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THE PROCESS OF ORIENTATION IN THE COLONIAL ORGANISM, GONIUM PECTORALE, AND A STUDY OF THE STRUCTURE AND FUNCTION OF THE EYE-SPOT

S. O. MAST

From the Zoological Laboratory of the Johns Hopkins University

SIX FIGURES

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1. INTRODUCTION

Among the most machine-like of the activities in organisms is the process of orientation. It is consequently not surprising that this process, which is common to so many different species, has received much attention in the investigations on behavior, with the result that a mass of highly interesting and important facts regarding it has been collected. These facts seem to show that the process of orientation differs fundamentally in different organisms and that it is far more complicated than has been assumed by some investigators, but that in general it facilitates the life processes in the individuals possessing it, and consequently tends to perpetuate the species. As to the reduction of the process to mechanical principles even in its simplest form, and as to its relation to conscious phenomena, little more can be said than that the field here is still wide open, although prospects are not altogether discouraging.

Among the questions associated with the process of orientation concerning which there is at present much contention is

that referring to the nature of the orienting stimulus. Loeb and some of his followers hold that this stimulus is, in all organisms, animals as well as plants, dependent, in a specific way, upon the amount of stimulating energy received by the sensitive tissue, in accord with the Bunsen-Roscoe law. That is, that a given amount of stimulating energy (which is the product of the intensity of the agent and the time it acts) always produces the same effect no matter how these two factors may vary. Thus according to this idea a weak agent acting a long time should cause the same response as a strong one acting a short time.

This is one of the essential characteristics of a theory of orientation which will be referred to as the 'continuous-action' theory.

Darwin and others maintain that, in some cases at least, the orienting stimulus is dependent upon the time-rate of change of stimulating energy. That is, that if there is no change in such energy there will be no response, no matter how much energy may be received. Response in accord with this idea constitutes the most important feature of a theory of orientation which will be called the 'change-of-intensity' theory.

Our observations on the process of orientation in *Gonium* strongly support the latter theory. They also support the contention that the eye-spots function as direction eyes essentially as do the eyes in some of the flat-worms.

The reactions of the colonial organisms have not received much attention. *Volvox* is the only member of this group that has been extensively studied, and in this form only the responses to light have been thoroughly investigated (Mast '07). It will be expedient to present, in this connection, the essential features of these responses since we desire later to compare them with those observed in *Gonium*.

Volvox, like most of the simple green organisms, responds very definitely to light. It orients fairly accurately, and is usually positive in light of moderate intensity and negative in that of high intensity. Orientation is direct, that is, if the position of the source of light is changed after the colonies are oriented they always turn at once toward the light again (provided they are

positive) never in the opposite direction, as frequently occurs in *Euglena*, *Stentor* and the like. The turning of the colonies is due to an increase in the effective stroke of the flagella on the shaded side. So much has been definitely established. As to the cause of the increase in the activity of the flagella on this side we are, however, not in a position to speak with so much assurance, but our evidence seems to indicate that it is dependent upon the time-rate of change of light intensity on the photosensitive tissue in the individual zooids. Let us briefly consider this evidence.

During the process of locomotion the colonies continuously rotate on the longitudinal axis; consequently, when they are not oriented and opposite sides are unequally illuminated, the zooids are continuously transferred from a region of higher to one of lower light intensity and vice versa. This results in a decrease of intensity on each zooid, when it reaches the shaded side of the colony; but there is another factor involved in producing changes of intensity, in all probability of more significance than this. In unoriented colonies the zooids are not only continuously subjected to a transfer from one intensity of light to another, but during the transfer different surfaces become exposed; for when they are on the more highly illuminated side of the colony the outer surface, and when they are on the opposite side the inner surface is directed toward the source of light. Since the zooids contain numerous translucent and refractive bodies this change in the surface exposed necessarily results in numerous changes of intensity on the different parts of each zooid. The eye-spots are the most prominent of the translucent bodies mentioned and they are consequently of greatest importance in producing changes of intensity within the zooids. The orienting stimuli, in my opinion, depend upon the time-rate of change of intensity thus produced.

This opinion is strongly supported by the reactions to light observed in *Gonium*. These reactions are essentially like those in *Volvox*, but before describing them it will be necessary to refer briefly to the structure of the organisms under consideration.

2. STRUCTURE OF GONIUM

Gonium is a thin flat rectangular structure somewhat over 0.1 mm. wide. It consists of 16 cells or zoöids loosely united with protoplasmic strands which penetrate a gelatinous substance found in the intercellular spaces (fig. 1). Each zoöid contains among other things, a relatively large chloroplast, a prominent

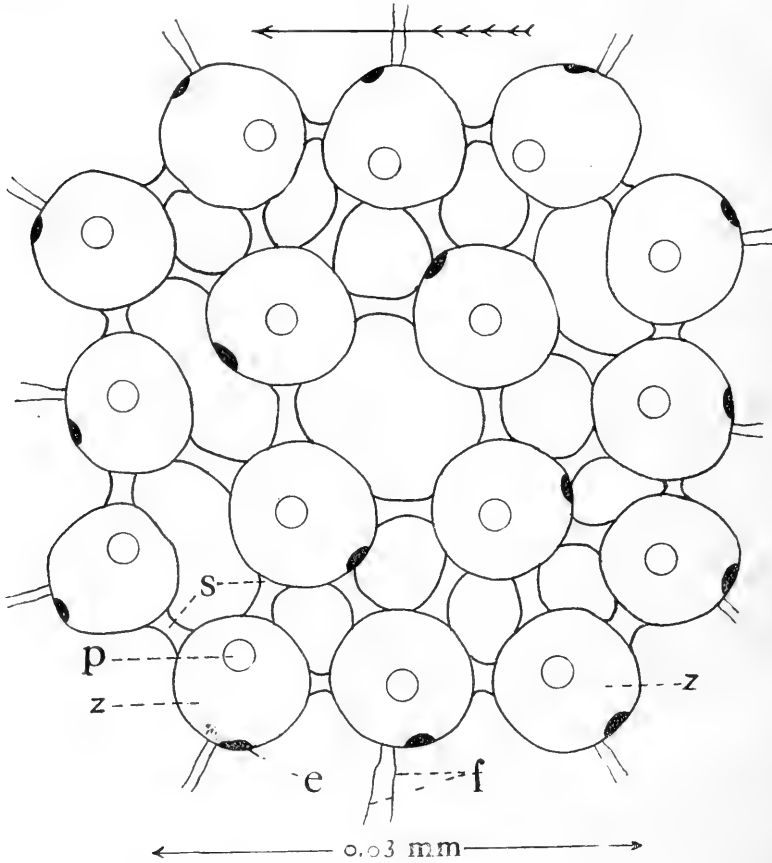


Fig. 1 Camera lucida drawing of Gonium as seen from the posterior surface. Each colony contains 16 zoöids, all situated in the same plane. *z*, zoöids; *e*, eyespot; *p*, pyrenoid; *s*, strands connecting the zoöids; *f*, flagella; *mm*, projected scale. The arrow indicates the usual direction of rotation. The eye-spots are located at the outer surface near the anterior end of the zoöids. Note that they are a little to one side of the middle of this surface.

eye-spot and two flagella which are about as long as the colonies are wide (fig. 2). The zoöids are slightly elongated and so situated that the longitudinal axes of all are nearly parallel to each other and approximately perpendicular to the flat surfaces of the colonies. The flagella extend from the anterior surface of the colony and the eye-spots are located near their base on the outer surface of the zoöids near the anterior end.

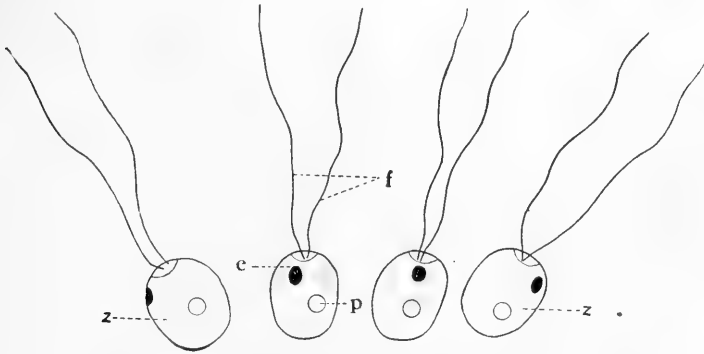


Fig. 2 Free hand sketch of *Gonium* as seen from one side. *z*, zoöids; *e*, eye-spot; *p*, pyrenoid; *f*, flagella. The eye-spots are situated on the outer surface of the zoöids near the anterior end. For details regarding the structure of the eye-spot, see figure 3.

3. STRUCTURE OF THE EYE-SPOT IN GONIUM, EUDORINA AND OTHER FORMS¹

Ever since the days of Ehrenberg ('31) the eye-spots or stigmata, as they are frequently called, have been looked upon by many as the most primitive eyes. They have consequently been of great interest especially to those concerned with the evolution of the visual apparatus in the higher forms. They have been described in many different organisms by various investigators. Among these Franzé ('93) probably made the most extensive studies. He investigated them in 31 different species.

Practically all of those who have worked on the eye-spots maintain that they consist of two essentially different substances, a

¹ This section is the result of histological studies made by Caswell Grave. It is a pleasure to acknowledge my great indebtedness to him for his generous assistance.

hyaline substance, globular or lenticular in form and a brownish opaque substance frequently somewhat cup-shaped. The latter, it is held, usually surrounds the former more or less completely.

Thus it appears that these structures resemble, somewhat, the eyes in turbellaria, rotifera and copepoda, and this is largely responsible for the conclusion frequently stated that the former are homologous with the latter. Franzé ('93, p. 162), however, opposes this contention. He says: "Die Augen der Turbellarien und Rotatorien sind keine Homologa der Stigmata, sondern die äusserliche Ähnlichkeit beider Differenzirungen wird durch die gleichen Funktionen bedingt." He, in common with a large proportion of other investigators, holds that the eye-spots function as light recipient organs.

