or an infusorian—we need, in order to understand its behavior, to think of it as a little engine of intense activity. The movements of the organism we know to be the results of the production of energy in these internal processes. We know further that these processes depend for their normal course upon each other, and upon the environment, and that this is as true of the movements as of the other processes. Disturbance of the internal processes we know to result in changes in the movements. In former papers ('05, '05a) I have given many cases of this and of the observed dependence of behavior on the relation of external conditions to these processes.

What then will happen if some external condition acts upon the organism in such a way as to modify one of these processes? Suppose, for example, that pressure acts in a given case in such a way as to interfere with the circulation—of the protoplasm in Paramecium or of the blood in the earthworm. The animal will perhaps first respond directly to the pressure as a primary stimulus, giving the reaction A. The pressure continuing uniformly soon impedes the circulation. We know that such interference with an internal process is in itself a cause of reaction; the animal, therefore, now responds to this interference, giving the reaction B. But circulation cannot be long impeded without interfering with respiration, and this again is a cause of reaction, giving us perhaps the new response C. The external conditions meanwhile remain uniform, but, as we see, the internal condition changes from one state to another. The interference with respiration is bound ere long to induce changes in assimilation; these necessarily entrain changes in dissimilation, and these changes in excretion. Excretion is likewise more directly impeded by the stoppage of the circulation. As a result thus of a uniform external condition modifying primarily but a single bodily process, the internal state of the animal passes from one change to another, since the internal processes are bound up in mutual interdependence. We know that the

bodily movements are the result of these internal processes, so that as one after another of these is blocked, it appears not merely natural but inevitable that repeated alterations of movement should result; we get the series of reactions A, B, C....Z. This, as I have shown in previous papers, is exactly what occurs in many lower organisms. *The action of a uniform external stimulus that affects any of the life processes is necessarily cumulative,* since a change in one process is bound to result in changes in the others. As a result of the cumulative internal changes we get repeated changes in movement, even though the external condition remains the same. There is no difficulty, therefore, in finding determining factors for such changes of behavior; they are induced by preceding changes in precisely what we know on other grounds to be the source of movement—the internal physiological processes.

There is thus no reason when we consider the fact that organisms are bundles of interdependent processes, for supposing that they should always behave in the same way under the same external conditions. Persistence in the same movement as one after another of the internal processes is disturbed would itself be a puzzle, requiring very precise internal compensatory regulations. Change of movement is what might be expected, and is what we commonly find. There is, of course, as much reason for finding such changes in the lower organisms as in higher ones, and this again corresponds with the results of experimentation.

"*Method of Trial and Error*"

In a previous paper ('04a)) I spoke of the reaction method in which the organism varies its movements under a given stimulus as the "method of trial and error," in order to bring out its similarity to the behavior for which that expression is commonly used in higher animals. The essential point which I intended to bring out by using this term in connection with the way in which these animals move toward or away from certain agents was the following: the organism performs varied movements, some features of

which are not determined[1] by the localization of the stimulus, but
by other factors; it then continues those movements which bring
it into or toward a certain condition, this condition being usually
either a greater or a less action of the stimulating agents, as
the case may be.[2] Holmes ('05, p.111) objects to the use of this
expression for some of the cases for which I employed it, as, for
example, the reaction to light in Euglena, while proposing to
reserve it for such cases as the earthworm. His reason for this is
that "Euglena does not react by a number of indiscriminate move-
ments until the right one is accidentally hit upon, but by a direct
reflex, whose effect is to bring the organism more nearly parallel
to the direction of the rays." He continues, "The phototaxis of
Euglena is not so manifestly the outcome of the trial and error
method as that of the earthworm. In the latter case light does
not cause directly a movement which makes for orientation. The
direct response may or may not have that effect. The successful
response is accidentally hit upon." He further suggests that the
expression "trial and error" be "limited to those cases in which
the adapted movements may be regarded as chance successes."

I have, of course, no desire to enter into an academic discussion
as to the proper definition to be given to the hackneyed expression
"trial and error," and I should be quite willing to drop that
expression, not only for Euglena but for the earthworm and other
lower organisms; it has perhaps gained in its history implications
of various sorts which find no corresponding factors in lower
organisms. But what does seem to me worth while is to try to make
clear the points on which the various methods of behavior that I
classified together under this name actually agree, these being the
points which impelled me to class them together. It appears to

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[1] The word *determine* is of use in experimental work only when it expresses a concrete experimental
result, namely, that when one factor varies, another (the determined one) varies in a corresponding
way. The factor A (*e. g.*, localization of the stimulus) is said to determine the factor B (*e. g.*, direc-
tion of movement) when a change in A involves experimentally a change in B. If B remains the same
even when A is changed or disappears (as in the case of the movements referred to above), then B is
not determined by A.

[2] The essential feature of this type of behavior could be expressed more generally, so as to
include other than *directed* reactions, as follows: the organism performs varied movements, some of
which do not tend to produce the result that is finally brought about by the behavior as a whole; of
these varied actions, those are followed up which do tend to produce this final result.

the writer most unfortunate to attempt to make a distinction on the basis of the emphasis of such terms as "indiscriminate," "accidentally," "chance." The "random" movements of the earthworm are of course no more a matter of chance than are the varied movements of Euglena. They are merely determined in a different way, and in very complex ways, as I have attempted to show in the present paper. Where the two agree is in the fact that they are partly determined by some other factor than the localization of the source of stimulus, and that they do not all tend to produce the finally resulting orientation. In both cases, if the stimulus were differently localized the first movements would still be the same. This is as true of the movements of Euglena as it is of those of the earthworm, in becoming oriented to light. Euglena when stimulated as a result of light coming from one side *swerves more than usual toward its own dorsal side.* This movement is at first just as likely to take the anterior end away from the source of light as toward it (see Jennings, '04). In precisely the same way, according to Holmes' account, the earthworm in reacting by the "random movement" method is as likely to turn its anterior end in one direction as in the other. But in both these organisms, after swerving for a certain distance in one direction, the creature swerves in the opposite direction. In Euglena this is due to the fact that the animal revolves on its long axis, so that the dorsal side, toward which it is swerving, soon reverses its position; in the earthworm it is due (partly at least) to the fact that a previous turn to the left itself serves to induce a succeeding turn to the right. The movement is fully determined throughout, in both cases equally. Now in both the organisms, since there is successive swerving in opposite directions, when the light is coming from one side one of the movements is bound to take the anterior end in general toward the source of light, the other away from it, and there is no more accident about this in one case than in the other. In both organisms, of these movements in different directions, "only those are followed up which bring the animal out of the undesirable situation" (Holmes).[1] In Euglena "light does not cause directly

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[1] That is to say, in both cases the following up of a certain motion *is* determined by the localization of the stimulus.

a movement that makes for orientation," any more than it does in the earthworm. Part of the movement is directly away from the position of orientation (see Fig. 23, Jennings, '04), and this very phase of the swerving is more pronounced when the animal is becoming oriented to light than in the usual spiral path when it is not reacting at all. Movever, this phase of the movement is a necessary one in bringing about the reaction, if the analysis I have given in the paper just cited is correct. The stimulus of light causes pronounced movements both away from and toward the source of stimulation, and the organism follows up more decidedly those which lead toward the optimum condition, just as happens in the earthworm.

Thus, while it appears to me immaterial whether we choose to call either or neither of these cases reaction by "trial and error," the essential point lies in the fact that in these as in many other organisms stimulation causes varied movements, which do not all lead toward the condition finally attained, and that those movements which do lead toward this final condition (the "optimum") are followed up more decidedly than the others. The behavior may perhaps be most accurately characterized as "selection from among the conditions produced by varied movements." In general we find that many organisms are so constituted that internal conditions (permanent or temporary) will produce under stimulation movements that are varied in precisely such a way as to subject the creature to as varied environmental conditions as possible, and thus give it an opportunity to select what is nearest the optimum.[1] Every one of these movements is, of course, as absolutely determined as is the most orthodox tropism, only the determining factor is not the localization of the stimulus (or other external factor) alone.

Certain recent writers have seemed to imply that there is a contrast between the "trial and error" method, and behavior that is definitely determined by structural and other internal conditions. It needs to be emphasized, perhaps, that the behavior which I and others have characterized by this phrase is very precisely determined by structural and other internal conditions; indeed, its dis-

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[1] As to how this selection occurs, see the author's paper on the "Method of Regulation in Behavior" ('05a).

tinguishing feature is the fact that it is thus determined by such conditions, rather than exclusively by the external conditions. It is, of course, very true, as Harper ('05) remarks, that definitely localized reaction methods are developed as we rise higher in the scale, yet it appears to be equally true that if we mean by "trial and error" the performance of varied movements, subjecting the organism to varied conditions, certain of which are selected, then this also becomes more highly developed and more used by organisms as we ascend the scale. We must not forget that this expression "trial and error" was originally based on the behavior of such highly developed organisms as the cat, dog and monkey; and doubtless there is no organism which uses this method to any such extent as does man. Whenever the external conditions do not furnish a precise determining factor for the movements, yet some sort of reaction is required, any organism is forced to have recourse to this style of behavior, performing varied movements till a condition is reached that relieves the organism of the necessity of continuing these movements. In its highest form we call this experimentation.

In his recent Experimentelle Biologie, v. Uexküll ('05) is unable to understand how we gain anything by characterizing certain behavior as "trial and error" (p. 128). Whether we gain insight by a certain procedure depends largely on what the problems are in which we are interested, and we find that v. Uexküll limits the problems of biology in so extraordinary a way that he could not possibly be expected to see any value in such a procedure. For him, "There is thus for animal biology only one point of view— to understand the purposive structure of every animal on the basis of its reflex arcs" (*l.c.*, p.96). He is, moreover, convinced *a priori* that we can never understand the purposiveness of organisms from a causal standpoint; "Die beiden Betrachtungsarten [Ursächtlichkeit und Zweckmässigkeit] auf einander zurückzuführen is unmöglich" (p. 129). When we add that v. Uexküll holds that *any* action of organisms may equally be characterized as trial and error,[1] it is evidently not surprising that he can make nothing of

[1] We have perhaps here further evidence that this expression is one that lends itself too readily to misinterpretation to make it the best characterization for the behavior in question.

the characterization of certain kinds of behavior as trial and error. On the other hand, some of us are interested in the question as to how it happens that organisms do those things that aid them in carrying on their physiological processes and thus keep them in existence—a matter which does not fall within the field of biology at all, as limited by v. Uexküll. To such as are interested in this problem it may very well appear that we have really made a certain advance when we recognize the fact that organisms do not in response to stimulation always perform at once a directly regulatory action; do not react merely by a stereotyped reflex, as has often been represented; but that on the contrary they perform varied movements, some of which are not directly regulatory, but which do subject them to varied conditions, certain of which conditions are selected, through cessation of the varied movements, thus resulting in regulation. If we are further able to find out the determining factors for setting in operation these varied movements, and for their cessation when a favorable condition is reached it will naturally appear to those interested in the problem sketched, that we have made a further advance. Recognition of a physiological law that results finally in the persistence of only the one movement that is directly regulatory, so that the animal later reacts by a stereotyped regulatory reflex, and no longer by the varied movements ("trial and error"), will seem a further advance to those interested in precisely the problem of how such definite regulatory reactions happen to exist. It is not necessary to hold that these determining factors and laws have been completely unveiled (see Jennings '05a, for a tentative statement of them), in order to recognize the importance and interest of the line of work that is concerned with their investigation. On the other hand, those for whom regulation is *a priori* an unsolvable problem, and who limit the field of biology to understanding the purposive structure of animals on the basis of their reflex arcs, will of course find these matters outside the field of their interest. But those whose interests are thus narrowly bounded are certainly in the minority; the writer does not happen to know of any biologist aside from Dr. v. Uexküll that holds to such limitations.

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THE PHYSIOLOGY OF REGENERATION

BY

T. H. MORGAN

WITH SEVEN FIGURES

Lest the title mislead someone expecting to find in the following pages an account of the processes of assimilation and of respiration, that presumably take place during regeneration, I ought to state that I shall deal only with the physiology of the growth process as shown in the regeneration of a new part. Morphogenesis does not express my meaning in all respects, for I am not concerned so much with changes in form as with the rate of growth and of differentiation. If I have taken a liberty in using the term physiology to cover these kinds of changes, my excuse must be that we are dealing with phenomena that lie on the borderland, where physiology and morphology overlap, and appear to merge into each other.

It is generally, if tacitly, assumed that when undifferentiated cells are supplied with food materials growth must follow, but I shall try to show, on the contrary, that whether or not growth takes place depends not so much on the available food supply as on a formative influence that regulates both the kind and the amount of growth. The nature of this formative influence is the most difficult and problematical factor with which we shall have to deal.

RATE OF REGENERATION IN STARVED AND WELL-FED SALAMANDERS

If the rate of regeneration is in any way connected with the food supply, the fact ought to become at once apparent by comparing the process in well-fed and in starved individuals. I have carried out such an experiment with the salamander, Diemyctylus viridescens. The results show that we must be careful to distinguish, in the use of the word *rate*, between a simple increase in size, and

the rapidity of the process of differentiation (or development in the narrower sense) of the new part.

Zeleny[1] has recently shown that the increase in size of regenerating arms of the brittle-star and of the legs of the crayfish is determined, in part, by the number of the appendages removed. The more parts removed, the faster each regenerates. Zeleny discusses amongst other factors, the possible relation of this result to the food supply, and points out that the larger the number of appendages removed the greater will be the temporary surplus of food, for the amount necessary to nourish the entire leg may be greater than that used at first in the growth of the small new part. Whilst pointing out the possibility of this interpretation Zeleny carefully refrains from committing himself to it as the only explanation of his results.

It seemed to me that this question might be tested in the following way. If the rate is determined by the food supply, then if two sets of individuals are selected, and one set starved and the other fed, the latter should be in a better condition for regeneration than the former. If the same number of parts is removed in each, the well-fed individuals should regenerate faster than the starved ones.

If fewer parts are removed from the well-fed individuals than from the starved ones, nevertheless the well-fed individuals should regenerate faster, for, the greater amount of food given ought to much more than outweigh the surplus in the starved ones due to the absence of more parts.

The experiment was carried out with salamanders collected in the autumn. They were in excellent condition when caught, although not so large as they soon became when fed on pieces of beef The individuals were kept for several weeks, without much food, before the experiments began. In some individuals one leg was removed, in others two, in others three, and in still others one, two, or three legs and also the tail, which was cut off near the base. Duplicate sets were prepared, each containing several of these different kinds of individuals. One lot was kept without food and the other fed about every other day on small pieces of raw beef.

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[1]Zeleny, C. A Study of the Regeneration of the Arms of the Brittle-Star. Biol. Bull., vi, 1903. Compensatory Regulation. Jour. Exp. Zoöl., ii, 1905.

As the new limbs developed they were carefully compared and in some cases measured. It was soon seen that no constant difference could be detected in the two sets, or between different kinds of individuals of the same set, if the regeneration of the new legs is measured by their rate of differentiation. Therefore, food does not seem to be the main factor in the result. But another fact also came to light. The new legs of the well-fed individuals were larger than those of the starved ones. This difference is correlated with the great difference in size between the two sets, for the well-fed animals grew to a large size, while the starved ones dwindled almost to a skeleton. By actual weight after eleven weeks one well-fed individual weighed 3.48 gms. and a starved one .67; the latter having, therefore, only one-fifth the weight of the former. This difference in weight is not due to the storage of fat; but all of the organs of the body, heart, liver, pancreas, intestine, skin, muscles, etc., are larger in well-fed animals. The new limbs also partake of the general condition of well being, so far as size is concerned; in other words, they developed in proportion to the size of the old part. Measurements of the new limbs show that those of the well-fed individuals have outstripped those of the starved individuals. The difference in diameter was especially marked, while the length of the new limb seemed to show less difference.

It has been stated that no difference in the rate of differentiation was found, but owing to the very considerable individual variation, small differences, if they exist, might have been easily overlooked, and while this must be freely granted, the main result was quite definite that no appreciable difference was seen, while the difference in size was quite apparent. There is another consideration in this connection. If the difference in size of the new parts, in relation to the number of parts removed, depends on the surplus of food, the detection of the difference might largely depend on the size of the part removed. In a form like the salamander, where the legs are relatively very small in proportion to the rest of the body, the difference in the amount of surplus food would be so small that we would not expect to detect any difference in the relative sizes, even if it exists, when one or when three legs are cut off. In fact, I could detect no such difference in these forms,

when individuals lacking one, two or three legs were compared
with each other; neither in the starved nor in the well-fed sets.
When the whole of the tail is removed the loss becomes proportion-
ately greater but still I failed to note any differences. It is to be
remembered that Zeleny's results show only an increase in size of
the new part, and not in rate of differentiation, and my own re-
sults show to some extent the same thing; at least, this difference
was found between the well-fed and starved sets, if not between the
individuals with one or with more parts removed. In the latter
case measurable results might depend, as stated above, on the rela-
tion between the relative size of the body and of the parts removed.

Zeleny's important discovery, regarding the relation between the
size of the new parts and the number of parts removed, bears a
close resemblance to another curious fact in regard to the rate of
regeneration. If the distal end of the tail is removed it regenerates
more slowly than when more of the tail is cut off. Thus the more
the material removed the greater the rate of regeneration of the
new part. Stated in this form the two results appear to be identi-
cal. This question may now be considered.

RATE OF REGENERATION OF THE TAIL OF DIEMYCTYLUS AT DIFFERENT LEVELS

If the tail of one individual is cut off near the base and of another
near the outer end, a great difference in the rate of growth of the
new tail becomes apparent. *The nearer the cut to the outer end
the slower the rate of regeneration.*[1] In general it may be said that
the rate of development of the new tail is directly proportional to
the distance of the cut surface from the distal end of the tail. A
few actual measurements will bear out this statement.

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[1]Spallanzani observed that it takes as long for the toe of a salamander to regenerate as it does for an
entire leg. King found in the starfish that regeneration is more rapid from the base of the arm than
from its tip. The results are in accord with the fact just stated for the tail of the salamander.

Increase in Length of Regenerating Tail

	Jan. 29,	Cut Off at Base		Cut Off Near Tip	
March 3	$3\frac{1}{2}$	4	$4\frac{1}{2}$	1	2
10	3	$3\frac{1}{2}$	4	$\frac{3}{4}$	2
17	4	$5\frac{1}{2}$	6	$1\frac{1}{2}$	$3\frac{1}{2}$
24	4	6	$6\frac{1}{2}$	2	4
31	$4\frac{1}{2}$	6	7	2	4
April 7	$4\frac{1}{2}$	6	7	2	4
14	5	6	7	2	4
28	6	7	7	—	$4\frac{1}{4}$

Increase in Length of Regenerating Tail

	Feb. 3,	Cut Off at Base		Cut Off Near Tip		
March 3	3	3	4	1	1	$1\frac{1}{2}$
10	4	$3\frac{1}{2}$	5	$1\frac{1}{2}$	1	$1\frac{1}{2}$
17	$5\frac{1}{2}$	6	7	2	$1\frac{1}{2}$	3
24	6	—	8	$2\frac{1}{2}$	2	—
31	7	—	8	$2\frac{1}{2}$	$2\frac{1}{4}$	—
April 7	$7\frac{1}{2}$	—	8	—	$2\frac{1}{4}$	—
14	7	—	$7\frac{1}{2}$	—	$2\frac{1}{4}$	—
28	$7\frac{1}{2}$	—	$7\frac{1}{2}$	—	3	—

What is the meaning of this result? Is it due to the larger amount of food-material available when more of the old tail is removed? This possibility was tested by starving one lot of animals. The results are not in accord with the assumption. For example: In the second half of the preceding table in the last two columns of the series "cut off at base" and in the last column of the series "cut off near tip," the records of individuals are given that were not fed after March 6. No decrease in the rate of regeneration is to be found. In fact these individuals as long as they lived did even better than the others. The salamanders were in a well-fed condition at the beginning of the experiment and the materials derived from their own bodies sufficed, during the time of the experiment, to give sufficient materials for maximum regenerative growth. In the experiment in which the legs as well as the tail were cut off, it was apparent that the new tails in the starved set were not as large, as in the well-fed set. In the starved animal the old tail also was very emaciated and much smaller in the ver-

tical and transverse diameters than was the tail of the well-fed individuals. The difference in length was not so apparent, which is probably due to the loss in the bones being less than that in the other tissues.

The normal tail of Diemyctylus is much bigger at its base than nearer its distal end, so that the cross-section of the base is larger than that of the tip. Can this difference account for the difference in the rate of regeneration from the two levels? At the beginning of the new growth this relation may account for the difference in size of the new part, because the amount of material proliferated from a large surface may be greater than that from a small one. This, in itself, would not account for the results, if, at each level, the new part, from beginning to end, were as broad as the old part, but such is not the case. The new part has more the shape of a somewhat flattened cone, with a broader base in one case than in the other, but tapering quickly to its apex. Therefore, if more material were proliferated from a broader base the cone would be longer *i. e.*, it would be in proportion to the base. On the other hand the period of proliferation is short, and the basal parts soon differentiate into their organs. Subsequent growth takes place near the tip. Hence after the first period is passed, the new tail must, in both cases, continue to grow in length through its own activity, and its increase in length must henceforth be due to this activity and not to proliferation from the base. It may appear that the difference in rate is due to some initial difference in the material at different levels of the old tail. If it were simply a question of material, *per se*, we should expect the new growth from a basal surface to be as rapid during the later stages of formation of the new tail as at first, since the material for both came from the same level, but this is not the case. Hence, I conclude, that the cause of the difference observed is not due to a difference in the old materials that go to produce the new part. The analysis leads here to the same conclusion as in other cases of posterior growth to be described, in all of which the result appears to be due to some retarding influence that appears as the growth approaches its natural terminus. The retardation is the same for the growth at the end of a new part (that arises from the base) and for the new growth

that begins from the old part near its end.   A discussion of this point will be left until other cases have been considered.

THE RATE OF REGENERATION OF THE EARTHWORM AT DIFFERENT LEVELS

In order to study the rate of posterior regeneration in the earthworm, Allolobophora fœtida, at different levels the worms were cut in two at the following places; (1), near the posterior end, removing 20 to 25 segments; (2) near the middle, *i. e.*, at about the 50th segment; and (3), just behind the girdle at about the 33d segment.   It is not advantageous to cut further forward, for, as I have shown elsewhere, the power of regenerating a posterior end ceases rather suddenly about the level of the 15th segment.   The following tables give the results of three experiments of this kind. Table I is for a set of worms 55 days old (September 30 to November 24).   Table II is for the same set 35 days old (September 30 to November 4).   Table III is another set 57 days old (December 28 to February 23).

TABLE I
September 30 to November 24
WORMS CUT NEAR POSTERIOR END, AT MIDDLE AND BACK OF GIRDLE.   ANTERIOR END INTACT

Posterior End		Middle		Behind Girdle	
Old	*New*	*Old*	*New*	*Old*	*New*
83	7	49	34	35	45
84	7	53	27	33	54
67	12	49	38	37	37
73	12			32	0
64	17			31	25
81	6			32	0
65	16			34	30+

If we consider the data given in these tables we find in the first series, Table I, that from the *posterior level* the number of new segments regenerated is small, the maximum number being 17.   In this case there were 64 old segments, showing that the regenerating end was not very near the posterior end, since about 36 segments must have been removed, or what is more probable some of the posterior segments pinched off after the operation.   In the other cases where

25 were absent six or seven new segments regenerated. From the *middle region* the number of new segments is 27, 34, 38; a much larger number than in the last case. From the region behind the girdle the number of new segments is 25, 30, 37, 45, 54; which is on the whole a still larger number than the last, although the difference is not very great. The highest number, 54, is higher than the greatest number for the middle region which is 38.

In Table II only one worm is recorded for the posterior level, and this has only 8 + new segments. In the middle region the numbers for the clearest cases are 26 +, 34 +, 34 +. In the region behind the girdle the only worm that regenerated normally had 52 new segments. The results in this table are few but they agree with those of the last one.

TABLE II
September 30 to November 4.
Worms Cut as in Table I. Intact Anterior Ends

Posterior End		Middle		Behind Girdle	
Old	*New*	*Old*	*New*	*Old*	*New*
77	8+	50	26+	38	52+
		55	34+		
		47	34+		
		51	13+		
		53	18+		

In the series recorded in Table III, the number of new segments for the posterior region is variable, owing in part to the fact that the levels from which the regeneration occurs are somewhat different, as shown by counting the old segments. There is one case with a very large number, viz: 24 (with 72 old segments) which gives almost the complete number, but in the other cases where the cut was made at about the 80th segment, only from 5 to 11 new segments regenerated. From the middle region the number of new segments is greater than for the last level, giving a maximum of 51, but most of the other cases produced about 30 to 40. From the region of the girdle the numbers are still larger, with a maximum of 61, the others varying from 31 to 40, or thereabouts. In this table also the data show the same relation between the rates at anterior and posterior levels as do the other two.

TABLE III

December 28 to February 23

TWENTY-FIVE WORMS IN EACH SET WERE CUT (A) IN FRONT OF GIRDLE; (B) BEHIND GIRDLE;
(C) AT MIDDLE; (D) NEAR POSTERIOR END

Posterior End (D)		At Middle (C)		Behind Girdle (B)		In Front of Girdle (A)	
Old	New	Old	New	Old	New	Old	New
72	13	about 50	42	about 34	61	19	0
76	8	about 50	35		37	19	0
74	10	about 50	33		31	20	0.
75	11	about 50	34		19 (abn)	21	22 (abn)
78	8	about 50	38		36–40 (abn)	23	17–20 (abn)
88	5	about 50	42		55 (abn)	26	23
71	17	about 50	11		Abn.	25	50
68	4	about 50	51		40+ (abn)		
72	24	about 50	40				
		about 50	24				
		about 50	30				

There is also another series, Table IV (56 days old), including only two levels. From the middle level of the worm the maximum number of new segments is 58 (with only 41 old segments present), while the other individuals have between 23 and 43. From the region in front of the girdle (22 to 26 old segments) the numbers are very variable; 40 new segments being the maximum. Comparison of these results with those in the other tables shows again that in the middle and anterior regions the number of new segments is large, and much greater in number than when the worm is cut in two nearer the posterior end.

TABLE IV

January 6 to March 3

ANTERIOR END INTACT; CUT AT MIDDLE OF WORM		A FEW ANTERIOR SEGMENTS CUT OFF ALSO CUT AT MIDDLE OF WORM.		INTACT ANTERIOR END, CUT IN FRONT OF GIRDLE	
Old	New	Old	New	Old	New
56	39	about 50	30	22	0
55	43	about 50	45	24	0
41	58	about 50	35	24	12+
47	44	about 50	18	24	3+
	23	about 50	50	24	40 (about)
	32	about 50	45	26	30 (about)
	38	about 50	26		
	30	about 50	42		
		about 50	48		
	30	about 50	30		
	30	about 50	48	48	
	24	about 50	23		
		about 35	55		

What is the cause of this difference in the rate of regeneration at different levels? The amount of food available at different levels might appear to furnish the most probable explanation of such differences. For instance, if the food is digested in the anterior part of the body, let us say in the stomach and the anterior part of the stomach-intestine, there will be the same amount present in the worms cut at the three levels; but since on the hypothesis the posterior end uses up more food than it digests, the surplus for regenerative purposes will be greater the less there remains of this posterior region. Hence at the level of the girdle, the regeneration will be more rapid than at the middle, and at the middle more rapid than at the posterior end. I tried to test this possible interpretation in the following way. The head ends of some of the worms were cut off, and also at the same time the posterior ends at the same three levels as before. For two or three weeks, or more, the worms were unable to obtain food, hence if the same difference in the rate of regeneration at the three levels were to occur the assumption that the difference is due to a food relation is disproven. Such was found to be the case, as the following data show. The series were made at the same time as were those given in the preceding tables with which, therefore, they are to be compared. Thus Table V should be compared with Table I; Table VI with Table II; and Table VII with Table IV.

TABLE V

September 30 to November 24

WORMS CUT NEAR POSTERIOR END, AT MIDDLE AND BEHIND GIRDLE; ALSO A FEW ANTERIOR SEGMENTS REMOVED

Posterior End		Middle		Behind Girdle	
Old	*New*	*Old*	*New*	*Old*	*New*
69	9	60	39	34	29
84	7	64	29	37	40
83	9	54	42	35	48
76	13	46	38	34	33+
87	5			33	35 (about)
64	17			34	49
79	16			34	35

As shown in this table there is a marked difference in rate of regeneration between the posterior and middle levels, but not between the middle and the girdle levels; in fact, rather in favor of

the latter, but since the same difference in rate is found between the girdle level and the tail level as is found in intact worms, the results show that the difference in rate is not due to a difference in the food supply.

In the next series, Table VI, the only survivors were those cut at the level of the girdle (except one worm cut at the middle which did not regenerate at all).

TABLE VI

September 30 to November 24

Worms Cut Behind Girdle and a Few Anterior Segments Cut Off

Old	New
35	58+
35	29
—	0
—	abnormal
—	very short

Owing to the difficulty in counting the segments in these worms the results are unsatisfactory; but so far as they go the results show, when compared with those of Table II, that the rate is about the same in both, yet while confirmatory the number of cases is too small to be of much value. In the middle columns of Table IV there are recorded other cases of worms cut in two at the middle, and at the same time some of the head-segments were also removed. The number of posterior segments that regenerated is about the same as in the corresponding set for the same level with intact anterior ends as recorded in the first columns of Table IV. These results, taken in connection with those given above, seem to show that whether the worms are with or without food for a considerable period of the time the number of the segments produced is about the same. Suppose we reverse the argument and assume that, since in the starved worms the materials for regeneration must be supplied by the reserve materials in the worms themselves, then the longer the piece the greater will be the sum total of the reserve supply and hence we should expect more regeneration; but the facts contradict this assumption, for the longer the worms the slower the regeneration. Here again we find that the results cannot be explained as due to the food factor. If we assumed that the anterior end is the storehouse for the reserve, and that the

posterior end merely uses up the food, and, therefore, the longer the piece the less material available for posterior regeneration, we might appear to offer a formal explanation of the results, but there are no facts in favor of this assumption, and the experimental results that are next to be described negative such a conclusion.

In order to examine the relation between the rate of regeneration and the size of the piece the following experiment was made. The worms were cut near the middle and at about the 20th segment from the posterior end in one series and in the other series at the middle and in front of the girdle. The pieces between these levels were used in both cases. In the former only a very few posterior segments regenerated (only one or two), and in the latter cases only 6, 7 and 12. The results are shown in Table VII. This is a distinct difference, to be sure, although the number of segments in both cases falls below those of the check series. The small number of cases is no doubt partly responsible for the few new segments at the middle region, as shown by other experiments to be described, but that this is not the whole question is also shown by the next series.

TABLE VII
January 6 to March 3

About 20 Posterior Segments Cut Off; also Cut at Middle of Worm. New Segments	Cut in Two at Middle and in Front of Girdle; New Segments from Middle Level New Segments
0	6
1	12
1	7
0	
0	
1	
0	
0	
2	
1	
1	
0	
0	

In order to obtain a series of still shorter pieces, the worms were cut into five pieces, each in the following way. Each worm was

first cut in two in the middle (about the 50th segment). The anterior end was then cut into three pieces, by one cut behind the girdle and by another halfway between the anterior end and the girdle. The posterior end was also cut into three pieces; thus, 20 segments were cut from the posterior end and thrown away, the remaining part was then cut in two. All of the pieces of one kind were kept together, and were killed and examined after two months (January 6 to March 3). The check set of longer pieces will be found in Table IV.

TABLE VIII

January 6 to March 3

WORMS CUT INTO SIX PIECES (TAIL PIECE THROWN AWAY). FOR DETAILS, SEE TEXT

E		D		C		B		A	
Old	*New*	*Old*	*New*	*Old*	*New*	*Old*	*New*	*Old*	*New*
13	15	12	18	14	14	26	15 (about)	19	knob
17	4	11	18	13	17			23	18
16	5	12	17	15	14			22	0
15	2	21	6	8	14			19	0
15	0	19	20	15	8				
17	0	16	14	15	knob				
17	0	16	35	14	14				
17	0	11	15	14	18 (about)				
		17	0	—	0				
		21	5	—	0				
		12	8	—	0				

For convenience the pieces will be called A, B, C, D, E; the first being nearer the anterior end, etc. If we examine the piece in the reverse order, beginning with E, we find relatively few new posterior segments in these pieces, although in one case the surprising number of 15 segments have developed. It seems not unlikely that in this case a piece was misplaced. In the D series the number of segments is very variable, ranging from 5 to 35, the latter number giving nearly the entire number lacking at the posterior end. In the C series, whose posterior end is at the middle of the worm, the number of segments is about the same as in the last case, except there is no such extreme case as that mentioned. In the B series only one piece was alive (of the 25 cut off) with about the same number of new segments as in the last cases. In the A series only one

piece had regenerated (from the posterior end of the 23d segment) with 18 poorly developed segments.

With the exception of the E pieces this series does not show any very marked difference for the different levels. There is a possible source of error in the pieces from the posterior region, for I have sometimes found these breaking up into smaller pieces, or pinching off pieces from the posterior end. The small number of the old segments in some of these pieces, where the expectation is about 15, may be due to this; but granting the possibility of such occurrences the main results cannot be due to this factor as will be seen by examining the number of old segments present in the pieces.

If we compare this table with the longer pieces of Table IV, comparing, for example, those cut at the middle of the worm in the two cases, we find that fewer segments regenerate as a rule in these very short pieces. It will be recalled that in none of these pieces does a head develop at the anterior end; on the contrary, most of the more posterior pieces develop a heteromorphic tail on the anterior end. How far this heteromorphic development may affect the result is not clear, but that the small number of posterior segments cannot be be due to this factor is shown by comparison with somewhat longer pieces that may also develop heteromorphic tails. The great mortality of these short pieces indicates that they are not under very favorable conditions, although the death rate is especially high at first, and while later the small pieces appear to be in a healthy condition and may live for several months, yet after two or three months they all die of starvation. One point, however, is fairly clear that although the small pieces lack the power to produce new parts at the maximum rate for a given level, yet the retardation is far from being proportionate to their small size. From this fact we may safely conclude that the amount of the food supply in the piece is not the main factor in its rate of regeneration, although when it has decreased below a certain point regeneration may stop or be much retarded for want of materials. These negative conclusions in regard to the rate of growth are useful in so far as they clear the way for the discussion of the main problem of what factors regulate the rate of growth, for, if it is not due to

food the road is clear to search in some other direction for the meaning of the facts. Before attacking this fundamental question I should like briefly to bring into relation with the foregoing results certain other facts that I have already published.[1]

I have shown that if 10 to 12 posterior segments are cut from the posterior end of a worm, and then the next 10 to 12 segments are cut off, the piece lying between these cuts does not as a rule produce any new posterior segments, even after three and a half months. Whole worms, however, that lack the last 10 or 12 segments regenerate a few segments in this time. In another experiment, long pieces from the middle of the worm, having the anterior end also removed, were compared with similar pieces with the head end intact. The rate of posterior regeneration was the same for both. Again, some worms with only a few posterior segments removed were compared with similar ones which lacked also the head end. The regeneration at the posterior end was the same in both after a month showing that the results do not depend on the question of the taking in of food. These and a few other experiments are in harmony with the results described above.

So far the results have been judged by the number of the new segments produced; in other words, by the *differentiation* of the new part. If, however, we test the results by the size of the new part certain differences are apparent. The most general result is seen at once when well-fed worms are compared with starving pieces.

The new part in the well-fed individual is larger, and this is especially noticeable in the diameter of the piece, that often approaches that of the old part. The individual segments are also larger in the well-fed worms, so that for an equivalent number of segments the new part is longer. In the starved worms the new segments are often very small, especially when the old part is a very short piece. In striking contrast to this difference in size, which is so apparent that it is not necessary to take measurements to discover it, the number of segments is, as we have seen, approximately the same in a well-fed and in a starved individual. These

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[1] Morgan, T. H. Regeneration in Allolobophora fœtida. Archiv f. Entw. Mech., 1897.

results show that while the size of the new part is dependent on the food supply, the growth and differentiation of the new part is to a large extent independent of this factor. The difference in the size of the new part in the two cases shows, nevertheless, that the new part is affected by the conditions of the food supply, and it is probable that the smaller number of posterior segments regenerated by very short pieces is the result of the lack of food for further growth, hence the pieces from different regions show approximately the same number of segments, but the difference in rate of regeneration of larger pieces at different levels cannot be accounted for in this way as the experiments first described have shown. So long as there is enough food material in the blood or other fluids of the body to allow growth to take place at all it goes on at a rate determined by the peculiarities of each level, and largely independent of the food supply.

If my conclusions from the data are correct, and the difference in the rate of development of the new parts at different levels is not, in the main, due to differences in the food supply, to what is the difference due? The answer that first suggests itself is that the difference must be due to differences in the materials of the worm at different levels. If by differences at different levels we mean the differentiation of the parts, or the kind of material that exists at each level, then the conclusion may express a part of the truth, but if we mean that each level possesses limitations in its powers of growth, not possessed by other levels the answer can be shown to be inadequate. For example, if the rate of regeneration from the middle of the worm were determined by certain peculiarities of its material, we should expect the same rate to continue until the entire missing part was replaced; and if we were then to cut off the tip of the new tail after it had completed itself it ought to regenerate as rapidly as does the new part from the middle of the worm, because its material has come from that level. While I have not actually carried out this experiment there can be no doubt that the newly regenerated tail would show at its tip the same retardation shown by the unregenerated tail. Furthermore, if this view were correct how could we explain the termination of the growth process when the normal number of segments has been

replaced ? Why should not the new part continue indefinitely to grow ? If we can find the explanation for the cessation of growth at the proper terminus we can probably find also an explanation for the difference in the rate at different levels, for, as can be shown, the two things appear to be one and the same. In other words, as the new part grows longer its materials change, and this change is of such a kind that it leads to the cessation of growth. Hence starting under different conditions at different levels the same end result will be reached in all cases, and when the terminus is reached the growth should slowly decline as we find in fact that it does. It is this idea that will be developed later.