While much of the work on the structure of the stigmata has been thorough, it was our opinion that with the application of modern histological technic it might be possible to discover elements in them that would throw light on their nature and function. With this in view colonies of *Gonium* and *Eudorina* were fixed in Bouin's and Fleming's fluids. Some were embedded in paraffin and cut into sections 2μ and 3μ in thickness and stained with iron haematoxylin and safranin. Others were mounted whole, some stained and some not. These preparations were thoroughly studied with a combination of No. 6 Comp. ocular and 2 mm. Apoch. Homog. Immersion objective and briefly with more efficient combinations. It was found that the eye-spots both in *Gonium* and in *Eudorina* consist of two parts, an opaque cup-like structure and a lens shaped hyaline structure (figs. 3 and 4) but no further details could be seen in them although the best lens systems made were used. This, of course, does not prove that there is no finer structure present. It merely indicates that if there is, it is ultra microscopic.

By referring to figures 3 and 4 it will be seen that the eye-spots in *Eudorina* are considerably larger than those in *Gonium*. These figures indicate that they are situated at the surface of the zooids with the hyaline portion outside. Careful observations seem to indicate that there is a thin protoplasmic layer, not represented in the figures, which is outside of this structure and

extends entirely around the zoöid and is continuous with the strands which connect them with each other (fig. 1). This layer of substance is more distinct in living colonies than in sections. Thus it is highly probably that all of the eye-spots in a colony have protoplasmic interconnections.

Superficially these eye-spots are very much like the primitive eyes in turbellaria and *Amphioxus* as will readily be seen by comparing figures 3 and 4 with figures 5 a and b. In the latter the

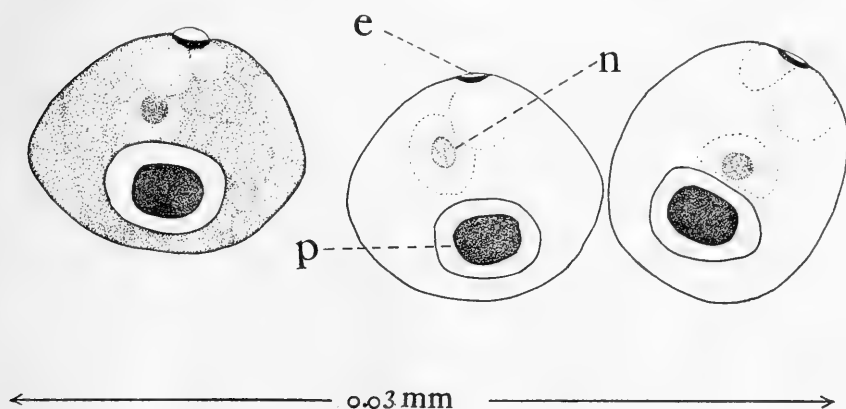


Fig. 3 Camera sketch of a section of *Gonium* taken perpendicular to the plane of the colony showing three zooids. Sections 3μ thick. No. 6 compensating ocular and $\frac{1}{12}$ homo. oil immersion objective. Enlarged 4 diameters with pentagraph. *e*, eye-spot; *p*, pyrinoid; *n*, nucleus; *mm*, projected scale. The eye-spot consists of an opaque saucer shaped structure and a hyaline lens-shaped body. It is less than 1μ in diameter. In the zooid to the left the razor passed nearly through the middle of the eye-spot; in the other two zooids it passed a little to one side of the middle, consequently the hyaline part appears relatively smaller in these. Drawn by Caswell Grave.

opaque part appears to function in restricting to certain areas, the field from which the sensitive hyaline position received light. Thus they seem to function as direction eyes. The eye-spots probably function in the same way. At any rate, these bodies in many species are so well differentiated and so similar in their structure and position in different individuals that they can not be looked upon merely as accumulation of waste products as is maintained by a considerable number of investigators.

4. PROCESS OF ORIENTATION IN GONIUM

The observations on orientation in *Gonium* were made in essentially the same way as those described in earlier works (Mast '11, pp. 92-96) it will consequently not be necessary to discuss methods here. The results of these observations follow:

Gonium swims in a fairly direct course with the flat surface perpendicular to the direction of motion. The surface with the

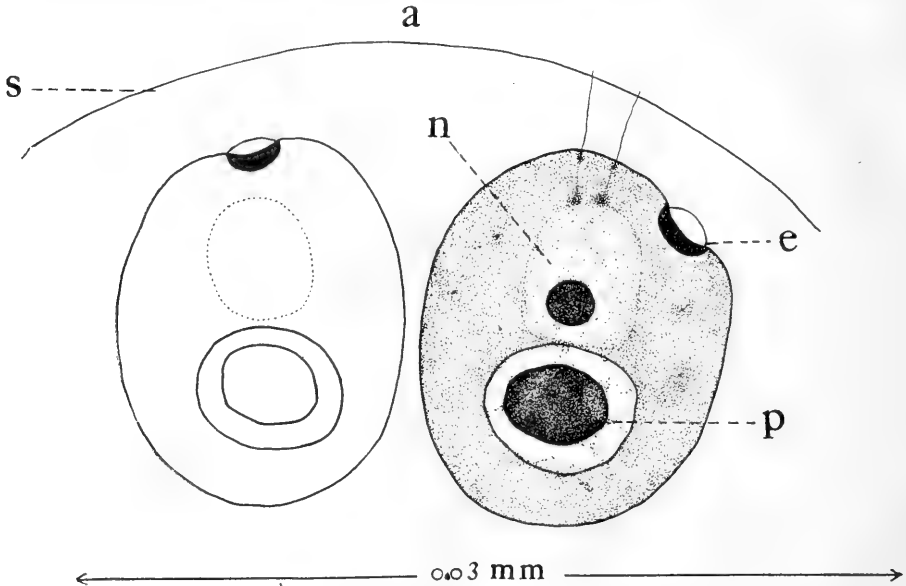


Fig. 4 Camera lucida sketch of a longitudinal section of *Eudorina* nearly through the middle showing two of the four anterior zooids. No. 6 comp. ocular; $\frac{1}{1\frac{1}{2}}$ homo. oil immersion objective. *a*, anterior end of colony; *s*, outer surface; *n*, nucleus; *p*, pyrinoid; *e*, eye-spot; *mm*, projected scale. The eye-spot in this form is essentially like that in *Gonium* but it is much larger and the two parts can be much more distinctly seen. The best lenses available fail, however, to reveal any differentiation in these two parts in either form. Drawn by Caswell Grave.

flagella is always ahead. As it proceeds it continuously rotates, usually counter-clock-wise as seen from the rear, although it reverses frequently and rotates in the opposite direction for short periods of time. It orients fairly accurately in light, being ordinarily positive in moderate and negative in strong illumination.

If the position of the source of light is changed after a colony is oriented so that the rays strike the anterior surface obliquely, it turns at once until the rays are again approximately perpendicular to this surface. The colony as a whole never turns in the wrong direction. Orientation is direct. The process is essentially the same as in *Volvox*. There is no indication of random movements or trial reactions in the colony as a whole.

The turning of the colony in the process of orientation is due to an increase in the activity of the flagellae of the zooids farthest from the source of light. The following evidence indicates that

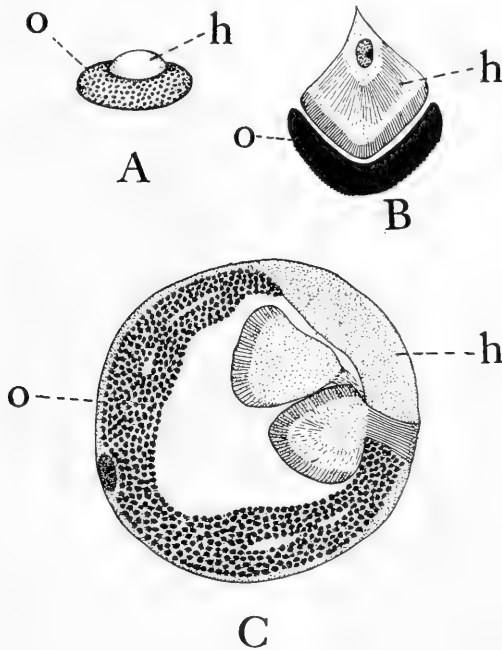


Fig. 5 A, Sketch of the eye of the turbellarian, *Ut. vulgaris* prepared by crushing a living specimen. After Wilhelmi (Taf. 15, fig. 4).

B, Sketch of a cross-section of the eye of *Amphioxus lanceolatus*. After Hesse (Taf. 24, fig. 8).

C, Sketch of a cross-section of the eye of the turbellarian, *Sabussowia diocia*. After Böhmig (Taf. 12, fig. 15). It can readily be seen that in all of these animals the eyes consist of two essentially different parts, just as do the eye-spots in *Gonium* and *Eudorina*, but that there is considerable differentiation in each part in the former while in the latter there is none that can be seen.

this increase in activity is due to a reduction of light energy on the sensitive tissue in the zoöids and that it is dependent upon the time-rate of reduction, not upon the absolute amount of reduction.

If the light intensity in a beam in which positive colonies are oriented is suddenly decreased without in any way changing the direction of the rays, the rate of movement for a short period of time suddenly increases, but if the intensity is suddenly increased there is no response. In negative colonies, however, just the opposite is true. They respond in precisely the same way to a sudden increase but not to a sudden decrease of intensity. This response of the colonies is very striking. It gives one the impression of a very marked forward spring, and seems to be in all essentials like the shock-reactions in *Euglena*. And just as in *Euglena* it does not occur if the light-energy is gradually changed. Obviously then, this response is dependent upon the time-rate of change of energy and not upon the absolute change.

The increase in activity in the zoöids farthest from the source of light, during the process of orientation, appears to be of precisely the same nature as the increase in activity of all the zoöids, due to a sudden decrease of the light-intensity in the entire field; consequently it would seem reasonable to conclude that it also is due to a sudden decrease of intensity on the sensitive tissue in the zoöids involved. How can this occur?