There is another point that must be mentioned here. The difference in rate is not so much due to an initial difference in the appearance of new material at different levels, as to the relatively greater slowness of the growth of a terminal part after it has been once started. This seems to mean that the stimulus for the formation of the new part is so much greater than the difference in the rate for each level, that the latter becomes entirely overshadowed in the formation of the first new tissue. Moreover, the proliferation of new material as the result of an injury may depend on a different set of factors than the subsequent rate of growth of a new part after the original stimulus that led to the proliferation has disappeared.

In order to compare the rate of regeneration at the beginning and toward the end of its period of growth some of the same lot of worms that gave the records of Table IV were kept alive for another month (January 6 to April 6) in order to see how much further the regeneration of new segments would continue. The results are given in the following Table IX.

TABLE IX

Cut at Middle		Cut Behind Girdle		Cut near Posterior End	
Old	*New*	*Old*	*New*	*Old*	*New*
48	52	33	13 (abn)	75	10
59	32	32	72	75	8
41	53	36	60	63	17
52	36	34	52		
51	40	34	53		
51	46	33	45		
53	42				

The number of new segments from the middle region is about the same as it was a month earlier; the full number having been nearly reached, although not quite in a few cases. More new segments are found in the worms cut just behind the girdle; the number approaching completion here also. In the cases where about 25 segments had been cut from the posterior end, the number removed had not yet been made good, and the same holds for the single case where about 37 old posterior segments had been removed. The results show a much smaller proportionate increase during the third month than during the two preceding ones. In other words, as the new part approaches completion its rate of regeneration declines.

REGENERATION OF THE CAUDAL FIN OF FUNDULUS

Experiments on the regeneration of the caudal fin of Fundulus were carried out during the summer of 1905, while occupying the Table of Columbia University at the Marine Laboratory at Woods Hole, and continued throughout the present winter at the New York Aquarium, where, thanks to the admirable arrangements and to the opportunity for scientific work extended by the Director, Mr. Chas. Townsend, it has been possible to carry on the work under the most favorable conditions.

In two former papers[1] dealing with the regeneration of the fins of Fundulus and of some other species of fish I have described the differences in rate of regeneration from the basal and distal parts of an oblique cut made through the tail. This difference I referred correctly, as further results have shown, to the retardation of the growth on the more distal parts. This retardation, however, did not seem to me to belong to the same category of facts as the retardation from a more distal squarely-cut surface, and it was primarily in order to account, if possible, for this difference that I again undertook a re-examination of the facts. The new results have cleared up this point satisfactorily, and I have gone further and been able to obtain data that bear on the more fundamental question of the rate of growth from different levels.

[1] Morgan, T. H. Regeneration in Teleosts. Archiv f. Ent. mech., x, 1900. Further Experiments on the Regeneration of the Tail of Fishes. Archiv f. Ent. mech., xiv, 1902.

This problem of the rate of growth at different levels would seem to be a comparatively simple one, but I have found it somewhat baffling, owing to the great individual differences in the rate of growth in different fish. Two methods of study have been followed. In the one, the tails were removed from a number of fish at two or three different levels, and the rate of regeneration measured from time to time in the living fish, or a few fish were killed at intervals and then measured. In the other case the records of the rate of growth of the same fish were kept which gives more accurate data.

The results may be briefly summarized in the following statements. The rate of growth is, *at first*, nearly the same whether the cut is made near the base or near the outer end of the tail. This period covers that of the first proliferation of new material. Very soon, the new parts grow more rapidly from the basal than from the more distal cut surface. In general, the nearer the new part approaches its completion, the slower its regeneration, so that the new part from a distal cut surface very soon grows with extreme slowness, while that from the basal cut continues for a longer time to grow, but it, too, as it gets longer, shows an ever increasing retardation in its growth.

In regard to obliquely cut surfaces, I can confirm my former statements, namely, that regeneration from the more distal part of the oblique surface is much retarded after the first period of proliferation is over. Moreover, this retardation is far greater than that seen in a cross-cut surface at the same distance from the end of the tail, and consequently is not due to the same factor. I can now give some further experiments that throw a great deal of light on this retarding factor in oblique regeneration.

If the tail of a fish is cut off in such a way (Fig. 1) that two cross-cut surfaces *a* and *b* are exposed at different levels, it will be found that on *a*, regeneration progresses much more slowly (after its first beginning) than on *b*. The rate of growth on *b* is as fast or faster than that on a cross-cut surface of the whole tail at this level, while that on *a* is very much slower, and seems almost to come to an end after a time unless the new part from *b* catches up to the

new part on *a*, after which the two new parts continue to grow forward together until the tail is completed.

In the reverse case the cut surface *a* is made the broader of the two, as shown in Fig. 2. Under these circumstances the retardation is smaller on *a*, while that on *b* is the same as in the former case, which is the same as that of an entire cross cut for this level. The results show that the retardation of the distal surface is in proportion to its height, while the growth on the basal cut surface is the same as the regeneration for an entire cross-cut surface at this level. The results are the same whether the distal partial surface is at the top or at the bottom of the tail.
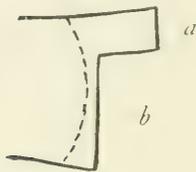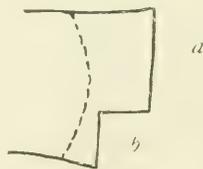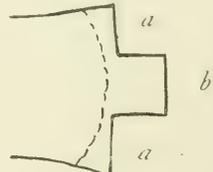
FIG. 1   FIG. 2   FIG. 3

It might be supposed that the retardation on the outer partial cut *a* is due to this part lying at one side of the tail, where it might be supposed to receive less nourishment. I tested and disproved this possibility in the following way. The tail was cut off as shown in Fig. 3. Here the middle portion has a small and independent cut surface. Regeneration takes place from all three surfaces, but the growth from the outer one *a* was markedly less than that from the other two. After a time the new upper and the lower parts, from the upper and the lower basal cut surfaces, catch up to the middle part, and after this has occurred the three parts, completely united, grow forward as a single organ to complete the length of the tail. In order to prevent this latter result I have cut away after a time the new parts above and below, leaving the middle part intact, and in some cases this was done two or three times before the upper and lower parts had caught up to the middle part. In this way I hoped to discover whether the middle part would finally grow out to its full length, or whether it would not go

beyond a size proportionate to the width of the piece from which it arose. It was found that the new middle part continued to grow, but with extreme slowness. Whether it would finally reach its full length I do not know, but it is not improbable that it would do so.

These results explain, in a way, the regeneration from an oblique surface. If we consider any part of the outer oblique surface by itself alone, it is in the same condition as the outer part in the preceding cases of partial cut surfaces, and the retardation of the growth in both would seem to be due to the same factors, whatever these may be. The outer part of an oblique surface cannot complete itself, except very slowly if at all, until the new tissue from the more proximal parts of the same cut surface has caught up, and set it free, so to speak. This is, in fact, what occurs. The nature of the retarding influence will be discussed after some further facts have been considered.

The details of the experiments in which the rate of regeneration at different levels was examined may now be given.

The first experiment was with Fundulus majalis. The operation was carried out on July 7, and the first measurements were made July 26. The same lot of living fish gave all of the measurements. The tails were cut near the outer or distal end, near the base, and obliquely between these two levels. From the basal cut surface the new parts measured $1\frac{7}{8}$, 2, 2, $2\frac{1}{4}$, $2\frac{1}{2}$, $2\frac{1}{2}$, 3, 3. From the distal cut surface the new parts measured 2, 2, $1\frac{7}{8}$, $1\frac{3}{4}$, $1\frac{7}{8}$, 2. Already at this time some of the basal cuts had regenerated faster than the distal ones. The oblique cut surfaces were measured near the top and near the base where the length was greatest. The two measurements for each fish are bracketed, the distal one standing above. They are $\begin{cases} 1 \\ 3 \end{cases} \begin{cases} 1 \\ 2 \end{cases} \begin{cases} 1 \\ 2 \end{cases}, \begin{cases} 1 \\ 2\frac{1}{2} \end{cases}$ The basal measurements correspond with those of the same level given above. The distal measurements are about half as much as the distal measurement given above. Five days later, August 1, the following measurements were taken.

Basal, $3\frac{1}{2}$, 4, 3, 3, $3\frac{1}{2}$, $4\frac{1}{2}$, $3\frac{1}{2}$, 4, 4, $4\frac{1}{4}$

Distal, 4, $3\frac{2}{3}$, $3\frac{1}{2}$, 3, $3\frac{1}{4}$, $3\frac{1}{2}$

Oblique, $\begin{cases} \begin{cases} 2\frac{1}{2} \\ 4 \end{cases} \begin{cases} 2\frac{1}{2} & 1\frac{1}{2} \\ 4\frac{1}{4} & 4 \end{cases} \begin{cases} 1\frac{2}{3} \\ 4 \end{cases} \begin{cases} 1 \\ 3\frac{2}{3} \end{cases} \end{cases}$

There seems to be little or no difference at this time between the basal and the distal cut surfaces. The oblique surfaces are much shorter at the distal part than at the basal, as in the last case.

Eight days later the following measurements were taken:

Basal, 7, 5, 6, 7, 5, 6, 6, 7, 6

Distal, 6, 5, 5, 2, 4, 4

Oblique, $\left\{\begin{array}{c}3\\6\end{array}\right.$ $\left\{\begin{array}{c}3\\6\end{array}\right.$ $\left\{\begin{array}{c}2\\5\end{array}\right.$ $\left\{\begin{array}{c}2\\5\end{array}\right.$ $\left\{\begin{array}{c}2\\4\end{array}\right.$

The new parts from the basal surfaces are noticeably ahead in most cases of those from the distal surfaces. The oblique cuts show the same relation as before.

Eight days later, August 17, the results were as follows:

Basal, 7, 8, 8, 7, 6, 5, 8, 6, 6

Distal, 5, 7, 4, 5, $4\frac{1}{2}$, 4

Oblique, $\left\{\begin{array}{c}2\\6\end{array}\right.$ $\left\{\begin{array}{c}3\\7\end{array}\right.$ $\left\{\begin{array}{c}4\\8\end{array}\right.$ $\left\{\begin{array}{c}3\\6\end{array}\right.$ $\left\{\begin{array}{c}3\\7\end{array}\right.$

The relation is the same here as before. It is noticeable that even at this time the distal cut surfaces had not regained their full length. Eleven days later the measurements were about the same, no appreciable increase in length being noted. As the fish were not in as good condition as at first the last results are no doubt due to this. Other results, to be described later, where the fish were under better conditions, show that the new growth continues after this time.

The next series of experiments were made with Fundulus heteroclitus. The tails were cut off on July 7 and the first measurements made July 20.

Basal, 2 +, 1, $1\frac{1}{2}$, to $1\frac{3}{4}$, $1\frac{1}{4}$ to $1\frac{1}{2}$, 2, 1

Distal, $1\frac{1}{4}$, $1\frac{3}{4}$, 2, $1\frac{1}{2}$, $1\frac{1}{2}$ to $1\frac{3}{4}$, $1\frac{1}{2}$, $1\frac{1}{2}$, $1\frac{1}{2}$, $1\frac{1}{2}$

Oblique, $\left\{\begin{array}{c}1\frac{1}{2}\\2\end{array}\right.$ $\left\{\begin{array}{c}1\frac{1}{2}\\3\end{array}\right.$ $\left\{\begin{array}{c}1\\2+\end{array}\right.$ $\left\{\begin{array}{c}1\\3\end{array}\right.$ $\left\{\begin{array}{c}1\\2\end{array}\right.$ $\left\{\begin{array}{c}1\\2\frac{1}{2}\end{array}\right.$ $\left\{\begin{array}{c}1\\2\end{array}\right.$ $\left\{\begin{array}{c}1\\2\frac{1}{2}\end{array}\right.$

$\left\{\begin{array}{c}1\\2\frac{1}{2}\end{array}\right.$ $\left\{\begin{array}{c}1\\3\end{array}\right.$ $\left\{\begin{array}{c}1\\2\frac{1}{3}\end{array}\right.$ $\left\{\begin{array}{c}1\\2\end{array}\right.$ $\left\{\begin{array}{c}1\\2\frac{1}{2}\end{array}\right.$

At this time, 13 days after the operation, there is nothing to show that there is any difference between basal and distal rates of growth. In the measurements from the oblique surface the basal growth is noticeably greater than the distal, and even also greater than the basal growth from the basal cut surface. Whether this is a

real difference, or one due to unintentional cutting nearer to the base in oblique cuts, is difficult to decide, but the latter view is the more probable. The following experiments were carried out on Fundulus heteroclitus in the New York Aquarium between December 8, 1905, and January 30, 1906. The water in which the fish lived was warmed to about 69° F. Measurements of the amount of old material cut off were made. They are given below:

Basal, 10, 10, 9$\frac{1}{2}$, 9, 9, 9, 9, 9, 8
Distal, 5$\frac{1}{2}$, 5$\frac{1}{2}$, 5, 5, 5, 5, 4$\frac{1}{2}$

Large fish were used in this experiment. The distance from the rounded end of the scales covering the base of the tail to the end of the tail measured, as shown above, about 10. Hence the basal cut passes just at the edge of the scales. It was found better not to cut closer than this level to the base; for further forward there is soon reached a region from which regeneration is abnormal and often delayed. The first measurements (of the tails of a few fish that were killed and put into formalin) after 21 days (December 29) were as follows:

Basal, 3$\frac{1}{2}$, 3, 3, 3
Distal, 3$\frac{1}{2}$, 3$\frac{1}{4}$, 2$\frac{1}{2}$, 2$\frac{1}{2}$

The rate at the two levels seems to be nearly the same at this time. The next measurements were made after 38 days (January 15).

Basal 5$\frac{1}{2}$, 5$\frac{1}{2}$, 5, 4
Distal, 4, 4, 3$\frac{1}{2}$, 3$\frac{3}{4}$, 3$\frac{1}{2}$

The new part from the base has now outstripped that from the distal level, although the latter has not yet reached its full growth.

The third and last set of measurements were made after 58 days, January 30.

Basal, 6, 6, 5$\frac{1}{2}$, 5, 5
Distal, 3$\frac{1}{2}$, 3

By this time the basal surfaces have regenerated nearly twice as fast as have the distal ones, although the latter still fall short of their full length, which they would have attained had they regenerated as fast as the basal cut surfaces.

In the last series the new tails had not regained their full length after 58 days. In order to obtain later stages for comparison a

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new series was started February 16 and continued until May 11, a period of 84 days. The results are given in the following tables. For comparison I give a few measurements of the lengths of the old pieces cut off February 16. The pieces cut off from the distal end measured in 10 cases 3, 3, 3¼, 3½, 3½, 3½, 3½, 3½, 4, 4½; and from the basal end 7, 7, 7¼, 7½, 8, 8, 8, 9. Subsequent examination showed that some of the distal cuts had been unintentionally made nearer the middle part of the tail (those measuring 4 mm. above, for instance) and these have been included in the tables in the column marked "Middle."

	Basal				*Middle*		*Distal*					
March 3......................	1½	2	2½	2½	1¾	2	1½	1½	1½			
17......................	2½	3½	4		3	3½	1½	1½	2			
31......................	4½	4½					2	2	2¼			
April 16......................	4¼	4½	4½		3	4	1	1¼	2	2	2¼	2¼
31......................	3¾	3⅞	4		2¾		1½	2	2	2½	2¾	
May 11......................	4	3½	3¾	3⅞	3		·1	2¼	2¼	2		

The results show that even after 84 days the new parts from the distal end had not reached their full growth, neither had the new part from the basal cut, although it was longer than the former, and enough had been produced to have completed the new parts of the distal cut had it regenerated as fast as the basal. There is for both levels a marked decrease in rate in the later stages which is the greater the more distal the cut surface.

The slow rate of regeneration from distal cut surfaces, shown in the preceding results, led me to examine this question further. A terminal piece was cut from the tip of the tail much shorter than in any of the preceding cases in order to see whether the same delay would manifest itself in this case, or whether the initial growth would replace at once so small a part. On March 3 a very narrow piece measuring 2 mm. was cut from the tip of the tail. On April 6 the new part measured ¾ mm.; on April 14, 1½; on April 28, 1 mm. · In a control, in which as much as 7 mm. was cut off, the new part measured 2 mm. on April 6, and 3 mm. on April 28. In both cases the fish had been kept in dishes in the laboratory and were under rather poor conditions, lacking food and sufficient air. Nevertheless the regeneration went on at about the same rate as when the conditions are better.

In another case, 2 mm. was cut off from the distal end of the tail on February 17. On March 1 the new part measured 1 mm.; on March 17 it measured $\frac{2}{3}$ mm.; on March 24, $1\frac{1}{4}$ mm.; on April 6, $1\frac{1}{2}$, on April 31, 2 mm. Thus it took two and a half month's to reproduce 2 mm. at the distal end, an amount that could be produced in two weeks from a basal cut. In another case $1\frac{1}{2}$ mm. was cut off on February 17. On March 17 the new part measured about 1 mm.; on March 24 it still measured 1 mm.; and on April 31, 1 mm. Thus at the end of this time the part cut off had not been completely restored.

In the same series as the one of December 8 to January 30, other kinds of operations were also carried out. In several cases cross-cut surfaces were made at two levels, as shown in Figs. 4 and 5. The rate of regeneration from the two levels is shown in the following figures, with the numbers attached. The first series is from December 8 to December 29.
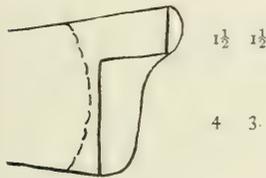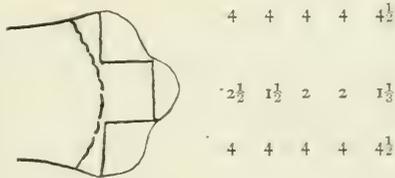
Fig. 4   Fig. 5

It will be seen if these results are compared with those of the preceding tables, in which entire cross-cut surfaces are involved, that the regeneration from the distal partial surface in these cases is greatly inhibited, being not more than one-third as much as from an entire surface at the same level. The statement holds both when the partial surface is at the edge and when it is in the middle of the tail. The next series (December 8 to January 5) gives further cases of this sort.
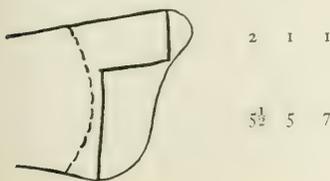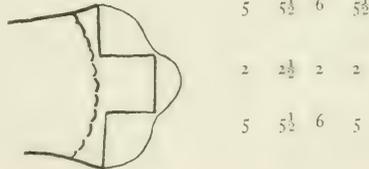
Fig. 4a   Fig. 5a

During the interval of 38 days between these two series the new part from the outer cut surface has grown very little, but that from the inner cut surface has grown as fast, and apparently a little faster, than from the entire cut surface. Here also the greater regeneration from the base is probably due to unintentionally cutting the partial basal surface nearer the base of the tail as examination showed. The third series (January 30) gave the following measurements:
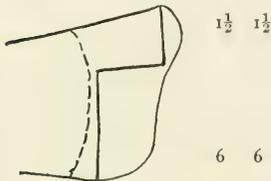
Fig. 4b
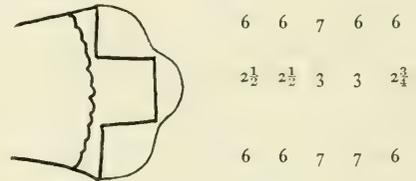
Fig. 5b

The new part from the outer surface is longer in some cases, and this is undoubtedly due to the new growth from the basal surfaces having reached and passed the level of the distal cut. Other series show that in this way the tail may finally complete itself.

Three other series of experiments gave essentially the same results, and, therefore, need not be cited. One point of some additional interest is furnished by these other series. The rate of regeneration from the outer partial cut surface is greater the broader, *i. e.*, the higher dorso-ventrally, the cut surface. This result shows that the retardation is directly connected with the height of the cut surface, and only secondarily with its distance from the base of the tail.

REGENERATION OF THE TAIL OF THE GOLD FISH CARASSIUS AURATUS

This fish has a forked tail which introduces some new factors into the problem. The most important fact connected with the regeneration of a forked tail is that the new part becomes forked very early, before, in fact, the new part has grown out to the level of the old notch. This shows that the distal end of the new tail

is very early laid down, although the later growth must take place very near the outer edge of the new tail; the tail retaining its forked form during the whole of the subsequent growth. The results for three cases in which the tails were cut squarely off, one near the base, the other two nearer the distal end, are shown in Fig. 6. The distance of the first from the notch is 8 divisions (= 4 mm.), and of the latter 2 divisions (= 1 mm.), and of a third 4 (= 2 mm.) The measurements give the rate in growth in the middle and at the upper and lower parts where the lobes grow out.

The results show that the growth is rapid at first, especially when we consider that the healing over of the cut surface and that the arrangement of the new materials take place during this time. The notch soon appears in the new tail owing to less rapid growth in this region. It will be noticed that the middle region is nearer its definitive end than the upper and lower parts of the tail and this may account for its retardation. It also seems, as far as I could judge, that the notch appears sooner when the regeneration is from a distal than from a more basal cut surface, and this is in accord with the idea just expressed. It is not improbable, however, that the appearance of the notch is due to other factors. The upper and the lower parts continue to grow longer after the middle part has reached or nearly reached its goal. At least this seems to be the case, although the middle part may also continue to grow, but so slowly that its progress was not observed in the somewhat rough measurements that I have made.

In another experiment with gold fish, measurements were not taken but sketches of each fish were made at intervals, and as the results illustrate some other points, they may be briefly mentioned here. In one set the exceedingly slow growth of the middle part was noted, the notch had not appeared after 45 days, although the middle had nearly reached its outer level. The upper and the lower parts had grown faster, but had not reached their limit at this time. By January 13 the notch was present, although the level of the old notch had not been reached. Its appearance is due to the greater growth above and below. In another set the notch appeared soon because the cut was made nearer to the old notch. The upper and the lower parts had grown past the level

T. H. Morgan
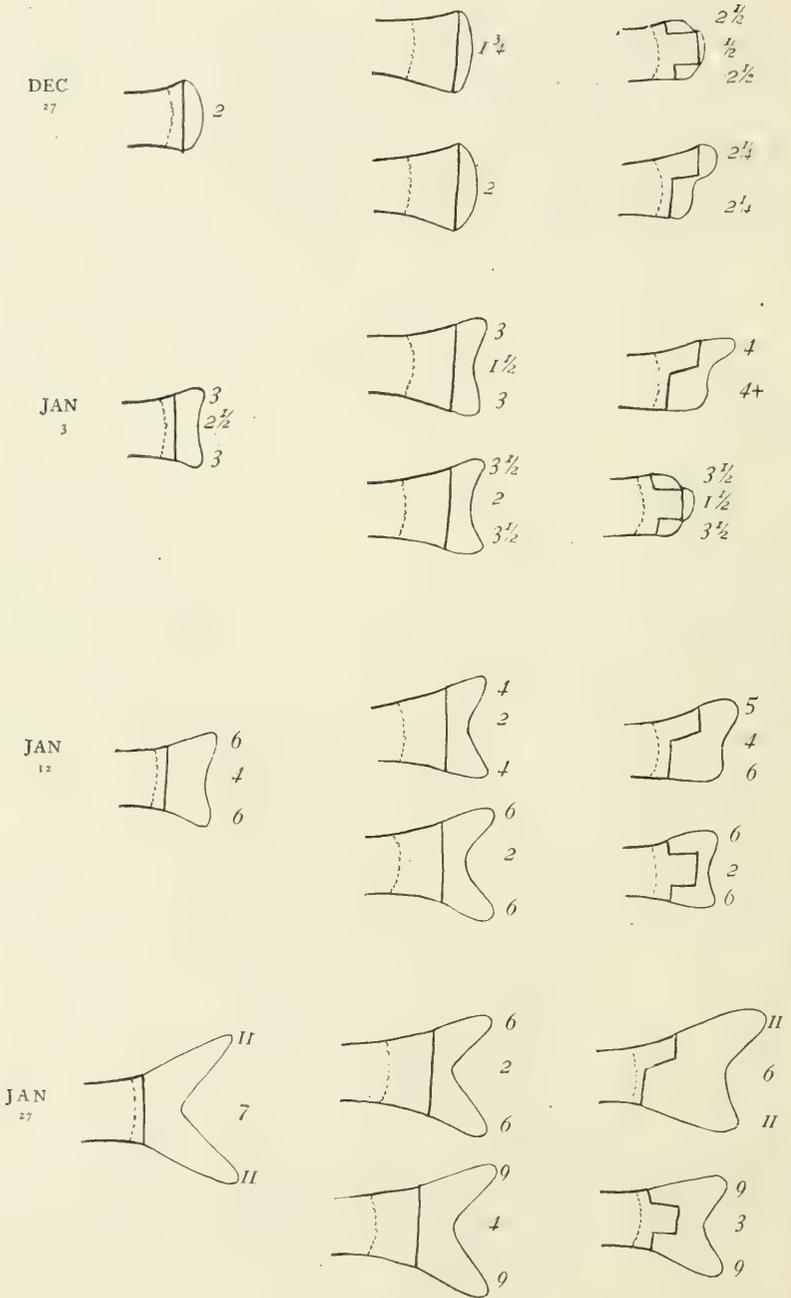
FIG. 6

of the old notch. In another case a small outer and a large inner surface was left. The new growth on the latter was removed once. The development was delayed, and even on February 15 it had not reached its full length. At this time the notch was present and the upper and the lower parts were of the same length.

AN EXAMINATION OF THE RELATION BETWEEN THE INCREASE IN SIZE IN NORMAL AND IN REGENERATING SALAMANDERS

The rate of growth at different stages of embryonic and larval development and its cessation in many species when a certain size or condition has been reached furnishes some of the most perplexing and important problems of morphology and physiology. It has proven difficult in the case of normal development to attack this problem except by measuring the different rates of growth at different stages of development, but this offers little opportunity to test experimentally any conclusions that the measurements may lead one to draw. It would seem that a chance to study the problem experimentally is afforded in the remarkable power to regenerate shown by many animals. The surprising fact has never, I believe, been sufficiently appreciated, that regeneration means a sudden and rapid renewal of the growth process, which takes place not only in those animals that have unlimited powers of normal growth, but also in those whose normal growth is limited within rather narrow boundaries. The fact that an animal that has ceased to grow larger will replace a lost part shows that its growth has come to an end not because of the loss of the power to grow, but because of some retardation of normal growth that has taken place.

It is commonly supposed in the case of an animal that has reached adult size that an equilibrium has become established between the amount of food that can be digested and the body weight. Suppose, if this is true, that a part of the body of appreciable weight is suddenly removed without affecting the surface of absorption of the digestive tract, will the animal quickly regain its lost weight before the missing part has regenerated? In the case of an animal that has not reached its upper limit of adult size, and

in the case of one having no well defined upper limit the problem is more complicated, but if the size at any one time is due to an equilibrium between the food digested and the body weight, we might anticipate on the theory that an animal lacking a part of the body would increase more rapidly in proportion to its weight than an intact animal. The salamander that I have used to test this point belongs to the latter class. The experiment was as follows: The tails of a few salamanders were cut off at the base, the animals being weighed both before and after the operation. They were then weighed at intervals of a week during the period of regeneration of a new tail. Control, intact animals were also kept under the same conditions and their increase in weight also recorded. The tailless animals have had their body weight suddenly decreased by the loss of the tail, while the organs of digestion remain as before, hence, since the tailless animals have less body weight to keep up, they might be expected to increase in proportion to their weight faster than the check series. If both sets increase in weight, as the results show to be the case, the body weight of both would be expected to increase at the same rate, but the amount of material that goes to nourish the tail in the tailed animals might go as additional increase to the body of the tailless ones. Hence, as I have said, we might expect at most only a greater increase in the tailless set, in proportion to the amount removed in the tail which is about one-seventh to one-tenth of the total weight. Unless the weights show a very constant although small difference it would not be possible to detect the supposed influence. Unfortunately, the outcome has shown that the increase is too variable to furnish very definite information on this question.

There is a further possible complication in the results. The presence of a regenerating stump may in itself react on the rest of the body, and cause the general growth to take place faster, in somewhat the same way that the presence of young in the uterus of a guinea pig, itself not fully grown, does not interfere with the normal growth of the parent,[1] but on the contrary, the powers of

[1] Minot, C. S. Seriescence and Rejuvenation, Jour. Physiol., xii, 1891.

assimilation seem to be increased, since under the conditions the mother digests enough material not only for her own normal growth but also for that of her rapidly growing progeny as well. In the case of the guinea pigs the result is complicated by the amount of food taken by the mother, which may be greater during pregnancy since the amount does not appear to have been regulated; but in the case of the salamanders the amount given was carefully controlled.

The salamanders were kept in flat dishes containing half an inch to an inch of water, and were fed on small pieces of raw beef about every other day. As they take food from the forceps the amount that each takes can be regulated, which would not be the case if the food were simply left in the dishes. As the animals grow larger they can swallow larger pieces, hence for some time their weight can be steadily increased by increasing the size of the piece of beef given to each.

The animals were not young, so that their increase in size is not to be ascribed to the increase from youth to age. The life history of this species insures the maturity of all the individuals, since Gage[1] has shown that they pass two years on land before undergoing the final changes that transform them into the aquatic Diemyctylus. The susceptibility of the animals to the amount of food is shown not only in their rate of increase, but also by the rapid loss in weight when food is scarce or absent, although they may be kept alive for several months deprived of food. Hence in weighing the animals care must be taken to weigh them at corresponding times from the last feeding. Failure in this respect is at once shown in the weight, as a few cases in the tables indicate. As a rule the last feeding was 24 hours before the time of weighing. The average of the five heaviest animals that I obtained by a steady and abundant feeding for three months was 3.14 grams and the minimum average weight for five starved individuals kept in a similar dish was 1.00. In the well-fed set there were considerable individual differences. Thus the largest individual in the same set weighed 3.25 and the smallest 1.62 grams or about half. I isolated one of

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[1]Gage, S. H.   Life History of the Vermilion Spotted Newt.   Am. Natural., xxv, 1891.

the smallest individuals in the hope of determining the cause of this difference and found that it took less food than the others. No doubt its smaller size is directly connected with this condition.

The most important fact connected with the increase in size of Diemyctylus is that the increase is not due to the storage of fat, as the weight of a man who had reached adult size might be increased by over feeding, but to an actual increase in size of nearly all of the organs of the body. The increase is, therefore, to be ascribed to growth, not the storage of reserve material. The starved animal may live for a long time at the expense of its formed tissues. This possibility indicates that an animal of average size, kept without food, may still be able to supply a regenerating part with materials for growth—the interesting point being that one part is rapidly growing at the expense of the rest of the body. The simplest interpretation of this result is, I think, that there must be present in the blood at all times a certain, although perhaps variable, amount of nourishing materials, and that a newly regenerating part has the power to take from the blood the materials that it needs for growth, even when the amount present in the blood has fallen so low that the rest of the tissues cannot maintain themselves, but break down to supply the blood with a certain amount of nutriment. If this idea expresses approximately the relation that exists, it follows that while the new part requires a certain amount of food in order to continue growing, it can take advantage of a condition that the older or differentiated tissues cannot make use of; in fact, when the latter slowly lose ground. There is apparently a similarity in this respect between an embryo and the newly regenerating part. Since in regeneration the new part is formed directly out of the old tissues we may assume that this property of young parts is something connected with their lack of differentiation, which is lost when differentiation takes place, and is regained again when the differentiation is lost.

In the first experiment the tails of six salamanders (three males and three females) were cut off near the base. In another set (also of three males and three females) the animals were intact. The former weighed 1.46 grams a piece (average weight) and the tails cut off weighed about 0.16 gram. Owing to a loss of blood, etc.,

the tailless animals were weighed again the next day, and their weight (1.28 grams) was found to be in this case about the same or a little greater, due perhaps to the absorption of water by the cut surfaces. The control set were at the beginning a little heavier, weighing 1.85 grams. The following table gives the average individual weight of these two sets of animals from December 12 to February 3. On January 5 one of the tailless animals died, and on January 10 another.

TABLE SHOWING THE INCREASE IN WEIGHT OF TAILLESS AND INTACT SALAMANDERS

TABLE A

	Tailless	Rate of Increase	Control Intact	Rate of Increase	Tailless	Rate of Increase
Dec. 12	1.46 (intact)		1.85			
12	1.22 (tailless)					
13	1.28	.06				
18	1.53	.25	1.74	—.11	2.28 (intact)	
27	1.57	.04	1.65	—.09		
Jan. 5	1.88	.31	1.98	.33	2.08 (tailless)	
13	1.95	.07	2.06	.08		
20	1.96	.01	2.13	.07	Feb. 4	
27	2.13	.17	2.36	.23	2.01	
Feb. 3	2.08	—.05	2.23	—.13		
10	2.18	.10	2.30	.07	2.12	.11
17	2.22	.04	2.34	.04	2.33	.21
24	2.36	.14	2.45	.11	2.34	.01
March 3	2.34	—.02	2.56	.11	2.41	.07
10	2.45	.11	2.43	—.13	2.51	.10
17	2.38	—.07	2.41	—.02	2.45	—.06
24	2.64	.26	2.78	.37	2.73	.28
31	2.66	.02	2.66	—.12	2.80	.07
April 7	2.83	.17	2.78	.12	2.84	.04

From this table it appears that both sets steadily increased in weight, but the increase was greater in the tailless set. The difference in rate during the first two weeks, before the new tail has appreciably developed is much greater in the tailless set. There is a gain of .35 for the tailless animals and a loss of .20 in the intact set. If the next week is taken into account when both sets made great gains the balance remains nearly the same. Their

differences may be due to uncontrolled factors—and in part I suspect this must be so, for there is no assignable cause for the decrease in the intact series—but if the difference is directly connected with the absence of the tail in one set it is obvious that the entire loss was made good, which is a greater increase than we should anticipate since the expectation would only be a proportionately greater increase in the tailless set.

The tables also show that after four months the tailless set had doubled the weight while the intact ones had increased only one and a half times.

The greater increase in weight in the tailless set might be supposed to be due to the stimulus of the growing part on the body as a whole, as said above; or the difference may be a purely fortuitous one, the tailless animals happening to be a faster growing set. By January 27 the new tails were about one-half to one-third of an inch long, and their increase in length continuously adds new weight to the tailless set. In order to see if the same result would follow if some of the intact individuals had their tails removed, they were separated in two lots; one lot kept intact and the other curtailed. The last two columns of the table give the rate of growth of the tailless set. The average weight before the operation was 2.28 grams; afterward 2.08 and the following day 2.01. The data are controlled by those of the middle columns. It will be noticed that the control, February 3, weighed a little less than the animals whose tails were to be cut off. During the two weeks following the operation the tailless salamanders gained .32 gram while the intact ones gained only .11 gram. If the third week is also taken into account the tailless animals gained .33 gram, and the intact ones .22 gram, which gives still a difference in favor of the former. When the experiment closed the tailless animals had gained .83 gram and the intact .55 gram.

Still not convinced that the difference in these two cases might not be due to uncontrolled conditions, I started two new sets on March 2. The results are given in the next two tables. The animals used had been kept all winter in the laboratory, and were under-fed, but for ten days before the operation they had been well-fed. Two of the animals in this set had had their legs cut off

some time before and were regenerating new ones, which introduces, perhaps, a disturbing factor in the result.

TABLES SHOWING THE INCREASE IN WEIGHT OF TAILLESS AND INTACT SALAMANDERS

TABLE B

	Tailless	Rate of inc.	Control	Rate of inc.
March 2..	⎰Intact 1.46			
	⎱tailless 1.32		2.05	
4..	1.30	—	—	—
10..	1.45	.15	2.41	.36
17..	1.46	.01	2.12	.29
24..	1.67	.21	2.22	.10
31..	1.96 one sick	.29	2.33 one died	.11
April 7..	1.93 one died	.03	2.57	.24
14..	1.93	.00	2.70	.13

TABLE C

	Tailless	Rate of inc.	Control	Rate of inc.
March 2..	⎰Intact 1.68	—	1.24	—
	tailless	.01	1.40	.16
	⎱1.48	.00	1.53	.13
3..	1.56	.20	1.63	.20
10..	1.57	.35	1.67	.04
17..	1.57	.04	1.67	.00
24..	1.76	.02	1.71	.04
31..	1.91			
April 7..	1.96			
14..	1.98			

The left hand table (B) shows that during the first two weeks the tailless set again gained faster, but there is a strange rise and fall in the control set that probably makes the result of little value. If we take another week into account when the disturbance may have had time to subside the rate of increase of the tailless set is still double that of the intact ones.

In the right hand table (C) the control animals increased in weight at first much faster than the tailless, but later the tailless ones gained much more rapidly than the control.

Our examination of the tables shows that it would be hazardous to ascribe the greater initial gain (in three of the former cases) in the tailless animals to the loss of the tail, although this may be the case. The difference when it occurs seems too great to be due to a *proportionately* greater increase as the result of the loss of a part; and if not due to variable factors, *i. e.*, accidental, it may mean that the changes taking place at the cut surface incite the digestive tract to greater activity or the cells of the body to greater assimilation.

In the first series (Table A) there is not only an initial greater

gain in the tailless set but an actually greater increase in weight
throughout the series. This might be attributed to the influence
of the regenerating tail on the growth of the rest of the body, but
as the difference is not found in the other three series, and in one
set, in fact, the intact animals grew faster, we must conclude that
there is no clear evidence in favor of the view that a regenerating
part has in its later stages at least an influence on the digestive or
assimilative changes that take place in other parts of the body.
The great powers of growth in a regenerating part may be local in
their influence and not transferable to other parts. The question
is, however, worthy of further examination.

<div align="center">CONCLUSIONS</div>

In connection with the description of the experiments a partial
analysis of the results has been attempted, and much of the ground
gone over need not be traversed again here, but the more general
bearings of the facts may now be discussed. The problems of
special interest are those connected with the rate of growth at differ-
ent levels, the rate of growth from partial as compared with entire
cut surfaces, and the rate of growth on different parts of the same
oblique surface.

The question whether the differences in rate can be explained as
due to the amount of food available at each level has been suf-
ficiently examined. Ample evidence was found showing that the
differences in rate of growth are not due to differences in the avail-
able food supply. It would be erroneous to conclude from this
that the available food supply has no influence on any of the phe-
nomena of regeneration, for it has been shown that the size of the
new part, for example, is affected by the amount of food, in the
same way as the rest of the body, and it has also been shown that
when starvation has gone beyond a certain point, even the forma-
tion of new parts may be delayed, or stopped before the animal
perishes from hunger. But despite these effects the experiments
show that the rate of formation of new parts as seen in the regen-
eration of the limb of Diemyctylus, and in the growth in length of
the tail of the earthworm and of the salamander takes place at the
same rate, whether the animal is fed or starved, provided there

still remains enough food for the formation of new material. The meaning of this relation seems to be that the greater power of assimilation of a young part makes it possible for this part to draw the necessary nourishment from the blood, although the amount present in the blood is below that which is necessary to maintain *in statu quo* the differentiated tissues. These slowly decrease in size and in the number of their cells, while the new part is increasing in size and in the number of its cells. The ultimate physiological-chemical basis on which this difference between differentiated and undifferentiated materials rests is entirely unknown at present. The most important consideration in this case is that the material of the new part is derived directly from that of the old, so that the difference is one of condition only, and is, therefore, a reversible process. In other words, because a tissue has become differentiated it has not lost the potentiality of becoming young again, provided it gives up its differentiation. This consideration has a bearing on the problem of the difference of rate at different levels, as will be apparent later on.

It has been shown in the fish that the rate of growth is retarded on a partial surface, provided the surface does not connect at one or at both ends with the rest of the tail. For instance if the tail is cut off, as shown in Fig. 7A, the outer free cut surface *a* regenerates more slowly than does an entire cross-cut at the same level; but if the partial surface is continuous with the rest of the tail at the same level as in Fig. 7A at *b*, it regenerates at the same rate as does the entire cut surface at this level. Results similar to the last are found when a square piece is cut out of the middle of the tail, as in Fig. 7B. The proximal, partial cross-cut surface *b*, continuous both above and below with the rest of the tail, regenerates as fast (or faster than) an entire cross-cut at the same level. It is to be remembered that the longitudinally exposed edge connecting the cross-cut surface *a* and *b*, does not proliferate in a vertical direction except in so far as to cover the exposed surface and to complete the structure as far as the next fin ray.[1] Additions to the

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[1] The explanation of this seems to be that fin rays cannot develop new ones except from the cut ends of the old ones. If one were split lengthwise it would probably complete itself, but not produce new ones.

new part from the basal cut surface are not made from this source, and the rate of growth of the basal part is not, therefore, increased in this way, but the growth from the base seems to be faster, nevertheless, along the line of the longitudinal cut surface, as seen for example in Figs. 7A and B, than at the opposite edge where the new part is free. In some way the presence of the new material along the longitudinal edge accelerates in its vicinity the growth of the new part from the base.
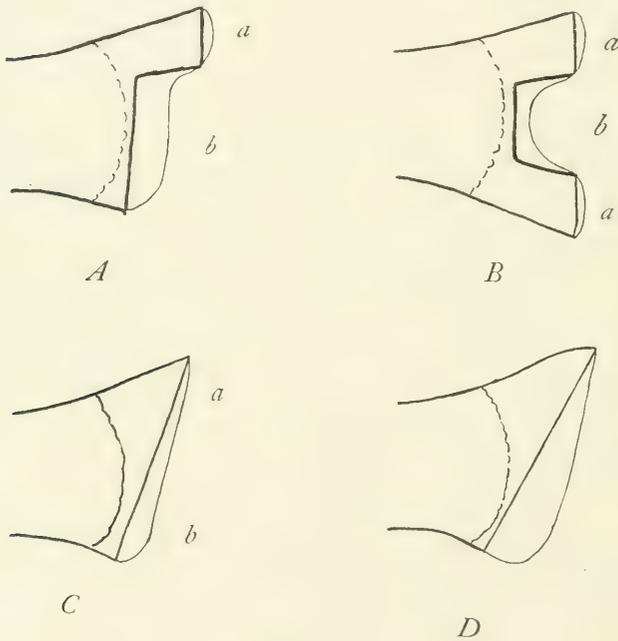
Fig. 7

What factor retards the development of a free, partial cut surface? The retardation is not due to the level, for it occurs at all levels alike. It is in proportion to the height of the free cut surface, hence the retardation must be in some way connected with the height of the base from which the new part arises. One might be inclined to interpret this result as due to a proportionate devel-

opment, by which I mean that the regulation of the growth is such that its rate is proportional to the base from which it arises. But even if this were the case, it would give no causal explanation of the results, unless it be assumed that proportionate development is in itself a vitalistic explanation and a causal one in that sense. But the facts do not seem to bear out this interpretation, for, while the growth on a free partial surface is undoubtedly delayed, there are some indications that it might continue slowly toward the natural terminus of its development. At least in the gold fish I have obtained evidence of this sort in two cases, where the lower lobe grew out slowly to its original length. In Fundulus the evidence is not so clear, but mainly on account of insufficient material.

Now the factor that seems to be responsible for the retardation in the growth from a free partial surface appears to be one involving the pressure relations in the new part. Observations show that the new part from a partial surface is rounded at the sides (as is also the new part from an entire surface), and this condition at the sides appears to be responsible for the retardation of the rest of the new part, for, since the rounding is nearly the same for a new part from a partial and from an entire surface, the retarding influence will be the greater the shorter the base. In contrast with this retardation from a *free* partial surface we have seen that when a partial surface is not free but continuous at one or at both ends with the rest of the tail, as at *b* in Fig. 7A, there is no retardation. In this case the side of the new part, that is continuous with the old part, is not rounded, but even with the rest of the new growth, hence the rate of growth is not retarded on this side, while on the free side the relation is the same as when the growth takes place from an entire cut surface. In fact, the growth of the new part on the side in connection with the old tail often seems to be accelerated, especially along the longitudinal cut surface that is also proliferating a little new material, and this condition is in accord with my interpretation.

The new part from an oblique surface also shows a retardation of growth on the more distal parts of the cut surface, and the difference between the rate of growth on the more distal and the more basal parts is in proportion to the degree of obliqueness of the

cut surface, as seen in Figs. 7C and D. The retardation in this case seems to call for the same explanation as that observed on a partial surface, for, if we consider by itself alone any part of the more distal surface it may be treated in the same way as a partial cut surface, because, despite the fact the parts lying next to it at a lower level grow faster than the part in question, yet owing to the fact that the part below is still behind that of the lower end of the postulated part, there will be a retarding influence on the growth of the latter. The pull or tension that exists will, on the theory, hold back the parts just above it. If we imagine this same influence existing throughout the whole of the new growth on an oblique surface, we can get an insight into the factor at work, and see how this case falls under the same head as that of the growth from a partial surface. Our analysis and experiments with oblique cut surfaces lead, therefore, to the conclusion that the slower growth over the more distal part of the oblique surface is not due to its more distal position, for comparison with cross-cuts at the same level disprove this interpretation, but the result is due to another factor. This factor is a formative one in the sense that the failure of the maximum potential of growth over the more distal part of an oblique surface is due directly to the new growth below it not having reached the same level, and owing to this difference there arises a pull or tension on the part that retards its maximum possible rate.

Before taking up again for further analysis the principal question of the retardation of growth at different levels, I should like to clear the way still further by referring briefly to the kind of regeneration from the anterior cut surface of the earthworm (or of Lumbriculus) and from the oral and basal ends of a piece of the stem of Tubularia. Both of these cases may appear to stand in contradiction to the conclusions so far reached in regard to posterior growth in Diemycytlus, Fundulus and Lumbricus. In reality, as I hope to show, there is no contradiction between the interpretation of the two classes of facts.

When one segment is cut from the anterior end of the earthworm, it is replaced by one; when two are cut off, two regenerate; when three are removed, three regenerate, and so on up to five, although for five sometimes only four come back. When more

than five are cut off only five (or sometimes only four) regenerate. This rule holds only for the anterior end. As the region of the gizzard is approached (about the 15th segment) the head, if it develops at all, is abnormal; and behind this level there regenerates a heteromorphic tail from the anterior end. In Lumbriculus, when from one to seven anterior segments are removed, the same number is regenerated; when more than seven, six or seven come back; and this rule holds for the greater part of the length of the worm, since there is no such regional limitation in this species for head formation as in the earthworm.

In the regeneration of the anterior parts in these two species there is to be observed no such difference in rate from different levels as seen in posterior regeneration, and this is the relation referred to above that may seem to be in contradiciton to the conclusions reached in the case of posterior growth. But it is to be recalled that we are dealing here only with the part that is first laid down, and not with subsequent growth after the terminal organs have been formed. The comparison should properly be made in the two cases only between the terminal organs. The formation of the head segments that are all laid down at the same time is comparable with the formation of the terminal posterior segments that are formed in all cases of posterior growth. I have no observations in the earthworm showing that there is any difference in the time of formation of the posterior terminal segment from cuts at different levels, and in the salamander I could detect no such difference. If such exists the difference is slight and in this respect the conditions are similar to the formation of a head at different levels, which also seems to take place at the same time, although the possibility of slight differences that were not detected must be granted for both cases. In both head and terminal segment the centripetal influences seem to be the predominating ones.

These considerations show that, in principle, there is no conflict between anterior and posterior regeneration. The difference found in the latter case is due to later growth in the posterior end, and no such growth takes place in the anterior end.

It has been shown in Tubularia that the time required for the formation of a new hydranth depends on the distance of the cut

surface from the old hydranth.[1]   The nearer the cut surface to the
oral end the quicker the regeneration.   The same law holds also for
the development of the aboral hydranth from the aboral end of a
piece.   In both of these cases we are again dealing with the devel-
opment of a terminal organ in the formation of which the centri-
petal influences predominate.   Therefore, the difference in the rate
of appearance of the hydranths at different levels involves only the
appearance of a terminal organ, which is, as I have tried to show,
a different problem from that of the growth of an organ as it ap-
proaches the terminus of its growth.   This difference in the forma-
tion of the hydranth of Tubularia is due, I believe, to the amount
of stem differentiation at different levels.   The gradation of this
differentiation is from the oral to the aboral end of a piece of the
stem.   Whether after the hydranth has emerged, the stalk grows
faster from a cut surface nearer the base remains to be examined.

Let us return now to the main problem of the factors involved in
the growth of the new part in the posterior regeneration of the
salamander, fish and earthworm.   As a result of removal of
the posterior end there is a proliferation of new materials, and this,
as we have seen, appears to take place at about the same time for
all levels.   The exposure itself may appear to give the stimulus
that calls forth the proliferation, but it seems improbable that this
is the immediate cause, since the greater part of the proliferation
takes place after the closure of the skin over the wound.   It seems
more probable that the real stimulus is to be sought for in the loss
of the connection with the old parts; in other words, to the loss of
the normal pressure relations essential for the normal equilibrium.
I base this inference mainly on the results of grafting experi-
ments in hydra where, when dissimilar regions are united, each
part completes itself at the line of graft, although the actual cut
surfaces are completely united, and the subsequent changes may
not take place for a week or more after the operation, when the
effects of the injury as such must have long since passed away.

The terminal part is quickly formed in the proliferated mate-
rials.   Between the terminal part and the old part there is also laid

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[1]This has been shown by Driesch, Stevens and Morgan.

down a growing zone that is a normal structure for the posterior end. I have already given my reasons for supposing that the growing region has the same potentialities for all levels,[1] and that it continues to grow until some retarding influence delays, and then prevents its further growth. We have also seen that the retarding influence is connected with the completion of the normal form, hence it is in the nature of a formative influence. I have also compared the retardation of a regenerating part to the retardation seen in the growth of the whole organism. The growth of many animals slowly decreases as the typical form or size is approached.

In the case of the posterior growth under consideration, the clue to the solution of the manner of growth is to be found, I think, in the relation of the new segments or parts to the parts lying proximal to them. At first this is the old part, and the first segment develops in relation to this part, the next one develops in relation to the first new one, and so on for the whole series. But what, it may be asked, is the nature of this relation that determines the formation of the successive parts? The old part has a certain differentiation as well as the potentiality of forming the whole of the distal or other regions. The relation in question must depend in some way upon differentiation, but differentiation in itself cannot be assumed to be a formative factor, since we know of no such influence extending from cell to cell. If, however, the differentiation is an expression of certain pressure relations that have determined the differentiation and which since they still remain, determine the pressure relations of the neighboring parts, and determine the kind of new differentiation that will take place, the new part thus formed will, in turn, influence the differentiation of the next new part that develops, and the process will continue until the completion of the typical form has been accomplished.

The new growth will come to an end when the last formed part has developed, whose differentiation is of such a kind that the resulting pressures, thereby established, no longer act as a stimulus on the growing region to produce another new part. In the forma-

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[1] An exception for tail formation must be made for the most anterior end, and for head formation in the region behind the gizzard of the earthworm.

tion of a new tail the pressure relation is a gradually decreasing quantity, and along with this decrease there goes a decrease in the stimulus to further growth that ultimately comes to an end. This analysis shows why there should be a gradual slowing down of the regeneration as the normal form is approached, and it is apparent that this retardation will be the same whether it occurs near the end of an old part, or, as a new part approaches completion; for, on the hypothesis, the conditions will be the same in each. The hypothesis gives at least a formal explanation of the facts, and I can find no other that will. The most problematical part of the hypothesis is, I think, the assumption regarding the nature of the influence of the formed part upon the unformed part. I have assumed this to be a pressure relation of some kind. Possibly some other condition may be found that expresses this relation more correctly, but the remainder of the argument may stand even if it be found that the nature of the influence is different from that which I have assumed. My assumption has, however, the advantage that it puts into the same category the influences that determinate the formation of a terminal part, and the subsequent growth of a posterior end, namely, a condition of pressure or tension. My pressure hypothesis has also the advantage, I think, that it involves only a known quantity. It appeals on the whole to phenomena that are known to occur in living things; for, response to pressure, or stereotropism in adult animals and plants is well known. That growth is influenced by pressure is also known. Less familiar perhaps is the assumption that differentiation is itself a response to a pressure relation rather than due only to the kind of material contained in a cell, although the latter also may be a factor that enters at times into the result.

I have expressed elsewhere the idea that polarity is an expression of the gradation of differentiated materials. We may now push the analysis further and refer the polarity to a gradation in the pressure relations, since these are the dynamical expression of the gradation of the materials, as shown in their differentiation. These differences can be traced to the egg where the differences in the pressure relations of the cells give rise to the later differentiation.

HYDRANTH FORMATION AND POLARITY IN TUBULARIA

BY

T. H. MORGAN

The two problems of hydranth formation and of polarity in Tubularia have much in common, yet, as I shall try to show they may sometimes involve fundamentally distinct factors. Failure to distinguish the proper field of each has led, I believe, to unfortunate and unnecessary confusion.

By polarity we mean in Tubularia the formation of a hydranth on the oral end, and of a stolon on the basal end of a piece. But since in Tubularia a hydranth not infrequently develops also on the basal end of the piece, it may appear that our definition of polarity has no real significance. I do not think that this is the case, although unquestionably the problem needs further analysis.

Inasmuch as a hydranth may develop at every level, it follows that every part has the capacity to produce this organ. Why then does it not develop equally on an oral or on a basal end? Theoretically both ends have the possibility of forming a hydranth, but there are two facts that show that the conditions are not the same on these two cut surfaces. First, when an oral and a basal end of halves of the same piece are exposed at the same level (the other ends respectively of the pieces being tied) the cut oral end develops its hydranth before the cut basal end. Second, while a stolon often develops from the basal end, and can easily be called forth, it is the rarest occurrence for a stolon to develop from the oral end, even when a hydranth is present on the basal end and a new cut is exposed near the oral end. Hence I believe we are warranted in using the term polarity in Tubularia, although the distinction between the two ends is less apparent than in other cases in which axial heteromorphosis does not occur.

In my last paper[1] on regeneration in Tubularia I gave a number of experiments to show that when a piece is tied at its oral and its basal end, and is then cut in two in the middle, the oral cut end of the basal half develops its hydranth before the basal cut end of the oral half. I have repeated this experiment again on a much larger scale. There was one difference in the conditions of the two experiments, viz: in that in the present case the basal piece was not tied at its basal end because another experiment had shown that a basal ligature has no influence on the rate of development of the oral hydranth. The basal piece developed in practically all cases its oral hydranth before the basal hydranth of the oral half appeared. The result is, therefore, the same as when the basal end of the basal piece is ligated.

THE CONDITIONS ON WHICH THE DEVELOPMENT OF THE ABORAL HYDRANTH DEPEND

In a former paper[2] I suggested that the suppression of the hydranth at the basal end of a piece, open at both ends, is due to the more rapid development of a hydranth at the oral end. If this hypothesis is correct we should expect the converse to be true and anticipate a delay in the formation of the oral hydranth if the basal hydranth could be made to develop first. This can be done by tying the oral end of a piece first, and then, as soon as the basal hydranth has begun to develop, or even after it has emerged, by cutting off the oral end of the piece below the ligature. Under these conditions I find that the development of the oral hydranth is delayed or suppressed for a time.

It would seem, therefore, that something is used up in the formation of the hydranth that is necessary for its development, and that whichever hydranth develops first uses up this something so that the other hydranth fails for the time to develop. But the conditions seem to be more complicated than this, as I pointed out, and as the following considerations will show:

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[1] "Polarity" considered as a Phenomenon of Gradation of Materials. Jour. Exp. Zoöl, ii, 1905.
[2] Morgan, T. H. An Attempt to Analyze the Phenomena of Polarity in Tubularia. Jour. Exp. Zoöl, i, 1904.

If a long piece is cut into a number of pieces 2 to 5 mm. in length, each will develop its oral hydranth in the same time that a long piece cut off at a corresponding level develops its hydranth. Therefore, a long piece must be capable of forming a far greater amount of the something necessary for hydranth formation than required for its oral hydranth alone. Why then does not the basal hydranth also develop?

By means of a separate experiment I found that a short piece (cut off below the hydranth) and a very long piece (cut off with its distal cut end at the same level) develop their oral hydranths in the same time. It might seem, therefore, that the effect produced involves only the immediate region near the cut surface, and the breaking down of the ridges in this region during the time of hydranth formation suggests that the active factor is the material of the ridges that is thrown into the circulation, but I have come to question whether this material is the something demanded; for as I have already pointed out, it is not obvious why the basal end might not also break down, and develop its hydranth by the use of the material thus set free, at the same time in which it develops its basal hydranth when the oral end is tied. In fact, in a certain small percentage of cases an oral and a basal hydranth develop on a piece at the same time—or very nearly so—and, more rarely, a basal hydranth may develop while the oral hydranth is delayed or suppressed. In very short pieces, less than the length of the normal primordium of the hydranth, double hydranths and partial double hydranths are of common occurrence. Therefore, I conclude that the basal hydranth fails to develop because it does not receive the stimulus necessary to start its development whatever the nature of this stimulus may be. Driesch has assumed that the stimulus calling forth the hydranth is the action of the salt water on the cut end. I tried to test this view by sticking the basal ends of long pieces into sand with only the basal part of the pieces surrounded by water. The oral ends were an inch or two above the water and surrounded by air. The air was kept saturated with moisture by covering the dish. Under these conditions the oral hydranth developed as rapidly, possibly more so, than when surrounded by water. The result does not conclusively

show, however, that the action of the water may not be an important factor, since the surfaces above water were of course moist; yet the results do seem to show that we must be cautious in accepting Driesch's explanation as the only interpretation.

Another experiment bearing on the question under consideration is the following. The oral end of a piece was tied and then after twelve hours, before the basal hydranth had developed, the oral end was cut off below the ligature and the basal end tied distal to the region that produces the hydranth. The oral hydranth did not develop any sooner (*i. e.*, in actual time) or at most very little sooner than it does when simply cut off. The result shows that in a tied piece the oral end does not undergo the changes preparatory to hydranth formation (as I thought probable at one time), and the more rapid development of the basal hydranth when the oral end is tied cannot be due to materials set free from the oral end that act as a stimulus for the basal development.

I must here go over again two experiments described in my last paper that appear to have a further bearing on this problem. I found that if pieces were cut off and allowed to remain with both ends open for four, six, eight or twelve hours, and then the oral ends were tied, the basal hydranths often developed as fast, or nearly as fast, as the control pieces tied at once. It might appear that changes are taking place in the aboral end of such a piece, open at both ends, that are leading to hydranth formation, and that ordinarily the changes are only retarded by the more rapid development of the oral end. That this is not the real explanation, plausible as it may seem, is shown by another experiment. If, as before, pieces are cut off and allowed to stand for several hours, then tied at the oral end as before, but at the same time several millimeters of the basal ends are cut off, these pieces produce their aboral hydranths in the same time, or nearly so, as do those whose basal end is not cut off after tying. The result shows that the acceleration of the aboral hydranth is not due to preliminary changes in that end, or at most only in a minor degree. Of course, if too much time is allowed to elapse before the oral end is tied, the control develops first, but a distinct hastening of the aboral development is nevertheless observable. I have found, for instance, that

if the tying is delayed for twelve hours, the aboral hydranth is generally later in appearing than that of the control, but not twelve hours later, and in very favorable cases I have found them developing at the same time.

Evidently then the results cannot be ascribed to changes in the aboral end, and probably not to changes in the oral end, but must be due to something that takes place throughout the entire piece. On this "something" depends the more rapid aboral development when the oral end is tied.

Another experiment, designed to test this supposition, is important, and although I have repeated it time after time I still feel the same doubts as to the result that I spoke of in my last paper. Pieces of the same length were cut off near the oral end of the stalk. It is of much importance to have the pieces of the same length and from the same region, since the rate depends on the distance of the aboral cut-surface from the cut end. For comparison, therefore, the cut ends must be at the same level; but in addition to this the rate differs in pieces of different ages, and although I have attempted to pick out similar pieces, it is practically impossible to determine accurately the age of the piece. A small piece that has newly arisen as a bud develops more promptly than one of the same diameter that has failed to develop fully owing to crowding by the larger pieces. Pieces of the latter kind are unfavorable for comparison. With this understanding concerning a source of error the experiment may now be described. After four, six, eight and twelve hours, or at other intervals, ligatures are tied around the pieces; in some of them near the oral end, in others near the middle, and in others near the base. The development of the basal hydranth in these three kinds of pieces was then compared. The results indicate that the basal hydranths in the three kinds of pieces develop at nearly the same rate, although the shorter basal pieces are often behind the other two, as I found in previous experiments of this kind, but the delay when it occurs does not seem to be in proportion to the relative lengths of the pieces between the ligature and the basal end. In my former paper I stated that the experiment might show whether the materials set free in the circulation affect the development at the basal end, because in the longer

piece tied near the oral end there would be more of such material shut off than in the shorter pieces tied nearer the base. This interpretation of the experiment now seems to me erroneous. There would be quantitatively (*i. e.*, absolutely) more material in the longer piece, yet the relative amount is the same and it is the relative amount to a given volume that must act as a stimulus on the basal end. Consequently we should not anticipate quicker stimulation in one case than in another. On the other hand if the substance is not given off equally from the entire wall of the piece and if relatively less is given off from the basal region than from the oral region some difference in the time of basal development might follow. It may be questioned, however, whether the difference, sometimes found, is due to this rather than to some other factor. It seems to me that more emphasis should be laid on that side of the results that shows clearly there is no proportion between the length of the shorter piece and the basal development, rather than on the apparently slight difference sometimes observed in the different cases.

Before coming to closer quarters with our problem a few additional results must be briefly given:

In a few cases a series of ligatures were tied at intervals around the oral end of a piece. If the changes induced by closing the anterior end introduces a factor that accelerates the development, a number of successive ligatures might be imagined to accelerate the aboral hydranth, but no acceleration was observed.

It has been stated that a single ligature at the basal end does not hasten the oral development. Also a series of successive ligatures, several hours apart, does not accelerate the oral development.

If the development of the oral end retards the basal development, it might be supposed, conversely, that, if any changes take place at the basal end, successive removals of the basal end might accelerate the oral development; but no such result was found. This observation is in accord with other experiments that seem to show that as a rule no development, or very little, takes place for a time at the aboral end.

On the other hand, removal of short millimeter pieces from the

oral end at intervals delays the development of the oral end but only a very little provided the intervals are not too long. For example, in one case a short piece was cut from the oral end of a long piece after five hours and another from the same piece after eight hours. The primordia appeared thirty hours after the stems had been removed from the colony, and at the same time (or so nearly so that little or no difference could be detected) in the control and in the pieces twice operated upon. In another experiment short pieces, 1 to 3 millimeters in length, were cut from the oral end of different pieces at different times, *i. e.*, not from the same piece, successively as before, at different times. The tips of some pieces were cut off after six hours, from other pieces after nine hours, and from other pieces after twelve hours. In the control a short piece of the same length was cut off at once. It was found, when the primordia appeared that the six and the nine hour pieces developed at nearly the same rate; the twelve hour pieces were somewhat behind, but not, apparently, twelve hours behind. The controls were like the six and the nine hour pieces, but possibly a little ahead of them. Thus it appears that some effect is produced by cutting off the oral parts, out of which the hydranth develops, but not in proportion to the intervals. There was another series of operations in the same experiment. Long pieces, similar to the last ones, were removed at a much greater distance from the oral end, entirely beyond the region of polyp-formation. It was to be expected that the new oral ends would develop more slowly, because the cut surface is more basally situated. It was found, in fact, that these pieces were somewhat behind the preceding ones, but the six and the nine hour pieces developed at nearly the same rate, while the eleven hour pieces were a little retarded. There was need of a control here that was not made, so that any deduction from the results is unsafe; but it did not appear that the six and nine hour operation had much delayed the development within the period of thirty hours.

From these last experiments it seems probable that a change takes place in the whole piece that leads up to the formation of the hydranth, as well as changes at the oral end itself. An acceleration in the formation of the hydranth results even when the oral

end out of which the hydranth develops is removed, provided the removal is not too long deferred.

The rate of development in most of these cases had been judged by the time of the first appearance of the primordia as indicated by the end of the piece becoming red. There is allowed here some leeway, unfortunately, for personal judgment. In all cases the time of emergence of the polyps was also recorded, and in general the two results are found to coincide. The latter method is more exact in some ways, but since the polyp may not emerge until twelve to twenty-four hours after the first appearance of the primordia, it has been found better, on the whole, to judge the rate by the appearance of the primordia rather than by the emergence of the polyp, since there is a shorter time between the operation and its result, so that the effects are less likely to be complicated by other conditions.

It has been stated that in a small percentage of cases, when the pieces were left open at both ends, there was, in this species, an almost simultaneous appearance of oral and basal primordia. We must assume that pieces of this kind were also present in the experiments and these may tend to confuse the results. It is, therefore, unsafe to rely on the result of one or two pieces in a series and a larger number of cases must be recorded. In practice this source of error is difficult to control, but I do not think it has vitiated the results seriously.

When pieces are cut at intervals from the oral end there is, a noticeable, and often a marked, increase in the number of basal hydranths, especially if the pieces are not too long. If very long, the time required for the basal end to produce its hydranth is so much greater than that of the oral end, that the latter is stimulated to produce a new hydranth before the aboral end can begin, hence the latter is kept in check. Very short pieces, especially those from the oral region, often produce "double hydranths," I have compared the development of such pieces with that of similar pieces producing only the oral structure, and, so far as I could make out, both developed at the same rate. In other words, the simultaneous development of the basal polyp does not seem to hold in check the oral polyp, provided both start at the same time. I

have also observed in longer pieces that when both oral and basal primordia appear at the same time that such pieces produce their polyps as soon as do pieces that make only oral polyps. The result seems somewhat paradoxical, but goes to show, I think, that the retardation of the basal polyp is ordinarily due to its failure to receive the proper stimulus to development, rather than to any inherent lack of latent food-producing properties in the piece. The stimulus once received, however, the development can go on simultaneously with that of the oral polyp, neither suffering retardation. If this interpretation is correct it brings us a step nearer the solution of our problem.

Such is the evidence that I have been able to collect. The approach of warm weather has prevented further experimentation, since the Woods Hole Tubularia develops poorly above a certain temperature. Enough has been gathered, however, to throw some further light on the two questions of polarity and hydranth formation.

CONCLUSION

The polarity of Tubularia has something to do, I think, with the differentiation and stratification of the materials as shown by the difference in the behavior of the two cut ends of a piece. The entire stem at every level has the potency to produce hydranths and stolons, but the kind of structure produced, and the rate of appearance of the hydranths at the oral and the aboral ends shows clearly that there is something in the sequence of the layers or in the direction that is a factor in the result. This influence may be overcome by more powerful factors as when a hydranth develops from a basal end, or, as occurs more rarely, when a stolon develops from an oral end. This reversal does not, however, mean that no polarity exists. The question arises as to the nature of the postulated stratification. At one time it seemed to me not improbable that it might relate simply to the relative age of the stem at different levels. The growth in length of the stem takes place apparently just below the hydranth so that the younger parts are always the more distal parts. Hence it might appear that the age of the material gives the stratification. I soon abandoned this idea because

Stevens and I found that when a newly formed stolon, whose growing end is its newest part, is cut off the new hydranth appears on the end originally nearer the old part; in other words on its oldest end. The new stolon shows, therefore, the same polarity as the stem; in fact, the stolon is only a continuation of the stem, and is a root only in the sense that it sticks to the substratum.

That younger pieces regenerate more quickly than older pieces was shown in an experiment in which old and young pieces from the same colony were compared. I also removed some long pieces with short lateral branches that arose near the base. The polyps were cut from the old stems and from the young branches. The young branches formed their primordia much sooner than the old stems. Each had been cut off just below the hydranths. This result shows that the old tissue of the stem becomes young again when it produces a new branch, at least so far as the material of the branch itself is involved.

The youthfulness of the stems is, therefore, an important influence in determining its rate of regeneration, but will it explain the phenomena of the polarity of the stem ? I have suggested that the stratification is due to the relative amount of hydranth forming material at every level, without attempting to define more precisely what this material may be. I can now, I think, give a more satisfactory definition of this relation. The farther the level of the stem from the hydranth the greater its differentiation as stem, hence its gradation of differentiated materials and hence the longer road it must retrace to produce another structure, the hydranth. This differentiation into stem means that the latent capacity to form a hydranth can be less easily called into action. That it can be awakened, however, is shown by the regeneration of a hydranth at each level when the stem is cut; and also by the formation of a bud, which means the local awakening of a hydranth at the expense of the old differentiated material. In the new branch, therefore, we also get a quicker response in hydranth formation than in the old stem at the same level.

It may appear that the behavior of pieces of the stolon, mentioned above, contradicts my hypothesis, because the part of the piece that develops its hydranth, while nearer, it is true, to the

old hydranth, is a new formation not connected with hydranth development at all, but with stem formation. In reality there is no contradiction here, because the tip of the stolon is a structure *sui generis*, and its stratification is from its tip inward. The part nearer the old stem has, therefore, less developed the stolon making qualities and more those of stem, hence the hydranth is more easily developed at this oral end where the conditions that call forth stolon formation are less active.

Loeb stated that when a ligature is tied at the oral end and an aboral hydranth develops the polarity of the whole piece has been reversed. That this is not the case was shown, convincingly, I think, by an experiment of Stevens and myself. We tied a ligature around the oral end, and then, when an aboral hydranth had developed, we cut the piece into small parts kept carefully oriented. Nearly all the small pieces produced new hydranths at the original oral ends, and the only exceptions were those from the region near the new basal polyp. In other words, the polarity has been changed only in the immediate vicinity of the new basal polyp, and the rest of the stem retains its original orientation or stratification. That its polarity might in time become changed is patent, but that it is not immediately changed by the presence of an aboral hydranth is shown by the experiment. In other words, the development of the basal polyp in a piece tied at the oral end is not due to a reversal of the entire polarity, but due to local conditions at the basal end, calling forth the development there of a hydranth, which leads to local changes in the material involved.

Loeb's theory[1] that polarity as an expression of the direction of the current in the digestive tract has been fully considered by Stevens and myself in an earlier paper. Loeb's idea that the red pigment is a hydranth forming substance has also been there considered. His rejoinder to our criticisms is that it is inconsequent on our part to imply that some of the red pigment may not be hydranth forming stuff because a large part of it is thrown away but this reply fails to show in the least that the red pigment assumed

[1]Loeb, I. Concerning Dynamic Conditions which Contribute Towards the Determination of the Morphological Polarity of Organisms. University of California Publications. Physiology, i, 1904.

to be remaining has had such a function. The reply of Loeb ignores also a number of other results that we obtained that indicate that the red pigment has no such rôle, for our criticism was not based on the ejection of the red pigment alone.

I have tried not to lose sight of the possibility that the polarity may be an expression of a fundamental stereometrical arrangement of the ultimate structure of the cytoplasm. To imagine a network of this sort running through the differentiated organs might form an attractive speculation, but would be simply fanciful in the present state of our knowledge. If we imagine a stereometric network as a part of the specialized structure, we must be prepared to admit that it changes at each level as the structure changes. Therefore, it seems to me simpler to base our hypothesis of polarity on the difference in differentiation itself, and not on an imaginary polarized system associated with the living materials.

The other question, with which the present experiments are more particularly concerned, relates to the factors that hold in check the development of the aboral polyp. This may seem a trivial question in itself, yet in principle it involves some of the most obscure points in regeneration, and for this reason I have studied it in detail, for it seemed to me that if we could give an answer to this question we have made a step in advance in the study of regeneration in general. While I do not pretend to have solved this problem, still the experiments permit us, I think, to push the analysis further than was possible before.

Without going over the ground already covered in the preceding account of the experiments let us attempt to scrutinize the results more closely. It has been shown that the development of the oral polyp is responsible for the retardation of the basal polyp. Conversely if a basal polyp is caused to develop first it may temporarily hold in check the formation of the oral polyp. Our problem has narrowed itself to the determination of the nature of this factor. The analysis seems to show that something must be set free in the stem that is necessary to stimulate the formation of the polyp, and also that the something is used up by the developing polyp. The stimulus is internal not external. If it were external we could not explain why the basal polyp is delayed when the oral polyp develops,

or why it begins to develop as soon as the oral end is tied.   It seems plausible that the stimulating agent is some material, set free either at the ends or by the entire wall, that is a storehouse of reserve materials.   To call it hydranth forming material begs the question, and introduces an unnecessary assumption since there is no need to postulate such a substance, inasmuch as the cells of the stem in every part are themselves capable of developing into a hydranth, the more rapidly the less they are differentiated in other directions. If we assume that a stimulating substance is set free, we must then assume that it is used up in the development of the hydranth, which after all is exactly the sort of thing a food substance is expected to do.

When the oral end is tied no hydranth develops there, hence the food substance accumulating soon starts the aboral development. Once started the development continues without further need of the stimulus, because possibly in the changes that have been initiated enough material has been set free to give all that is needed for development, or possibly because a process of this kind once started can and must continue even if the stimulus that started it is removed.

This view is necessary in order to explain the simultaneous development of oral and aboral hydranths that sometimes occurs.

It may be asked whether the stimulating material is set free only near the cut ends or throughout the piece.   Since the piece has potentially the power of starting a dozen or more polyps, as shown when it is cut into many pieces, I think that it is more probable that the materials are set free throughout the piece, although possibly more near the cut ends.   The material must be soluble and pass into circulation, for otherwise the basal hydranth would develop irrespective of what is taking place at the oral end.   The experiment of cutting off short pieces from the oral end after certain intervals shows that the region involved must be more extensive than that occupied by the primordium of the hydranth.   The greater frequency of basal hydranths under these conditions shows that initial changes have taken place at the basal end also, but that they are not very great is shown by the experiment of cutting off the basal end, and finding that the basal hydranth of a piece tied at the oral end still develops almost as fast as when left uncut.

Why, it may be asked, does the basal hydranth begin to develop when the oral end is tied? Must we suppose that whether it develops or not (as when the oral hydranth is developing), it is still setting free materials? To answer this question we must turn to the experiments that involve tying off different lengths of basal ends. These experiments show that a longer tied-off part produces a basal hydranth only a little sooner than a shorter tied-off part. If these results are confirmed on a larger scale with more abundant and favorable material they would seem to mean that the material set free, that acts as a stimulus involves a greater part of the stem than that of the immediate hydranth forming region. That the stem has the capacity to set free much more than this has been indicated. It appears, therefore, that we are dealing here with one of those characteristic cases of organic equilibrium, not uncommon in growth phenomena and starvation periods. The stem, isolated from its feeding organ, the hydranth, slowly sets free in the fluid food materials from its reserve supply. This material is drawn upon by the first hydranth to develop, usually the oral one. The balance must be continually made good by the stem until the hydranth is finished. Should two hydranths start at the same time double the material is used up, and in order to maintain the equilibrium, double the amount must be set free by the rest of the stem. But if the amount set free is used up at the same rate by the oral hydranth, the aboral hydranth does not get the stimulus necessary to begin its development. Should it once begin, however, it proceeds without regard, or with little regard, to the amount set free by the stem, which will tend nevertheless to become greater, the greater the difference between the amount in reserve and that in the circulating fluids.

A number of experiments were made to test whether when a piece (open at both ends so that the oral end begins to develop) is tied after a time, the basal development is hastened even more than when the oral end is tied at once. This seems to be the case, but I do not think that it can be due to the materials set free at the oral end. Whatever materials are set free must be used up by the oral end as soon as it is in excess, otherwise the basal end would start.