In unoriented colonies the light strikes the anterior face obliquely (fig. 6), and as these colonies rotate it is evident, just as in *Volvox*, that in each zoöid the surface exposed to the light continuously changes. This necessarily causes changes of intensity owing to the movement of the shadows cast by the translucent bodies in the zoöids, particularly the opaque portion of the eye-spots. By referring to figure 6 it will be seen that in the zoöids on the side of the colony nearest the sources of light the hyaline portion of the eye-spot is fully exposed, while in those on the opposite side this structure is shaded by the opaque portion. There is consequently a great reduction in the intensity of the light on it as the zoöids, owing to the rotation of the colony, are transferred from the former to the latter position and an equally

great increase as they are brought back to the original position again. If, then, the photo-sensitive tissue is largely confined to this hyaline substance as it seems to be in *Euglena*, we should

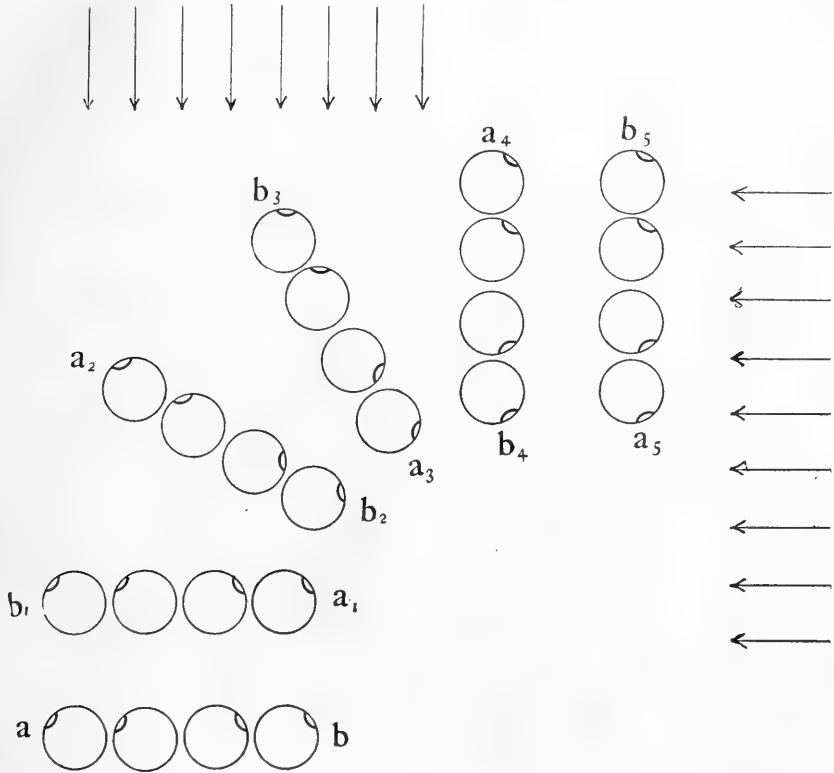


Fig. 6 Diagrammatic sketch representing the process of orientation in a colony of *Gonium* as seen from the side. Each circle represents a zoöid; the small arcs represent the eye-spots, the arrows the direction of the rays of light, and *a-b*, *a₁-b₁*, *a₂-b₂*, etc., different positions assumed by the colony during the process of orientation. Only 4 of the 16 zoöids in the colony are shown. The colony rotates on its antero-posterior axis as it proceeds. This causes the hyaline portion of the eye-spot to become alternately fully exposed to the light and shaded. The turning of the colony is due to an increase in activity of the zoöids as they are transferred to a position in which the hyaline part of the eye-spots is shaded, that is, the positions represented by *a*, *b₁*, *a₂*, *b₃*, etc. The hyaline part is probably highly sensitive to light, and the increase in activity mentioned is probably dependent upon the time-rate of reduction in illumination on this part.

expect in positive specimens a shock-reaction in the zoöids on the side of the colony farthest from the source of light, owing to the shading of this substance, but none on the opposite side where this substance becomes exposed to the light. This is precisely what is observed in the process of orientation. In colonies in the negative state, on the other hand, we should expect just the reverse and this is also in accord with our observations. Moreover, as the colony turns toward the light the change of intensity on the hyaline portion of the eye-spot becomes gradually less and when it has turned enough so that it directly faces the light, that is, when it is oriented, there is no longer any change, and consequently on the basis of our assumption no more shock-reactions and no further turning would be expected. This is again in accord with our observations. If, then, the photo-sensitive substance is confined to the hyaline portion of the eye-spot, or largely so, and if the orienting stimulus is dependent upon the time-rate of change of light-intensity on this substance, we can account for the observed reactions in the process of orientation, in *Gonium*, and this is in full accord with our explanation of orientation in *Stentor*, *Euglena*, and a number of other organisms (Mast, '11, pp. 80-135). Moreover, if our assumptions are correct, it is no longer necessary to hold, as in earlier publications (Mast '11, p. 133), that the function of the eye-spot is not the same in all organisms. But how on the basis of these assumptions is it possible to explain the fact that after the colony is oriented it continues in a fairly direct course toward the light?

This question has already been answered, in part, in the statement that after the colonies are oriented changes of light intensity on the hyaline substance in the eye-spots cease, and consequently, if our explanation of orientation holds, no further turning would be expected, and the colonies should therefore remain oriented, unless some factors other than the light in which they are oriented cause them to turn. And if this should occur the orienting stimulus would, in accord with our explanation, again act, and result in reorientation. This explanation is based upon the well known principle that organisms not subjected to lateral stimulation tend to move in direct paths. To account for con-

tinued orientation; it is consequently not necessary to assume that the orienting stimulus continues to act after orientation as well as during the process of orientation, as is demanded by the continuous-action theory. Organisms are partially isolated dynamic systems and much that they do is dependent upon changes within, quite independent of immediate environmental factors.

5. DISCUSSION

We have in this and in previous publications presented a considerable amount of evidence in favor of the change-of-intensity theory of orientation. Let us now briefly consider the evidence that favors the continuous-action theory.

In the reactions of the unicellular and the colonial forms very little has been discovered that supports this theory. In fact practically all of the favorable evidence is found in Bancroft's work on *Euglena* ('13). Bancroft maintains that he has demonstrated that in this form orientation occurs in accord with Loeb's continuous-action theory; at any rate that the change-of-intensity theory does not hold. I need not here enter upon a discussion of Bancroft's interesting observations, for I have elsewhere shown ('14) that his results must be confirmed under conditions more thoroughly controlled before much dependence can be placed upon them and that if his contentions are valid they actually oppose the theory that he substitutes for the one he claims to have overthrown; that is, they oppose the continuous-action theory.

Thus we see that the evidence in support of the continuous-action theory found in the reactions of the forms mentioned is exceedingly weak. In the reactions of some of the more complex organisms there is, however, some evidence indicating that this theory holds at least in part. Blaauw ('08), Fröschel ('08 and '10), Arisz ('11), and Clark ('13) have demonstrated that photic orientations, in a number of different seedlings, is within certain limits, dependent upon the amount of light energy received; that is, that long exposure in weak light produces the same effect as short exposure in strong light. Mast ('11, p. 163) reached

conclusions regarding the orientation of Eudendrium which are in harmony with the work just mentioned, and Loeb and Ewald ('14) support these conclusions. Ewald ('14) also maintains that certain responses in *Daphnia* are proportional to the amount of stimulating energy; at any rate that they are not dependent upon the time-rate-of-change of such energy. Patten ('14) comes to the same conclusion regarding orientation in blow-fly larvae. He says (p. 272): "Orientation in the blow-fly larva depends to a large extent on the stimulating effect of constant intensity. The reaction to light of constant intensity follows the Bunsen-Roscoe law."

Both Ewald and Patten base their conclusions upon the fact that the reactions observed at the intersection of two beams of light were the same when the light in one beam was intermittent, as they were when it was constant in both, provided the relative amount of energy in the two beams was the same under both conditions, and provided that the intermission was relatively frequent and continuous.

It seems to me that these results do not show that the reactions referred to in *Daphnia* and the process of orientation in the blow-fly larvae are necessarily dependent upon the continuous action of the light as maintained by Ewald and Patten. All that they actually demonstrate is that if the intermission is of sufficient frequency, periodic illumination acts the same as continuous illumination. This is true for the human eye and yet no one holds that this in itself precludes the possibility that stimulation is dependent upon time-rate of change of energy. Moreover, Patten in his work on the fly larvae did not eliminate changes of intensity on the sensitive tissues in the larvae due to the alternate extension and retraction of the anterior end, consequently the reactions observed in the process of orientation in these animals may have been due to these changes without reference to the amount of light energy received.

Patten's conclusion (p. 272) that "orientation to light from two sources depends on the relative amount of stimulation received by symmetrically located sensitive areas" is equally precarious. As a matter of fact, all of the responses which he main-

tains favor this conclusion could be accounted for on the basis of the change-of-intensity theory, even if the photo-sensitive tissue were confined to a single median spot such that there could be no balancing of effects on symmetrically located tissues.

This author, moreover, comes to another conclusion that seems to be supported by neither logic nor fact. He says (p. 271): "Bancroft's ('13) work, in which he showed not only that there was a distinct reaction to constant intensity present in *Euglena* but that it was largely the reaction to constant intensity which determined its orientation, shows the untenability of Mast's sweeping statement in one of the forms on which Mast himself worked." What is this sweeping statement? Our author quotes it as follows (p. 270):

Mast ('11, p. 234) says: There is no conclusive evidence, except perhaps in animals with image forming eyes, showing that light acts continuously as a directive stimulus, that symmetrically located sides are continuously stimulated (p. 235). Light no doubt acts on organisms without a change of intensity much as constant temperature does, making them more or less active and inducing changes in the sense of orientation; but there is no conclusive evidence showing that light acting thus ever functions in the process of orientation.

Is it not perfectly obvious that the results of Bancroft's investigations presented in 1913, assuming that they are as quoted above, do not have the slightest bearing on the validity of this statement, made in 1911? Does the discovery of a certain response at a given time make untenable the statement that it had not previously been discovered?

Whatever the final conclusion may be regarding the two theories of orientation in question the fact that many reactions in animate systems depend upon the time-rate-of-change of stimulating energy is well established. These reactions are of great interest, partly because they are exceedingly rare in inanimate systems, and a thorough study of them cannot fail to yield interesting results.

6. SUMMARY

1) The eye-spots in both *Gonium* and *Eudorina* consist of an opaque cup-shaped part and a hyaline lens-shaped part. The latter is partially surrounded by the former and it is probably relatively very sensitive to changes in light-intensity. These changes are probably largely due to shadows produced by the opaque part.

2) Orientation in *Gonium* is direct. The colonies never turn in the wrong direction, as often occurs in *Euglena*, *Stentor*, and many other forms. The turning which results in orientation is due to an increase in the activity of the flagella on the zooids farthest from the sources of light. In these zooids the hyaline part of the eye-spot is shaded by the opaque part at the time the activity of the flagella increases.

3) If the light-intensity of the field is suddenly decreased, the rate of locomotion, in positive colonies, suddenly increases. But if it is slowly decreased or if it is increased there is no response. In negative colonies, however, just the reverse is true. They respond to a sudden increase in rate of locomotion if the illumination is decreased but not if it is increased.

4) The increase in the activity of the zooids on one side of the colony during the process of orientation is apparently of the same nature as the increase in the activity of the whole colony when the illumination is changed. This indicates that orientation in these organisms is dependent upon the time-rate of change of light energy on the photo-sensitive substance, probably the hyaline portion of the eye-spots, and not upon the absolute change or the continuous-action of light.

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RESULTS OF CONTINUED SELECTION IN HYDRA

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In a recent paper¹ I reported an attempt to obtain a modified clone of *Hydra viridis* by the continued selection of variates differing in the number of tentacles. In that experiment a clone was bred from a single wild polyp and two groups of its descendants, each composed of 25 lines, were selected for variations in tentacle number in opposite directions from the mean of the clone. Selection was continued for several generations, then the number of tentacles of all buds produced by the last selected generation of the 50 lines was recorded and the averages of the two groups were compared. A slight difference, in the direction of selection, was found in the averages of the earlier buds of the two groups but this difference did not persist in the buds produced later by the same parents; regression was complete in a single generation.

The chief criticism of such a negative result in a selection experiment is based upon the supposition that in a single character of a species some continuous variations are, others are not inherited. If this is true, any selected individual may be either a germinal variate, in which case it will contribute to the progressive change in the racial character; a somatic variate, in which case its selection will not alter the racial type; or a variate in which both somatic and germinal variations are combined but active in opposite directions, thus leading to an effect of selection the reverse of that expected from the somatic character. It is further assumed that somatic variation follows more frequently than it contradicts germinal variation, giving natural selection an opportunity to produce evolution when applied to a large

¹ Inheritance in the Asexual Reproduction of *Hydra*. Jour. Exp. Zoöl., vol. 19, pp. 157-210, 1915.

number of individuals. When selection involves only a few individuals the chance selection of a somatic variate or a composite variate of the third type above may counteract the effect of previous selection. Only when enough individuals are selected to average out the composite variates can the results be relied upon.

It should be pointed out that if this criticism is carried out consistently it results in practical experimental indeterminism, since it may be said of any experiment that it is much less extensive than the course of evolution and is therefore not conclusive. Perhaps the only answer that can be given to the criticism is a selection experiment extensive enough to make the chance reversal of selection improbable and throw the burden of proof upon those who advance the criticism. The selection experiment with *Hydra* has been prolonged until it now seems to meet this requirement.

EXPERIMENTAL DATA

Essentially the same technique was used as in the earlier experiment. Each polyp was kept in a separate Stender dish; the food supply, *Cyclops*, was distributed as uniformly as possible to all polyps; the dishes were cleaned and sterilized and the culture fluid renewed every second day, except during the winter months when the polyps were not budding. New buds were removed from the cultures and recorded every second day.

The first experiment, that previously reported, was brought to an end by the freezing of the food pond and for the ensuing three months very little food could be obtained for the polyps. As a result only 6 of the original 50 lines survived the winter, these being represented by members of the last selected generation. When food was again available all descendants of these polyps were kept until 24 members of each of the groups, varying in the required direction, were obtained. From these, 48 lines were established, half of which were selected in each direction from the mean number of tentacles of the entire clone. During the heat of the summer many lines died and were replaced from others of the same group so that at the end of the experiment only 20

TABLE 1.

Relationships and initial numbers of tentacles of all selected Hydras in the lines which survived throughout the experiment. The successive figures from left to right represent the numbers of tentacles of the parents in successive generations. The braces show where two or more offspring of a single parent were retained. The number of tentacles of the final generation from which the unselected buds were obtained is given at the extreme right.

7-7-7	6-7-7-7-7-7-7-7-7	6-7.....	7
		6-8.....	8
	5-7-7-7-7-6-7	7	7
		6	7
	6-7-7-7-7	6-7.....	7
		7	7
	6-7-7-7-7-7-7	7	7
		7	7
	6-7-7-7-7-7	7	7
		7	7
	6-7-7-7-7-7-7	6-7.....	8
		6-7.....	7
	6-7-7-7-7-7-7	6	7
		6	7
	7-7-7-7-7-7-7	7	7
7		7	
6-7-7-7-7-7	7-7.....	7	
	6-7-7.....	7	
6-7-7-7-7-7	7-7-7.....	8	
	7-7 7.....	8	
6-7 7-7-7-7-7	6	7	
	7-7.....	7	
6-7 7-7-7-7-7	7-7.....	7	
	7.....	7	
6-6-5-4-6	7-5-5-5-5-6-4-6-6-6-6-6-5-6-5	5	5
		5-6-4-6-6-6-6	6
	6-5-5-5-6-6-5-5-4-5-5-6-6-6	6-6-6-6-6-6-6-6-6	6
		6-6-6-6-6-6-6-5	6
	6-6-6-6-5-6	6-6-6-6-6-6-6-6	6
		6-6-6-6-6-6-6-5	6
	5-6-6-5-6-6-6-6-5-5-5-6	5-6-6-6-6	6
		6-6-6-6-6	6
	8-6-5-6-6-6-6-5-6-6	6-6-6-6-6	6
		6-6-6-6-6	6
	6-5-6-6-6-5-6-6-6-5-6-6	6-6-5-6-6-6	6
		5-5-6-5-6-6-6	6
	5-6-5-4-6-6-6-6-6	6-6-6-6-6	6
		6-6-5-5-6	6
	6-5-6-6-6-5-6-6-6	5-6-6-6	6
6-6-6		6	
6-5-6-5-6-5-5-4-6-6-5-5-6-6	6-6-6	6	
	6-6-6	6	
6-5-5-6-5-4-4-6-6-6-6-6-6-6	4-6-6-5-6-5	6	
	5-6-6-6-6-6-6	6	
6-6-6-4-6-6	5-6-6	6	
	6-6-6-5-6-5	6	
5-6-6	5-6	6	
	6-6-6-5-5	6	
6-6-6-5-3-5	6-6-6	6	
	5-6-6-5-3-5	5	

distinct lines of descent were represented. The complete history of selection is given in table 1.

When approaching winter again threatened a reduction of the food supply, selection was stopped and the initial numbers of tentacles of all buds produced by the last selected generation were recorded, until at least ten buds had been obtained from each of the 48 lines. These were bred under conditions as nearly uniform for the two groups as it is possible to make them for animals with such complex food habits as Hydra. The data obtained from these buds furnished the basis for a trial of the effects of the preceding selection.

The entire experiment extended over somewhat more than a year. After the test previously reported, selection was continued for an average of 17.13 generations in the group selected for 6 or less tentacles and for an average of 9.38 generations in the group selected for 7 or more, an average of 13.25 generations for the two groups. The greater number of generations in the group selected for few tentacles is due to the fact that buds with few tentacles are produced early in each fraternity while buds with many tentacles appear only after the parents have fully matured. Before the first test an average of 6 selected generations was obtained and this, with the 13.25 later selections, gives an average of 19.25 generations rigidly selected during the experiment. The average number of tentacles of all selected parents of the minus-selected group is 5.567, that of the plus-selected group is 6.888, giving an average difference of 1.321 tentacles per generation as the extent of selection. A total of 366 buds was obtained from the last selected generation of the group selected for 6 or less tentacles, and of 358 in the group selected for 7 or more. The distribution of variations in the initial number of tentacles of these unselected buds is given in table 2. The constants determined from the table are:

	<i>Mean</i>	σ
For the group selected for 6 or less.....	6.584 \pm 0.022	0.6408 tentacles
For the group selected for 7 or more.....	6.544 \pm 0.023	0.6569 tentacles
Difference.....	0.040 \pm 0.031	

TABLE 2.

Distribution of variations in the number of tentacles of buds produced by the last selected generation

NUMBER OF POLYPS	NUMBER OF TENTACLES						
	4	5	6	7	8	9	
Ancestry with 6 or less tentacles		6	162	177	20	1	366
Ancestry with 7 or more tentacles	1	12	152	177	16		358

The difference in the average number of tentacles (0.040) is not significant but the little that appears is in a direction the reverse of that to be expected if variations within the clone are inherited.

In a consideration of diverse races the range and extent of variation may be of equal importance with the mean. The standard deviations (σ), measures of the amount of variability, are not significantly different for the two groups. The range of variation must be considered in two ways; first, with respect to individual polyps, second, with respect to variation in the mean number of tentacles of different lines. As appears in table 2, the polyp with the fewest tentacles (4) appeared in the group selected for a large number, that with the most (9) in the other group. The range of variation of individual polyps, like the difference between the averages, is the reverse of that which should appear if selection were effective. The mean number of tentacles of all buds produced by each parent in the last selected generation is given in table 3, where the constants are arranged in the order of magnitude. Here again the line with the highest average belongs to the group selected for few tentacles and that with the lowest to the group selected for many, although the differences are not significant.

Most theories of evolution by continuous variation assume that the range of variation increases as the mean of the selected group diverges from the racial mean, so that the average of the selected group may be brought beyond the original range of the race by the continued selection of extremes. In this experiment no widening of the range was found: the extent of selection, the differ-

TABLE 3.

Mean number of tentacles of all buds produced during the time of cultivation by each parent of the last selected generation (last column, table 1). Not less than ten buds were obtained from each parent

NO. OF TENTACLES OF PARENTS	7+	6-	7+	6-	7+	6-
Mean number of tentacles of buds.	6.00	6.18	6.44	6.47	6.69	6.66
	6.05	6.28	6.46	6.50	6.72	6.66
	6.08	6.33	6.61	6.50	6.75	6.67
	6.20	6.43	6.65	6.50	6.75	6.68
	6.29	6.43	6.66	6.60	6.84	6.75
	6.37	6.43	6.66	6.61	6.85	6.83
	6.40	6.46	6.66	6.63	6.90	6.86
	6.42	6.46	6.68	6.65	6.92	6.93

ence between the selected members of the two groups, remained fairly constant throughout the experiment.