It seems much more probable that the basal acceleration is due to changes having been initiated in the stem that leads to the rapid formation of materials that have been taken from the fluids by the oral end. That end being suddenly closed the surplus becomes quickly sufficient to stimulate the basal polyp. But, as explained, the difficulties and uncertainties of this experiment make it undesirable to lay too much stress upon its results.

My analysis leads, therefore, to the following interpretation of polyp formation. The regeneration is due to changes set up in the stem resulting from the separation of the old polyp. The stimulus is largely internal, although another factor, the presence of an open end, is also essential, as shown by closing the end by means of a ligature in which case no polyp develops.[1] The oral end develops first, both because it is a younger part (*i. e.*, less differentiated stemward), and because it has the direction of differentiation for hydranth formation. Its development holds in check for a time the basal hydranths, because the hydranth that first develops uses up or may even deplete the circulating fluids of its surplus food supply. It is well known in other cases of regeneration that a growing part will grow at the expense of old parts. When the oral end is tied the food supply in the fluids of the stem soon rise to a point sufficient to start the basal development. The growth process once started is powerful enough to draw from the common body fluids or other sources sufficient material for its further development.

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[1] If tied very near the old polyp, where the cuticle is thin, an oral polyp sometimes develops behind the ligature.

STUDIES ON THE DEVELOPMENT OF THE STARFISH EGG[1]

BY

D. H. TENNENT AND M. J. HOGUE

WITH FIVE PLATES

INTRODUCTION

The studies made in the preparation of this paper have led to the view that a conjugation of sperm and egg chromosomes takes place soon after fertilization in eggs which have been treated with CO_2 and subsequently fertilized. That this process takes place in normally fertilized eggs is suggested by the similarity in shape of chromosomes in eggs of these two classes, but no detailed study of the processes occurring in normally fertilized eggs has been made.

This interpretation is made with caution and it is recognized that its truth can be determined only by a reinvestigation of the processes occurring in normally fertilized eggs, or better, by a careful study of the changes taking place during the formation of the germ cells.

Inasmuch as most of the accounts of the cytological processes occurring during artificial parthenogenesis have been based on experiments performed on eggs which had given off their polar bodies while the egg was still within the ovary, it seemed that further observations on an egg which might be subjected, either before or after the extrusion of the polar bodies, to influences capable of causing parthenogenetic development might be of interest.

[1] The experimental work on starfish eggs, described in this paper, was done by the senior author in the summer of 1905, at the Marine Biological Laboratory, at Woods Hole. Some of the material then obtained has been studied during the year 1905-06 in the Biological Laboratory of Bryn Mawr College. Miss Hogue has studied the eggs developing as a result of treatment with CO_2 sea-water and has written the account of the nuclear changes, seen in sections of these eggs, given in section 1 of this paper. For the remainder of the paper the senior author is responsible.

The eggs of the starfish (Asterias forbesii) lend themselves to such observations. These eggs, as is well known, if ripe when removed from the ovaries and allowed to remain in sea-water, soon mature, and further, these eggs, like the eggs of other Echinoderms and unlike the eggs of Molluscs and Annelids, may complete their maturation phenomena before the entrance of the spermatozoon.

Delage's accounts ('02, '04) of the use of CO_2 in the treatment of the eggs of Asterias glacialis naturally suggested that his convenient method might be found useful in experiments on the eggs of Asterias forbesii.

Delage ('02) made use of a siphon in which sea-water was charged with CO_2 by means of sparklet bulbs. His best results were obtained with eggs which were subjected when in the ''stade critique'' to the action of charged water for an hour, after which the eggs were removed to ordinary sea-water.

During the work of which this paper is an account it was soon recognized that the duration of immersion mentioned by Delage as most favorable for the eggs of Asterias glacialis is much too long for the eggs of Asterias forbesii. The eggs of this starfish when allowed to remain in CO_2 sea-water[1] for more than half an hour were apparently killed, as they disintegrated without undergoing development.

Eggs in various stages of maturity were subjected to the action of CO_2 sea-water for varying periods of time. The details of these trials are unnecessary. It is sufficient to say that the egg of Asterias forbesii, like the egg of Asterias glacialis, is in its most favorable condition during the time that elapses between the extrusion of the first and of the second polar body. With the eggs of Asterias forbesii it was found that uniformly the best results were obtained when the eggs were subjected to the action of the CO_2 sea-water immediately after the appearance of the first polar body as a protrusion from the surface of the egg.

The best length of time of immersion was about five minutes. Good results were obtained by the immersion of the eggs in CO_2

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[1] Throughout this paper the term "CO_2 sea-water" will be used instead of the longer phrase "sea-water charged with CO_2."

sea-water for from three to ten minutes, but five or six minutes gave uniformly the best results. However, in one lot of eggs in which the time was ten minutes, fully 95 per cent of the eggs segmented regularly and gave rise to normal swimming embryos which were kept alive and under observation for more than a month.

It seemed that the best results were obtained when the sea-water was charged and allowed to stand in the siphon for from ten to twelve hours before using.

Method of Treatment

The eggs were shaken from the ovaries into large dishes of sea-water, in which they were allowed to remain until the first polar body had made its appearance. They were then drawn with a pipette from the bottom of the dish in which they had partially matured and transferred gently and with as little water as possible to finger-bowls. The CO_2 sea-water was then run slowly into the finger-bowl until the bowl was about half-filled. During the whole operation, care was taken to avoid violent agitation of the eggs.

The eggs settled to the bottom of the bowls in about three minutes. At the end of the desired period of immersion, the CO_2 sea-water was withdrawn and replaced by ordinary sea-water, this in turn being changed for fresh sea-water as soon as the eggs had again settled.

The method is convenient in its application and for use with the starfish egg offers an ideal reagent.

The Scope of the Investigations

Since the method is so sure in its action, and since many of the developmental processes reproduce so faithfully the processes occurring in the normally fertilized egg, it seemed that we might have in the starfish egg treated with CO_2 the means of imitating the processes of normal parthenogenesis occurring among rotifers, crustaceans and insects.

Delage ('01) as a conclusion from experiments in which the eggs of Asterias glacialis were subjected while in the critical stage (in this case the time when the germinal vesicle loses its membrane and prepares for the emission of the polar globules),

to the action of a solution of KCl, expressed the idea that artificially parthenogenetic eggs, like those in which parthenogenesis occurs naturally, emit but one polar body, and that the agent producing parthenogenetic development acts by the inhibition of the formation of the second polar body, the second polar body playing the role of a spermatozoon.

Delage in 1902 found that eggs submitted to the action of CO_2 during the time between the disappearance of the nuclear membrane immediately preceding maturation and of the return of the nucleus to the resting condition ordinarily preceding fertilization, developed independently of the polar globules. Parthenogenesis ('02, p. 231) resulted whether the egg had given off neither, or one, or both of the polar globules.

Subsequently ('04), by a somewhat more complicated method of treatment, he was able to produce parthenogenetic development, using CO_2 as a reagent, in sea urchin eggs in which he knew that both polar bodies had been given off.

In my work on the eggs of Asterias forbesii it became apparent that although the second polar body could not be seen in many of the eggs, it made its appearance in many, and that the eggs developed into swimming embryos in both cases. It was found later that the second polar body was formed in every case, although it might remain within the egg membrane in a cup-shaped depression in the surface of the cytoplasm.

But, although both polar bodies were extruded, thus removing the possibility of imitating exactly the processes of normal parthenogenesis, there was as yet no evidence that part of the chromatin normally extruded in the second polar body was not retained within the egg and that this might later assume the functions of the sperm nucleus.

In consequence of this possibility, it seemed that if it were possible to fertilize the egg after its subjection to the action of CO_2, this retained chromatin might be rejected or at least some series of changes might be caused that would be of interest when compared with the normal maturation and fertilization stages and with those occurring in the egg which had been induced to develop by treatment with CO_2 sea-water.

This idea involved the question of the comparative effectiveness of the CO_2 solution and of the starfish spermatozoon on the egg of the starfish.

After determining that it was possible to fertilize eggs after they had been immersed in CO_2 sea-water, the question quite naturally arose: What will be the result of treating fertilized eggs with CO_2 sea-water?

The results of the investigations may be discussed to the best advantage in three sections.

Section 1 embodies the observations on living eggs after their treatment with CO_2 sea-water and the data obtained from a study of the sections of the eggs so treated.

Section 2 contains the data obtained from a study of eggs which were treated with CO_2 and subsequently fertilized.

Section 3 gives the result of the examination of eggs that were fertilized and subsequently subjected to the action of CO_2 sea-water.

I. UNFERTILIZED EGGS TREATED WITH CO_2 SEA-WATER

a. *Observations on the Living Eggs*

The time required for maturation varies, as is well known, with the condition of the egg, the surrounding temperature, etc., so that any facts which were observed as to variations of this kind are without value in this connection, but it is of importance to notice the influence of CO_2 sea-water in delaying the completion of maturation.

In one lot of eggs the first polar body was given off one hour and ten minutes after the removal of the eggs from the ovary. In the eggs of this lot treated with CO_2 sea-water the second polar body appeared one hour and fifteen minutes after the first had been extruded or two hours and twenty-five minutes after removal from the ovary.

In the stock dish a quantity of the same lot of eggs were allowed to complete their maturation undisturbed and the second polar body appeared thirty-five minutes after the first or one hour and forty-five minutes after the eggs were removed from the ovary.

The CO_2 sea-water is thus seen to have delayed the process of maturation forty minutes.

In some lots of eggs, but not in all, a distinct membrane, somewhat thicker than the ordinary fertilization membrane, was formed and pushed out from the surface of the eggs when these were transferred from the CO_2 sea-water to ordinary sea-water, this activity recalling the facts observed by Lefevre ('05, '06) on eggs of Thalassema treated with acid solutions. This membrane carried the first polar body out with it, the second polar body being extruded into the space between the surface of the egg and the membrane.

In the average lot of eggs the series of changes preceding the first cleavage follows very closely, those described by Wilson ('01) as occurring in Toxopneustes eggs, the important difference being that in the eggs of Asterias forbesii that have received the best degree of treatment with CO_2 sea-water no cytasters were to be observed. In eggs which had received too prolonged a treatment, numerous cytasters might be seen.

In about three and a half hours after removal from the CO_2 sea-water the cytoplasm becomes coarser and looser in appearance, apparently becoming more fluid in character in the region of the nucleus. Radiations appear stretching out from the vaguely defined clearer area into the denser cytoplasm. As a result of these changes the nucleus becomes very distinct.

These primary radiations then become fainter and new radiations growing in from the sides of the nucleus are seen, the nuclear membrane breaks down and the definite mitotic figure is seen to be forming, its centers gradually enlarging as the figure becomes definitely established. The division of the egg into two cells is completed in about four hours after removal from the CO_2 sea-water. On one or two occasions, the temperature being low, the first division was completed only after five hours.

It is of interest to notice that in the eggs of Asterias glacialis observed by Delage ('02) segmentation commenced about three hours after removal to the ordinary sea-water, the eggs having remained in the CO_2 sea-water for an hour, that is, segmentation commenced four hours after the beginning of the treatment, while

in the eggs of Asterias forbesii, observed by me, the segmentation began in approximately the same time, about four hours from the beginning of the treatment, although the eggs had remained in the CO_2 sea-water for but five or six minutes.

b. A Study of the Nuclear Changes as Seen in Sections

Delage in his work on Asterias glacialis did not make a detailed study of these phenomena. The purpose of his work being finally ('04) to raise the larvæ until they would metamorphose.

Morgan's ('99) observations on the eggs of Asterias forbesii when treated with solutions of NaCl, MgCl and KCl ('99, p. 499) although brief, since the eggs were immature, are of interest. He mentions the appearance of areas of cyanoplasm and the fact that in some unfertilized eggs that had been in a solution of magnesium chloride for three hours, stars with delicate rays made their appearance in these areas of cyanoplasm.

Kostanecki's work on the eggs of Mactra ('04) is of especial interest in the present connection, since in this egg, which normally does not extrude its polar bodies until after the entrance of the spermatozoan, he traced a series of phenomena analogous to those observed in the starfish egg.

The eggs of Mactra were treated with solutions of KCl, NaCl, CaCl or with concentrated sea-water. The most normal development was obtained in eggs which remained in a weak solution of KCl thirty minutes. Here both polar bodies were given off ('04, Fig. 34) and from the chromosomes remaining in the egg a nucleus was formed quite as in the fertilized egg, although it contained but twelve chromosomes, one-half the normal number (twenty-four), as was later shown in the first segmentation spindle.

The polar spindles developed deeper within the cytoplasm and the polar bodies were larger than those of fertilized eggs. In the manner of division of the segmentation nucleus and in the absence of centrosomes and centrosome-like structures in these divisions, the eggs of Mactra differ from those of Asterias forbesii.

Scott ('06), in his observations on the parthenogenetic development of Amphitrite eggs after treatment with salt solutions, shows that the ripeness of the eggs, the strength of the solutions,

etc., were the factors determining whether development would be nearly normal or very abnormal.   If the Amphitrite eggs are ripe, normal polar bodies are given off in a weak solution of calcium nitrate, although the subsequent segmentation is abnormal.   Here, again, little comparison can be made between these eggs and those of Asterias forbesii, the most important point of agreement being that both polar bodies are given off in each case.

Lefevre's ('06) work on artificial parthenogenesis in the eggs of Thalassema shows many points of agreement with my observations on starfish eggs, the formation of the "fertilization membrane," the extrusion, as a rule, of both polar bodies may be mentioned here and further comparisons reserved for a later mention.

In the present experiment the eggs, after the extrusion of the first polar body, were covered with sea-water charged with carbon dioxide.   They remained in this four minutes and were then transferred to ordinary sea-water.

The series consisted of twenty-five stages, the earlier numbers of which were fixed at five minute and the later at ten minute intervals in Boveri's picro-acetic acid.

The eggs were cut in sections 3 microns thick and stained with Heidenhain's iron-hæmatoxylin, long method.   Eosin, erythrosin, and Bordeaux red were tried as counter-stains but the iron-hæmatoxylin gave the clearest and best effects.   Total mounts were stained by Conklin's hæmatoxylin method.

Throughout the series a few eggs were found with the germinal vesicle intact, which is due to the fact that the nuclear membrane had not begun to fade when the eggs were treated with the CO_2 sea-water, *i. e.*, they were not in Delage's "critical stage."

After the first polar body is given off, a cone of cytoplasm is often seen projecting from the surface of the egg at the side of the first body during the time that the second polar body is forming. This cone frequently persists until the first segmentation spindle is well formed (Figs. 21 and 26) and is occasionally seen in the two-cell stage at the edge of the cleavage plane.

The polar bodies are of the same size as those given off in fer-

tilized eggs, differing in this respect from those observed by Kostanecki in the artificially parthenogenetic eggs of Mactra.

The cleavage is normal, dividing the egg into halves and the second forming four equal-sized blastomeres.

Shortly after the extrusion of the first polar body the chromosomes are found lying on the spindle fibers, which have already begun to degenerate at their polar ends (Fig. 1). In a later stage, eighteen chromosomes may be counted (Fig. 2), and with them are seen a few spindle fibers. These fibers disappear and the chromatin is left free in the cytoplasm (Fig. 3), more or less massed together. In the cytoplasm directly beneath the first polar body is a darkly staining region which is not represented in the drawings. It does not take the chromatin stain.

The formation of the second polar body proceeds slowly and the time of the division of the chromosomes varies. In an egg from stage 5 (Fig. 4) the chromosomes, undivided, are still attached to the fibers of the first polar spindle. In stage 11 (Fig. 5) the chromosomes are divided for the second polar body although they are still attached to the fibers of the first polar spindle.

From a study of the preserved material, it seems evident that the second polar spindle is formed tangentially to the surface of the egg (Fig. 6) and that it later revolves until it has taken a radial position (Figs. 7 and 8). The centrosomes, single and double, may be seen clearly in these spindles. (Figs. 6, 7 and 8.)

In the early anaphase of the second polar spindles (Fig. 9) the chromosomes are scattered irregularly over the whole spindle. In the late anaphase (Fig. 10) they are collected in plates at the two poles of the spindle, one or two of the chromosomes being later than the others in taking their positions.

After the second polar body is formed, the chromatin is more or less free in the cytoplasm, some of it lying on the astral rays as though passing down these into the cytoplasm (Fig. 12), and the remainder forming five or six vesicles which fuse to form the female pronucleus. At each division of the nucleus, chromatin is thrown out into the cytoplasm. Occasionally the chromatin is found lying free in the cytoplasm, without a trace of astral radiation or of vesicle formation (Fig. 11).

The newly formed nucleus now moves to the center of the egg and at the same time begins to divide. The walls of the vesicle become indented (Fig. 13), and on the side nearest the surface of the egg two centrosomes appear and move around the nucleus until they lie one at each end of the slightly elongated nucleus. This process differs from that described by Lefevre ('06) for Thalassema in which the cleavage asters with their centers arise simultaneously at opposite poles of the egg nucleus.

At the same time the astral fibers are forming in the cytoplasm. This growth apparently begins at the nuclear membrane and extends into the cytoplasm of the egg as described by Wilson ('01) and Morgan ('99) for Echinoderm eggs. Later, these radiations collect at the two ends of the nuclear vesicle whither the centrosomes have migrated.

Following this stage the nuclear membrane breaks down and particles of chromatin, seen in Fig. 15 on the astral rays, pass into the cytoplasm.

As the nuclear membrane disappears (Fig. 16), the conspicuous nucleolus unravels and its chromatin, together with that derived from the chromatin network of the nucleus becomes broken up into short threads, the discharge of chromatin into the cytoplasm continuing meantime. The study of the sections has not shown whether all of the chromosomes of the equatorial plate are derived from the nucleolus. The spindle fibers grow into the nucleus and may be seen stretching from the poles to the center of the nucleus.

At this stage the nuclear sap has been poured out into the cytoplasm and a difference in staining reaction of the areas indicated by the dotted line may be seen.

Figs. 14 and 17 represent later stages of the first segmentation spindle.

In the late anaphase the chromosomes become enclosed in vesicles (Fig. 18), which fuse to form the daughter nuclei. The nuclear membrane forms as the astral rays are disappearing. During this process chromatin rejection continues, the rejected chromatin being seen enclosed in little vesicles lying in the cytoplasm (Fig. 22).

After the daughter nuclei are formed they move apart and the constriction appears which divides the egg into halves. It is interesting to note that the cytoplasm does not begin to divide until the daughter nuclei are completely formed. Figs. 23 and 25 represent the spindle and equatorial plate of the second segmentation.

Mathews ('95) was unable to find centrosomes in the segmentation spindles of Asterias forbesii. In the material from which these sections were made two kinds of centrosomes were found; one granular, containing several small deeply staining bodies (Figs. 17 and 23); the other, sometimes a single body, more often two, from which the radiation extend (Fig. 16). In Fig. 18 the double centrosomes appear in the cytoplasm while the astral rays are disappearing.

It has been impossible to count the chromosomes in the segmentation spindle as they do not take definite form (Fig. 14) until they line up in the equatorial plate, ready for division. Here, again, it was useless to try to count them since they were massed together and of irregular shape. Often part of one chromosome is in one section and part in another. Again, some divide before others and frequently when the majority of the chromosomes have separated, a long chain of chromatic material, as yet unsegmented, extends down the middle of the spindle. A few chromosomes seem to have a characteristic form.

It will be remembered that in the second polar spindle there were eighteen chromosomes (Figs. 1, 2 and 8). From Figs. 9 and 10 it seems evident that at least eighteen chromosomes will be left in the egg after the second polar body is given off. Fig. 21 is drawn from a section showing the two polar bodies at the surface and the equatorial plate at the center of the egg. This is shown again drawn under higher magnification in Fig. 20. While it is impossible to count the number of chromosomes exactly, it is evident that there are at least eighteen here. Another egg (Fig. 24) shows the two polar bodies at the surface and the equatorial plate at the center, with the spindle fibers in cross-section. This equatorial plate is shown again in Fig. 19.

In this the chromosomes have divided for the daughter nuclei of the first division.

Delage maintained a doubling in the number of chromosomes in the parthenogenetic eggs of Strongylocentrotus lividus. The eggs had given off both polar bodies when treated with the solution and yet later they contained the normal somatic number of chromosomes. He held that at some time, as yet undetermined, the chromosomes divide again and so establish the normal number (autoregulation), these in turn dividing to form the chromosomes of the daughter nuclei. Boveri has since shown that eighteen is not the somatic number of chromosomes for Strongylocentrotus lividus, but the reduced number.

In the parthenogenetic eggs of Mactra, Kostanecki ('04) found the reduced number of chromosomes in the segmentation spindle when the egg had given off both polar bodies. These eggs did not segment many times.

Boveri ('04) experimenting with the fertilization of nucleated and non-nucleated fragments of sea-urchin eggs, found that when a non-nucleated fragment is fertilized it contains less chromatin; *i. e.*, the nuclei are smaller than the fertilized nucleated fragments. Morgan ('95) performed similar experiments with similar results.

Stevens ('02) while working on the eggs of Echinus microtuberculatus which were cut into pieces while in the anaphase of the first division, found that fragments containing a centrosome and a small number of chromosomes may divide five or six times without the chromosomes returning to the constitutional number.

Wilson ('01) showed that in parthenogenetic Toxopneustes eggs the number of chromosomes is eighteen, one-half the number occurring in fertilized eggs.

The evidence at present, seems in favor of the permanence of the number of chromosomes occurring in the unfertilized egg which is caused to develop parthenogenetically after two polar bodies have been given off.

Another idea of Delage's, that the presence of two polar bodies might be due to the division of the first polar body, seems incorrect. In Asterias forbesii the formation of the second polar body

has been traced and its actual cutting off has been noted. The second polar body is slightly smaller (Figs. 21 and 24) while the first polar body contains the greater amount of chromatin (Fig.11).

One egg was found in stage 24 (Fig. 24) in which the second polar body had been formed but not extruded from the egg before the nucleus began to divide. Here it is certain that the polar body does not again enter the nucleus. This retention of the second polar body within the egg is not the usual method of procedure but may be accounted for by the tardiness with which this egg began development. There is, of course, a possibility that later the chromatin of this retained polar body might mix with that of the egg nucleus in a manner similar to the behavior of the sperm nucleus in partial fertilization as noted by Boveri.

One cannot but be impressed with the normal procedure of development in the eggs treated by Delage's CO_2 method. In the thousands of eggs examined in the study of these sections, there were not more than a dozen abnormal structures. (It is to be remembered, of course, that this series which has been studied with greatest detail was selected primarily because of its perfection.)

Two cases of multipolar spindles were observed, one with three poles, the other with four. A few eggs, undivided, contained three nuclei, each with its amphiaster ready for division. There were also two or three eggs which had divided into three blastomeres. The cleavage in the early stages was normal. No cytasters were formed in the eggs.

SUMMARY

1 Two polar bodies are given off.

2 Eighteen single chromosomes are left in the egg after the extrusion of the second polar body. (The number counted varies within slight limits.)

3 Centrosomes are present in the polar and segmentation spindles.

4 Cleavage is normal though slower than in fertilized eggs.

II. OBSERVATIONS ON EGGS WHICH WERE FIRST TREATED WITH CO_2 SEA-WATER AND SUBSEQUENTLY FERTILIZED

Description of the Experiments

Since it was not known whether the eggs were capable of fertilization after removal from the CO_2 sea-water, some preliminary work was necessary in order to determine, first, whether it was possible to fertilize such eggs, and, second, the optimum time for such fertilization after the eggs had been removed from the CO_2 sea-water.

A set of eggs was subjected immediately after the appearance of the first polar body, to the action of CO_2 sea-water for six minutes and then transferred to a large dish of sea-water and used as stock from which at five minute intervals during a period of two hours, eggs were taken and treated with active sperm.

A series of these eggs (24 stages in all), each stage taken ten minutes after the addition of the sperm, was fixed for sectioning and the remainder of each stage were allowed to continue their development. The study of these sections is now but partially completed.

Some data of this work may be of interest. I give brief notes of observations on the stock and four stages from the twenty-four which were under observation.

Stock—Eggs remained in sea-water for one hour and eleven minutes when the first polar body was seen pushing out from the surface of the majority of the eggs (9:15–10:26).

(1) Eggs subjected to CO_2 sea-water for 8 minutes (10:26–10:34)

(2) 11:15, still but one polar body.

(3) 11:30, two polar bodies and irregular membrane.

(4) 3:00, ten per cent in two-cell stage.

Stage A—Stock eggs treated with sperm at 10:40 (six minutes after removal from CO_2 sea-water).

(1) 10:43, fertilization membrane distinct.

(2) 11:35, two polar bodies, one inside, one outside of fertilization membrane.

(3) 12:00, beginning two-cell stage.

(4) 3:00, twenty per cent past eight-cell stage.

Stage C—Stock eggs treated with sperm at 10:50 (sixteen minutes after removal from CO_2 sea-water).

(1) 12:08, beginning two-cell stage.

(2) 3:00, twenty per cent past eight-cell stage.

Stage D—Stock eggs treated with sperm at 10:55 (twenty-one minutes after removal from CO_2 sea-water).

(1) 12:10, some segmented.

(2) 3:00, thirty-five per cent segmented.

Stage H—Stock eggs treated with sperm at 11:15 (forty-one minutes after removal from CO_2 sea-water.)

(1) 12:15, beginning two-cell stage.

(2) 3:00, thirty per cent eight-cell stage.

In A eggs began to divide one hour and twenty minutes after fertilization.

In B eggs began to divide one hour and eighteen minutes after fertilization.

In D eggs began to divide one hour and fifteen minutes after fertilization.

In H eggs began to divide one hour after fertilization.

In this experiment the highest percentage of dividing eggs was obtained from those treated with sperm between twenty and forty-five minutes after removal from CO_2 sea-water. After 11:15 through the four succeeding stages 11:20, 11:25, 11:30, 11:35, the percentage of eggs dividing in approximately one hour after fertilization rapidly diminished, being practically zero at 11:35 (5 minutes after the second polar body had been given off in the CO_2 stock).

In all of the succeeding stages, segmentation began between 2:20 and 2:40 or roughly four hours after treatment with CO_2.

From these observations it seemed possible to conclude that eggs treated with CO_2 sea-water were capable of fertilization during the hour immediately succeeding removal from CO_2 sea-water. After that time had passed the spermatozoa, although still exceedingly active, were either not able to enter the egg or entering it produced no effect and the eggs proceeded to their parthen-

ogenetic division.   Fresh spermatozoa were tried but without avail.

For the sake of comparison, a table showing the percentage of eggs developing from a set allowed to mature for one hour and forty minutes (10:00–11:40), in sea-water (until the extrusion of the first polar body), and then fertilized at the intervals noted, is given.   These eggs were not treated with CO_2 but were simply fertilized with sperm.

Fertilized 11:40, ninety-five per cent segmented.

Fertilized 12:03, ninety-five per cent segmented.

Fertilized 12:25, ninety per cent segmented.

Fertilized 1:40, thirty per cent segmented.

Fertilized 3:00, no eggs segmented.

Fertilized 4:00, no eggs segmented.

In this set of normal eggs a considerable percentage was capable of fertilization for more than two hours after the extrusion of the first polar body.

Comparison of these sets of results, then, show that apparently treatment of the eggs with CO_2 sea-water shortened the time during which the sperm might be effective.

From the observations mentioned it was thus found that the most normal results were obtained by fertilizing the CO_2 eggs from twenty to forty minutes after their removal from the CO_2 sea-water.   These eggs when successfully fertilized commenced their segmentation in approximately one hour and thirty minutes after fertilization.   The treatment with sperm shortened the time elapsing between the treatment with CO_2 sea-water and the beginning of segmentation, or, stating it more concretely:

The time usually required for segmentation of CO_2 eggs was about four hours from the extrusion of the first polar body.

The time required for the beginning of segmentation of CO_2 eggs subsequently fertilized was about one and one-half hours.

The eggs that had been treated with CO_2 and subsequently fertilized gained in time, then, two and one-half hours.

In work with other lots it was found that the greatest percentage of fertilized eggs was to be obtained, if the CO_2 eggs were treated

with sperm twenty or thirty minutes after removal from the CO_2 sea-water.

Several sets of eggs were thus treated and from these, series were fixed which have served as the basis of the cytological study.

It is of considerable interest to notice that the thick vitelline membrane pushed out from the egg, did not prevent the entrance of the spermatozoan, and that in eggs fertilized after the appearance of this membrane, a second membrane, somewhat thinner than the first, was formed, so that the eggs were surrounded by concentric membranes.

In several cases, the actual passage of the spermatozoan through the outer membrane was observed, while in another lot of parthenogenetic eggs which were treated with sperm when in the two- and four-cell stages, the spermatozoa were seen swimming actively between the blastomeres.

The sections of these eggs have not been studied so that it is impossible to state whether or not the spermatozoa entered the blastomeres, or their effect on subsequent developments.

The Study of Sections of the Eggs

The facts determined by the study of sections of eggs treated with CO_2 sea-water and later fertilized, show that the processes occurring are so like the normal that a detailed description is unnecessary. I shall, therefore, content myself with a description of the figures and later make a comparison between the facts brought out by Miss Hogue in her study of the parthenogenetic development and those determined in my own study of the eggs which have received the double treatment.

In some of the eggs sectioned, the first polar body was in process of formation. Here, there seemed absolute evidence of the longitudinal division of bivalent chromosomes.

Fig. 30 shows a section through the remains of the first polar spindle immediately after the extrusion of the first polar body. In the polar body and in the egg the dumb-bell-shaped bivalent chromosomes are seen (Fig. 31). In some cases a tetrad effect (Fig. 32) due, I believe, to the grouping of the double chromosomes was to be seen.

In some cases the chromosomes of the second polar body remained within the egg (Fig. 33), clustered beneath the first polar body where they apparently degenerated, since in slightly later stages they were seen to be broken into small fragments, and in still later stages a few slightly stained granules were found in this position. There was absolutely no evidence that as chromosomes they took any part in subsequent nuclear transformations.

The best evidence shows that in the second maturation division the bivalent chromosomes are divided to form univalent chromosomes (Figs. 34–37), as has been described by Mathews ('95). The division is transverse. I have been unable to find evidence of the double longitudinal division which has been described by Bryce ('02) for Echinus esculentus.

In many cases abnormalities occur (Figs. 38–42). In some cases tripolar spindles are seen, in which event it seems possible that both polar bodies are being formed in the same division.

The chromosomes remaining within the egg soon form five or six vesicles which are drawn together and fuse to form the female pronucleus which lies surrounded by some of the degenerating fibers of the second polar spindles (Figs. 44, 48, 49 and 50). Meantime the sperm nucleus, which has become vesicular, has been moving, accompanied by its aster toward the egg nucleus, the centrosome and aster dividing as the two approach (Figs. 45 and 46), the two nuclei finally fusing to form the segmentation nucleus.

In a very few cases the egg nucleus seems to be provided with an aster of its own (Fig 44). In most cases it seems simply lying among some of the fibers remaining from the second polar spindle.

The segmentation nucleus remains in a resting condition for some time, during which the progression of the asters to opposite sides of the nucleus may be observed. From their inner sides fibers may be seen projecting into the nuclear membrane (Fig. 58). and in slightly later stages these fibers may be seen within the nucleus although the membrane seems intact. The nucleus has meantime become decidedly elongated in outline.

Succeeding this stage the conditions represented in Figs. 59–61 rapidly succeed one another. The nuclear membrane apparently dissolves first at the poles and finally dissolves throughout,

during which process the achromatic figure increases greatly in size.

The chromatic reticulum and the nucleolus break down into coarse threads which in succeeding stages becomes finer, these threads ultimately becoming broken up into short rods. (Figs. 54–56). These rods become rounded (Fig. 57) and during the formation of the equatorial plate are replaced by bivalent structures (Figs. 62–68), which lie with their long axis at right angles to the long axis of the spindle.

All of the observations point to a conjugation of rounded univalent chromosomes to form elongated bivalent chromosomes.

In Figs. 54 and 55 the chromosomes are seen to be rod-like. In Fig. 57 the form has changed, all of the chromosomes having become rounded, a few showing the bivalent form. In Figs. 62 and 63, 66 and 67, it is seen that the bivalent form has become more common while the actual number of chromosomes has diminished, while in Fig. 68, which represents all of the chromosomes of the equatorial plate, it is seen that the number of chromosomes has been still farther reduced and that with the exception of six univalent chromosomes all of the chromosomes show the bivalent form.

These chromosomes retain their bivalent form during the segmentation divisions (Figs. 64 and 65 and 61 and 69). In Figs. 61 and 69 it is seen that some of the chromosomes are still of the univalent form. This was true of all of the segmentation stages examined.

The division of the chromosomes in the segmentation is longitudinal, the chromosomes which are at first placed with their long axes at right angles to the long axis of the spindle becoming pulled out so that their long axes lie parallel to that of the spindle (Figs. 64, 65 and 53).

The daughter nuclei are formed by the fusion of chromosomal vesicles.

Granules of chromatin are thrown out into the cytoplasm from the time of the breaking down of the nuclear membrane preceding segmentation until the formation of the daughter nuclei is completed.

Further reference will be made to some of these changes in the general considerations at the end of this paper.

III.   EGGS FERTILIZED AND SUBSEQUENTLY TREATED WITH CO_2

In these eggs the sperm was allowed to act for ten minutes and the eggs were then transferred to CO_2 sea-water for five minutes.   I give the data of but one lot.

Fertilized eggs placed in CO_2 sea-water (12:28–12:33).

No eggs segmented until 3:40 or three hours and seven minutes from the time of removal from the CO_2 sea-water.

Since the sections of these eggs have not yet been studied it is impossible to say definitely whether the eggs developed as a result of the CO_2 treatment or as a result of fertilization by the sperm.

It is probable that since segmentation began within three hours after the eggs were removed from the CO_2 sea-water the sperm fertilization was effective, in which event the action of the CO_2 sea-water was simply to delay development.

SUMMARY

The observations on the behavior of eggs treated with CO_2 may be summarized as follows:

1 Unfertilized eggs subjected to the action of CO_2 sea-water for from 3 to 10 minutes commenced segmentation about four hours after treatment.

2 (*a*) Unfertilized eggs subjected to the action of CO_2 sea-water for from three to ten minutes and fertilized twenty to thirty minutes after removal from the CO_2 sea-water began segmentation about one hour after fertilization.

(*b*) Unfertilized eggs subjected to the action of CO_2 sea-water for from three to ten minutes when treated with sperm one hour or more after removal from the CO_2 sea-water segmented four hours after removal from the CO_2 sea-water.

3 Fertilized eggs subjected to the action of CO_2 sea-water segmented three hours after removal from the CO_2 sea-water.

GENERAL CONSIDERATIONS ·

As to the cause of the parthenogenetic development of eggs treated with CO_2, whether it is by reason of a change of osmotic pressure, or because of agitation, or of the exposure to some specific chemical substance, or is due to the presence in the sea-water of new compounds formed in reactions which may take place between the CO_2 or impurities which may be present in the materials used and substances present in sea-water, this paper has nothing to say.

The simple fact remains that eggs treated with CO_2 as has been described, segment regularly and develop into embryos which in form and structure cannot be distinguished from embryos obtained from fertilized eggs.

Whether the reagent acts as a stimulus, or a shock, or a poison, the author cannot say. The evidence, however, is sufficient to show that Delage was correct in his view that maturation is arrested temporarily by the action of the CO_2.

It is in the comparison of sections of CO_2 eggs with those of CO_2 eggs which were subsequently fertilized and with those of normally fertilized eggs that the most interesting facts come to light. All behave in essentially the same manner in the maturation processes, these processes apparently agreeing with those described by Mathews ('05) but which he has not figured in detail. In the material studied, which it is to be remembered must be regarded as having received somewhat artificial treatment from the beginning, the number of chromosomes remaining in the egg, after the extrusion of the second polar body, varies slightly, the number ranging from eighteen to twenty-three, although eighteen seems to be the common number.

It is seen that the maturation processes in both the CO_2 eggs and in the CO_2 eggs which were subsequently fertilized, differ remarkably from those observed in compressed eggs by King ('06). Here, although the first polar body may be extruded, the second is retained, all of the retained chromatin going into the formation of one or several vesicles which unite to form the egg nucleus, but "the retention of chromatin that is normally extruded

in the polar bodies does not lead to a parthenogenetic development of the egg." In compressed eggs which were fertilized, great differences in size in the two pronuclei were noted, a phenomenon which does not occur in CO_2 eggs subsequently fertilized. The polyspermy observed in fertilized compressed eggs has been noted in CO_2 eggs subsequently fertilized which did not receive the treatment which results in normal development.

It is worthy of note again that in the CO_2 eggs which were subsequently fertilized and in ordinarily fertilized eggs the chromosomes during the maturation divisions are bivalent (Mathews, double chromosomes) and at the close of the maturation divisions eighteen univalent (Mathews, seventeen [?] single) chromosomes remain within the egg.

It will be noticed that the straight CO_2 treatment evidently has some influence on the maturation processes, for while the number of chromosomes remains the same as in the fertilized eggs, the shape of the chromosomes varies.

The process of reconstruction of the egg nucleus is by the formation and fusion of chromosomal vesicles in eggs of the three classes mentioned.

The appearance of the segmentation nucleus in the CO_2 eggs and in the CO_2 eggs which were subsequently fertilized shows no difference. This nucleus breaks down in the same manner in both cases, the nuclear reticulum becomes threadlike and breaks up into fragments, the mass of chromatic material being the same, so far as the eye can judge, in the one case as in the other.

In neither case can the exact number of fragments be counted, but it is clearly to be seen that they are not to be regarded as individual chromosomes since the numbers counted are more than double those found at any earlier or later stages when the count may be made with reasonable certainty, and since many of these fragments may be seen in various stages of withdrawal into the cytoplasm.

But at the time of the completion of the equatorial plate, fundamental differences between the chromosomes of the CO_2 eggs and the eggs which were subsequently fertilized may be seen. In the one case the chromosomes are irregular and in the other, of a

distinct bivalent form; a form which is preserved during the longitudinal splitting of the chromosomes as it divides to form the daughter nuclei, a form which persists in the later divisions of the egg so far as observed and a form which is again seen in the maturation divisions until the final separation of bivalent into univalent chromosomes during the formation of the second polar body.