DISCUSSION

Clearly this experiment offers no positive evidence for evolution by the accumulation of continuous variations. It remains to determine its value as evidence against such a method of evolution. This may best be done by a consideration of the sort of hereditary changes which such an experiment should reveal. The figure, 1.321, determined as the average extent of selection in each generation, makes it possible to compute roughly the theoretical effect of selection through any number of generations for any given strength of heredity, provided that selection of somatic variates does not interfere during the experiment. The number of buds obtained from the last selected generation is great enough to give a probable error for the difference between the groups of only 0.031 tentacles. From this, the probability that any expected difference between the selected groups would be revealed by the experiment may be computed. If we assume that the offspring inherit one-tenth of the variation of their parents, the theoretical result of 19 generations of selection at a difference of 1.321 tentacles per generation is a difference of 1.176 tentacles between the offspring of the last selected generations of the two groups. This is 38 times the probable error of the dif-

ference found, which means that the true value of the difference could fall so far from the value given by the sample only once in some billions of samples; a certainty that no such difference was produced by selection in this experiment. Even with a strength of heredity of only 0.01, nineteen generations of selection result in a diversity of more than 0.2 tentacle which is almost 7 times the probable error so that the chances are more than 400,000 to 1 that no such diversity would be obtained in any given experiment of the same magnitude. If all variations are inherited to the same extent, if selection of every variation contributes to evolution in proportion to the extent of the variation from the mean of the race, then the strength of heredity in Hydra must be considerably less than that represented by a coefficient of ancestral inheritance of 0.01.

If not all variations are inherited, the present experiment may have failed to reveal a greater strength of heredity than this, but if even three-fourths of the variates selected were strictly somatic, a regression of 0.05 would almost certainly have been revealed: The inclusion of so many somatic variates would reduce the number of effective selections to one-fourth and a regression of 0.05 should result in a diversity of 0.29 tentacle after five selections. If it is assumed that regression is as great as 0.30, which is approximately that ascribed to Hydra by Pearson, the percentage of variations which are inherited to this extent must sink below 10 per cent to escape detection by this experiment.

These figures are of interest chiefly for a comparison with the theory of homotyposis and are sufficient to disprove, for Hydra, the theory as advanced by Pearson in 1901 and 1910,² and make it possible to state with assurance that if inheritance of variations in number of tentacles occurs within the clone of Hydra it does not follow biometrical laws. For other theories, which hold that continuous variations are inherited but do not define the method or extent of such heredity, it can only be pointed out that if any hereditary variations occur in Hydra they must

² On the Principle of Homotyposis. Phil. Trans. Roy. Soc. London, vol. 197, pp. 443-459, 1901. Darwinism, Biometry, and Some Recent Biology. Biometrika, vol. 7, pp. 368-385, 1910.

amount to less than one one-hundredth of the total variation. The difficulty of accounting physiologically for such a condition, upon other than the mutation theory, and the lack of any positive evidence of such heredity suggest that the most tenable position is one which assumes that the continuous variations of Hydra are not inherited in asexual reproduction but are wholly the result of the interaction of the constant reaction-norm of the clone with a fluctuating environment.

THE EFFECTS OF THE REMOVAL OF THE NASAL PITS IN AMBLYSTOMA EMBRYOS

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FOURTEEN FIGURES (THREE PLATES)

INTRODUCTION

Many of the difficulties encountered in the study of the nervous system by the usual methods are obviated by experiments upon embryonic material in which nerve fibers are not yet developed and the blood has not begun to circulate. In this way it is possible to observe the effect of the removal of an end organ upon the central nervous system without undue complication of the factors involved, and furthermore such a method makes possible the study of the formative influence one part may exert on another during ontogeny.

The number of investigators who have applied this method is relatively small.

In 1906 Braus extirpated the forelimbs of the larvae of *Bombinator* before the outgrowth of the brachial plexus, with a view to determining the effect of the absence of the limb on the ventral horn of the spinal cord at the level of the brachial plexus. The experiment showed that there was at first no observable effect on the cord. The brachial nerves grew out into the surrounding tissue and ended more or less blindly. The size and number of the motor cells of the ventral horn was in no distinguishable manner changed. But this investigator found that when the operated larvae were kept alive until just before metamorphosis and then killed, a distinct reduction in the size of the cells of the ventral horn was discernible. Hence, he concluded, as a corollary to the theory proposed by Roux in 1885, that the development of the central nervous system was readily divisible into

two periods, in the first of which growth and differentiation was independent of functional activity, but in the second of which functional activity determined whether or not growth would continue. But as Forel had previously pointed out, the atrophy which made the distinction in size of the cells evident was an exceedingly slow process as evidenced by the fact that this distinction did not occur until just before metamorphosis.

Two other investigators may be mentioned here, who have worked along this line. In 1909 Shorey performed a similar series of experiments on chick and amphibian embryos. She found in the chick an almost immediate effect of the destruction of the limb bud on the size of the ventral horn at the limb level. But whereas Braus concluded that the neurones were self-differentiating and that the reduction in size was a secondary one due to the absence of function, Shorey argued that the neurones did not differentiate without the stimulus of function and environment.

So far as it has been possible to ascertain, Dürken ('11) is the only investigator who has attempted to carry this question further to a consideration of the effect of the absence of an end organ on the gross morphology of the brain. He found very extensive changes in the shape and organization of the brain of *Rana fusca* and *R. esculenta* when the fore or hind limbs were extirpated at an early age. The abnormalities affected not only the somatic motor areas, but also parts of the diencephalon and telencephalon, notably in the roof. As will be seen, the experiments with which the present report is concerned show no such fundamental upheavals.

The last two named investigators, while using embryonic material, worked with individuals in which the peripheral nervous system had already partly developed, and it is suggested that the fundamental discrepancy between their results and those that follow may be due to this factor.

From the experiments of Braus it is evident that there is a possibility that Gudden's atrophy, when it can be induced, would make possible the tracing of a given system of nerve fibers from its peripheral ending to its cortical origin with all its collateral

connections. The problem lies in making a lesion that will cause Gudden's atrophy, and then in keeping the animal alive sufficiently long for the atrophy to reach its height.

In the spring of 1911, Dr. R. G. Harrison suggested that the removal of the nasal placode of *Amphibia* would be a practical method to attack such problems. Frog larvae were first used for the experiments but as Bell showed in 1907 the placodes often regenerated, a fact which would destroy the value of the material for this study. Bell found this regeneration occurred in a large number of cases, even where it was believed the placode had been entirely removed. This regeneration may have been due to the fact that the regenerating area about the placode was not removed.

In *Amblystoma*, the placode can be easily extirpated. The nasal Anlage is readily distinguished from the surrounding tissue, and hence complete removal is a relatively simple matter.

In the course of the experiments it was found that the extirpation of the nasal placode made possible not only a study of the neurological problems involved, but also a part, at least, of the developmental mechanics of the skull. Since the material for this morphological work was kept alive for some months, a study of the reactions to food stimuli of the operated and normal forms was also undertaken. These three aspects of the problem form the body of the following report. Owing to the difficulty encountered in keeping operated forms alive through metamorphosis, the report of the histological changes in the telencephalon as a result of the operations must be deferred until later.

MATERIAL AND METHODS

Amblystoma larvae 5-6 mm. long, were used for all of the operations, of which two series were performed. In the first the nasal placode of the right side only was removed; in the second both were extirpated. The material obtained from the first series served as a basis for the morphological investigation—that from the second for the physiological experiments and as a check for the morphological study.

The operations were performed with a pair of iridectomy scissors under the binocular microscope. During the operation, and for the subsequent twenty-four hours, or until healing was accomplished, the embryos were kept in a 0.2-0.4 per cent salt solution. They were then removed to individual dishes in which the water was kept fresh. When the larvae were ready to feed they were removed to aquaria, balanced largely with *Lemna* and *Ceratophyllum*. Records were kept of the history of each operated specimen.

Care was taken in all of the operations not to injure the underlying forebrain, since any injury causes noticeable defects in the telencephalon.

In the series of experiments under consideration a large number of unilateral operations were performed. The material thus obtained was killed at frequent intervals, the oldest larvae being about six months old dating from the time of operation, and the youngest only a few hours old. The killing fluid used was sublimate acetic. Ehrlich's Haematoxylin and Congo-red were used to stain the 10 μ sections. Normal material was subjected to the same technique for controls. From the sections of the oldest larvae a wax reconstruction was made of the rostral part of the skull and brain by the Born method.

The experimental investigations that have up to the present time been reported on Amphibia, have tended to show that the Anlage of the nose readily regenerates when parts are removed. Bell ('07) goes so far as to assert that the complete extirpation of the nasal anlage in the frog does not prevent the regeneration of a nasal sac. In the few cases he reports, what he believed was complete extirpation resulted in every case in a regenerated sac, sometimes perfectly normal in size and structure, and in other instances, smaller than its fellow.

The present series of experiments started with the extirpation of the nasal placode of *Amblystoma*. It became evident at once that careful and complete extirpation of the rudiment was not followed by regeneration. Of the two hundred and thirty-two operations performed, only four showed any external evidences

of a regenerating capsule. Later study by means of sections added two more to the number.

A number of experiments were then performed on the frog embryo to see if there was any difference in the behavior of the nasal anlage in two such closely allied forms. Great care was taken to remove all of the nasal placode. It was apparent from the outset that the placode of the frog is not nearly as compact nor so clearly differentiated from the surrounding ectoderm and not so easily separated from the underlying mesenchyme as it is in *Amblystoma*. Hence the complete extirpation is a much more difficult and delicate matter. External inspection showed twenty-two regenerating capsules out of seventy-six operations. Sections of a number of the remaining fifty-four taken at random showed an occasional abortive pit. It is evident then, that complete extirpation in the frog does preclude regeneration quite as completely as in *Amblystoma*. It is possible that here, as in the regeneration of the limbs, lens and gills, there is a circumscribed area about the anlagen which on the removal of the anlagen may regenerate it. Apparently in the frog this regenerating area is less restricted than in *Amblystoma*, if any such is present in the latter.

REACTIONS OF NORMAL AND OPERATED LARVAE

Turning now to a consideration of the experimental study of *Amblystoma*, it is obvious that by removing the nasal placodes of both sides previous to the formation of nerves, it is possible to obtain larvae which have never possessed functional olfactory organs. Thus is afforded excellent material for a comparative study of the reactions of noseless larvae and normal larvae to olfactory stimuli without the introduction of secondary factors due to shock or discomfort as a result of the operations.