These facts, it seems to me, are to be explained only by the suggestion that a conjugation or synapsis of egg chromosomes and sperm chromosomes takes place immediately before the formation of the equatorial plate of the first segmentation spindle.

That this process takes place seems very probable when, after comparing Figs. 19 and 20, representing sections through the equatorial plate in CO_2 eggs, with Figs. 54, 56 and 57, representing sections through the equatorial plate of CO_2 + sperm eggs, one follows the series of changes shown in Figs. 62, 63, 66, 67 and 68, in which the rounded chromosomes are shown, some lying free, some drawn closely together in pairs and still others showing the completed bivalent form. This form is retained from the equatorial plate stage (Fig. 68), through the division figures (Figs. 64, 65, 69, 61) of the CO_2 + sperm eggs and may be seen in the division figures of the normally fertilized eggs (Figs. 28 and 29).

As a result of such a conjugation the *number* of chromosomes would remain the same in the parthenogenetic as in the fertilized eggs, with the difference that in the parthenogenetic eggs there remain eighteen univalent chromosomes and in the fertilized eggs eighteen bivalent chromosomes.[1]

[1]During the summer of 1906 the experiments which have been described in this paper were repeated and some further observations made. In this work it was demonstrated satisfactorily that the equatorial plate in the eggs from some individuals contained, within slight variations, thirty-six chromosomes. The Woods Hole starfishes thus show a variation as to the number of chromosomes similar to that pointed out in Echinus microtuberculatus by Stevens ('02). In such eggs as in the eggs that have been considered in this paper the number of chromosomes remains the same in both fertilized and in parthenogenetic eggs. A point of disagreement between these eggs and those obtained during the previous summer is that dumb-bell-shaped chromosomes were found in parthenogenetic eggs. Little dependence, therefore, as has been suggested by several authors recently, can be placed upon the shape of the chromosomes as an indication of valency.

It is possible that the objection will be made that the activity of the CO_2 sea-water is such as to change the shape of the chromosomes in the equatorial plate of the first segmentation spindle, since we have seen that the shape of the chromosomes in the maturation divisions was thus influenced.

In replying to such an objection, it can only be pointed out that the number of chromosomes, although these chromosomes vary in size, shape, etc., remains apparently the same in both the CO_2 eggs and in the CO_2 eggs which were subsequently fertilized, and this in spite of the fact that we should expect that the number in the fertilized egg to be doubled.

An additional point of interest comes out in the comparison of these eggs, and this in respect to the cleavage asters. The cleavage asters with their centers have the same appearance in both the CO_2 eggs and in the CO_2 eggs which were subsequently fertilized. In eggs which were fertilized at the optimum time these were the only asters formed, while in eggs fertilized later than this time many cytasters and additional sperm asters were formed, the subsequent divisions being exceedingly abnormal. The study of these phenomena has been partly completed and its results will be submitted in a later contribution.

Bryn Mawr College
    June, 1906

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DESCRIPTION OF PLATES

The drawings were all made from camera lucida sketches. Zeiss compensating ocular 12, with 2 mm. oil immersion objective, giving a magnification of 1500 diameters, were used for all of the figures except those hereinafter noted. All of the drawings are reduced one-half in reproduction.

(Figs 1–27 from CO_2 eggs)

PLATE I

Fig. 1. First polar body formed. Remains of degenerating spindle. Eighteen chromosomes present.

Fig. 2. Later stage of degenerating spindle. Eighteen chromosomes.

Fig. 3. Complete disappearance of spindle. Additional chromosomes were in next section of the egg.

Fig. 4. Chromosomes remaining on a half spindle.

Fig. 5. Same as Fig. 4, except that the chromosomes have divided.

Fig. 6. Second polar spindle tangential to surface of egg.

Fig. 7. Radial position of second polar spindle as seen in later stages.

Fig. 8. Second polar spindle with eighteen chromosomes.

Figs. 9 and 10. Early and late anaphase of second polar division.

Fig. 11. Two polar bodies given off; chromatin lying free in cytoplasm.

Fig. 12. Formation of female pronucleus through fusion of vesicles.

Fig. 13. Female pronucleus preparing to divide; chromosomes and fibers appearing.

Fig. 14. Late prophase of first segmentation division.

Figs. 15 and 16. Early prophase of first segmentation. Ocular 4, 2 mm. oil immersion objective.

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PLATE IV

Fig. 43.   Second polar spindle formed near center of egg.

Fig. 44.   Female pronucleus formed by fusion of chromosomal vesicles.   Part of second polar spindle lying in cytoplasm beneath first polar body.

Fig. 45.   Male and female pronuclei.   Aster divided.

Fig. 46.   Male and female pronuclei.   Centrosome divided.

Fig. 47.   Segmentation nucleus.

Fig. 48.   Male pronucleus approaching partially formed female pronucleus.   Ocular 4, 2 mm. oil immersion objective.

Fig. 49.   Same as Fig. 48.

Fig. 50.   Vesicles which are to fuse and form female pronucleus.

Fig. 51.   Segmentation nucleus elongated; a chromatic fibers are to be seen within the nuclear membrane.   Ocular 4, 2 mm. oil immersion objective.

Fig. 52.   Male pronucleus.

Fig. 53.   Portion of first segmentation spindle.

Figs. 54 and 55.   Adjoining sections showing thread broken into short, rod-like segments.

Fig. 56.   Part of section through nuclear material.   Three bivalent chromosomes present.

Fig. 57.   Rod-shaped chromosomes rounded up into spherical structures.   Some bivalent chromosomes.

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SOME EXPERIMENTS ON THE DEVELOPING EAR VESICLE OF THE TADPOLE WITH RELATION TO EQUILIBRATION[1]

BY

GEORGE L. STREETER, M.D.

Associate, Wistar Institute of Anatomy

WITH TWELVE FIGURES

The eventual object of the experiments reported in the following paper was the rearing of some tadpoles which had been deprived of their auditory vesicle and acoustic ganglion, either on one side alone or on both sides; that is to say, an artificial production of a unilateral and bilateral absence of the acoustic apparatus. This was done in the expectation that it might be possible to trace the central acoustic path, in this new way, and perhaps throw further light upon its course and relations. The absence of these sense organs, however, produced such definite abnormalities in the behavior of the growing larvæ and in the development of their swimming abilities that it became at once apparent that I was dealing with valuable evidence in respect to their function and its bearing on the mechanism of equilibrium. It is, therefore, deemed advisable to restrict the following paper to the physiological features of these experiments, and reserve the study of the central nervous system of the reared specimens for a later communication.

What we already know concerning the function of the vertebrate ear is based principally on experimental sectioning or stimulation of the semicircular canals, or the nerves to their ampullæ, in adult birds and fishes.[2]

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[1] Read in part before the Section of Anatomy of the British Medical Association, at the meeting held in Toronto, August 21-25, 1906.

[2] For experimental work on fishes we are for the most part indebted to Lee ('93 and '98) and Lyon ('oo), both of whom carried on their experiments at the Woods Hole Laboratories. Further work on fishes has just been completed at the same place by Professor Parker, whose paper I am told is now in press and will appear in the Bulletin of the U. S. Fisheries Bureau. An abstract of part of his work was read before the American Zoölogical Society (Parker, '05). A voluminous literature exists concerning experiments on higher vertebrates, particularly the pigeon, but it need not be considered here.

The fact that it is possible to experiment on the embryo and to produce at will practically a congenital absence of this organ, besides serving as a control over the experiments on adult animals, introduces a direct advantage both as regards the ease with which the operation is performed and as regards its completeness and permanence and freedom from injury of adjoining structures, the latter point being of particular importance to those who are still in doubt as to how much is due in the experiments on adults to injuries and stimulations associated with the operation and how much is purely the result of the cessation of the stimuli which normally originate in the labyrinth. Furthermore, since the labyrinth is removed during the early formative period at a time when it may be presumed that the various organs possess their greatest adaptability, it will be seen that such embryonic interference affords a most complete test of the power of functional compensation on the part of other organs.

Behavior of Normal Tadpoles

In analyzing the behavior of operated specimens it was found necessary to make a preliminary study of control tadpoles, in order to determine the normal development of motor reflexes and their coördination and the consequent establishment of equilibrium. This was done by removing the larvæ from their gelatinous capsule shortly after fertilization and following their development in tap water. In this way it was seen that in the process of learning to swim they pass through three periods, which may be named as follows:

1. Stage of non-motility, first three days.

2. Stage of spinal reflexes, fourth to sixth days.

3. Stage of equilibrium, sixth day to maturity.

The first stage, with a favorable temperature, lasts from the time of fertilization to the third or fourth day. During this time the larvæ, aside from the movement due to cilia, lie motionless on their side on the bottom of the dish and do not respond to stimuli. The second stage begins at the time when they first respond to

mechanical stimuli by flexion of the body and tail.[1] These reactions consist of simple motor reflexes at first, but they soon become combined and coördinated so that by a series of such body flexions they are able to wiggle rapidly forward on the bottom of the dish. This manner of progression evidently consists entirely of spinal cord reflexes and is not controlled by higher centers. In order to perform it, it is necessary for the tadpoles to touch the bottom or
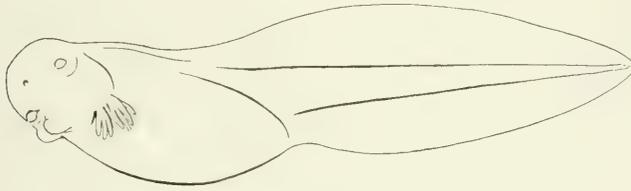

FIG. 1
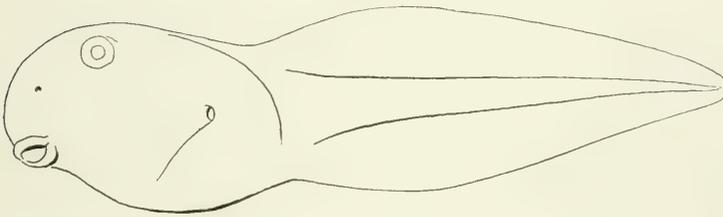
FIG. 2

Fig. 1. Outline drawing of normal tadpole (Rana sylvatica) of the second stage or stage of spinal reflexes. Enlarged 8 diameters.

Fig. 2. Outline drawing of normal tadpole (Rana sylvatica) at the beginning of the third stage. This specimen had the power of equilibration, although sections of the ear vesicle showed that the development of the semicircular canals was not yet complete. Enlarged 8 diameters.

side of the dish; when they are driven up into the free water with a pipette, where there is no contact with solid objects, they make no effort at movement, but sink inertly to the bottom; on striking the bottom they run forward again. The third stage begins when they are first able to move freely about without touching solid

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[1] I have been informed by Dr. R. G. Harrison that it is just at this time that the motor nerve roots make their appearance, and this may determine the onset of the second stage. According to his observations the power of muscle contraction follows almost immediately after the development of the motor roots; but it never precedes their development, as is maintained by some. He has found the motor root present in specimens that had not yet moved.

objects. At this time a new control over their movements is developed, in virtue of which they become able to leave the bottom of the dish and swim up into free water with maintenance of what may then be called equilibrium. The form of the tadpole during the latter part of the first stage is shown in Fig. 3. The second and third stages are shown in Figs. 1 and 2.

The correlation between the histological development of the labyrinth and the development of the power of equilibrium was studied by selecting specimens of the second and the beginning of the third stages, carefully noting their behavior, and then cutting them in serial sections.[1]

From these series it could be seen that shortly before the animal enters the stage of equilibrium the labyrinth consists of a closed epithelial sac incompletely subdivided into compartments and possessing differentiated nerve endings which are connected with the brain by the acoustic nerve and ganglion. That at least one such apparatus is essential for equilibrium will be seen when I describe the behavior of tadpoles that have been completely deprived of the same. As regards the semicircular canals it is a different matter; they can already be seen in the process of development, but are not completely pocketed off until after equilibrium is already established. Consequently the semicircular canals as such are not an essential factor in equilibration.

Method of Operation

Larvæ of Rana sylvatica measuring about 3 mm. long were selected as being most suitable for the operation. Their general form at this time is shown in Fig. 3. There is a distinct tail bud, and on the head the eminences caused by the optic cup and head ganglia are visible. The structure that is to form the future labyrinth is situated just dorsal to the ganglionic eminence and is shown

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[1] The correlation between the histogenesis of organs and the development of their functional activity forms a fruitful field which has been explored by comparatively few investigators. It may be approached both through ontogeny and phylogeny. Prentiss ('01) by this means worked out important facts regarding the crustacean otocyst. Many details concerning the vertebrate ear which do not belong to the scope of the present paper could doubtless be learned in the same way.

in Fig. 3 by the mark +. It consists of a cup-shaped mass of cells (auditory cup) which have differentiated themselves from the deeper layer of epidermis, and are just in the process of closing in at the edges to form the completed ear vesicle. In size this ear cup or ear vesicle is about one-half that of the optic cup.

For performing the operation it is not necessary to anesthetize the specimen as it is still in the non-motile stage and does not respond to stimulation. After removing the larva from its gelatinous capsule it is placed under a binocular microscope and an incision made near the place indicated in Fig. 3. The edge of the incision is then raised a little and the auditory cup is picked out with a needle. After a little

Fig. 3. Outline drawing of Rana sylvatica at the time suitable for operation, just at the end of the non-motile stage. The tail bud is present and on the head are seen the eminences due to the optic cup and head ganglia. Above the latter is the point of operation shown by a cross. Enlarged 8 diameters.

practice one learns to make the incision directly at the edge of the cup so that it comes away easily and intact, resembling somewhat a thimbleberry. Lying just in front of it is the acoustic ganglion which is not so sharply outlined. This is also removed and, in order to make sure that it is all taken out, the surrounding mesoderm is cleaned out as far in as the brain. Where but one vesicle is to be removed the operation is then complete, and the specimen is left to proceed in its development. The wound immediately closes of itself and heals in the course of a few hours leaving no trace of the operation. Where both sides are operated on, the same procedure is carried out on both sides. The ear vesicle never regenerates following complete removal.

The ear vesicle was removed on one side from thirty specimens and on both sides from twenty specimens. The animals were then kept under observation and their behavior recorded through the whole larval period and until the completion of metamorphosis. The following notes were selected from these records.

Removal of One Ear Vesicle

Twenty-four hours after operation: Specimens are 5.5 mm. long and show presence of gill buds. In appearance and behavior, no difference can be detected between them and normal tadpoles. They lie on their side and on stimulation flex their body, but make no attempt at swimming.

Forty-eight hours after operation: Specimens are 7 mm. long, gills are branched and the blood can be seen circulating through them. In appearance and behavior they still show no departure from that seen in normal control specimens. While at rest they lie on their side. On stimulation (sunlight, jarring the dish, or touching with needle) by a rapid flexion of the body and tail from side to side they swim forward, 5–10 cm., on the bottom of the dish in a straight or slightly curved line, and then come to rest on their side, and remain so until a new stimulation excites another such excursion. Their course is directed either by the side or bottom of the dish. When forced up into free water the flexions stop and they sink inertly to the bottom.

Third day after operation: Specimens average about 8 mm. long, abdominal epidermis differentiated from that of the dorsal parts of the body by being less pigmented. Appearance and behavior is still practically normal. They begin to show a tendency to assume the upright position while at rest, but no great importance can be attached to this feature as throughout the early days of the tadpole period, preserved specimens lie in the same positions as living ones. Their posture in water may be entirely determined by their body proportions. Their movements remain of the spinal cord type seen on the previous day, the response being more prompt.

Fourth day after operation: Specimens 9–9.5 mm. long. In appearance the operated specimens are the same as the normal ones, but in behavior they present a difference. The normal ones still confine their movements to the bottom or side of the dish; when stirred up into free water, though most of them still roll about inertly, some of them are able to maintain a direct course. On the other hand the operated ones, as soon as they are driven from the bottom, swim in a spiral or circular manner as shown in the

accompanying Fig. 4. The tendency is to swim with the operated side under, and in the rolling movements around the long axis of the body it is from the operated side under to the opposite. When these same specimens touch the bottom they are able to direct their course as on the previous two days. Evidently, a functional union is normally established at about this time between the ear vesicle and the spinal cord reflex centers, upon which the individual is dependent for maintaining its position in free water, and it is not until this occurs that the removal of the ear vesicle causes any symptoms.
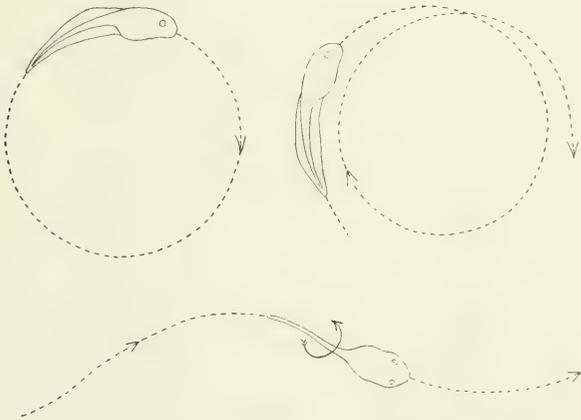
Fig. 4. Sketch showing three typical swimming movements made by specimens on the fourth day after removal of their left ear vesicle.

Sixth day after operation: Specimens about 12 mm. long, and have commenced to nibble at food and pass fæces. The characteristic movements which first appeared on the fourth day have become stronger and stand out in more marked contrast to the behavior of normal specimens which at this time can swim easily up into free water with accurate maintenance of equilibrium.

Seventh day after operation: The operated specimens show distinct improvement in swimming ability; many of them are now able to maintain a fairly direct course in free water, but on excitation they renew the spirals and circles which characterized the fourth, fifth and sixth days.

Eighth day after operation: nearly all the specimens now swim freely and directly in all parts of the water, and irregularity of swimming is only elicited by excitement.

Tenth day after operation: Swimming is practically normal. Their movements are under such control as to enable them to support themselves in free water and nibble at floating stems and leaves. It can be seen, however, that in swimming they lean
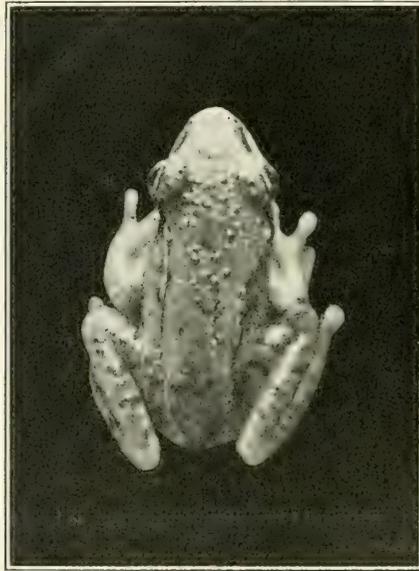
Fig. 5. Photograph of a frog whose left ear vesicle was removed when a tadpole 3 mm. long. The only asymmetry noticeable is the absence of the ear elevation on the left side normally caused by the labyrinth and its cartilaginous capsule; the lateral line on that side is straight from the eye back, while on the right or normal side it is deflected. The posture is normal. Enlarged 3½ diameters.

slightly toward the operated side, a sympton which persists throughout their larval period.

Twelfth day after operation: Specimens are normal as regards size, nourishment, and symmetry, except for the absence on the operated side of the elevation which is caused normally by the labyrinth and its cartilaginous capsule. In behavior they differ from the normal only in the slight leaning toward the operated side

and a momentary loss of equilibrium which can be elicited by excitement.

Three months after operation: The specimens passed through a normal metamorphosis at the end of the third month. A photograph made of one of them a few days after the completion of the process is shown in the accompanying Fig. 5.

As long as they continued as swimming tadpoles the slight leaning toward the operated side persisted and it was possible through excitement to cause a momentary disturbance in equilibrium, but the latter became gradually more difficult to demonstrate. As soon as they commenced to make use of their legs the character of the swimming changed; it then became a series of leg strokes instead of the sinuous flexions of the body and tail. After that it was no longer possible to detect the leaning toward the operated side; both when swimming and when at rest their behavior was to all appearance normal. When taken out of water they jumped normally and came to rest in a normal posture. When turned over on their backs they righted themselves promptly.

The fact that the slight disturbance of equilibrium, which could be still detected in the tail-swimming tadpole, could no longer be seen in the leg-swimming frog, a change completed within four or five days, probably does not signify the cure of the condition, but rather that under the latter circumstances a slight defect is more difficult to recognize. The corollary of this would be that equilibrium in the swimming tadpole is a more delicately balanced mechanism than in the kicking and jumping frog.

Removal of Both Ear Vesicles

During the first three days after the operation the appearance and behavior of these specimens are the same as seen in the normal ones, and in those from which one ear vesicle was removed. The response to stimuli is perhaps a trifle less prompt, but otherwise they could not be distinguished one from the other.

Fourth day after operation: It was seen that in one-sided operations the specimens commenced about this time to make excur-

sions into free water, and in doing it they departed from the normal by swimming in spirals and circles. Tadpoles with both ear vesicles taken out make no such excursions and show decidedly less activity. Occasionally they flex their body and tail from side to side producing a snapping effect which does not result in any forward progress. Like the other specimens they are, however, able to wiggle along in contact with the side and bottom of the dish.

Seventh day after operation: The specimens are smaller and are retarded in development as compared with the normal and one-sided specimens. They are, however, symmetrical in form and are normal as regards the appearance and movements of the eyes, mouth, heart and intestine. They are decidedly less active and stimuli produce irregular attempts at swimming, sometimes somewhat spiral in character but usually nothing more than a series of awkward flexions of the body. These flexions also occasionally occur with no apparent stimulus. They make a partially successful effort at nibbling on the bottom of the dish.

Twelfth day after operation: Absolutely no improvement in swimming; any attempt at it results in a series of somersaults. they throw their body up into the water and then promptly sink to the bottom in almost any position. When at rest, they lie on their side, back, or normally on their belly, depending apparently on whether their intestine is filled with sand, etc., to properly balance the body. The intestine is very apt to be empty because of the difficulty they experience in feeding. They do not wiggle along on the bottom as well as they did on the fourth and fifth days.

Two months after operation: The specimens could not be carried much beyond this point, the difficulty apparently being starvation from inability to wander around and collect food. Perhaps also the respiration was involved, for they were unable to go to the surface for oxygen as the normal tadpole does.

In behavior they show no improvement. For the most part they lie stiff and inert in various positions on the bottom, and their occasional attempts at swimming have never developed into anything more successful than was described on the seventh and twelfth

days after operation. Their appearance departs from the normal principally in the small contracted character of the abdominal region. In volume they are about one-third as large as the normal specimen, varying from 2.5 to 4 cm. in length. They have a hind leg bud 2.5 to 3 mm. long. As some of them commenced to die at this time the rest were put in preserving fluids for microscopial purposes.

A *summary* of the above notes on the operated individuals may perhaps be best formulated by making the following comparison with the three stages of normal behavior.

First stage: The operation was performed during the latter part, while the animals were still non-motile.

Second stage: During this period they behave exactly like normal specimens, both those having one vesicle removed and those that have been deprived of both vesicles. They respond to stimuli and learn to wiggle along in contact with the bottom of the dish in the normal manner.

Third stage: It is at the beginning of this period that they depart from the normal. It can be plainly seen from their conduct that something has happened to that controlling influence from above, which they require in order to leave the bottom and to swim and maintain their position in free water. In case but one ear vesicle is gone they swim in spirals, circles, or straight while rolling around their long axis. This, however, lasts only a few days and then it is gradually overcome. From then on they swim almost perfectly; there may be a slight tilting toward the operated side and on excitement a momentary loss of equilibrium, but this would only be seen on careful examination. It is a different matter where both labyrinths are absent; the animals in that case are completely and permanently incapacitated for swimming. There is no apparent sense of equilibrium and they never develop any. The animals were kept alive about two months, at the end of which time their movements were as irregular as at the beginning.

Transplantation of Ear Vesicle After Bilateral Removal

From the above experiments it became evident that a tadpole having but one labyrinth proceeds in its general growth and

develops swimming abilities about as well as the normal animal; but specimens deprived of both ear vesicles never learn to swim and never develop any sense of equilibrium. The next step was to see if it would be possible to remove both vesicles and at the same time transplant one of them into a new position, having in mind the successful results obtained by Lewis ('04) in transplantation of the optic cup.

After that operation if the tadpole succeeded in developing equilibrium and the power of swimming then it would prove that a transplanted ear vesicle could establish new connections with the central nervous system and develop its normal functions; the ship would simply be sailing with its compass set up in a different place.
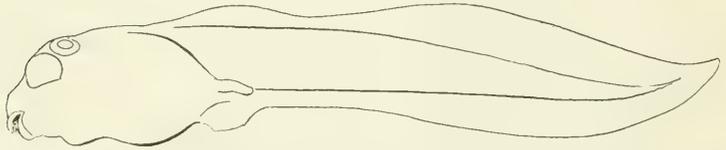
Fig. 6. Tadpole showing elevation in front of eye caused by the transplanted left ear vesicle, the right ear vesicle having been entirely removed. Drawing made three months after operation. Enlarged 4½ diameters.

The operation was one that could be performed without difficulty. A tadpole about 3 mm. long is selected and the ear vesicle taken out on one side in the manner described above. The specimen is then turned over and the opposite ear vesicle is uncovered and loosened from the epidermis. Before actually removing it a straight incision is made with scissors or needles in front of the eye and a pocket is created by gently spreading the subjacent mesoderm apart until the brain is exposed. The loosened ear vesicle is then lifted from its natural place and slipped into this pocket. If the incision is carefully made the edges of the wound close at once and on the following day there is no trace of the operation left. Nine operations of this kind were made and seven of the tadpoles successfully reared. While they were growing it could be seen from a surface view that the transplanted vesicle was developing and causing a corresponding elevation in

front of the eye.   A sketch of one of these at the end of the third month is shown in Fig. 6.

Their behavior during the first week following the operation was identical with that of specimens deprived of both vesicles as was to be expected.   Toward the fifth and sixth days they could make progress while touching the side or bottom of the dish, but any attempt at swimming in free water resulted only in irregular flexions of the body and somersaults.   It was hoped that the transplanted vesicle might then begin to function and make it possible for them to perceive their position while in free water, but this did not occur.   They continued to behave in all respects like tadpoles having no labyrinth and never gave evidence of possessing any trace of equilibrium.

At the end of the third, fourth and twelfth weeks specimens were killed in preserving fluid and prepared in serial sections. Examination of the sections showed that in six out of the seven specimens which were cut, the transplanted vesicle had developed to a greater or less extent, and it was these vesicles that formed the surface elevations that had been macroscopically visible in front of the tadpole's eyes.   Graphic reconstructions of them are represented in Figs. 7 to 12.   It will be seen that none of these constitute a perfect labyrinth, but on closer study it is found that they all possess certain features which are characteristic of it.   In the first place, that which was transplanted in the form of an open auditory cup developed after the operation into a closed vesicle containing endolymph.   This did not then remain a simple vesicle, but exhibited the tendency to subdivision into two or more compartments, the utriculus and sacculus, as seen in Figs. 7, 8, 12.   In the walls of these compartments there are areas of specialized epithelium representing the maculæ acusticæ.   In Fig. 7 there opens out of the more dorsal compartment a distinct endolymphatic appendage. A typical semicircular canal is not present in any of them; but what may be called a canal tendency is seen in Fig. 8, where there is a tube uniting the two principal compartments.   The small blind pouches leading off the main vestibule, three of which are present in Fig. 10, doubtless represent abortive canals.   In transverse section they are perfectly round and look like typical

canals. It may be recalled that Rüdinger ('88) described the semicircular canals as developing in the form of blind tubes sprouting out from the general vesicle. It is quite possible that he was dealing with an abnormal embryo and had the same form of canals that we see in Fig. 10.

The ear vesicles are more or less completely enveloped in connective tissue membranes and they are partly incorporated in masses of cartilage, some of which belongs to the normal cartilaginous cranium and some of it is the regular cartilaginous capsule of the labyrinth, the two fusing together in some places.

In four cases (Figs. 7, 8, 9 and 10) a group of ganglion cells and nerve fibers are attached to the median side of the vesicle near its caudal end and extend toward the central nervous system. In one instance (Fig. 7) the nervous connection between vesicle and brain at the junction of olfactory lobe and fore-brain, is complete, though it is only a few fibers that actually enter the brain. As the acoustic ganglion at the time of the operation is attached to the auditory cup some of its ganglion cells are undoubtedly carried along with it, and it is probable that it is these cells that furnished the nerve connections just described. At the time the transplanted ear cup was slipped into its pocket the adherent ganglion cells must have been lodged in various positions as regards the ear cup and the fact that they all come finally to lie on the median side of the vesicle and lead toward the brain must be explained by some theory of an attraction existing between brain and nerve.

When we have to deal with a transplanted labyrinth that has reached a development equal to those that function in young tadpoles, and has established communication with the central nervous system, we might expect that it would show some sign of physiological activity. The failure of it to do so is perhaps best accounted for by the fact that the point of entrance into the brain is so far away from the hind-brain centers and the spinal cord that connections with these are not established. If the experiments were varied and the vesicle transplanted to some point in the neighborhood of the occipital nerves this difficulty would be obviated.
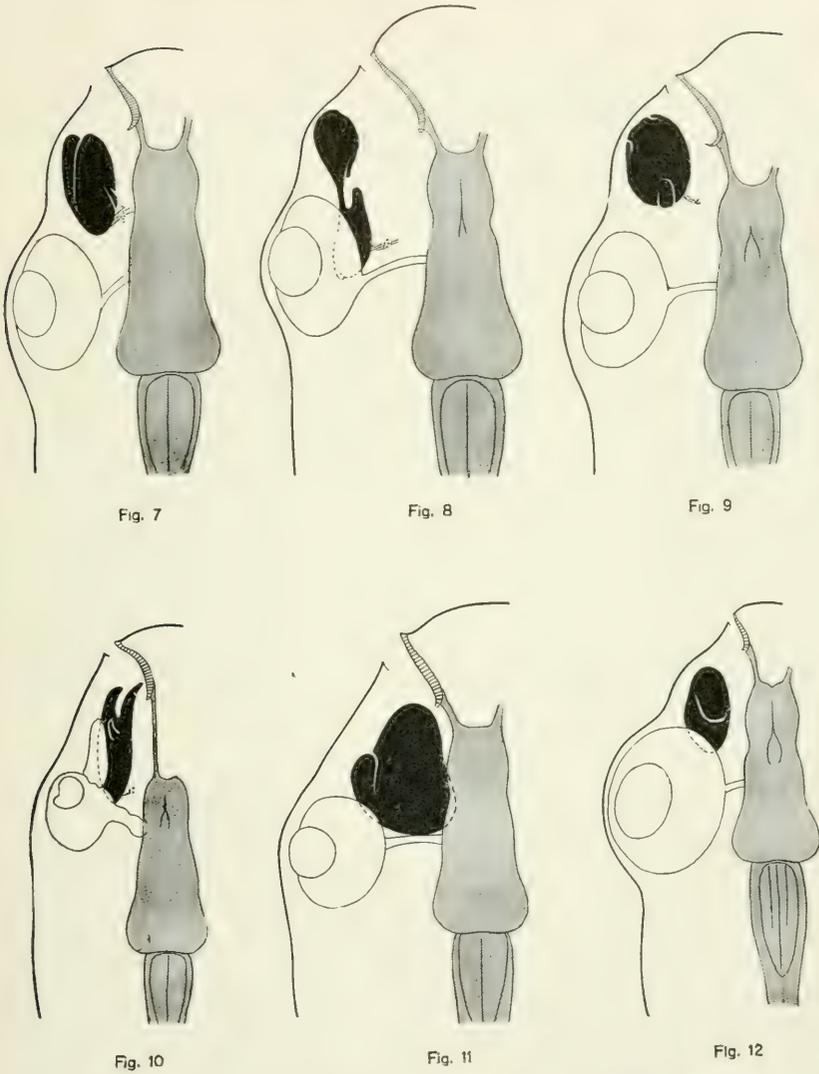
Figs. 7 to 12. Graphic reconstructions showing the form and relations developed by transplanted ear vesicles, one to three months after the operation. In all six cases the right ear vesicle was removed and the left vesicle transplanted into a subdermal pocket between eye and nostril. In Figs. 7, 8, 9 and 10 the acoustic nerve and ganglion extended from ear vesicle toward brain; in Fig. 7 the connection was complete, the fibers entering at junction of fore-brain and olfactory lobe. Central nervous system, shaded; ear vesicle, solid black.

Conclusions

In the tadpole the ear vesicles are essential for the development of the power of equilibration, but the study of normal specimens shows that well developed equilibration may be present before the completion of the semicircular canals; the latter as such are therefore not essential.

When both vesicles are removed no other organ compensates for their loss and the animal is completely and permanently helpless as regards the maintenance of equilibrium. When only one ear vesicle is taken out the remaining vesicle is capable of performing the work of both so perfectly, that the casual observer would mistake them for normal individuals.

Transplantation of the ear vesicle shows that the group of cells forming the auditory cup or primitive ear vesicle is specialized to that degree that although removed from their natural relations and placed in a new environment they still continue to differentiate themselves into a structure approximating the normal labyrinth. A nerve and ganglion develops, and complete nervous connection may be established between the transplanted vesicle and the brain at an abnormal place. Where the latter occurred it did not give evidence of any functional ability.

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THE RELATION BETWEEN FUNCTIONAL REGU-
LATION AND FORM-REGULATION

BY

C. M. CHILD

The phenomena of regulation in the organic world have received much attention in recent years and it has become more and more evident that a consideration of these phenomena involves some of the most fundamental problems of biology. It is perhaps not going too far to say that the solution of the problems of regulation will constitute a solution of most of the other problems which underlie physiological biology. Moreover, we have, in the possibility of analyzing and modifying regulatory processes by experimental conditions, a means of attacking the problems involved, which is more exact and has already proven more fruitful than other efforts directed toward the same end.

Inorganic life structure and form are to the observer perhaps the most salient features and so constitute the most available criterion for distinction and recognition not only of the component parts of the organism but of different organisms. Any method of procedure which permits the control, analysis, and modification of the processes which give rise to structure and form is, therefore, of the greatest importance, since it affords an insight into some of the phenomena most characteristic of and peculiar to organic life. The experimental method as applied in the field of form-regulation is of this character, and it has already demonstrated that many of the formative processes are dependent upon conditions which can be altered experimentally. Thus we are able in many cases to modify and alter the form and structure very widely. We are at present only on the threshold of this field of investigation but the outlook for the future is most promising. As our methods improve and as we come to comprehend more clearly the character of the phenomena with which we are dealing the

field within which these methods are applicable will extend and the value of the results obtained will increase.

Certain workers in the field of form-regulation have, it is true, reached somewhat disappointing conclusions. Some maintain that the data are as yet insufficient to permit any interpretation, others that the phenomena of regulation belong to a totally different category from those of the inorganic world and that, therefore, we cannot hope to interpret them in terms of physics and chemistry. The reason for these conclusions is, the writer believes, to be found in the fact that these authorities assume the formative processes to be a special series or complex of processes in the organism differing in nature from other so-called functional processes. The organism is regarded as possessing two groups or complexes of activities, the one giving rise to structure, the other concerned with the dynamic activities in the structure. Any such distinction seems to the writer entirely artificial and without basis in fact. Numerous examples of the dependence of structure for its existence upon dynamic or functional conditions are before us, and the only reason why the data are not still more abundant is that those already obtained have failed to attract due attention.

It becomes increasingly evident that the organism is primarily a dynamic or functional complex and the structure and form are merely visible expressions of the dynamic conditions. The writer has attempted in various papers during the last three years ('02–'06a) to develop this idea and apply it to specific cases of form-regulation but it seems worth while to present in more general form some of the conclusions reached, together with some of the facts on which they are based, and to show how the ideas involved may be applied to other cases.

The process of form-regulation is commonly regarded as consisting essentially in the replacement of a lost part—hence the term regeneration is used by some authors as synonymous with form-regulation. But form-regulation includes not only the replacement of lost parts: it may also involve hypertrophy or atrophy of parts remaining, the substitution of one part for another, the development of a part widely different from that lost, partial replace-

ment or no replacement at all. The recognition of the fact that these various phenomena, some of which are widely different from regeneration in the original sense are fundamentally similar justifies the use of a term which has not the disadvantage of possessing another and different meaning. Moreover, the term regulation serves or should serve to call attention to the fact that there is something similar in these processes to what has long been known as regulation in functional processes.

In the following paragraphs some of the writer's conclusions are presented, perhaps often in somewhat positive fashion, but the positive form of statement has its value in the presentation of hypotheses.

The Initial Factor in Replacement of a Lost Part

Driesch ('01) maintains that the initial factor in regulation is a disturbance in function or in constitution. While the writer cannot agree with Driesch in regard to the sharp distinction which he makes between function and constitution, it is sufficiently obvious that the initial factor in the process of replacement consists in some change in that part of the dynamic system which remains.

Have we any data which afford a clue as to the character of this change and the regions involved? In this connection certain experiments on flatworms may be mentioned first. In Stenostoma (Child, '02) or Cestoplana (Child ,'05b) after removal of the posterior end of the body at any levels except immediately posterior to the cephalic ganglia the posterior portion of the remaining part is used by the animal in much the same manner as the posterior portion of the part removed. The functional activities of this region which are involved in locomotion are readily observable since it forms the chief region of attachment and is employed almost constantly during progression. These forms possess means of adhesion along practically the whole length of the ventral surface but under normal conditions the posterior end is most used and the organs of adhesion are most highly developed there. This functional substitution is often very imperfect at first, but rapidly becomes more perfect and after a few hours

the posterior end of the piece is employed in much the same manner as the original posterior end, though adhesion is apparently not as firm.