Parker ('10) was the first to show conclusively that aquatic animals react positively to the olfactory stimulus of food. By suspending two bags in an aquarium, the one containing cheese cloth, the other bits of worm, he was able to detect distinct positive reactions of *Ameiurus* to the bag containing the food mate-

rial. When on the other hand, the olfactory nerves were severed, no positive reaction occurred. Since then Copeland ('12) has confirmed Parker's results with other fish.

Reese ('12) was the first to attack the problem in Amphibia. Working with *Diemyctylus*, he showed that the adults would follow and snap at moving bits, whether food or not. They would also make characteristic snapping movements when beef juice was squirted over the external nares. No attempt was made to control the sense of sight. He tried to control the sense of taste, however, by introducing a bit of cotton soaked in cocaine into the mouth. The results were conflicting and inconclusive. Animals in which the olfactory nerves were cut failed to respond to stimulation.

Copeland ('13) repeated the experiments of Reese, using more exact methods. Control of the visual sense was accomplished by stimulating the olfactory epithelium from a motionless source. By dividing the total reaction into two periods, during the first of which an approach was made to the source of the stimulus, and during the second, the object was snapped at or taken into the mouth, he came to the conclusion that the approaching reaction was due entirely to the sense of sight, the seizing alone to the sense of smell.

The object of the following experiments was to test this problem in *Amblystoma*, comparing the reactions of the normal larvae to food with those of the noseless.

There are in general three groups of sensory organs which conceivably may receive stimuli from the source of olfactory stimulus. These are, in the order of relative importance, the eyes, the taste buds and the lateral line and general cutaneous systems.

Stimulation of the latter systems can be eliminated by using a motionless source, since such stimulation is brought about by currents in the water. Such a source would at the same time give relatively little stimulus to the visual sense.

Control of the stimuli of the taste buds is hardly practicable experimentally without rather serious operations on adult forms. Fortunately it is known that the sense of taste is operative over

relatively limited areas and for concentrated fluids only, and hence the danger of stimulation may be minimized by keeping the source of the stimulus at a distance from the mouth (Herrick '08) and Sherrington ('06).

The most important sense, then that must be controlled, is the visual. This may be accomplished in two ways, (1) as stated above by keeping the source of the stimulus motionless and (2) by removing the optic vesicles at an early stage.

In the study of the reactions of *Amblystoma* to olfactory stimuli, more particularly to the stimulus of food, three sources of stimuli were used, two of which were olfactory and the third purely optic. Pieces of freshly killed *Amblystoma* larvae and live entomostraca were used to stimulate the olfactory centers; grains of sand, which possess no powers of olfactory stimulus, for the optic centers.

It was necessary to test as exactly as possible the visual reactions of the larvae since, as is evident to the most casual observer, under ordinary conditions, that sense is the most active in the capture of food. Under normal conditions where the food supply is abundant, individual larvae rarely move about, but remain motionless until a small crustacean comes within striking distance. The reactions of larvae under such conditions are quite characteristic. Resting motionless on the bottom of the aquarium, with body held above the debris and head elevated, it will, with a sudden contraction of the trunk muscles followed by a quick forward lurch, snap and engulf some particular crustacean whose movements carry it within striking distance.

When on the other hand, the supply of moving food is reduced, the larvae will forage the aquarium for food. Under these conditions the reactions are quite as characteristic as in the well stocked aquarium. The young *Amblystomas* crawl slowly around the aquarium, nosing here and there. The attitude is strikingly like that of a dog following a scent, and suggests that now in the absence of food that is moving, the sense of smell is actively used. Support is given to this suggestion by the fact that the larvae may often be seen to snap up some bit of the debris.

In the experimental study of this problem, sixteen normal and twenty-four operated larvae were used. Sixteen of the latter were noseless and eight were eyeless. These last were kindly loaned by Dr. Henry Laurens who performed the operations. As soon as the operated individuals as well as the normal ones isolated at the time of the operation began to feed regularly, experimentation was begun. At this time the larvae are about 1 cm. in length. The yolk has completely disappeared, the gills are plume-like arching forward over the head, the forelimbs are tridigitate, the rudiments of the fourth digit just beginning to appear, and the hind limbs are just noticeable as a slight elevation on either side of the cloaca. Experimentation with these young larvae was difficult because of the very great activity which they exhibited whenever there was any disturbance in the water. For that reason the following report deals with somewhat older larvae, the age varying from one and a half to six months.

The first problem was to determine the relative importance in the obtaining of food of the visual and olfactory sense. For this purpose grains of sand were used, dropped from a capillary pipette so as to fall within striking distance of the larva. A single individual was tested at a time. Four noseless and four normal larvae were subjected to these tests. The first were made when the larvae were five months old, dating from the operation. Another set of exactly similar tests were performed one month later.

The forty tests on the four normal larvae resulted in twenty reactions in which the sand grain was snapped at and engulfed, such reactions being designated as positive, and twenty in which no attention at all was paid to the sand. The percentage of positive reactions then, was fifty. Fifty-four tests on four noseless larvae resulted in thirty-nine positive reactions, a percentage of seventy-two. The same eight larvae tested one month later gave for the normal larvae eleven positive reactions out of forty tests, or 28 per cent and for the operated twenty-two out of forty-four, or 50 per cent (table 1).

TABLE 1
Reactions to moving sand grains

CONDITION OF LARVAE	REACTION	NO REACTION	PER CENT POSITIVE
Normal; 5 months.....	20	20	50
Normal, 6 months.....	11	29	27.5
Noseless, 5 months.....	39	15	71.7
Noseless, 6 months.....	22	22	50

It is quite evident then, that larvae, whether normal or operated, will snap at almost any object which in any way simulates the movements of the food, no matter whether it stimulates the olfactory sense or merely the visual. An interesting fact was noted in connection with the reaction of the noseless and normal larvae to the sand grains. As will be seen in table 1, there is a marked diminution in the total number of positive reactions of the older larvae as compared with the younger. While the number of reactions is far from sufficient to serve as a basis for any definite conclusion, the difference in behavior suggests that the larvae gradually adapt themselves to the new situation.

The second problem was to determine experimentally whether the olfactory sense played any part at all in the quest for food. Bits of beef were placed in the bottom of aquaria containing normal and operated individuals. The normal specimens would soon nose out the beef and engulf it; the noseless ones never. Exact records were not kept of these tests because the larvae did not thrive on the beef. They would often gorge themselves, much to their own detriment.

Since entomostraca make up the bulk of their natural food, these were used in a series of tests to determine the reaction to olfactory stimulus. By careful handling with a capillary pipette, it was possible to deposit individual Daphnids two or three millimeters from the head of the larvae, so that they would remain motionless on the withdrawal of the pipette. Ninety-five tests were performed on eight normal larvae; eighty-seven of these resulted in positive reactions, a percentage of ninety-two. One hundred and nineteen such tests performed on eight noseless

larvae resulted in not a single positive reaction, the individuals would invariably swim off without paying any attention to the Daphnid, or after a time the entomostracan would move away. The above experiments were performed on larvae varying from one and a half to four months old, dating from the time of operation, and indicate that these larvae do make use of the sense of smell in obtaining their food. This was further corroborated by the following tests on the eyeless forms (table 2).

TABLE 2
Reactions to motionless entomostraca

CONDITION OF LARVAE	REACTION	NO REACTION	PER CENT POSITIVE
Normal.....	87	8	91.6
Noseless.....	0	119	0.0
Eyeless.....	119	10	91.8

The eight eyeless larvae were subjected to the same tests. Out of one hundred and twenty-nine, one hundred and nineteen were positive, giving a percentage of ninety-two, a very close correspondence with the normal. Here the olfactory sense was the only one held in common by the normal and eyeless larvae that could receive stimulation.

A curious phenomenon, to be investigated later, was observed in connection with the eyeless individuals. They were very much more sensitive to currents in the water than the normal larvae. A pipette waved in the vicinity of the tail of the eyeless larvae would cause it to turn sharply around and snap at the pipette. No such extreme sensitiveness was observed in the normal larvae. The latter would snap at a pipette but only when waved with the characteristic jerky movement of its normal food. It is suggested that this peculiar action of the eyeless larvae is due to a compensatory hypersensitiveness of the lateral line and general cutaneous systems.

In the spring of 1914, one year after the above experiments were performed, a number of larvae, probably *A. opacum*, were brought into the laboratory from the field. These were about 30 mm. long—evidently developed from eggs laid the previous

season. Five of these were isolated and their feeding reactions tested with small bits of freshly killed *Amblystoma* larva. Sixty-three tests were made, fifty-two resulting in positive reactions, a percentage of eighty-three. The food was deposited by means of a capillary pipette some 5 to 6 mm. from the head of the larva. Copeland has pointed out that the reaction of *Diemyctylus* to food is a complex one involving an approaching reaction as a result of a visual stimulus and a seizing reaction due to the olfactory stimulus. In the present experiments on *Amblystoma*, it was often evident that the placing of the food attracted the attention of the larva. But the stimulus thus imparted was seldom followed by approach to the source. When it was, the test was excluded from the tabulation. If the larva became quiescent after the visual reaction, it would react positively in the majority of cases to the olfactory stimulus by snapping at the food, as indicated above.

In addition to the above tests, bits of larvae were placed in the aquarium as far from the individual under observation as possible, so that stimulation of the optic sense could be practically eliminated. In all of the cases, there was no immediate reaction, though the food was eventually discovered and eaten.

An interesting observation deserves mention in connection with these experiments. Powers in 1907, in studying variations in *A. trigrinum*, found that certain of the individuals collected, possessed a remarkable width of head with a correlated expanse of jaw and unusual development of teeth. After watching these individuals, he concluded that the differences "were due to the cannibalistic" habits of the larvae. They would quickly devour young larvae and snap off projecting parts of their fellows. He found only a few such individuals, and concluded the variation was rare.

The larvae in the laboratory at New Haven are probably of the species *punctatum*, though none have as yet been raised through metamorphosis. But all of these, whether reared from eggs in the laboratory or brought in already hatched, would readily eat one another, or such parts of limbs, gills and tail as were obtainable. This cannibalism did not, however, appear in

the larvae until some time after feeding had commenced. When it appeared, there seemed to be no correlation between it and the amount of normal food, entomostraca and plant life, available. Of those in the aquaria in the laboratory there were a few that could not be induced, even after starving, to touch parts of other larvae. The older larvae brought in were almost all maimed in some part or other. On being isolated, the missing parts were soon regenerated, producing perfect specimens. It is obvious, then, that cannibalism is not as rare in *A. punctatum* as Powers believed it to be in *A. triginum*.