In short, the animal has altered its behavior and in response to the new conditions has "learned" to use this part in place of the part removed. This altered function involves not merely the portion immediately adjoining the cut surface, but other parts for a greater or less distance anterior to this (Child, '05b). In other words, a portion of the piece becomes functionally posterior even though its original position may have been far from the posterior end. If we follow the process of form-regulation which succeeds this change in function we find that this part becomes in the course of time by a process of redifferentiation structurally as well as functionally a posterior end. In Stenostoma the process of formation of the new posterior end is very rapid, and if we prevent the piece from attaching itself in the characteristic manner form-regulation is delayed (Child, '03a). In this latter case then we have demonstrative evidence that the functional conditions connected with the use of the part in a certain manner are important factors in form-regulation. Moreover, facts which cannot be cited here lead us to the same conclusion in regard to Cestoplana.

In some of the flatworms, *e. g.*, Planaria maculata (Morgan, '98, etc., and others) a new head region can be formed at any level of the body, in others, like Dendrocœlum, only in the more anterior regions (Lillie, '01), and in still others, Leptoplana (Child, '04c), Cestoplana (Child, '05b), etc., only from levels immediately posterior to, through, or anterior to the cephalic ganglia. Lillie was first to note that the pieces of Dendrocœlum capable of producing a new head react much more like the normal animal than those incapable of such regulation. The writer has observed the same difference in reaction in Leptoplana, Cestoplana, and many other Turbellaria. Undoubtedly in these cases the character of the reactions is dependent in large measure on the physiological character of those portions of the nervous system which are present. In all of these pieces where formation of a new head is possible the anterior end of the piece behaves in some degree, often very slightly it is true, like a head. We find, more-

over, that this "head-likeness" increases as time goes on and long before the new head is fully formed the behavior of the growing parts is very distinctly head-like.   In this case the piece is "learning" to use the region as a head and the morphological development of the head follows.

In these cases as well as in many others the behavior of the pieces and the use of the parts affords us valuable evidence as to what is really taking place.   It must not be supposed that the movements themselves are the only or most important factors involved.   They may and undoubtedly do play a part in many cases where particular regions are subjected to particular mechanical or other conditions in consequence of characteristic movements (Child, '02, '03a, '04a, '04b, '04c, '05a, '05b), but their chief value is that they serve as an index to internal conditions. and changes.

These cases are perhaps sufficient to illustrate the point of view and to afford a certain basis in fact for it.   Other facts which bear upon the same point have been cited and discussed in the papers above referred to.   According to this point of view the first step in the process of form-regulation is functional regulation, an alteration in internal dynamic conditions in consequence of the disturbance of what we may call the physiological equilibrium of the system.   The process of functional regulation is not, however, fundamentally a process of "trial and error" (see pp. 578, 579), for its nature is in large measure predetermined by the dominant reactive capacities of the system.

But can we bring such cases as the regeneration of an arm in the starfish, a leg in the crayfish, or the amphibian, into the same category?   Is there any functional regulation in the direction of substitution of a part remaining for a part removed.

In such cases as these the first visible change is the closure of the wound either by a mass of coagulated blood, leucocytes, etc., or by cellular material and in all cases sooner or later by a mass of tissue composed of cells capable of division and growth.   This process of wound-closure, while regulatory in nature in that it is the result of reactions in response to new conditions is not directly connected with the replacement of the lost part, but is primarily

the result of purely local conditions. As is well-known, it occurs not only where replacement takes place later but also in those cases where there is no approach toward replacement. It is evident then that *the closure of the wound is not, strictly speaking, the first step in replacement of the lost part, although it may be the first morphological step in regulation* This distinction is important, for, as was pointed out above, regulation and even form-regulation involves much more than the replacement of lost parts.

But the small mass of growing, physiologically plastic tissue which closes the wound may stand, as soon as it forms, or strictly speaking as soon as it begins to form, in a relation to the whole system or organism more or less similar to that in which the part removed or some portion of it originally stood. This region must be subjected to many conditions—internal and sometimes external—similar in greater or less degree to those to which the part removed or some portion of it was subjected. The nerves which formerly led to the part or some of them now lead to this region and the regeneration of the nerves begins very early. It stands in the same or somewhat similar relations to other parts of the body as the part removed and must, as it forms, use a certain amount of energy which is derived from other regions in much the same manner as the part removed derived its energy. In short, this region may by virtue of its relations to the whole become the physiological representative in greater or less degree of the part removed. In case this physiological substitution takes place up to a certain degree further regulation occurs and the region begins to develop into the part removed. In case the substitution does not occur or is insufficient in degree to bring about further growth no replacement of the lost part occurs. As growth proceeds the dynamic conditions are more and more modified so that the substitution becomes more and more complete until the missing part is fully formed.

In many cases the growth of the regenerating part may proceed at least for a time without actual exercise or use of the part, though complete differentiation apparently does not occur without some degree of use. The regenerating arthropod appendage

is coiled beneath the closed end of the stump until the first moult following the operation. It is clear, therefore, that the conditions which determine the earlier stages of the growth and differentiation of the part are not identical with those to which the part is subjected later though they are just as truly functional. The growth of the new leg is not the result of the attempt to use the leg which is missing. The growing tissue begins to develop into a leg because its relations to the other parts of the system are in some degree similar to those of the leg removed. As it grows, the conditions approach more and more nearly those to which the normal leg is subjected, *i. e.*, there is a gradual return of the functional conditions to the normal.

This condition, functional substitution in the tissue closing the wound, constitutes the one extreme as regards the process of replacement and results in the formation of the lost part wholly by the outgrowth of new tissue. At the other extreme are the cases similar to those first mentioned where the functional substitution involves a considerable portion of the piece and the process of replacement is one of redifferentiation of this region without the outgrowth of new tissue except that closing the wound. Between these extremes are found the various intermediate forms of form-regulation.

The Common Methods of Form-Regulation

If the functional substitution for the part removed is accomplished within the part remaining, the region involved undergoes a process of "redifferentiation" (Child, '06a) and the conditions determining the growth of new tissue from the cut surface may be largely or wholly absent except so far as closure of the wound is concerned. This is the case in Stenostoma (Child, '02) and in posterior regulation in Cestoplana (Child '05b). If, on the other hand, the substitution is confined to the region immediately adjoining the cut surface or to the tissue closing the wound form-regulation is almost wholly or wholly a process of regeneration. The substitution in the old part may, however, be incomplete and the distal portion of the part removed may be formed by the

growth of new tissue, *i. e.*, regeneration, while its basal portion is formed by redifferentiation as in Planaria maculata (Morgan, '98, etc.; Child, '06b). Again the substitution in the old part may be more complete at one level than at another and the relative amounts of regeneration and redifferentiation will vary at different levels. This is to some extent the case in Planaria maculata, for example, where the regenerated anterior region is shorter and the redifferentiated region is longer in pieces from levels near the head, while the reverse is true in pieces from the middle regions (Child, '06b). The difference is still more marked in Polychærus where the writer has found[1] that the new posterior end is formed almost wholly by regeneration in pieces from levels near the head and almost wholly by redifferentiation in pieces from levels near the posterior end, and the relative amounts of redifferentiation and regeneration show all intermediate conditions in pieces between these two extremes. And finally in some cases form-regulation at the posterior end may be largely or wholly a process of redifferentiation and at the anterior end a process of regeneration as is the case in Cestoplana (Child, '05b). In all these cases of "mixed" form-regulation the redifferentiation occurs in the region which is physiologically similar to the part removed to such a degree that substitution occurs readily and with relative completeness, while regeneration occurs where the substitution is confined to the tissue closing the wound or to regions immediately adjoining the cut surface.

In short, the greater the physiological similarity between the old part or a given region of it and the new, the greater the amount of redifferentiation and the less the amount of regeneration. The reverse is also true up to a certain point. When the part remaining is so widely different from the part removed that no substitution is possible in any region not even regeneration occurs except in closure of the wound, and the missing part is not replaced. All intermediate stages between complete regeneration and mere wound-closure may occur in a single individual at different levels. This is well illustrated in Leptoplana (Child, '04c) and in Cesto-

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[1] Not yet published.

plana (Child, '05b), where the regeneration of the head is complete from levels anterior to or through the cephalic ganglia and in Cestoplana also from levels immediately posterior to the ganglia, and becomes increasingly incomplete with increasing distance from the ganglia, until in pieces from the posterior regions scarcely more than wound-closure occurs. The absence of regeneration beyond wound-closure in many higher forms is doubtless due largely to the fact that the physiological specification of the tissues is so great that no appreciable substitution occurs in any region after removal of a part.

But form-regulation may occur, nevertheless, even in such cases and may be represented by hypertrophy of other parts. A good illustration of this is the hypertrophy of one kidney following the removal of the other.

A process of destruction often occurs during form-regulation. This is commonly the case in redifferentiation where the structures formed and maintained by a given complex of conditions cannot persist under the altered conditions and so degenerate or atrophy. In a piece of Cestoplana from the prepharyngeal region, for example, the new pharynx appears a considerable distance from the posterior end of the piece and all the intestinal branches posterior to the new pharynx disintegrate and are replaced by others formed anew. It is probable that the movement of intestinal contents during contraction and extension plays an important part in this rather remarkable change. The data in this case are as yet unpublished.

The Rate of Form-Regulation and the Limit of Size

The development of a part reproduced by regulation is often greatly accelerated as compared with normal ontogenetic development. This rapid development can be due to nothing but a disproportion between size and intensity of physiological conditions to which the part is subjected. It is a familiar fact that increase in intensity of functional conditions brings about increase in size or hypertrophy of the part involved and decrease in intensity a decrease in size or atrophy. The new part in regeneration and often also in redifferentiation is at first much "too small," *i. e.*, the

intensity of the physiological conditions to which it is subjected corresponds to a region of much larger size and hence increase in size takes place very rapidly. During growth the relative intensity of the physiological conditions decreases and so continually approaches that existing in the original part before removal. Hence unless other factors prevent, the part will grow with decreasing rapidity until it attains approximately the size of the part removed.

In the flatworms where form-regulation will occur in starving pieces we find that the increase in size often ceases even before the new part has attained the proper proportions with respect to the old. In these cases the new part grows at the expense of the old and may undergo absolute as well as relative increase in size while the old part is undergoing absolute decrease. In such cases an equilibrium between old and new parts different from that existing in the normal animal is attained. As the new part grows the conditions for further growth decrease in intensity and as the old part is reduced its demand for nutritive material becomes relatively more intense and a larger proportion of the material available is used up in its own activities. Therefore, growth in the new part must cease before it reaches normal proportions, even with respect to the reduced size of the old part.

The Relation between Rate of Regulation and the Degree of Injury

Comparison of two series of pieces of Leptoplana, the one set consisting of the anterior one-fourth or one-fifth of the body, the other of the anterior four-fifths or three-fourths, both kept without food, shows that the pieces of the first series regenerate from their posterior ends very much more rapidly and a much larger amount of new tissue than the pieces of the second series (Child, '04b). Yet the pieces showing the greater and more rapid regeneration are only one-fourth as large as the others.

If we compare the behavior of the two sets of pieces during regulation, especially during the earlier stages after the regeneration has begun, we find that in the first series the new part is used to a much greater extent than in the second. Not only is this the case but the activity in the old parts also is much greater in the first series than

in the second or in the normal animal. The movements are more violent and the irritability appears to be greater. In short, to all appearances, marked modification in the dynamic conditions has resulted from the removal of the posterior four-fifths and a scarcely appreciable modification from the removal of the one-fifth.

When the new tissue has appeared it becomes in the first series the functional representative of the four-fifths removed, and in the second only of the one-fifth. The early stages of its growth are of approximately the same size in both cases but in the first series the intensity of dynamic conditions must be much greater in this region, since its role in the complex is much more extensive and important than in the second. It must demand and receive a much greater proportion of the energy of the complex. Consequently growth or hypertrophy is much more rapid and greater in amount. Moreover, if it is true that removal of the four-fifths has brought about an increase in the intensity of dynamic processes in the old part this will doubtless also tend to increase the rapidity of growth in the new part, especially during earlier stages, since it is involved in the various reactions. As the new part develops, the intensity of activity appears to decrease.

Here as in other similar cases it is not simply the degree of movements or exercise of the part that determines the rapidity and amount of growth, though this of course may constitute one factor. It is rather the conditions underlying the motor activity, the nerve stimuli, the metabolic conditions, etc., of which the motor activity is an index (Child, '04b, '05b).

Zeleny has recently obtained somewhat similar results in several species of decapod crustacea and in a species of ophiurid (Zeleny, '05a, '05b). In all of these cases the rate of regeneration increases with the degree of injury up to a certain point. These cases differ, however, from the case of Leptoplana in that here regeneration occurs from two or more different regions while there only one region is involved. Strictly speaking the case of Leptoplana is comparable to removal of larger or smaller parts of a single arm in the starfish or of a single leg in the crustacea. But if we remove three or four arms from the starfish or several legs from the crayfish each one of the new primordia represents, when it appears, no larger

portion of the complex than does the primordium of a single arm or leg cut off at the same level, yet it regenerates more rapidly.

It was noted above that the short piece of Leptoplana showed much more intense activity than the long piece. It is perhaps not too much to say that this increased activity is a response or reaction to the absence of the four-fifths. On receiving a stimulus the piece reacts but reaction in the normal manner is impossible and the result is "irradiation" of the stimulus and the appearance of other more or less violent reactions. This fact of "irradiation" has long been recognized in nerve physiology and Jennings' recent experiments on the modifiability of behavior show that it is an important factor in behavior.

Zeleny has been unable to observe any characteristic difference in activity connected with the degree of injury. But the reaction to the injury need not necessarily appear as actual motor activity; it may take the form of increased rapidity of metabolism or other forms. In those cases where increased motor activity is present it serves as an index to internal conditions but its absence does not necessarily indicate the absence of increased dynamic activity of other kinds.

There can be no doubt that a normal reaction normally carried out has a certain effect on the individual and that an attempt and failure to carry out the reaction has another and very different effect, viz: in the direction of altered character and increased intensity of reaction. The old experiments upon the reflexes of the decapitated frog are sufficient demonstration of the fact. The larger the number of arms or legs removed in the cases cited above, the more impossible is the accomplishment of the normal reactions and consequently the more intense the effect on the animal as regards other reactions. As the new tissue appears it becomes a more or less complete functional substitute for the parts removed—very incomplete at first no doubt. In the conditions of intensified activity resulting from the operation the functional conditions to which the regenerating structures are subjected must increase in intensity with the degree of injury. This increase will be all the greater because their presence is the first step in the approach to a normal method of reaction to which the system is, so

to speak, "seeking." As regeneration goes on the functional substitution becomes more complete, and reactions take place more nearly in the normal manner, functional conditions become relatively less intense, and the rapidity of regeneration decreases.

Compensatory Hypertrophy

In a number of cases among the Crustacea (Przibram, '01, '02, '05; Zeleny, '05a), where an asymmetery of the chelæ exists, removal of the more highly specialized chela is followed by a more or less complete transformation of the other into the more highly specialized type, and the regenerating chela develops into the less specialized form. Thus a reversal of asymmetry results. If the less specialized chela alone is removed no reversal occurs. Zeleny ('05a) has obtained somewhat similar results with certain species of serpulids where two opercula, one large and functional, the other small and rudimentary, are present. Removal of the functional operculum results in a reversal of the asymmetry while no reversal occurs after removal of the rudimentary operculum. When the whole head region, including both opercula is removed both regenerate in the form of the functional structure. Wilson ('03) has found in Alpheus grounds for believing that when the nerve to the chela is cut the reversal does not occur, but further experiments along this line are needed.

A satisfactory interpretation of these cases on a functional basis appears possible. When the specialized chela or operculum is removed the disturbance of the system thus produced results in changes in reactions and it seems probable that the easiest and most natural change will be such that the less specialized or rudimentary organs will receive stimuli and be subjected to conditions which originally affected the specialized or functional structures. In fact, it is difficult to see how any other interpretation can be made to serve. Zeleny ('05a) postulates for the serpulids the existence of a "retardation stimulus" from the functional operculum which, so long as this is present, inhibits the development of the other. The nature of such a retardation stimulus is highly problematical. It seems to the writer much more probable that the failure of the rudimentary or less specialized part to develop

beyond a certain point under normal conditions is due rather
to the absence of adequate stimuli than to a positive inhibitory
stimulus.   So long as the other more highly specialized part
is present the animal reacts in a characteristic manner which
involves the less specialized part only to a certain degree; and the
structure of that part expresses the character of the conditions to
which it has been subjected.   Removal of the less specialized part
does not alter the reactions to any such extent as removal of the
other, hence the part removed regenerates in the same form.
Removal of both places the new primordia on equal terms and both
may produce the more highly specialized or the functional structure.
Here, as elsewhere, the functional conditions involved are not
primarily those connected with use or exercise of the part but the
sum total of its dynamic relations to the whole system.

Polarity and Axial Heteromorphosis

Physiological polarity in the developed organism may be defined
provisionally as a habit of reaction resulting from past or present
relations.   This polarity of the organism may be a consequence of
the polarity of the egg and this in turn a consequence of the rela-
tions of the egg-cell to the body of the parent or of other con-
ditions affecting the egg.   At any rate there seems to be no good
ground for believing that polarity is a permanent and funda-
mental property of the cell or of protoplasm, although certain
authorities have urged this view.

Polarity may be rendered visible by structural differentiation
along the axis or it may not, but in any case the structure is prima-
rily a result not a cause.   It may, however, become the determining
condition in that it determines the character of the reactions.

The original polarity is commonly maintained in form-regula-
tion simply because each of the parts reacts most readily in a
manner approaching that in which it has reacted in the past.   But
this habit of reaction can be altered in certain cases.   In Tubu-
laria, for example, pieces very commonly produce a hydranth at
both ends, the oral hydranth, first the aboral later.   The delay in
the appearance of the aboral hydranth represents the time neces-
sary to alter the character of the reaction or in other words to

change the habit.   Moreover, when the oral hydranth is pre-
vented from forming, the aboral hydranth appears more quickly
than when the oral hydranth is permitted to form.   Here we have
an example of the case discussed above in which failure to carry
out a "normal" reaction or the reaction most readily performed
results in increased intensity of other reactions.   That the habit
is really changed is shown by the fact that a second aboral hy-
dranth develops more rapidly than the first.   Undoubtedly struc-
tural changes in the various regions result from the changes in
reaction but these are not visible in this form except in the struc-
tures produced at the cut surfaces.

Axial heteromorphosis can be brought about in various ways in
many of the lower forms.   The isolation of very short pieces is per-
haps the simplest method.   In these cases the piece is not large
enough to represent the whole complex and the reaction in which the
piece has been most intimately involved in the past becomes the
chief or only possible reaction and similar structures are produced
at both ends.   We find, therefore, that pieces of this kind often
produce at both ends the structures characteristic of the regions
with which they have been most closely associated in the past.
Sometimes other special factors modify this result, *e.g.*, preparation
for fission in Planaria maculata (Child, '06b), or as in Tubularia
and some other hydroids the dominance of a particular reaction-
complex throughout the individual.   This latter case requires a
word of comment: pieces of Tubularia from any part of the stem
or from the stolons produce hydranths much more frequently than
they do stolons when the ends are not in contact with a surface or
solid.   Polarity in this form is commonly indicated by the relative
size and rapidity of formation of the hydranths from different ends
and at different levels of the piece rather than by the formation of
typically different structures, though occasionally stolon-forma-
tion occurs at the aboral end even without contact.   It is possible,
however, as the writer has found, to increase experimentally and
without contact the intensity of the stolon-forming reaction to such
an extent that almost every piece will give rise to stolons at the
aboral end.   Many of these stolons will after a considerable time
produce hydranths at their tips.   The writer is inclined to believe

that the hydranth-forming reactions may be interpreted as primarily a reaction to lack of nutriment. This reaction occurs most readily of course in those parts which have been most closely associated with it in the past.

In a number of forms short pieces are not necessary for the occurrence of heteromorphosis. These include Tubularia and various other hydroids, some other cœlenterates, the earthworm and some other forms. In some of these as in Tubularia one form of reaction is dominant throughout, and in others, like the earthworm, this dominance is regional. In the earthworm, for example, pieces from the posterior region commonly produce tails at both ends (Morgan, '02). The head-forming reactions can occur only in the more anterior regions. The very close relation between head-formation and the nervous system in this as well as in many other cases indicates that important factors in polarity are situated in the nervous system.

Heteromorphosis may also occur in various forms when a short piece is grafted in reverse position on a much longer piece. In such cases the short piece produces at its free end the structures characteristic of the end of the longer piece with which it is united. These are undoubtedly simply cases of physiological dominance of the larger component. The greater intensity of its reactions alters the conditions in the small piece so that this becomes functionally a part of the other. This is doubtless accomplished by the passage of stimuli from the larger into the smaller piece and it may be by the actual growth of nerves in some cases.

The regulation of a piece into a complete individual with typical axial differentiation and maintenance of the original polarity depends upon the relative difference in reactive capacity in the different regions. In all such cases the piece must possess the power to react in some degree like the whole. Its isolation brings into play potentialities not evident while normal relations were maintained, and the regions best fitted by past associations, for particular reactions perform them most readily and thus the original polarity persists.

The Relation Between the Nervous System and Form-Regulation

This relation which is very general, though of course not universal, affords the strongest evidence in favor of the view that form-regulation is a functional process. No good ground has been discovered for assuming the existence of a peculiar trophic or formative influence originating in the nervous system. Goldstein, in a recent paper ('04), gives a most interesting discussion of the subject and concludes that the relation between the nervous system and growth and differentiation is essentially functional in character.

It must be remembered, however, that the nervous system adds nothing fundamentally new to the phenomena of life. The transference of stimuli in definite directions and along more or less definite paths occurs where no visible nervous system exists. Indeed the development of the nervous system itself is probably in greater or less degree the result of such conditions. The problem of form-regulation is, therefore, not necessarily different in its fundamental features in those cases where no nervous system exists or where regulation occurs in the absence of visible nerves, as compared with those where visible nervous structures are present.

Conclusion

Regulation in general may be defined as a return to physiological equilibrium after such equilibrium has been altered by external conditions (Child, '06a). Holmes ('04) has recently given a very similar definition which he developed from the idea of symbiotic relations or "social pressure." As the writer has attempted to show in a previous paper ('06a) the idea of symbiotic relations does not afford a basis for the replacement in form-regulation of a part similar to that removed since removal of an element or a group of elements in the symbiotic complex must, according to Holmes' assumption, alter the condition of the whole in such manner that the "social pressure" upon the part which becomes the substitute for the part removed will be different from that originally exerted upon this part, and something else rather than a duplicate of the part removed must be formed. But a characteristic feature of form-regulation is a return or approximation to the original con-

dition and any interpretation must recognize this fact. In the
paper above referred to the writer has called attention to the fact
that replacement of the missing part cannot occur unless that
portion of the system remaining is capable of reacting in a manner
essentially similar in some degree to the part removed. But mere
capacity to react in this manner is not sufficient in most cases.
This method of reaction must be the predominant or characteristic
method, for if it is not, then a structure different from the part
removed or nothing at all is replaced. This idea affords a basis
for understanding why the ability to replace lost parts is limited in
many cases. When by removal of a part the system is altered to
such an extent that the previous method of reaction becomes
impossible or is no longer the dominant method, replacement can-
not occur. In such cases, however, provided the system is not
altered to such an extent that continued existence is impossible,
some other form of regulation may occur and a new equilibrium
may be established differing more or less widely from the old. Thus
when we remove the ganglia from Leptoplana and various other
polyclads the processes characteristic of the head-region can no
longer occur and the head is not replaced. In Planaria, on the other
hand, removal of the ganglia does not alter the complex to such an
extent as in Leptoplana since the nervous system is not as highly
cephalized here and the headless piece or certain parts of it still
retain the ability to react like a head in sufficient degree to initiate
the process of head-formation. When this process is once started
the ability to perform the characteristic reaction increases and
the return to the original condition is gradually accomplished.
The removal of the part brings into play various potentialities of
reactions which exist in the remaining piece by virtue of its past
relations to the whole and those processes which are predominant
determine the character of form-regulation. But objection may be
raised that a region in the middle of the body of Planaria, for exam-
ple, has not been associated in the past with reactions or processes
characteristic of the head and tail regions. If we observe the living
animal, however, we find that the more intense motor reaction of
either end may visibly involve the middle region in greater or less
degree. They must also involve it in many ways not visible. It is

probably true that the middle region does not while still a part of the whole *initiate* such reactions, because whenever conditions which give rise to such reactions are present other parts of the body react much more readily than this. But when we sever its connection with other parts we find that it is visibly capable of initiating such reactions, though of course very imperfectly and much less promptly than the original head and tail regions. The ability to develop a new head and tail cannot arise from anything else than this ability. In this particular case—Planaria—this ability evidently depends in large measure upon the nervous system. This idea does not conflict in any way with that developed in the foregoing sections, viz: that a functional substitution of some degree must precede and initiate the process of replacement. It is evident that such functional substitution cannot occur unless the system retains the ability to react to some extent like the part removed. The substitution may alter the reaction of a larger or smaller part of the piece but the very fact of substitution depends on the occurrence in the system of reactions resembling in greater or less degree those of the part removed.

In connection with the views expressed above a recent paper by Jennings ('05), and also certain parts of his book ('06), are of interest. Jennings' recent work has demonstrated the high degree of modifiability in the behavior even of the lower organisms and here he attempts to apply the conclusions reached to other fields. His views on the individual adjustment or regulation of behavior are based on the following premises.

"1 Definite internal processes are occurring in organisms.

"2 Interference with these processes causes a change of behavior and varied movements, subjecting the organism to many different conditions.

"3 One of these conditions relieves the interference with the internal processes so that the changes in behavior cease and the relieving condition is thus retained."

The selection of the process or reaction which relieves the interference depends upon the fact of relief.

Jennings attempts to apply this "method of trial and error" in reaction to the phenomena of regulation in other fields, pointing

out the possibility that the selection of the apparently advantageous reaction is to be found in the fact that it relieves disturbing conditions.

It is perhaps sufficiently evident from what has been said above that Jennings' views and the writer's are somewhat similar in certain respects. The physiological equilibrium which forms the basis of Holmes' and the writer's definition of regulation corresponds to the condition postulated by Jennings in which the various physiological processes are occurring without interference: the alteration of equilibrium is brought about by some interference—in the cases under discussion in this paper usually by removal of a part. In consequence of this interference a more or less extensive rearrangement of dynamic processes occurs which becomes manifest in alteration of the localization and perhaps also of the character of the reactions: because of the occurrence and continuation of certain of these processes a return to equilibrium occurs; but the writer does not believe with Jennings that the continuation of particular processes is necessarily due to the fact that they "relieve the interference;" their relation to relief may, however, bring about their intensification.

There is one point in connection with form-regulation which Jennings has not discussed but which is of considerable importance in this connection and that is the frequency of return or approximation to the original condition. This is so characteristic of regulation that certain authors like Driesch ('01) have made it the basis of their definition. In the regulation of behavior and of chemical processes it cannot be supposed that only a single method of reaction exists whereby the interference can be removed or diminished. In many cases, indeed, this is visibly not the case and the organism may attain in consequence of the interference an equilibrium widely different from the original. It seems probable, for example, that the formation of a new head at the posterior end and a tail at the anterior end or of these structures on the right and left side respectively of the piece in Planaria would remove or diminish the interference and so constitute a return to equilibrium, but this does not usually occur. It is necessary to recognize the fact that the possibility of reaction is limited somewhat strictly by the past relations of the part or, in other words, by its reactive capacity. In short, the

reaction leading to replacement of the lost part is usually the reaction which the piece can perform most readily, *i.e.*, that for which it is best adapted.   The endeavor has been made to direct attention to this fact in the preceding paragraphs.   Hence the process of trial and error does not occur to any great extent in most cases and a very high degree of uniformity in the processes of form-regulation exists.   Moreover, a particular reaction-complex must continue fairly constant for a considerable period of time before it becomes manifest in such extensive structural development as appears in form-regulation.   If the characters of such reactions were not very strictly limited we should expect a much greater range of variation in the processes of form-regulation than occurs.   That variation in the results does occur has been pointed out by various authors. The writer has called attention to the fact that in Leptoplana (Child, '04c) some individuals are more capable than others of producing head-like structures in the absence of the ganglia, as well as to numerous other cases.   In a recent paper (Child, '06b) a rather remarkable case of this kind has been noted: in certain species of Planaria very small pieces from the middle region of the body may produce any one of five different results, viz: single tail-less anterior regions, single headless posterior regions, double anterior regions, double posterior regions, or normal animals. This region is apparently ''indifferent,'' *i.e.*, neither anterior nor posterior reactions are dominant in particular parts of the piece though both are possible in some degree, and the result depends apparently upon a "chance," *i.e.*, upon certain internal conditions not at present recognized.   These contribute to determine what reactions shall become dominant in each piece.

But the variation in processes of form-regulation in general is not sufficiently great to indicate that "trial and error" plays any important rôle.   The reactions which bring about form-regulation do not continue *because* they "relieve the interference" but because they are dominant in the part in consequence of its past relations to other parts.   In the regulation of behavior there is doubtless often a greater range of possibilities, but even here it appears probable that the character of the regulatory reaction is determined, in many cases at least, not by the fact that it "relieves the

interference" but by the character of the "apparatus" or system under the existing conditions.

All the evidence points us to the conclusion that the phenomena of form-regulation are not essentially different from other dynamic phenomena in organisms and that they must be interpreted on a dynamic basis. As the writer has maintained in various papers the organism is primarily a dynamic complex and visible structural features are primarily incidents or by-products of the dynamic processes. Formative processes, so-called, do not differ fundamentally from processes of behavior.

Objection to such interpretation may be made on the ground that it is not really an explanation but rather a step away from explanation in that it groups phenomena apparently relatively simple in the same category with the most complex phenomena of life. In answer to this it may be said that the first step in true interpretation is the recognition of the nature of the problem. With increasing knowledge of vital phenomena it is becoming more and more evident that we cannot select a single group of these phenomena and "explain" them without reference to other groups. The organism is not a mosaic of independent complexes acting in different ways. There is not a particular group of dynamic processes concerned in building the machine and another in keeping it going. We shall find similar laws governing all whatever may be our final conception as to the nature of these laws. Semon ('04) has recently proceeded from a somewhat similar idea, in his exceedingly interesting attempt to interpret the phenomena of heredity, habit and memory on a common basis.

Recognition of the fact that the fundamental problems are the same in fields before regarded as distinct or only remotely related is always an advance and when different observers in these different fields recognize this fact independently of each other, the significance of the conclusions is necessarily greater than it would be otherwise.

Hull Zoölogical Laboratory
University of Chicago
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A STUDY OF THE SPERMATOGENESIS OF COPTO-CYCLA AURICHALCEA AND COPTOCYCLA GUT-TATA, WITH ESPECIAL REFERENCE TO THE PROBLEM OF SEX-DETERMINATION

BY

W. N. NOWLIN

WITH TWO PLATES

Very little work on the germ cells of the Coleoptera had been done until last year, when the form Tenebrio molitor was investigated by Dr. N. M. Stevens. The results obtained were so suggestive from the standpoint of sex-determination that she is at present engaged in examining a number of species of this order of insects.

The following paper is a contribution to this series of investigations, and I take pleasure in acknowledging my indebtedness to Dr. Stevens, both for material used and for direction in the work.

I. MATERIAL AND METHODS

The beetles under discussion are Coptocycla aurichalcea and Coptocycla guttata, both collected from Convolvulus arvensis at Woods Hole, Mass., September 5, 1905.

The testes were fixed in Flemming's strong solution, Hermann's platino-aceto-osmic, and Gilson's mercuro-nitric, each of which gave excellent results.

Heidenhain's iron-hæmatoxylin method was used for most of the preparations. Other stains, employed principally for chemical tests, were thionin, Delafield's hæmatoxylin and orange G, and with the material fixed in Hermann's and Flemming's fluids, the safranin-gentian stain of Hermann. The iron-hæmatoxylin slides were often counterstained with orange G.

II. OBSERVATIONS

The testes of these beetles consist of many follicles radiating from a central duct, the vas deferens, into which are poured the spermatozoa. We should expect then to find ripe spermatozoa at the inner end of the follicles and spermatogonia at the outer, with the intervening stages between. This is true in most cases but it is by no means an inviolable rule. Cysts containing ripe spermatozoa may be found mingled with those containing first or second spermatocytes, and second spermatocytes may occur in the same cyst with the first. The classification then cannot be made by the location but purely from the character of the cell itself.

1. *Coptocycla aurichalcea*

The equatorial plate of a spermatogonium of Coptocycla auri-chalcea contains twenty-two chromosomes, most of which are V-shape, and radially arranged with the concave side outward (pl. I, Fig. 1). Near the center usually, though it may take any other position, is a very small chromosome, which is the small member of an "idiochromosome" group, and while we cannot here be sure of its mate we are safe in assuming it to be one of the five smaller chromosomes. An attempt was made to arrange the chromosomes in pairs according to form and size, but it soon became evident that they differed in different plates; the form, whether rod-shaped or V-shaped, depending largely on position with respect to the other chromosomes. As is unusual the behavior of the differential chromosomes in the spermatogonium is in every respect like that of the others: each divides longitudinally and passes to the poles, where condensed masses are formed.

The earliest spermatocyte shows a true condensation stage or bouquet stage [synizesis of McClung ('05)], in which the chromatin is in threads closely looped together at one side of the nuclear space (Fig. 2). The number of loops is here very evidently more than the reduced number and probably each loop corresponds to a spermatogonial chromosome. In a slightly later stage the loops straighten until many free ends are seen above the condensed mass (Figs. 3 and 5). Each of these strands appears to be a single

chromosome, and this idea is strengthened when in the next phase the distal ends are seen to bend toward one another in pairs and unite (Figs. 5 and 6). Fig. 4 is a cross-section of a nucleus at this stage. Thus synapsis takes place in the early part of the growth period soon after the last spermatogonial division. This agrees with Montgomery's ('03) description of the synapsis stage in Amphibia and furnishes more detailed evidence of the process.

Up to this period the idiochromosomes have been indistinguishable from the others, but a few very clear sections were obtained which showed at the base of the loops, close to the nuclear membrane, the larger member of the unequal pair (Fig. 5). This suggests the probability that the idiochromosomes retain their characteristic form during the greater part if not all of the synizesis and synapsis stages. All the chromatin has a strong affinity for stains during these periods, and the spireme is most distinct when first formed (Fig. 7), but almost immediately it becomes very faint (Fig. 8). The differential chromosomes still stain deeply, however. The spireme soon breaks up into separate rods, each showing a longitudinal split (Figs. 9,10), even at this time there seems to be a characteristic form, one chromosome assumes a U-shape, one a triangular form, and one that of a nearly closed ring. These show still more clearly in the later prophase (Fig. 12).

The split disappears (Fig. 11), the chromosomes condense, growing slightly thicker and shorter (Fig. 12), until we get such forms as appear in Fig. 13. The centrosomes are large and distinct in this material, and at this stage two may be seen lying close against the nuclear membrane. In Fig. 13 we see them nearly 180° apart, and as yet no spindle fibers have appeared. Throughout the spireme stage the idiochromosomes are dense spherical bodies. In many cases only the larger member is visible (Figs. 7, 8, 9). In Fig. 11 both are plainly seen. In the later stages while the ordinary chromosomes are undergoing great changes the differential chromosomes remain the same (Figs. 12, 13).

An equatorial plate of the first spermatocyte (Fig. 15) shows eleven chromosomes, and since the spindle fibers are very large, these may often be seen in cross-section attached to the periphery of the chromosomes (Fig. 15). When the material is faintly

stained one chromosome stands out prominently, due to its retention of the stain (Fig. 14). This is the larger member of the differential group, as may be seen in a lateral view of the spindle (Fig. 21). The small idiochromosome fails to show here, due probably to the angle at which the pair is lying; in late prophase (Figs. 17, 22) the pair is easily distinguished. While in numerous metaphases the small chromosome is difficult to discern, there are a few cases in which it is clearly seen (Fig. 18). This unequal pair exhibits no difference in behavior during mitosis, but like the others, and at the same time, with the exception of one which always divides early (Figs. 18, 21), it divides transversely, the large member passing to one pole and the small member to the other (Fig. 25).

We have in this division one of the clearest cases of reduction division possible. In the spermatogonium the chromosomes are V-shaped. During the condensation stage they elongate into loops, at first closely massed together, but later these straighten and unite in synapsis to form longer loops. This is followed by the formation of a continuous spireme (Figs. 7 and 8) which soon breaks up into segments, each split longitudinally (Figs. 9, 10). In the prophase of the first spermatocyte many of the chromosomes are thick rods, which upon examination are seen to be two V's joined end to end, giving in profile a typical E-shape (Fig. 16, *e*). The spindle fiber is attached at the vertex of each V of the bivalent chromosome and in anaphase this element separates at the original place of union, at right angles to the initial longitudinal split (Figs. 18, 20). The V-shaped chromosomes observed in the spermatogonium are thus restored and carried into the second spermatocyte. Exactly the same thing is true in the separation of the components of the cross-shaped chromosome (Figs. 16, *a—d*), and of the ring-shaped one (Fig. 16, *l—o*, and Figs. 22, 23, 24).

There is no rest stage between the two generations of spermatocytes, but almost immediately the massed chromatin in the late anaphase (Fig. 26) separates into chromosomes which begin to arrange themselves in the equatorial plate (Figs. 27, 28, 29). In Fig. 30 we see this in progress, the small chromosome being very distinct. In Figs. 27 and 28, we again see eleven chromosomes

of which one is the small idiochromosome. Having seen that in the first division the large idiochromosome goes to one pole and the small to the other, we should expect to find half the daughter cells containing the large and half the small chromosome of this pair. This is exactly the case. Fig. 29 shows a representative of the cells possessing the large member. This is especially clear in material very much destained, for here as in earlier stages this peculiar chromosome holds the color much longer than the others. In other respects the differential chromosomes are not different, but divide longitudinally with the ordinary chromosomes.

In early anaphase each V shows a distinct split which lies in the plane of the equatorial plate (Fig. 31). That this is the original longitudinal split seen in the early prophase (Figs. 9, 10) there can be no doubt. One chromosome which maintains its identity from the early prophase through the first spermatocyte as a cross is sufficient evidence. In the late prophase and even in metaphase this chromosome (Fig. 16, *a—c*) may be seen to be longitudinally split (Fig. 16, *d*). The line of separation comes at right angles to this split and the univalent chromosomes pass to the poles as V's, each possessing a longitudinal split, though it closes and cannot be seen again until the beginning of the following anaphase. The chromosomes are typically arranged with the vertices attached to the fibers and pointing inward. In division they are drawn apart, at first with the ends of the V's pointing poleward (Figs. 31, 32) but later the vertices turn toward the poles, though disorder often reigns until a comparatively late anaphase (Fig. 33).

In the spermatids there is a great amount of archoplasm, and in the late telophase of the second division it occupies the greater part of the cytoplasmic area (Fig. 36). At a slightly later stage the mass loses entirely its fibrillar structure and, condensed somewhat, lies as a gray sphere (iron hæmatoxylin) against the nucleus (Fig. 38). This is the so-called *nebenkern* of the spermatid. The entire cell now elongates, and with it the axial filament. The archoplasm has assumed the form of a large pennant attached to the nuclear membrane, and the axial fiber runs throughout its length and often beyond (Fig. 39).

A cross-section of the tail at this time shows that the archoplasmic sheath is folding about the axial fiber (Fig. 50, *a*). When the transformation is nearly complete the two cannot be distinguished from each other, the sheath lies close against the filament with a thin layer of cytoplasm outside (Fig. 50, *c*). Often in cross-section the sheath appears to be split (Fig. 50, *b*). Since longitudinal views of the tails reveal nothing of the kind we are left to conjecture that this appearance is due to oblique sections of the trough-shape sheath. The fact that many of these appear together does not alter the explanation, for the spermatozoa and late spermatids lie parallel in large numbers.

There is one peculiar but apparently typical stage in the development of the tail: while the head is yet round, though the chromatin is much condensed, the axial filament has assumed a vacuolated appearance (Fig. 40). This lasts until the head has begun to elongate, and is just over in Fig. 41. This is not to be confused with the rare occurrences of double filaments seen in Fig. 48. These are the products of giant cells, due to a failure of one of the divisions of the spermatocytes or the spermatogonia to complete itself. They consequently have two centrosomes and two axial filaments. Paulmier ('99) found similar conditions in Anasa. As in other insects the axial filament in the Coleoptera seems to arise in close relation with the centrosome.

After the vacuolated stage the tail narrows and lengthens much more and we see it in its final form in Fig. 44. All this time the head of the spermatid has been changing. The dense chromatin mass begins to lose its affinity for hæmatoxylin until a deep gray results. A structure is now revealed in the nucleus, not heretofore seen, a small densely staining nucleolus-like body (Fig. 36). The nucleus now widens its circumference and the chromatin condenses around the membrane in the form of a ragged border (Fig. 38). The nucleolus-like formation is not seen at this stage, but the supposition is that it is merely obscured by the dense patches of chromatin, for in the next stage it again appears and occupies a most characteristic position: the chromatin is arranged in a crescent shape and the nucleolus lies in a clear area between the arms of the crescent (Fig. 37). The chromatin becomes diffuse and min-

gles with the karyolymph until a uniform light gray results (iron hæmatoxylin). The nucleolus is unaffected, changing neither in size nor in staining reaction. It makes a change, however, in position about this time, moving from a point opposite the centrosome around the periphery of the nucleus until it often lies very near the centrosome (Fig. 49).

The head of the spermatid lengthens and becomes vacuolated (Fig. 41), but as the elongation increases the vacuoles disappear and the deeply staining nucleolus-like body is apparent, lying closely against the nuclear membrane (Fig. 45, *y*). Very soon this single body breaks up into two, three or four parts and later, it disappears (Figs. 46 and 47). The head condenses, lengthens, stains intensely (Fig. 42), and in its final form is spirally twisted, resembling the Bacterium spirillum (Figs. 43, 44).

It is impossible at the present time to say just what may be the function of this peculiar, deeply-staining spermatid element. It suggests the accessory of Orthoptera which can be seen in one-half the spermatids (McClung, '99, '00, '02a), and at first it seemed probable that it might be one of the idiochromosomes in the beetle as it, in one stage, is about the size of the larger member of the pair. Instead of being found in half the cells, however, it is without doubt in all, so that this explanation was relinquished. It is characteristic of the spermatids of all the beetles thus far studied and of some of the Orthoptera.

2. *Coptocycla guttata*

In external appearance Coptocycla guttata is very different from C. aurichalcea, but in their germ cells there are many points of resemblance. Guttata has the smaller number of chromosomes, eighteen in the spermatogonia instead of twenty-two, but the size and form are much the same in the two species (Figs. 1 and 51). The unequal pair is also present here.

The suggestion that synapsis takes place immediately after the condensation stage in C. aurichalcea is strongly confirmed by guttata. In the condensation or synizesis stage, the chromatin is in

the form of loops with the ends against the side of the nuclear membrane (Fig. 52). This point Montgomery calls the distal pole of the nucleus and the opposite point on the nuclear membrane, the central pole. The process is the same as in aurichalcea; the loops straighten, thrust the free ends toward the central pole (Fig. 53), bend toward each other in pairs and unite end to end (Fig. 54). The end of the chromatin thread either bears an enlargement or stains more deeply in cross-section for there is at this point the appearance of a deeply staining bead. This dark spot thus conveniently marks the place of union in the bivalent chromosomes, and we are led to the conclusion that the short loops first observed in the synizesis stage are univalent chromosomes. The idiochromosome pair is very distinct at the base of the loops as shown in Fig. 52.

The growth stages are not unlike those of C. aurichalcea. A spireme (Fig. 55) is formed which varies in its staining reactions exactly as in C. aurichalcea. The thread finally breaks up into bivalent elements which stain deeply and exhibit a longitudinal split, and at last condense into forms which they retain during the first division. In Fig. 57 are seen two bivalent chromosomes assuming the form of rings, and in Fig. 58 these have closed together. This figure also exhibits crosses which later change to the form found in aurichalcea. Most of the chromosomes of this species are dumb-bell-shaped in the first spermatocyte mitosis and of the usual V-shape in the second. The idiochromosomes maintain their spherical form throughout all the stages.

An equatorial plate of the first maturation division shows nine chromosomes arranged usually in the order seen in Fig. 60, with seven in a circle about the other two. All nine chromosomes may be seen in lateral view in Fig. 59, where they are just coming into the spindle. The dumb-bell shape of four is here quite evident and the unequal pair is conspicuous. Three of the others appear as straight rods and the other one bent in V-form.

During metaphase the chromosomes arrange themselves with their long axes parallel with the axis of the spindle, and later they divide at right angles to their length. In other words, they exhibit qualitative division, bivalent chromosomes separating at the point

of union made during synapsis. As a result the small chromosome of the idiochromosome pair goes to one pole while the large member goes to the other (Fig. 63). The equatorial plates of the second division confirm this, half possessing nine V-shaped chromosomes of approximately equal size (Fig. 71), the other half having eight large and one small chromosome (Fig. 65). Fig. 66 shows the second maturation spindle with this small chromosome in metaphase, and in Fig. 64 it is seen dividing.

Here, then, as in C. aurichalcea and Tenebrio molitor (Stevens, '05) half the spermatids will possess the small idiochromosome, and half the large.

The transformation of the spermatid is essentially the same as in aurichalcea. The deeply staining nucleolus-like body is present but no clue to its function or nature is given. The ripe spermatozoa are not spirally twisted but resemble that stage of aurichalcea seen in Fig. 42.

Owing to lack of material it was impossible to study the dividing somatic cells of the male and female forms. That they would exhibit the same conditions found repeatedly in other beetles there is no doubt. By permission I have reproduced four drawings from Dr. Stevens' originals, which show the chromosome of (Fig. 67) a somatic cell from the digestive tract of a male pupa of Tenebrio molitor, and (Fig. 68), a female somatic cell of the same form found in the egg follicle; (Fig. 69) a female somatic cell of Trirhabda virgata from the egg follicle, and (Fig. 70) a male somatic cell taken from the larval body of the same species.

Here, as in other forms that Dr. Stevens has investigated, the small idiochromosome goes to the male and the large one to the female.

SUMMARY OF OBSERVATIONS

Coptocycla aurichalcea

1 The spermatogonial number of chromosomes in Coptocycla aurichalcea is twenty-two, twenty-one of which are V-shaped and one very small one, spherical in form.

2 Synapsis takes place in a manner, so far not described but also observed in certain beetles now being investigated by Dr. N. M. Stevens. The loops seen in the synizesis stage, which represent individual chromosomes, straighten and unite in pairs by the free ends which are pushed up into the nuclear space. Pseudo-reduction, therefore, occurs just after synizesis and just before the formation of the spireme.

3 The first maturation mitosis is a transverse, reducing division, the second longitudinal, occurring along a lengthwise split formed early in the prophase.

4 There is present a typical pair of idiochromosomes [according to Wilson's definition ('05 and '06)] which, with the others, divides qualitatively in the first division and quantitatively in the second, the small member, therefore, going to one-half the spermatozoa and the large member to the other half.

5 The chromosomes show marked individuality from the beginning of the prophase, one having the form of a ring, two of a cross, several the shape of an E, and finally the unequal pair.

Coptocycla guttata

1 The spermatogonial number of chromosomes is eighteen, seventeen large V-shaped chromosomes and one which is small and spherical.

2 All other observations on this species confirm those on aurichalcea.

GENERAL DISCUSSION

Individuality of the Chromosomes

Convincing results have been published in regard to the individuality of the chromosomes by Boveri ('02), who found a difference in their function; by Sutton ('02), who found a difference in size; and by Baumgartner ('04), and others, who have discovered a difference of form.

The idiochromosomes indicate so clearly a difference in function as well as in size that it is unnecessary to go into detail on this

point. The ordinary chromosomes also confirm the size difference; though no careful measurements were made, it is obvious that such differences exist at least in most cases (Fig. 19). However, the difference in form is most evident in Coptocycla aurichalcea, and furnishes another strong support for the doctrine of the individuality of the chromosomes.

Of the eleven bivalent chromosomes several possess forms characteristic enough to mark them as the same in different generations; there are two or three crosses, several E's, a ring and an unequal pair (Fig. 16). There is, doubtless, a size difference that separates those of a group though this is not as obvious in some cases as in others. The ring form occurs only once in this species, but twice in guttata and maintains its identity of shape from an early prophase, being much more expanded then, however, than later. It is formed by the V-chromosomes of the spermatogonium uniting by both ends, as is plainly shown in Fig. 16, *l*, where two ends are not firmly joined. In a late metaphase the bivalent pair seems to elongate slightly; this closes the opening and gives the appearance seen in Fig. 23. In profile the ring assumes an oval form, with the central opening much smaller (Fig. 16, *n*), but this is distinguished from the actually elongated phase by the length (Fig. 23).

Mendel's Law

Assuming that the idiochromosomes are sex-chromosomes, or represent the sex-characters, they furnish excellent opportunities for speculation on the application of Mendel's law to chromosomes.

If the Mendelian principles of segregation apply to sex, there should be in the second generation 25 per cent males, 50 per cent hybrids, and 25 per cent females. Castle ('03) concludes that there are no individuals pure in regard to the sex-character, but only hybrids are produced. Now to apply this theory to the beetles. We know by actual observation that the male somatic cells possess the small idiochromosomes, and the female somatic cells the large one. Below are given in a schematic representation

two kinds of eggs and two kinds of spermatozoa with the dot above to indicate the idiochromosome and the letter to show the sex character.
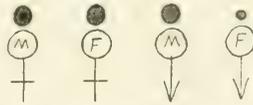
There are four possible combinations here:

(1) $= $ (FERTILE MALE) $= \frac{1}{4}$

(2) $= $ (INFERTILE MALE) $= \frac{1}{4}$

(3) $= $ (INFERTILE FEMALE) $= \frac{1}{4}$

(4) $= $ (FERTILE FEMALE) $= \frac{1}{4}$
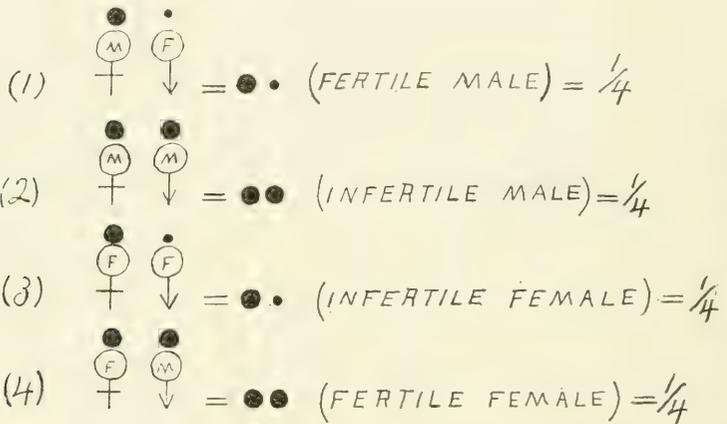
Now by actual examination of the male and female somatic cells we know that no such combinations as (2) and (3) exist. Our observations, therefore, strongly support the theory of selective fertilization, only gametes bearing opposite sex-characters fusing.

While the beetle furnishes no explanation why femaleness as a character often dominates (Castle '03) yet its chromosomes indicate why it is recessive a part of the time in these insects. In the 50 per cent of spermatozoa that carry the female character in the form of the small idiochromosome, this is overpowered by the much larger male idiochromosome in the eggs with which they unite. When the chance is even, *i. e.*, when the idiochromosomes are equal in size, then femaleness invariably dominates.

The Idiochromosomes

But two groups of animals thus far investigated are known to possess the idiochromosomes; these are the Hemiptera and the Coleoptera. It is probable that they exist in other forms and have been wrongly classified as was the case with Paulmier's ('99) work on Anasa, and Montgomery's ('01) on Cœnus delius and Euschistus tristigmus. Paulmier failed to distinguish between the accessory and the microchromosomes, while Montgomery saw the small idiochromosomes in the resting stages and the different number of chromosomes in different cysts, but misinterpreted them.

To Wilson is due the correct interpretation of these idiochromosomes in the Hemiptera heteroptera, as a distinct pair of chromosomes with a definite and characteristic behavior in the different generations.

Slightly earlier than this and independently Dr. Stevens working on one of the Coleoptera, Tenebrio molitor, found an unequal pair of chromosomes, which proves upon comparison to be essentially the same as those of the Hemiptera.

In details of behavior, however, these chromosomes differ markedly in the two groups. As in Lygæus and Cœnus (Wilson, '05) the idiochromosomes of Coptocycla maintain their identity throughout the growth period. They are first distinguished in the synizesis stage as a compact, spheroidal body at the base of the chromatin loops (Fig. 52). The small member has not been observed at this stage, but it is doubtless present and is obscured by the larger of the idiochromosome pair. As this pair appears in the spheroidal form, so early in the growth stage, it is a question whether it ever assumes the form of loops with the other chromosomes.

In Lygæus in the synizesis stage the larger differential chromosome is elongated and bent U-shape. In the post-synaptic phase instead of lengthening and splitting longitudinally as in Lygæus, both of the idiochromosomes of Coptocycla maintain their compact, rounded form. In early prophase these chromosomes in Hemiptera exhibit a bipartite structure and sometimes remain separated while the ordinary chromosomes have fused in synapsis.

In Coptocycla the unequal pair has fused, but neither member shows any tendency toward being bipartite. In fact, at no time is there a hint of a longitudinal split, unless the vacuolated appearance of the large idiochromosome in the early growth stage may be considered as such.

The later behavior of the idiochromosomes is markedly different in the two groups. In the Hemiptera they remain separated and univalent in the first maturation mitosis, but at the close of this division their products conjugate to form a dyad, which in all but one form, Nezara, is asymmetrical. In the Coleoptera the behavior during mitosis is exactly like that of the ordinary chromosomes; having united in the synapsis stage to form a bivalent, they divide transversely in the first mitosis and longitudinally in the second. The result is, of course, the same in both cases; the spermatids are of two kinds as regards the idiochromosomes, half possessing the small and half the large one. The distribution of these chromosomes to the somatic cells of the two sexes is also the same in the two groups; the male cells contain the smaller, the females cells the larger idiochromosome.

Sex-determination

The observations recorded in the present paper add nothing new to the subject of sex-determination, and their chief value consists in their confirmation of the very suggestive work on Tenebrio molitor (Stevens, '05).

Since McClung ('00) advanced his theory that the accessory chromosome of the Orthoptera is a sex-determinant, numerous investigators have sought evidence for or against it. The first in its favor was that of Sutton ('02), who found the odd chromosome present in the male somatic cells and absent in the female cells of Brachystola magna (23 in ♂ cells, 22 in ♀ cells). Since that time, however, Wilson has found the reverse true for Hemiptera, *i. e.*, the additional chromosome in the female somatic cells (Anasa 21 ♂, 22 ♀), and this leads him to question Sutton's count for the Orthoptera. Wilson suggests that the accessory chromosome of Anasa, Protenor and the Orthoptera is the homologue of the larger

member of the idiochromosome group found in Cœnus, Lygæus and certain other Hemiptera, and its missing mate is the homologue of the small idiochromosome. He thus supports the view of Paulmier and Montgomery in regard to degenerating chromosomes.

Knowing from his observations on Anasa that the accessory goes to the female in the Hemiptera, he, therefore, conjectured that the larger of the idiochromosomes would be found in the female somatic cells, and the small one in the male somatic cells, and he later found positive proof of this.

As stated before Dr. Stevens while investigating a form of beetle, Tenebrio molitor, found that an unequal pair of chromosomes is present, the large one of which goes to the female somatic cells, and the small one to the male somatic cells. Since then she has confirmed this in other species of Coleoptera, so that at last there seems to be much indisputable evidence for the chromosome-sex-determinant theory.

The question arises, what is to be done with such forms as Thermopsis (Stevens, '05) and Banasa (Wilson, '05). In the former there are no chromosomes which have any external peculiarity that would mark them as sex-determinants; in the latter there are two sets of chromosomes that we interpret as sex-determinants when they appear separately in other forms.

The solution for Thermopsis is perhaps less difficult than for Banasa; one pair of the chromosomes may bear the sex-character although there are no external differences. This does not conflict with present views. In Banasa, the situation is different. Here we have a pair of idiochromosomes as well as a typical accessory. In other cases Wilson has suggested that the large idiochromosome is the homologue of the accessory inasmuch as both are members of pairs, one member of which is degenerating or has already disappeared. This seems plausible and if taken from the standpoint of degeneration only, there is nothing conflicting in the fact that two pairs of chromosomes even in the same cell are undergoing the change. In fact, as Wilson suggests, it adds weight to the idea of degenerating chromatin, for we have no reason to suppose that degeneration is necessarily limited to one pair.

However, taken from the standpoint of sex-determination it seems to offer serious difficulties. While we are perhaps not justified in assuming that the sex character is confined to one chromosome, or to one pair of chromosomes, all recent observations point that way; then on *a priori* grounds it seems improbable that the same function should be assigned to two pairs of chromosomes in the same cell. With the numerous characters to be transmitted it would seem more likely that one pair of chromosomes must be the bearer of many qualities.

In Banasa there are four classes of spermatozoa which contain:

(1) the small idiochromosome + the accessory;
(2) the large idiochromosome + the accessory;
(3) the small idiochromosome — the accessory;
(4) the large idiochromosome — the accessory.

If the chromosome relations in the male somatic cells of Banasa correspond to those in Lygæus and Anasa, only one of these classes of spermatozoa (3) can be used. Any combination of (1), (2), (4) would refute the idea of homology of these two types of chromosomes. The same thing is true as to the production of females; but one kind of spermatozoon (2) can be functional.

This means that three-fourths of the spermatozoa are functionless as regards production of males, three-fourths as regards production of females and one-half absolutely functionless. Such a condition seems most improbable, but, of course, the only way of determining it is to study the male and female somatic cells of Banasa. This promises interesting results as it will either refute the homology theory (for this form, at least) or reveal new facts in regard to selective fertilization and a functional and non-functional condition of spermatozoa.

While Castle believes there is no hard and fast rule for dominance in the sex character in diœcious individuals, it seems that in the beetles we have a clear case of female dominance. In cells where the male and female sex chromosomes are equal in size, femaleness invariably dominates. Where they are unequal (the female small, as in Coleoptera and some Hemiptera, or entirely missing as in Orthoptera) then the female character is visibly

recessive, due merely to its reduced strength or entire disappearance in the male.

The results pointed out for Tenebrio and confirmed in this paper are briefly as follows: an unequal pair of chromosomes is present which, we have strong evidence for believing, transmit or determine the character of sex. The fact that the small one invariably occurs in the male somatic cells and the large one in the female somatic cells seems on first thought to mean that the small one carries maleness and the large one femaleness. But Wilson's thorough analysis of the conditions in Hemiptera ('06) has shown that the larger idiochromosome which alternates between the sexes must bear the male character, while the small idiochromosome, which is confined to the male sex, must represent the recessive form of the female character.

While it has been proved beyond doubt that certain chromosomes are concerned in the determination of sex among insects, a general application of the theory cannot yet be made, but it must for the present be limited to those forms possessing the accessory or the idiochromosomes. Without doubt further investigation will reveal either similar differential chromosomes in all forms, or show something homologous to them. It may not be a difference of size or shape that will distinguish them from the ordinary chromosomes, but probably one of behavior. Even in case the last difference is not found it need not disprove the chromosome-sex-determinant theory, for the sex character doubtless can be carried like other pairs of antagonistic characters without affecting the chromosome visibly. The eggs of comparatively few forms have been investigated, and it is possible that a dimorphism of the chromosomes in the matured oöcytes may be found in certain groups.

Biological Laboratory, Bryn Mawr College
April 30, 1906

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DESCRIPTION OF PLATES

All drawings were made with the aid of a camera lucida. A Zeiss oil-immersion 2 mm. objective and oc. 12 were used throughout, and the drawings have been reduced one-third.

PLATE I

Coptocycla aurichalcea

Fig. 1. Equatorial plate of spermatogonial mitosis, 22 chromosomes.

Fig. 2. Bouquet-stage or synizesis, showing univalent chromosomes in loop form.

Fig. 3. Later stage in which loops are straightening.

Fig. 4. Cross-section of the stage in Fig. 2.

Fig. 5. Synapsis. Fusion of univalent chromosomes end to end at *y*. Large idiochromosome *x*, at base of loops.

Fig. 6. Late synaptic stage, showing the relative lengths of the bivalent elements.

Fig. 7. Formation of spireme; *x*, the large idiochromosome.

Fig. 8. A slightly later stage; spireme very pale, *x* deeply stained.

Figs. 9, 10. Very early prophase, showing the longitudinal split in the chromosomes, idiochromosome excepted.

Fig. 11. Still later prophase. Both members of the unequal pair visible (*x*).

Figs. 12, 13. Prophase in which the chromosomes are contracting into their characteristic forms.

Fig. 14. An equatorial plate of the first division showing nine of the eleven chromosomes. The idiochromosomes retain the stain much longer than the others.

Fig. 15. An equatorial plate of the first mitosis with the full number, eleven chromosomes, present. The large linen fibers are seen attached to the chromosomes.

Fig. 16. Three types of chromosomes, found in C. aurichalcea, from various points of view, *a—e*, the cross-shaped chromosome; *a*, between a front and side view; *b*, front view when the longitudinal split is closed; *c* and *e*, profile; *d*, front view showing split, *f—h*, the E-shaped chromosome *f*, front view; *g*, profile, bivalent chromosome dividing; *h*, profile; *l—o*, ring-shaped chromosomes: *l*, the two univalent chromosomes not well fused; *m*, probably later stage in which union is more complete; *n*, ring closed and seen in profile; *o*, central opening growing smaller.

Fig. 17. Late prophase of first spematocyte; chromosomes coming into equatorial plate; *x*, the idiochromosome pair.

Fig. 18. Equatorial plate formed and one chromosome divided ahead of the others.

Fig. 19. Shows the size difference in some of the chromosomes.

Fig. 20. Anaphase of the first division, showing distinctly the form of the univalent chromosomes, and the manner of separation.

Fig. 21. Side view, first spindle, showing the densely staining idiochromosome when the others are very pale.

Figs. 22–24. Different views of the ring chromosome.

Fig. 25. Idiochromosomes dividing in first mitosis; *l*, large chromosome; *s*, small chromosome.

Fig. 26. Late anaphase of first spermatocyte.

Figs. 27, 28. Equatorial plates of second mitosis, showing 10 large and one small (*s*) chromosome.

Fig. 29. Equatorial plate of same mitosis, showing 11 large chromosomes, *l*, the large idiochromosome.

Fig. 30. Prophase of second mitosis, showing 10 large chromosomes and the small idiochromosome.

Fig. 31. Anaphase in which the large idiochromosome is dividing.
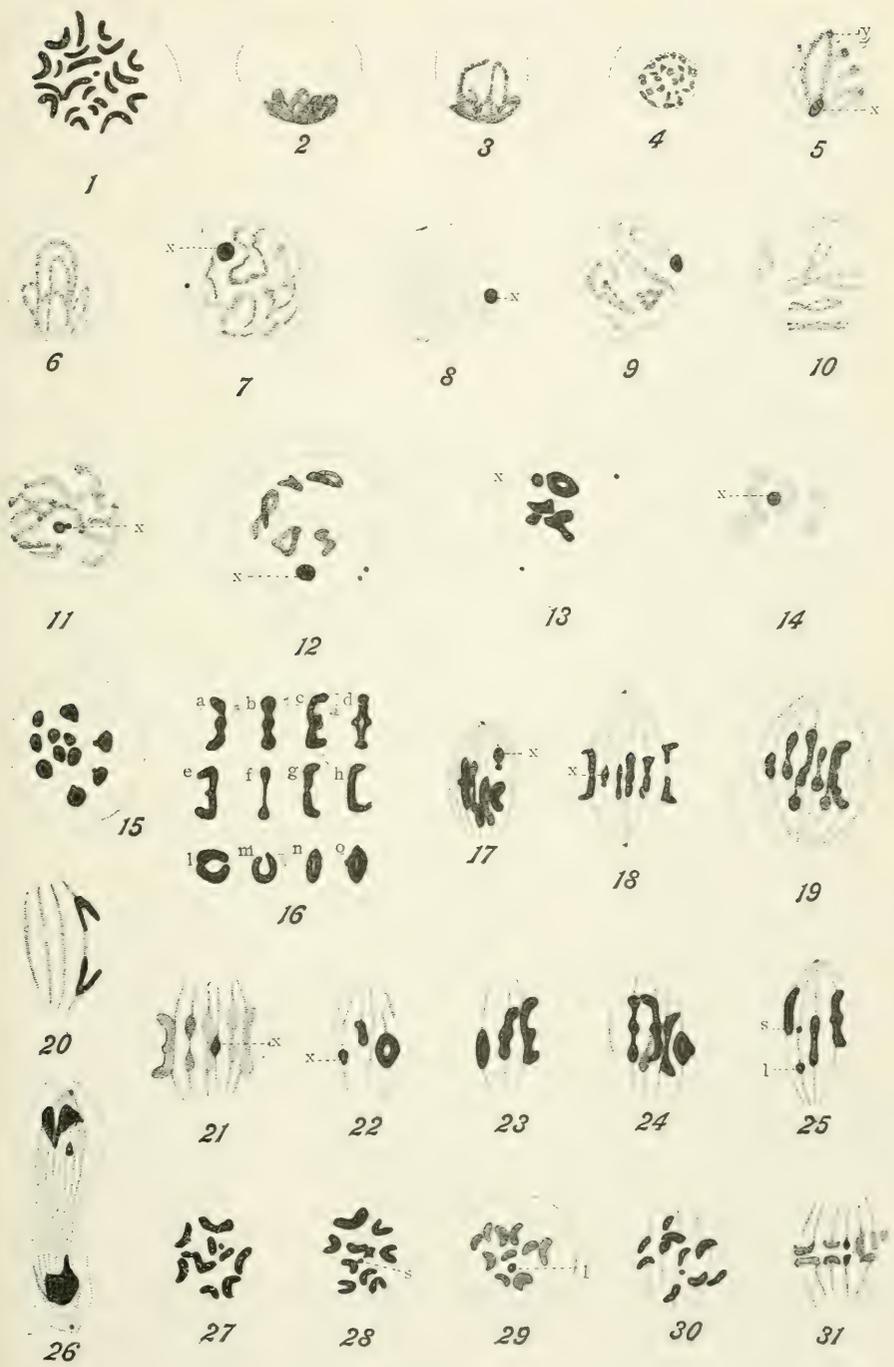
PLATE II

Coptocycla aurichalcea

Fig. 32. Anaphase of second maturation division, showing the position assumed by the chromosomes when first separated.

Fig. 33. Late anaphase; small idiochromosomes dividing.

Fig. 34. Late anaphase; unusual case in which the form of the chromosomes is not obscured by massing.

Fig. 35. The usual late anaphase.

Fig. 36. Very early spermatid showing a great amount of archoplasm.

Fig. 37. Nucleus at a slightly later stage, showing the peculiar nucleolus-like body.

Fig. 38. Archoplasmic substance in the form of a sphere applied to nuclear membrane.

Fig. 39. Archoplasm elongated into a pennant form with axial filament running throughout.

Fig. 40. Vacuolated stage in formation of the tail.

Fig. 41. Vacuolated appearance of head *c*, centrosome.

Figs. 42–44. Later stage in the transformation of the head. Ripe spermatozoa (44) possess a spirally twisted head.

Figs. 45–47. Later behavior of the nucleolus-like body.

Fig. 48. Giant spermatozoa, with two centrosomes and two axial filaments.

Fig. 49. Shows the migration of the nucleolus-like body.

Fig. 50. Cross-sections of the tail of the spermatid, *a—b*, at about the stages shown in Fig. 41; *c*, in Fig. 44.

Coptocycla guttata

Fig. 51. Equatorial plate of spermatogonial mitosis; 18 chromosomes.

Fig. 52. Synizesis stage showing univalent chromosomes in loop form. Large idiochromosome at base of loops.

Fig. 53. Later stage in which loops are straightening and bending toward each other.

Fig. 54. Synapsis. The dark bead at the highest part of loop marks point of union.

Fig. 55. Spireme stage; chromatin very pale with exception of unequal pair (*x*).

Fig. 56. Same stage, showing small idiochromosome somewhat removed from the large member and connected with it by a chromatin strand.

Figs. 57, 58. Formation of ring and cross-shaped chromosomes.

Fig. 59. Late prophase in which all nine chromosomes are seen from a side view. *x*, the idiochromosome pair.

Fig. 60. Equatorial plate of the first maturation mitosis; 9 chromosomes.

Figs. 61, 62. Prophase and metaphase, respectively, showing the idiochromosome pair (*x*).

Fig. 63. Anaphase of first mitosis; the idiochromosome pair, dividing qualitatively. *s*, small idiochromosome; *l*, large.

Fig. 64. Very early anaphase of second mitosis, showing division of the small (*s*) idiochromosome.

Fig. 65. An equatorial plate of the second spermatocyte in which the small idiochromosome (*s*) is present.

Fig. 66. Metaphase of second spermatocyte. The small idiochromosome (*s*) undivided.

Fig. 67. Equatorial plate from dividing somatic cell of male pupa (Tenebrio molitor), showing nineteen large and one small chromosome.

Fig. 68. Equatorial plate of a dividing cell of follicle of a young egg (Tenebrio molitor), showing twenty large chromosomes.

Fig. 69. Equatorial plate of a dividing follicle cell of a young egg (Trirhabda virgata), showing twenty-eight large chromosomes.

Fig. 70. Equatorial plate from the somatic cells of a male larva (Trirhabda virgata), showing twenty-seven large and one small chromosome.

Fig. 71. An equatorial plate of same spermatocyte in which all nine chromosomes are of approximately equal size.
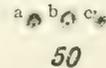
TORSION AND OTHER TRANSITIONAL PHENOM-ENA IN THE REGENERATION OF THE CHELIPED OF THE LOBSTER (HOMARUS AMERICANUS)

BY

VICTOR E. EMMEL

WITH TWO PLATES

INTRODUCTION

In the course of a series of experiments and observations on the phenomena of regeneration in the lobster, which necessitated a detailed study of the external morphological characteristics of the regenerating cheliped ('05, '06), it was noticed that in the successive stages of development there occurred a gradual torsion of the regenerating chelæ.

This and some other closely related phenomena do not seem to have been hitherto recorded, although the regeneration of the crustacean limbs has been the subject of extensive investigation by Herrick ('95), Morgan ('02), Przibram ('01, '02), Steele ('04), Schultz ('05), Wilson ('03), and other writers. These phenomena offer new material for the study of the phylogenetic significance of the regenerative process.

The problems of development are now being approached by the experimental method, and interpretations are based on physiological and mechanical princlples. And yet while the processes of development may be essentially physiological in nature and affected by mechanical factors, still the important truth remains that the organization of the cells is an "inheritance from the past," and that consequently the *method* of development may still furnish evidence of phylogenetic value. Wilson's ('94) valuation of the embryological phenomena may apply equally well to those of regeneration: "the idioplasm of every species has arisen through the modification of pre-existing

idioplasm, and every response that it gives to stimulus is an expression of its past history. Hence, we need not despair of ultimate success in the attempt to decipher the meaning of the embryological record, and to find in ontogeny a real criterion of homology, and it is here that we find encouragement, if any is needed, not to relax our efforts to investigate the normal phenomenon of comparative embryology on the largest scale and down to the minutest detail" (p. 123). It is with the purpose of contributing something to the facts bearing on these problems that the following observations are presented.

These observations were made at the Anatomical Laboratory of Brown University, and at the Experiment Station of the Rhode Island Commission of Inland Fisheries, where the lobster hatchery furnished unsurpassed facilities to obtain material for experimental work. I desire here to express my thanks to Dr. Mead for his interest and helpful suggestions in the present study.

I.   ON THE REGENERATION OF THE CHELIPED

a.   Method

In order to trace the transformations involved in the regeneration of the cheliped, a series of drawings have been made to illustrate successive stages in the process.

The material for this was obtained from about 75 fifth-stage lobsters. These lobsters were practically equal in size and age, and had all molted to the fifth stage, July 30 and 31. On August 1, the right and left chelipeds were autotomously removed from each individual. Every twelve hours, two or three of these lobsters were killed in corrosive acetic and preserved in alcohol. This was continued until August 13, when some of the remaining lobsters had begun to moult to the sixth stage. By this method, regenerating limbs were obtained in a series, ranging from minute papillæ to fully formed functional structure. These preserved lobsters were then carefully examined and eight specimens selected which presented typical stages in the differentiation and development.

The data for these eight specimens may be tabulated as follows:

No.	Stage	Removed Chelipeds	Killed	No. of days regenerating	Drawing
1	V	Aug. 1, 8 p.m.	Aug. 2, 8 p.m.	1 day	Fig. II
2	V	Aug. 1, 8 p.m.	Aug. 5, 8 a.m.	$3\frac{1}{2}$ days	Fig. III
3	V	Aug. 1, 8 p.m.	Aug. 6, 8 p.m.	5 days	Figs. I, IV, XV
4	V	Aug. 1, 8 p.m.	Aug. 7, 8 p.m.	6 days	Fig. V
5	V	Aug. 1, 8 p.m.	Aug. 8	7 days	Fig. VI
6	V	Aug. 1, 8 p.m.	Aug. 9, 8 p.m.	8 days	Fig. VII
7	V	Aug. 1, 8 p.m.	Aug. 13, 8 p.m.	12 days	Figs. VIII, XVI
8	VI	Aug. 1, 8 p.m.	Aug. 15, 8 a.m.	$13\frac{1}{2}$ days	Figs. IX, XVII.

Camera lucida drawings were made of a right cheliped of each lobster. The drawings were taken as nearly as possible from a constant plane (Fig. I), which shows a regenerating bud (*reg*) on the right basipodite (*bs*) of lobster No. 3. Each lobster was laid flat on its left side upon the microscopic stand, thus giving in every case, a postero-lateral view of the basipodite and regenerating cheliped.

b. *Torsion of the Regenerating Cheliped* (Plate I)

First Day (Lobster No. 1, Fig. II). During the first and second days after the removal of the cheliped, only a very slight regeneration, if any, can be detected over the exposed surface of the basipodite (*bs*). The first external indication of activity among the regenerating cells, is a minute light colored papilla pushing up through the dark clot of blood in the region (*bk*).

Third Day (Lobster No. 2, Fig. III). By the third day a distinct bud has appeared. Seen from the exterior, it is a simple club-shaped mass enclosed within an epithelial membrane or sac. The only indication of future segments and joints is a slight cleavage or groove (*a*), forming at the apex of the bud. This groove marks the first development of the claw; *i.e.*, the dactylopodite (*dc*), and propodite (*pr*).

Fifth Day (Lobster No. 3, Fig. IV). At this stage of development the outlines of the dactylopodite (*dc*), and propodite (*pr*) are becoming more definite, as indicated by the constrictions for the

first (1) and second (2) joints, (counting from the distal segment). The groove (a) for the two jaws of the claw has grown deeper.

Sixth Day (Lobster No. 4, Fig. V). The anlage of all the segments of the appendage have appeared; *i. e.*, the dactylopodite (*dc*), propodite (*pr*), carpopodite (*cp*), meropodite (*me*), and ischiopodite (*is*). The dactylopodite is now more completely differentiated from the propodite.

It is interesting to note here that the direction of differentiation is from the distal portion backward toward the proximal region of the bud, a method of differentiation characteristic of regeneration in many animal forms (see Zeleny, '06; Child '06, p. 410).