MORPHOLOGICAL EFFECTS OF OPERATION

I. The skull

Normal development. The normal development of the chondrocranium of the Amphibia has been fully described. Platt ('97) has considered carefully the origin and early history of cartilage in the head of Necturns. The later development has been studied Winslow in 1898, by Wilder in 1903, and Gaupp, 1905. The normal development of the nasal cartilages, particularly in *Amblystoma* has been fully described by Terry in 1906.

In order to grasp fully the effect of the absence of nasal epithelium on the formation of cartilage in the head region, a brief account will be given at this point of the normal development as outlined by Terry and confirmed from the controls of the present series of experiments. Occasion, has been found, however, to change the nomenclature used by Terry, preference being given to the terminology of Gaupp ('05).

The first evidence of cartilage in the head region is the appearance of two centers of chondrification on the latero-ventral aspects of the diencephalon. From these centers the mesenchyme becomes chondrified in an antero-posterior direction forming two rods of cartilage, the trabeculae. The posterior growth does not concern us here and further consideration of it will be omitted.

Chondrification proceeds anteriorly until a cartilaginous rod is formed reaching to the anterior face of the olfactory sac. At

the same time the mesenchyme interposed between the eye and the brain is converted into a flat plate of cartilage, the crista trabeculae, by chondrification, which, starting from the tabecula, proceeds dorsally. In the early stages this plate does not extend anteriorly beyond the caudal end of the olfactory sac. With the formation of these cartilages, there appear two processes, one a lateral process—the antorbital, and the other a median process. The antorbital appearing first as a more or less straight rod projecting outward, eventually becomes hooked anteriorly about the posterior nares.

The median process projects medially from the trabecula, just in front of the anterior end of the telencephalon. It eventually meets and fuses with its fellow from the opposite side. This solid bar, whose caudal margin is later thinned out into the thin floor of the anterior part of the cranium, becomes the ethmoidal or anterior trabecular plate.

With the advancing chondrification of the trabeculae is correlated the formation of a cartilaginous plate underlying the nasal sac, supporting it and Jacobson's organ. This *solum nasi* extends under only the anterior two thirds of the olfactory organ.

During all these earlier stages of development the nasal placode is closely applied to the latero-ventral surface of the telencephalon. As growth proceeds, the sac begins to push anteriorly and at the same time laterally, leaving thus a clearly marked olfactory nerve. In the mesenchyme which lies above this early olfactory nerve there appears as a separate chondrification a small rod of cartilage—the ethmoid column. This grows in an antero-posterior direction, becoming fused posteriorly with the crista trabeculae. Anteriorly, it grows toward the trabecula sending a short process to meet it before attaining to its maximum length. In front of this process is formed the narrow slit, the medial incisure through which passes a branch of the *nasalis internus*. Subsequently this slit is reduced to the *foramen apicale*. The growth of the ethmoid column posteriorly to unite with the crista and anteriorly to join the trabecula forms a bridge over the olfactory nerve, which constitutes the roof of the *fenestra olfactoria* (fig. 8 *fo*). At the same time there appears the medial

nasal process. As is shown in figure 8 *mp.*, this is a thin plate of cartilage that lines the medial and anterior surface of the nasal capsule, between the sac and the premaxilla.

Correlated with the posterior growth of the ethmoid column is a lateral chondrification of the mesenchyme overlying the medial-dorsal aspect of the nasal sac. In this manner is formed a roughly rectangular plate of cartilage whose lower surface is concave to fit the dorsal aspect of the sac, and whose posterior edge marks in a general way the anterior boundary of the orbit. This is the tectum nasi (fig. 8 *tc.*).

The cartilages formed in the above manner almost completely surround the olfactory sac, only the region about the external and the internal nares is left uncovered.

Development after operation. The first visible effect of the removal of the nasal sac is a sinking in of the skin in the nasal region. In the case of the bilateral operations this is so marked as to give a veritable 'pug dog' effect, which increases with age. Figure 10, a drawing of a section through the head of a larva with a unilateral operation, shows how marked this caving in is.

A study of sections of these unilaterally operated larvae shows at once that most of the capsular structures that arise in connection with the olfactory sac are absent. The ethmoid column, the tectum nasi, and the medial nasal process are all absent. There is, however, a slight indication of a solum nasi. The anterior trabecular plate is apparently regularly formed, though thicker than is normally the case on the operated side. The antorbital process is also formed, though, as a glance at figure 6 will show, it is atypical in form and position.

The trabecula of the operated side is very much thickened (fig. 11). In addition the ascending process of the premaxilla has become connected laterally with the maxilla by a thin plate of bone, bridging over the region that would have been occupied by the external nares, figures 5 (*bp*) and 10. The formation of this unusual bony connection has apparently drawn the maxilla in, so that it no longer possesses the normal curvature (fig. 6). The line of the lower jaw follows that of the upper perfectly. There is in the lower jaw a compensating curvature to that of

the upper, so that where the upper jaw curves in as a result of the operation, the lower jaw follows. This brings the teeth into apposition at all times. The maxillary teeth are unaffected by the operation. They are normal as to both size and number on the operated side.

The process by which the above changes in structure are formed is relatively simple. In the operated larvae, the trabecula of the operated side appears simultaneously with that of the unoperated side and for some little time no difference can be observed in the development of the two sides.

Soon after the larva has begun to feed, it becomes evident that the trabecula of the operated side begins to lag behind its fellow in the advancing chondrification. At the same time that this observable retardation in longitudinal growth occurs there is a corresponding increase in the cross section of the trabecula. The antorbital process appears at this time though it is somewhat farther forward than its normal fellow. As development proceeds it becomes more and more atypical, finally appearing as an irregular projection from the trabecula at the level of the posterior terminus of the maxilla.

Coincident with the growth of the cartilages in the region of the posterior nares, there appears laterally the plate-like chondrifications which eventually form the tectum nasi and the solum nasi. The appearance of these on the unoperated side presages a marked thickening of the trabecula of the operated side. The end result of this is shown in figure 11 (*t*). Eventually the discrepancy in the length of the trabecula mentioned above is compensated for, both reaching forward to the premaxilla.

It is evident then that correlated with the absence of the nasal sac, there is an almost complete absence of the normal structures. The process of this reduction may be largely explained on a mechanical basis.

In the early development of the head region, the brain, together with the anlagen of eye and nose fill practically the whole of the anterior part of the head. There are, however, certain inequalities in the contour of the organs which are filled with mesenchyme, thus giving the smooth oval of the head. For

example, a section through the head of a young larva at the level of the eyes, shows roughly three circles, the two optic vesicles and the diencephalon enclosed in a fourth, the skin. Between these organs above and below lie two triangular depressions filled with mesenchyme. From the two ventral masses arise the trabeculae. As the eyes become separated from the brain the mesenchyme filling the depression above shifts, gradually becoming chondrified in the process and forming the crista. Exactly the same procedure may be imagined to occur at the level of the olfactory placodes. Here, however, owing to the greater extent of the olfactory nerve, no crista is formed, the mesenchyme of the upper depressions becoming secondarily chondrified to form the ethmoid column.

If now the placode of one side is removed all of the mesenchyme filling the depression simply and naturally joins the trabecular mesenchyme. Hence, no ethmoid column is formed, the mesenchyme from which it is normally developed becoming united with that of the trabecula, greatly adding to its mass.

Figures 5 and 8 from drawings of the model will show that the crista too, of the operated side is not so high as its fellow. It is quite evident that some of the mesenchyme tissue destined to form the cartilage of the crista has, owing to the absence of the placode and olfactory nerve which usually serves as a dam to keep it in position, simply slid forward, reducing the crista forming mesenchyme and adding again to the trabeculae.

The presence of the antorbital process on the operated side indicates that the removal of the nasal sac has not eliminated some factor that determines its formation, though the irregularity in shape and position shows the lack of some formative influence.

The presence of a rudimentary plate of cartilage in the general position of the *solum nasi* seems to point to a formative influence still present in that region. A careful study of the early stages shows that this plate does not develop until the bony connection is established between the ascending process of the premaxilla and the maxilla. This latter plate in all probability molds into shape laterally a portion of the mesenchyme which later chondrifies to form the rudimentary *solum nasi*.

The structure of the normal nasal cartilages shows quite clearly that they are an almost perfect 'cast' of the underlying nervous tissue. The removal of the 'core,' the nasal sac, of the capsular cartilages causes a collapse of the whole structure with the subsequent settling of the cartilage forming material to the lowest point.

It may be concluded from these experiments that there exists in the head a certain amount of mesenchyme tissue destined to form cartilage. The general shape of these cartilaginous structures is determined by the form of the underlying organs. The removal of any such underlying parts results not in any reduction in the mass of cartilage formed, but in the rearrangement of the cartilage forming tissue to meet the change in the form of the 'core' about which it is formed.

II. Development of naso-lacrymal duct

In addition to the modifications of the capsular structures, it was noted that the naso-lacrymal duct present in the 33 mm. larva, on the unoperated side was entirely absent on the operated.

At present the most widely held theory of the formation of the naso-lacrymal duct is that it is a solid cord of cells growing centripitally toward the nasal epithelium from the point of origin in the ectoderm on the dorso-anterior aspect of the optic anlage. This solid cord becomes secondarily canalized.

Figures 10 and 11 (*nl*) show respectively the junction of the cord with the nasal epithelium and the surface ectoderm. It is rather thicker at the point of union with the nasal sac than where it meets the ectoderm and seems to run more gradually into the sac than it does into the ectoderm. There is also a marked continuation of the cavity of the sac into the cord indicating that the canalization occurs from this end. Born in 1876 worked out the development of this duct. He found that it first appears as a solid cord of cells reaching from the surface ectoderm to the nasal sac and that it is derived from the former. The work of Schaeffer in 1912 on man has shown that the duct is at first a solid cord derived from the inner layer of the epidermis.