Seventh and Eighth Days (Lobsters Nos. 5 and 6, Figs. VI and VII) The four joints and five segments are clearly defined and the whole bud is now assuming a forward curvature.

The important fact for our present purpose in the six stages just described, is the position or spatial relations of the two terminal segments (*i. e.*, the dactylopodite and propodite) with reference to the basal segment of the limb.

It should be observed that each figure represents a nearly constant point of view—the postero-lateral aspect shown in Fig. I. The plane of the paper thus presents a common plane of reference and comparison in each case. Let this plane be called the "reference plane."

Now if in Figs. III and IV, we compare the position of the two segments of the claw (*dc* and *pr*), it may be seen that the plane of cleavage for the two jaws of the claw, or groove (a) is practically at right angles to our "reference plane." In other words, if we compare the "biting plane" of the claw at this early period with the normal position of the body, *the claw will open almost vertically upward*.

The same is practically true for Figs. V and VI. In Fig. VII, however, the plane of cleavage (a) is no longer vertical to the "reference plane." This is due to the fact that a slight twisting or torsion of the terminal segments has begun. The dactylopodite (*dc*) and propodite (*pr*), and possibly the carpopodite (*cp*), are gradually turning in such a way as to throw the dactylopodite farther inward toward the median line of the body; so that at

this stage, the "biting plane" of the claw is inclined away from the "reference plane" at an angle of about 20°. The later phases of this torsion appear in the further development.

Twelfth Day (Lobster No. 7, Fig. VIII). At this period of the regenerating cheliped the lobster is rapidly approaching the molt. The segments and joints are now almost completely developed. The regenerating limb is assuming a rounded, plump appearance, as the result of the large growth of tissue compressed within the epithelial membrane. The significant fact here is the position of the claw. The torsion has progressed to such a degree that the plane passing through the dactylopodite (*dc*) and propodite (*pr*) or "biting plane," is now inclined at an angle of about 45° to its original position.

Thirteenth Day (Lobster No. 8, Fig. IX). This phase of the development marks the culmination of both the regenerative process and the torsion of the chela. At this stage the lobster has moulted its old shell, and Fig. IX, represents the completely regenerated limb with its joints and segments expanded to their normal shape.

The complete torsion of the chela is now readily perceived. Instead of being parallel, as in Figs. III and IV, the "biting plane" of the dactylopodite (*dc*) and propodite (*pr*) is at right angles to the "reference plane." Thus it is clearly evident, that beginning with the earliest phase of the regenerating cheliped, with the claw nearly vertical in position, a torsion of the terminal segments has gradually taken place during the development, so *that the dactylopodite and propodite have rotated through an angle of 90°, and attained the horizontal position of the normal claw.*

c. Relative Size of the two Segments of the Claw (Plate I)

In the earliest period at which the differentiation of the claw is apparent in the regenerating bud (Fig. III), the partially developed propodite (*pr'*) of the claw is relatively smaller than the opposing dactylopodite (*dc*), a relation which is just opposite to that which holds in the adult structure.

On the fifth day (Fig. IV) there is less difference in size, although

the dactylopodite (*dc*) is still clearly larger and farther developed than the propodite (*pr'*) element of the claw. By the sixth day, however (Fig. V), this relative inequality has disappeared and the two segments are practically equal.

The seventh day (Fig. VI) marks the transition to a relation in which the propodite jaw (*pr'*) is slightly more developed, and by the eighth day (Fig. VII) it clearly forms the larger segment of the claw. During the remaining development, the propodite and dactylopodite gradually acquire the proportions of the normally developed chela (Figs. VIII and IX). Thus, according to these observations on the regenerating chela, it is evident that in the course of its differentiation the claw develops from an early stage in which the propodite portion of the claw is *proportionately smaller*, to a stage in which it is *proportionately larger* than the opposing dactylopodite.

d. Transitional Characters in the Regenerated Crushing Claw
(Plate II)

In the lobster the chelipeds are differentiated into two types of claws, the "toothed" or "nipping" claw, and the "crushing" claw. When the "crushing" cheliped has been amputated and another cheliped regenerated from the breaking plane, the claw of the latter frequently displays such a close resemblance to a "nipping" claw that it is not easy to distinguish the regenerated crusher from a normal "nipping" claw.

Figs. XXI, XXII and XXIII represent the normal and regenerated chelipeds of a seventh(?) stage lobster.[1] The normal left "nipping' and the right "crushing" claws are shown in Figs.

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[1] At this stage the two types of claws are clearly differentiated, although it should be observed that the older the animal the more highly developed the "crusher" becomes. This specimen was one of a number of lobsters used in an experiment upon the reversal of the chelæ, and in this individual the "crusher" had been removed later than the "nipper" in order, if possible, to give an advantage to the regenerating "nipper." It might be added that although the regenerated chelipeds frequently appeared so much alike that it was necessary to wait until another moult before it could be determined whether a crusher had been developed or not, still no conclusive evidence was obtained that "reversal" ever occurs in either young or old lobsters, a result similar to that of Przibram's ('02) on the European lobster.

XXI and XXII, respectively, and the regenerated right "crusher" in Fig. XXIII.

Both chelipeds were autotomously removed, the left on July 30 and the right August 4. Twenty-seven days later, the lobster moulted and measured 26.5 mm. Both chelipeds had regenerated, but in this individual as well as in many of the other lobsters used in the experiment indicated in the footnote, the claws looked very much alike. The regenerated left chela ("nipper") had all the characteristics of the original "nipper;" the right chela, on the contrary, was unlike the original "crusher" and showed characters transitional between the original "nipping" and "crushing" claws.

In a morphological comparison of the regenerated right "crushing" claw with the original "nipping" and "crushing" claws the following contrasts may be observed (see Plate II)

	ORIGINAL LEFT CLAW (Fig. XXI)	ORIGINAL RIGHT CLAW (Fig. XXII)	REGENERATED RIGHT CLAW (Fig. XXIII)
Form of claw	Proportionately elongated and slender	Proportionately thicker and more massive	Proportionately elongated and slender
Ratio of breadth to length of claw.......	$\dfrac{2.6\,mm.}{10\,mm.} = .26$	$\dfrac{3.3\,mm.}{9.8\,mm.} = .34$	$\dfrac{2.5\,mm.}{9.3\,mm.} = .27$
Dactylopodite	Slender and nearly straight	Distinctly crooked and stubby	Almost straight and comparatively slender
Dentition	Pointed cutting teeth, grouped in periodic sequence (p,p) with formula 1:3:5:7*	Characteristic broad crushing tubercle † (t.t. etc.), with only a rudiment of periodic sequence (p')	Cutting teeth predominate. Sequence of 1:3:5:7 well marked (p)
Number of spines (sp) on inner border of propodite...........	5	3	5
Tactile hairs on inner and outer border of claws	Very numerous	Sparse ‡ .	Quite numerous
Dominant morphological characters	"Nipping" type	"Crushing" type	Transitional between "nipping" and "crushing" types

*According to Stahr's schema. (Stahr, '98, p. 459)
†Formed by a fusion of periodic teeth. (Herrick, '05)
‡In an older lobster, tactile hairs on the crushing claw almost entirely disappear.

From this comparison, it is evident that the claw on the regenerated right cheliped differs from the original "crusher" and closely resembles the "nipping" claw, in general form and proportions, the character and arrangement of teeth, and even in the number of spines and tactile hairs. The conclusion seems clear that *the cheliped, which regenerates after the removal of the "crusher," is a type transitional between the original "nipping" and "crushing" claws.*

In regard to the universal character of this rule it has already been intimated that the transitional type may not always be present in the regenerated "crusher," especially in the older lobsters. This may be partially due to the conditions under which the limb was removed; *i.e.*, if it is removed early in the interval between two moults, the regenerating bud will have a longer time to develop, and may consequently be farther advanced in its differentiation toward the original type before the next moult occurs.

II. COMPARISON WITH THE NORMAL DEVELOPMENT OF THE CHELIPED

a. *Torsion in the Larval Stages* (*Plate II*)

The torsion of the limb in regeneration which has just been described, was discovered before I had seen Herrick's ('05a) work on normal torsion. I have since gone over the larval development of the lobster on some carefully preserved material which Mr. P. B. Hadley kindly gave me, and have verified Herrick's work and made drawings to facilitate comparison with the regenerating appendages.

In the first larval stage; *i.e.*, just after hatching, the claw of the cheliped, as well as the claws of the first and second thoracic legs, opens in a vertical plane with a slight inclination outward. A dorsal view (Fig. XVIII) shows the dactylopodite (*dc*) in a position vertically above the propodite (*pr*).

In the second larval stage a change in position begins, and in the third stage (Fig. XIX) the dactylopodite (*dc*) and propodite (*pr*) have turned over and inward, so that the claw now opens at an angle of about 45° to its former plane. The rotation of the

cheliped is completed at the fourth stage (Fig. XX), the dactyl-opodite (*dc*) and propodite (*pr*) are at right angles to their original position and the claw now opens inward on the nearly horizontal plane characteristic of the normal limb. At the same time it may be observed that the claws of the first (1′) and second (1²) thoracic legs have retained their original vertical position. Thus "the position of the great forceps has been reversed by rotation through 90°, in consequence of which their inner or anterior faces have become their under sides" (Herrick, p. 130).

A direct comparison of the rotation of the regenerating cheliped with the torsion in the larval stages may be made by means of Figs. XV, XVI and XVII. These figures represent the dorsal view of the regenerating lobsters, Nos. 3, 7 and 8, respectively (see p. 605). The dactylopodite and propodite on the fifth day stage of regeneration (Fig. XV) lie in a plane vertical and outward similar to the position for the normal claw in the first larval stage (Fig. XVIII). On the twelfth day (Fig. XVI) the two segments of the claw (*dc* and *pr*) lie in a plane at 45° from their former position and correspond closely to the third larval stage type (Fig. XIX). On the thirteenth day the regenerated claws of the moulted lobster open inward on the horizontal plane shown in Fig. XVII.

Thus in both the regenerative and the ontogenetic development, the "great claw" of the cheliped rotates through an angle of 90° to the horizontal plane of the normal claw.

b. *Relative Size of the Dactylopodite and the Opposing Propodite in Normal Development (Plate I)*

Having observed that in the regenerating claw the propodite part of the claw which was at first smaller, gradually surpassed in size the opposing dactylopodite, the question naturally arose whether this, too, might be a feature of the ontogenetic development. If so, it was evident that it must be sought in the larval stages, for at the fourth stage the propodite, part of the claw, is already slightly larger than the dactylopodite.

The larval specimens upon examination showed just such a morphological transition in the development of the claw. In the first larval stage the propodite part of the claw (Fig. X, *pr′*)

is nearly a third *smaller* than the opposing dactylopodite (*dc*). In the second stage (Fig. XI) the propodite jaw (*pr'*) is only slightly the smaller, while in the third larval stage (Fig. XII) the two jaws of the claw are practically equal in size. At the fourth stage (Fig. XX, Plate II) the propodite jaw (*pr'*) is *larger* than the opposing dactylopodite, and the claw is now assuming characteristic normal proportions. Thus early in the development of the cheliped, the *dactylopodite* is the larger of the two jaws of the claw. Then by a gradual transformation the *propodite* jaw becomes equal, and finally in the adult structure is much the larger of the two segments of the claw.

Nor, indeed, is this an unexpected course of ontogeny. For the comparative morphology of the crustacean appendages certainly indicates that the cheliped is merely a highly modified leg appendage. In the lobster a transitional series, almost equal to a demonstration, exists between the terminal segments of the thoracic legs and the claw of the cheliped. The third and fourth legs have no claws (Fig. XIII), but in the first and second thoracic legs (Fig. XIV) an elongated process (*pr'*) has grown out from the distal part of the propodite, thus forming a jaw to meet the opposing dactylopodite (*dc*). In the cheliped this process becomes the propodite segment of the highly developed claw. That this propodite part of the claw is a true process which has grown out from the second segment independently of the joint for the dactylopodite, is clearly shown by the well marked groove or shoulder (*g*, Figs. XIV, X, XI,) between the propodite jaw (*pr'*) and the dactylopodite in the early stages of the developing chela. From this series it is evident that the "great" claw of the cheliped has been evolved by a distal elongation of the propodite process or "claw-like" projection, against which the terminal segment "bites," thus forming a claw with greater facilities for grasping prey.

c. Development of the "Crushing" Claw

The development of the external morphological features of the chelipeds has been carefully described by Herrick ('05), and my observations do not add anything of an original character.

But a brief description seems necessary here in order to complete the comparison between the regenerative and normal method of developing this appendage.

In the adult lobster two distinct types of claws are found, the more primitive "toothed" or "nipping" claw, and the larger and phylogenetically younger (according to Stahr, '98; and Przibram, '01) "crushing" claw. But in the larval stages and up to the fifth moult, on the contrary, both claws are "similar" and of the "toothed" type. At about the sixth stage (Hadley, '05) the "crushing" claw begins to differentiate. The claw becomes wider, broad tubercle-like teeth develop, and the tactile hair of the toothed type (Plate II, Fig. XXI) gradually disappear in successive moults. Thus the adult "crushing" claw comes to be characterized by the almost entire absence of tactile hairs, and the presence of the broad crushing teeth; by a dactylopodite relatively smaller, and by being on the whole larger and more massive than the "toothed" or "nipping" type.

These facts in the ontogenetic development serve to show the relation between "nipping" and "crushing" claws, and give significance to the fact that the regenerating crusher for a long period has a form intermediate between these two types.

III.   RÉSUMÉ

Three characteristic phenomena may be observed in the regeneration of the cheliped, each of which resembles corresponding stages of the ontogenetic development.

(*a*) In the very early stages of differentiation the cleavage of the claw first appears at the apex of the regenerating bud in such a manner that the dactylopodite is vertically above the propodite. The claw would, therefore, open upward and outward. In the later course of development a marked transition in position takes place. The terminal segments gradually rotate over and inward, so that when the moult occurs, a torsion of about 90° has been produced, and the claw now opens inward on the naturally characteristic horizontal plane.

(*b*) Contemporaneously with this torsion, a transition in the form of the claw also occurs. At an early stage the dactylopodite

is larger than the opposing segment of the claw. Later the propodite part of the claw rapidly develops, and becomes equal and finally larger than the dactylopodite. By the time of moulting the terminal segments have practically assumed their normal proportions.

(*c*) During the regeneration of the "crushing" cheliped it was observed that after the first moult, the regenerated "crushing" claw could not always be recognized as such, but is of a type intermediate between the "nipping" and "crushing" claws.

The ontogenetic development of the cheliped to which this series of changes correspond is as follows:

(*a*) At the first larval stage the claw of the cheliped together with the claws of the first and second chelate legs open upward and outward, the dactylopodite being in a position vertically above the propodite. The chelæ legs retain their original vertical position, but in the cheliped a gradual change occurs during the second and third larval stage, so that at the fourth moult the claw has rotated through 90° and assumed a horizontal position.

(*b*) In the first larval stage the propodite jaw of the claw is much smaller than the opposing dactylopodite, but in the second and third stages it increases in size and ultimately becomes proportionately much the larger of the two segments of the claw.

(*c*) In the first four larval stages both claws are similar in type. At about the sixth moult one claw begins to differentiate toward a "crushing" claw. During ensuing moults this claw passes through transitional stages and is finally completely transformed into a normal "crushing" claw.

In a word, *the torsion and other transitional morphological changes in the regeneration of the cheliped are parallel to similar phenomena in the ontogentic development of this appendage.*

IV. DISCUSSION

In view of the fact that there is such a close similarity or parallelism between the regenerative and ontological processes just described, the question arises whether this parallelism has any phylogenetic significance.

The general question of the repetition of phylogenetic and ontogenetic processes in regeneration is still open. Such facts as the regeneration of an ancestral type of claw in the shrimp (Müller, '80); the reproduction of a walking leg in place of a maxilliped in Portenus (Przibram, '01); the formation of a lens from the iris in Urodeles (Wolff, '94); the regeneration of a five, instead of four-fingered hand in the Axolotl (Barfurth, '94); have led to a diversity of interpretation. Weismann ('02), on the basis of his "determinant" theory, speaking of the differences between the regenerated and the original structure, holds that "there remains nothing for it but the assumption that the regeneration determinants have remained at a phyletic lower level, while the determinants which direct embryogenesis have varied and either developed farther or retrogressed" (p. 28).

On the other hand Herbst, Driesch, Morgan, and others, do not see a dependence of regenerative processes upon fundamental biogenetic laws, but rather hold that a similarity in development is due to a similarity in conditions; "that the mistake is not in stating that the two processes are sometimes similar or even identical, but in stating the matter as though the regenerative process repeats the embryonic method of development," and "that it may be entirely misleading to infer that ancestral characters have reappeared" (Morgan, '01, pp. 213, 214).

Quite recently, Schultz ('05) has reaffirmed the atavistic significance of the process in a striking case of regeneration in the claws of five species of Russian crayfish: A. fluvitalis, A. pachypur, A. colchicus, A. kessleri and A. leptodactylus. The regenerated claws in each species were not only unlike the normal type of claw in each case, but, moreover, the remarkable fact was observed that they all resembled more or less closely a single type of claw, namely, the type characteristic of A. leptodactylus. Schultz's atavistic interpretation of the case is supported by the recent work of Skorikows, whose study of the genetic relationship among the crayfish from the standpoint of geographical distribution, etc., makes A. leptodactylus var. colchica the parent form of all Russian crayfish. Schultz accordingly maintains that "we see here a dependence of regeneration upon fundamental biogenetic laws, a dependence which

Herbst, Delage, Driesch, and others, can vainly deny or slight, as "explaining nothing" (p. 46).

In regard to this controverted question, the present observations on the lobster seems to favor a recapitulation theory in regeneration. Since it is difficult adequately to account for the similarity between the ontogenetic and regenerative processes on the basis of a "similarity of conditions" in the organism. The ontogenetic torsion and proportional development of the claw take place in the larval stages of this crustacean. At this period the organic conditions must be widely different from those under which the same phenomena occur in the regenerative process of the adult lobster, for during the larval stages the organism undergoes such an extensive metamorphosis in both function and form (Hadley, '05) that the fourth moult of the young animal "marks the most surprising leap in the whole history of development" (Herrick, '05). Even the differentiation of the claws into the "nipping" and "crushing" types, which occurs after the larval stages, marks the change from a "symmetrical" to an "asymmetrical" "equilibrium" in these appendages, and is correlated with the establishment of the "bottom-living" habits of the lobster (see Przibram, '05, p. 238; Herrick, '95, p. 180).

This parallelism between the regenerative and normal development, therefore, seems to be more adequately interpreted by the conclusion that *in the regenerative process of the cheliped we meet with a recapitulation of characteristic phases of its ontogeny;* phases which, if investigators are at all correct in interpreting the origin of the crustacean cheliped through a modification of the thoracic leg appendages, are themselves in turn the recapitulation of a phylogenetic development.

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a—Cleavage or groove between the two jaws of the claw.

bs—Basipodite.

c—Cheliped.

cp—Carpopodite.

dc—Dactylopodite.

ex—Exopodite.

g—Groove or projection between the dactylopodite and the claw-process of (*pr'*) the propodite in early development.

h—Tactile hairs.

is—Ischiopodite.

l'—First thoracic leg.

l'—Second thoracic leg.

me—Meropodite.

p—Group of teeth in periodic sequence.

p'—Indications of a former sequence in the teeth of the "crushing" claw.

pr—Propodite.

pr'—Propodite-part of claw opposing the corresponding jaw or dactylopodite.

sp—Spines on lateral edge of propodite.

t—Tubercle-like teeth one crushing claw. *1, 2, 3* and *4*, respectively, the first, second, third and fourth joints of the cheliped.

PLATE II

XV-XVII—Torsion in the regeneration of the right cheliped. Dorsal view of fifth stage lobsters, showing different phases of the regenerating limb.

XV—Regeneration, 5 days, dactylopodite (dc) outward and above the propodite (pr^2). (Lobster No 3) 4.5$\times$

XVI—Regeneration, 12 days, "biting plane" of claw turned inward at angle of about 45° to its original position. (Lobster No 7) 4.5$\times$

XVII—Cheliped completely regenerated, 13.5 days. Lobster had just moulted and the complete torsion of the cheliped permits the claw to open inward on a nearly horizontal plane. (Lobster No 8) 4.5$\times$

XVIII-XX—Torsion of the right cheliped in normal development.

XVIII—First larval stage; claw of cheliped (c) opens upward and outward. 9$\times$

XIX—Third larval stage; claw opens inward at an angle of about 45° to its original position.   9$\times$

XX—Fourth stage; claw opens inward on nearly horizontal plane. 4.5$\times$

XXI-XXIII—Transitional characters in the regeneration of a "crushing" claw. Lobster in seventh (?) stage. (Dorsal view)

XXI—Original left "nipping" claw. 4.5$\times$

XXII—Original right "crushing" claw. 4.5$\times$

XXIII—Regenerated right "crushing" claw. 4.5$\times$
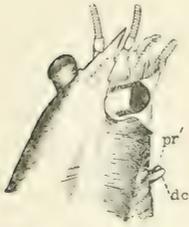
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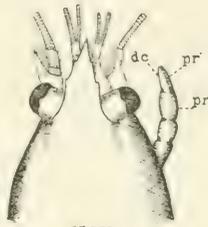
XVI

XVII
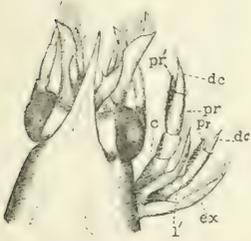
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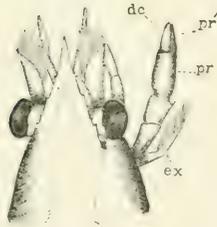
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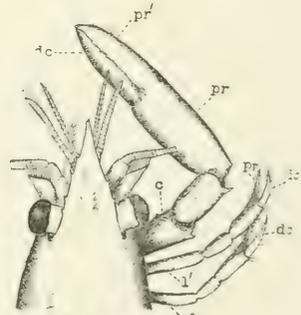
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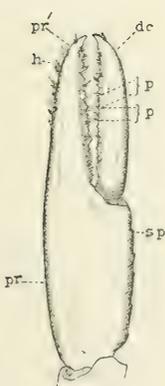
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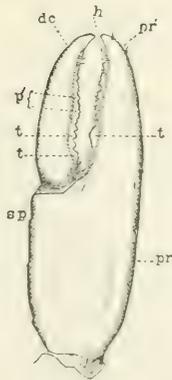
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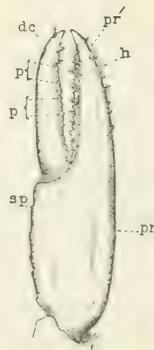
XXIII

THE INFLUENCES OF GASES AND TEMPERATURE ON THE CARDIAC AND RESPIRATORY MOVEMENTS IN THE GRASSHOPPER

BY

EULALIA V. WALLING

The object of the experiments recorded in the following pages was to ascertain the influences of certain gases upon the cardiac and respiratory movements of the grasshopper, both in the uninjured animal and when the heart and respiratory centers were isolated from all or part of the body.

The principal parts of the respiratory organs are the paired spiracles, with their air sacs, and the tracheal tubes, that ramify from these to all parts of the body. The mechanical respiratory movements consist of an expiratory and an inspiratory phase. During the latter, the active contraction of the abdominal muscles increases the breathing space by enlarging the antero-posterior and dorso-ventral diameters of the abdomen. In consequence of the lowered pressure therein produced, air passes into the tracheal tubes. During expiration the muscles relax and air enters the air sacs.

The heart extends as a delicate tube longitudinally above the intestine, directly beneath the chitin, along the whole length of the abdomen. When entirely exposed to view it is seen to pulsate rhythmically throughout its whole extent. By a system of valves it is divided into a series of eight chambers, that communicate through slits or ostia with a surrounding pericardial sinus. Through the aorta, which arises at the anterior end of the heart, and the aortic branches, blood is conveyed to all parts of the body, along the paths of the tracheal tubes.

It was demonstrated by Ewing[1] that the respiratory movements persist not only in decapitated animals but also in the isolated abdominal segments, in which are contained the ganglia of the ven-

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[1] Ewing, H. Z.   Kansas University Science Bulletin, 1904, Vol. ii, p. 305.

tral nerve cord, that are the centers for the reflex and respiratory movements of the special segments to which they belong.   I found that the heart also will continue its rhythmical contractions for hours, when left attached to a narrow strip of chitin along its mid-dorsal line and entirely removed from the body; moreover, that segments cut from the heart will continue their pulsations for long periods of time, especially if kept moist with Ringer's solution. Whether there are dorsal ganglia as described by Carlson[1] for Limulus, is a question that I am now investigating.   Certain it is, that electrical stimulation on the dorsal side accelerates, and burning this region with a very fine needle destroys the action of the special segment that was injured.

More than a thousand grasshoppers of different species were employed in this investigation.   Besides normal, decapitated and deviscerated specimens, abdominal segments and isolated heart pieces were employed.   In the deviscerated animals, the viscera and air sacs were removed, thus leaving the heart, ventral nerve cord and respiratory muscles intact.   The experiments were repeated many times and the data for each set of experiments represent average results.

I take this opportunity to express my thanks for valuable suggestions and help to Professor Hyde, under whose direction this work was conducted.

Vitality Varies with Conditions.—When kept under the most favorable conditions; that is, when placed in a wire cage containing sod and growing grass, the animals live from seventeen to twenty-five days, according to the species; they live on an average three days, and certain species five days, under the same conditions when decapitated or without food.   Respiration and heart action last about thirty-three hours in deviscerated animals and abdominal segments.   When the isolated heart is kept moist in a moist chamber, or with Ringer's solution, it pulsates two days, but it becomes dry and stops beating in nine hours when exposed to the air.   Post-mortem examinations showed, as a rule, that heart action outlasts that of respiration.

[1] Carlson, A. J.   American Journal of Physiology, 1904, vol. xii, p. 67.

Effect of Vitiated Air.—In each of these experiments, a single grasshopper was placed in a hermetically sealed tube ten inches long and one-half inch in diameter. One grasshopper was confined with each isolated heart, to furnish the vitiated air.

The results from many experiments were, that normal animals continued to breathe forty-two hours; those of one species (Arphia) lived four days. Often when placed in fresh air, the hearts continued their movements nine hours after the breathing had ceased, and frequently both cardiac and respiratory activities were resumed in fresh air, after they had stopped for many hours in vitiated air. The decapitated animal and the isolated heart not protected from drying, lived as long as in fresh air when not kept moist and often when the isolated heart was moistened with Ringer's solution, after removal from the vitiated air its activity would return and continue ten hours longer. It was interesting to learn that both the respiration and heart action could be resumed after they had ceased for many hours.

Ether.—One end of a glass tube containing the grasshoppers was placed under water and the other end was joined to a bottle partly filled with commercial ether. Through this system air was forced under constant pressure. Both normal and decapitated animals stopped breathing in this atmosphere of ether in from five to ten minutes, indicating that the brain exercised no influence under these conditions. Three hours' exposure to the ether did not inhibit the heart action, but after four hours the ether had injured the tissue so that neither respiration nor heart action returned when the animals were placed in fresh air. Isolated hearts ceased beating in one minute in the ether and when exposed longer than forty minutes, were past recovery in fresh air. In the uninjured animal the ether reaches the heart indirectly during respiration. When that ceases, it is fed with venous blood, as in the previous and following experiments, with the addition, however, of some ether in solution.

Carbon Dioxide.—The carbon dioxide, which was purified by passing through solutions of sodium carbonate, alkaline and acid potassium permanganate and alkaline pyrogallic acid, constantly flowed through the glass tube in which the grasshoppers were

confined. All connections were tested and made perfectly air tight. The exit end of the tube dipped under oil.

Observations from many experiments showed that, although the respiratory movements in the normal animal ceased in from twenty to sixty seconds, the heart action continued about six hours longer. Moreover, if the grasshoppers were subjected fifteen hours to the gas, the respiration would return, provided the animals were then removed to fresh air; and what is more remarkable, the heart action, but not the respiration, will be resumed in fresh air after an exposure to the carbon dioxide for forty-eight hours. In some cases only a few minutes, in others several hours, and in a few instances, it was necessary to expose them to fresh air for twenty-four hours before the heart resumed its rhythm. Isolated hearts directly exposed to the gas unlike those in the uninjured body cease their activity in from twenty to sixty seconds and will, like those in the body, resume their rhythm in fresh air after an exposure of forty-eight hours to the carbon dioxide gas. The gas inhibits the respiratory movements and those of the isolated heart in exactly the same time. The results indicate that the tissue was not injured by long exposure to the gas, but probably its oxidative or metabolic action was inhibited.

Carbon Monoxide.—Carbon monoxide was obtained by heating a mixture of sulphuric and oxalic acids, and purified by passing through the same solutions that were employed to purify the carbon dioxide. The gas passed under a slight pressure constantly through the glass tube containing the grasshoppers, into a vessel containing a solution of cuprous chloride. Grasshoppers exposed to this gas behave much the same as do those in carbon dioxide. Respiration stops in the same time in both gases, but animals may recover respiratory movements after an exposure of thirty hours to carbon monoxide, while they cannot recover if they remain in carbon dioxide more than fifteen hours. On the other hand, the heart action in the normal animal and in the isolated hearts, directly exposed to carbon monoxide for forty-eight hours, will not recover in fresh air, indicating that carbon monoxide is more damaging to cardiac activity than is carbon dioxide.

Hydrogen.—Hydrogen obtained by the interaction of HCl on

zinc, was passed through solutions of sodium carbonate, sodium hydroxide, acid and alkaline potassium permanganate and alkaline pyrogallol and further through sulphuric acid when dry hydrogen was needed. Under slight pressure the hydrogen passed constantly through the glass tube containing the grasshoppers. The open end of the tube was drawn out into a long point that dipped under oil. The connections were all sealed, tested, and made perfectly air tight.

A study of the data obtained from many experiments on the influence of hydrogen upon the respiratory and heart movements, reveals interesting results. In some of the experiments the cardiac and respiratory activity continued five days in an atmosphere of dry hydrogen. It will be remembered that this is as long as these functions persisted in the grasshoppers kept in fresh air without food. The average duration of activity, however, is not as great in hydrogen as it is in air, because the animals are often in a comatose condition. That is, they are very restless and active for a few minutes, then they become quiet and respiration seems to stop for from five minutes to two hours. Occasionally an appendage is moved and then they revive, become active and move about in the tube, so that periods of activity and lethargy alternate until they die.

Isolated hearts directly exposed to the hydrogen, behave in somewhat the same manner. The first effect is an acceleration of the heart beat. For example, the heart may contract sixty times a minute before it is put into the hydrogen, but one hundred times per minute after one minute exposure, eighty-six per minute after five minutes, only thirty times per minute after ten minutes, and in about twenty minutes all contractions have ceased, only to begin again in from two to four hours. They then continue to beat in the dry hydrogen for about twenty-seven hours or in moist hydrogen twenty-three hours. It was interesting to note, that the hearts exposed to the hydrogen did not become dry although they were not moistened with Ringer's solution.

It would appear from these experiments, that the absence of oxygen is not so injurious to the heart and nerve tissue in the grasshopper as might have been inferred from the experimental results on higher forms.

Oxygen.—The gas from a cylinder of oxygen was purified by washing through solutions of sodium carbonate, sodium hydroxide, acid and alkaline potassium permanganate and distilled water, respectively. To obtain dry oxygen, the gas was further washed through sulphuric acid. As in the other experiments, the gas passed constantly under slight pressure through the glass tubes containing the grasshoppers, and the free end of the tube dipped under oil.

An inspection of the many results secured from the influence of oxygen upon the cardiac and respiratory movements, disclosed the fact that these functions persist longer in animals kept in that gas than when living in fresh air without food. In some normal grasshoppers, these functions continued eighty-nine hours, while in fresh air, the same species lived but seventy-two hours. There was not so much difference in the effects of dry and moist oxygen as there was between the dry and moist hydrogen.

Isolated hearts, moistened with Ringer's solution when first placed in oxygen, continued to pulsate actively for about thirty hours. They could not be kept moist as could those which were placed in moist chambers in fresh air, and which contracted on an average forty-eight hours; therefore, a direct comparison between these and those subjected to oxygen cannot be made.

While the grasshoppers are inactive, breathing faintly and periodically in hydrogen, they are active, moving about in the tube frequently, in an atmosphere of oxygen, but at times do not breathe at all, being in what appears to be an apnœic state. Possibly if they had had food, they would have lived much longer than did those in air.

High Temperature.—In these experiments the grasshoppers and isolated hearts moistened with Ringer's solution were observed through the glass door of a digester in which the temperature could be carefully regulated and observed.

In one series of experiments, the temperature was gradually raised during three hours from 25° C. to 68° C. It was a noteworthy fact that as the temperature was raised, the first effect upon the isolated heart was to retard its contractions for a few minutes. A heart that contracted rhythmically sixty times a minute at 25° C.,

contracted only forty-eight times a minute at 30° C. and then the action increased rapidly, so that at 48° C., it was beating at more than twice its normal rate. In Coloptena femur rubrum the pulsations were increased to about two hundred per minute. As the temperature was raised beyond 48° C., the contractions became slower and stopped entirely in temperatures between 62° C. and 68° C. On an average the respiratory rate increased from 48 per minute gradually to 66 per minute at 41° C., then the rate decreased to 60 at 43° C. It then increased to 112 at 51° C., from there on decreased and ceased at 57° C.

In another set of experiments, the isolated heart was removed from a temperature of 25° C., in which the contractions were on an average fifty-six per minute, and subjected directly to a temperature of 57° C. Here the heart's action was instantly increased to one hundred and fifty contractions per minute. During the next hour the temperature was gradually raised from 57° C. to 68° C., with the result that the rhythmical contractions decreased and ceased entirely at 68° C., but when the heart was then placed in a temperature of 25° C., its action was again resumed.

In a third set of experiments, normal grasshoppers, abdominal segments and isolated hearts were subjected to a temperature of 14° C., that was gradually raised during four hours to 63° C., and then lowered again to 14° C., or room temperature. In a temperature of 14° C., there were, in the normal insect, on an average forty respiratory contractions per minute and in the abdominal segments twenty-five contractions per minute. In that time the isolated heart contracted about thirty-six times. As the temperature was raised from 14° C. to 54° C., the respiratory movements in the normal animal, and in the abdominal segments increased from 40 to 110 and 50 contractions per minute, respectively, then decreased and ceased at 59° C., while the isolated heart increased its action from 36 beats in a temperature of 14° C. to 160 beats in a temperature of 48° C., then gradually slowed, and stopped entirely at 62° C., one-half hour after the respiratory movements had ceased. The specimens were then subjected to a gradually but rather rapidly falling temperature, but the cardiac and respiratory activities were not again resumed.

Low Temperatures.—When the effect of a gradually lowered temperature was to be studied, the grasshoppers were placed with a thermometer in a test tube around which the temperature was gradually lowered, first by placing the tube with its contents near a large dish containing a mixture of chopped ice and salt, and, as the temperature became lower in the tube, it was gradually submerged in the salt and ice mixture, until a temperature of —17° C. was obtained. For studying the influence of still lower temperatures the tubes were placed in a liquid air chamber, where a temperature of —100° C. was obtained.

The following are the results of many experiments upon normal and decapitated grasshoppers and upon abdominal segments and isolated hearts.

The respiratory and heart action becomes slower and fainter as the temperature falls from 20° C. to 5° C. At this temperature, the animals are usually inactive and breathe faintly only five or six times a minute, if at all, while the isolated heart ceases to contract. The respiratory movements in the normal animal, however, may not stop until the temperature falls to 0° C. Normal animals and isolated hearts kept in a temperature of —10° C. for about one hour will recover their activity if the temperature is very gradually raised to that of the room. Several nymphs, young immature grasshoppers, were kept for one-half hour in a temperature of —30° C., then the temperature surrounding them was gradually raised during the following eighteen hours to that of the room. Respiration did not return but the heart action did. Probably those that were subjected to —30° C., or even —100° C. would have recovered if the temperature had been more gradually raised during several days to that of the room.

SUMMARY

Normal grasshoppers can live under most favorable conditions in the laboratory twenty-five days, and under the same conditions but without food three to five days; whereas the isolated heart beats nine hours if unprotected, but if kept moist with Ringer's solution it will beat for two days.

· In vitiated air in sealed tubes the normal grasshopper lives two days, but the heart beats as long as it does in fresh air only more slowly and at irregular intervals.

In carbon dioxide the heart and respiratory movements cease within a minute and may remain inactive as long as two days, and then when the specimens are placed in fresh air for about two hours the movements are again resumed. Carbon dioxide may act upon the cardiac cells and nerve centers as a reaction product, inhibiting enzymes or metabolic activity in the cells. Carbon monoxide is slightly more toxic than carbon dioxide in its effect upon the heart and respiratory centers.

Hydrogen has a most remarkable effect upon the respiratory and cardiac action. For the first few hours it has an inhibitory influence upon the heart and respiratory movements both in the normal and isolated states. After the comatose condition has lasted about four hours, the respiratory and cardiac actions revive, and in some cases the movements continue for four days. The contractions are conspicuous in consisting of long intervals of cessation alternating with shorter ones of activity. In moist hydrogen the activities cease sooner than in dry hydrogen. If the energy of the nerve, heart and muscle cells depends upon oxidative processes, then the necessary oxygen for the four days activity must have been supplied by an amount stored in some manner in the cells.

In normal grasshoppers and isolated hearts the contractions of the heart continued in some cases longer in oxygen than in air. It was observed, however, that periods of inactivity alternated with periods of strong respiratory contractions.

It is interesting to note that the cardiac cells can contract in a temperature as high as 66° C., and cease at about 0° C. Moreover, that they can withstand for at least half an hour, a temperature of —30° C., and probably if the thawing was carried on very slowly, they could withstand a temperature of —100° C.

Whether the heart tissue in the isolated heart is capable of contraction independent of the influence of intrinsic nerve cells, I have as yet not been able fully to determine, but hope soon to obtain definite proofs in regard to this question.