The suggestion is made here that it would be possible by very careful operating to remove the nasal analge without disturbing the duct rudiment, and vice-versa. The material at hand rather indicates that the duct is derived not from the surface epithelium but from the cells of the nasal sac. The above operations would make it possible to arrive at a definite conclusion experimentally so far as *Amblystoma* is concerned as to the exact origin of the duct.

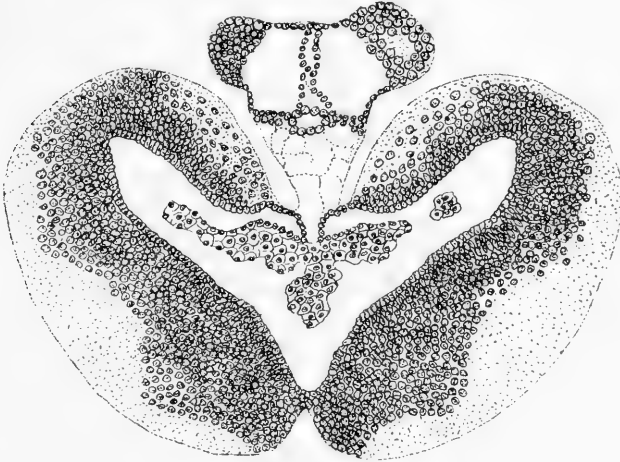
III. The Brain

Owing to the difficulties encountered in keeping noseless larvae alive up to metamorphosis, the report on the effect of the absence of the olfactory organ on the fiber tracts of the forebrain must be postponed until later. The effect on the gross morphology can, however, be given in its entirety at this time. For the sake of clarity the description of the brain of the six months old larvae will be given first, since, as will be shown, the effect of the removal is not immediate.

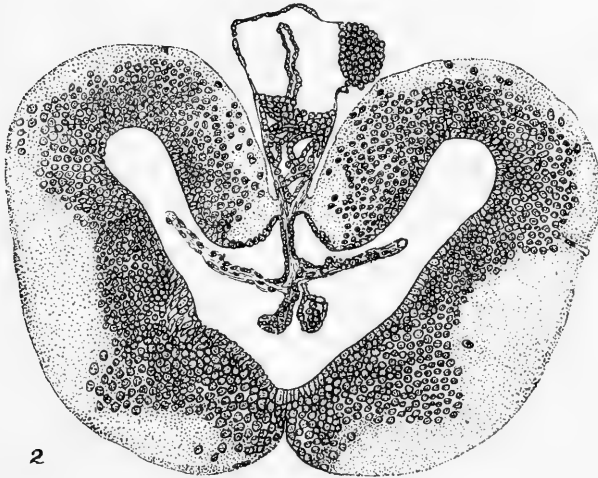
Figure 7 will make evident at once the effect of the absence of the nasal sac on the gross morphology of the telencephalon. The hemisphere of the operated side is markedly smaller than that of the unoperated. This reduction in size, however, is related largely to the anterior portion of the telencephalon. In other words, the olfactory bulb as such is greatly reduced in size—a reduction which is apparent also for some distance caudally. In the region of the primitive hippocampus, on the other hand, the two hemispheres are equal, barring slight unevenness in the sections (text figures 1, 2 and 3).

A study of the cross sections of brains of operated larvae show quite as strikingly the above facts. Figure 12 through the olfactory bulb of the unoperated side shows no sign of the hemisphere of the operated side. Figure 14 from a section some 170 μ caudad shows the right is greatly reduced in size. A section still further caudad at the level of the interventricular foramen shows no distinguishable differences in the two hemispheres other than that in the size of the ventricles. A comparison of

text figures 1, 2 and 3 will make evident that there is a progressive enlargement of the ventricles from the normal through the brains of the unilaterally operated larvae to the bilaterally operated larvae. It is not possible at this time to explain the significance of this difference if any exists.

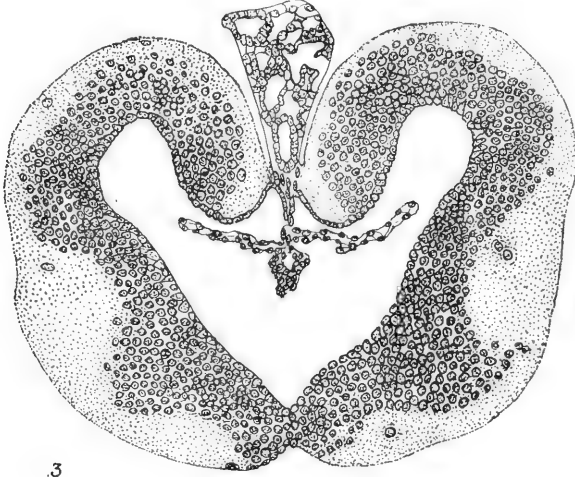


1

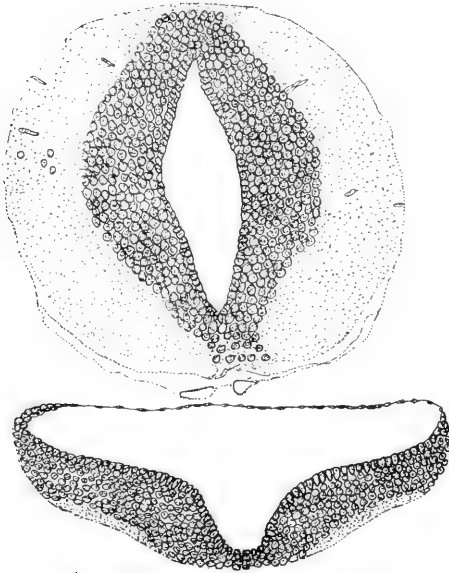


2

Sections through the brain, at the level of the hypothalamus, show absolutely no asymmetry in any of the operated forms (text fig. 4).



.3



4

It becomes apparent then that the absence of the nasal sac has here resulted in a reduction in the size of the telencephalon of the operated side, the reduction being limited to the anterior portion of the hemisphere.

It is perhaps best at this point to mention one of the results of the operations on the histogenesis of the brain that has been brought out by the technique employed in studying the gross morphology. It is a well known fact that the growth of the olfactory nerve is centripetal,—from the olfactory placode to the telencephalon. The terminations of the ingrowing nerve fibers are known as glomeruli. As would be expected, the absence of the olfactory nerve results in the complete disappearance of the glomeruli. So that there are present in the telencephalon of the operated side only secondary and tertiary tract fibers, the primary fibers being entirely absent.

The material so far studied has as yet shown no discernible effects on these secondary and tertiary tracts. Gudden's atrophy is necessarily a slow process, and it is evident from the material at hand that if they are to show this type of atrophy the operated larvae must be kept at least to metamorphosis. It is probably, too, that the atrophy would continue with age so that metamorphosed larvae would show the most marked differences.

A study of entire brains dissected out of normal and operated larvae, together with a careful inspection of transections of the brains of parallel stages shows that the reduction in size is not immediate. The first four weeks of development after the operation show no differences in the size of the telencephalon. That is, the growth of the two hemispheres during the first part of larval life, three weeks of which have been dependent on yolk and the fourth on the food in the environment, has not been in the least affected by the absence of the nasal sac. From the fourth week on, however, the hemisphere of the unoperated side begins to grow more rapidly than its fellow of the operated side, finally outdistancing it to the extent shown in the drawing of the model.

The fact that the difference in growth did not occur until active feeding had commenced suggested at once that here was

a correlation between structure and function (page 34). The physiological experiments showed that larvae over four weeks old could detect food by the sense of smell. Correlated, then, with the beginning of functional activity of the nasal epithelium, there exists in operated forms the beginning of a difference in the growth rates of the two hemispheres.

The above facts suggest that there is inherent in the tissue of the central nervous system a certain potential for development and differentiation. This potential carries the growth up to a certain point at which all the parts of the brain are present. The further growth then becomes dependent on the functional activity of the parts.

The conclusion stated above can not be considered final. A more extensive study with special methods, of the fiber tracts involved must be engaged in before any final judgment can be reached. At present such a study is under way. It is planned to carry the operated larvae through metamorphosis as it is evident the effects become more pronounced with age.

The writer takes this opportunity to express his appreciation of the criticism and suggestions made by Dr. Harrison during this investigation.

SUMMARY

The physiological tests of the reactions to food of normal and noseless larva show:—

- 1) That normal larvae will react positively to sand grains that move in the water.
- 2) That normal larvae will react positively to food that is not moving.
- 3) That noseless larvae will react positively to sand grains but not to motionless food.
- 4) That eyeless larvae will react positively to food that they cannot see and will not react to moving sand grains.

The effect of the absence of the nasal sac on the form of the skull is shown in the complete collapse of the cartilages that normally surround the sac, this collapse being due to the absence

of the support given by the nasal placode to the mesenchymal tissue in which the cartilage is formed.

The effect of the absence of the nasal sac on the brain is a reduction in size of the telencephalon.

The removal of the nasal epithelium deprives the developing forebrain of a stimulus necessary for its complete development. This is evidenced by the fact that the forebrain of the six months old larva from which one placode had been removed, showed considerable differences in the size of the two hemispheres, the operated side being the smaller. This difference however is found only after the nasal sac of the unoperated side has become functional. This fact indicates that there exists in the central nervous system as, indeed, in other organs a "potential for differentiation" which carries the development of the parts to a point at which all parts are present although not in their final form or size, the further growth being dependent on the functional activity of the end organs of the brain.

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PLATE 1

EXPLANATION OF FIGURES

- 5 Three quarter view of model of skull of 33 mm. larva from the right side.
- 6 Dorsal view of above model. Drawn to smaller scale than figures 5, 7, 8.
- 7 Dorsal view of model of brain of unilaterally operated 33 mm. larva.
- 8 Three quarter view of model of skull of unilaterally operated 33 mm. larva from left side.

ABBREVIATIONS

<i>ao</i> , antorbital process	<i>fo</i> , fenestra olfactorius
<i>ap</i> , anterior trabecular plate	<i>m</i> , maxilla
<i>apm</i> , ascending process of premaxilla	<i>mp</i> , medial nasal process
<i>bp</i> , bony plate connecting premaxilla and maxilla	<i>pm</i> , premaxilla
<i>ct</i> , crista trabeculae	<i>sn</i> , solum nasi
<i>ec</i> , ethmoid column	<i>t</i> , trabecula
	<i>tc</i> , tectum nasi

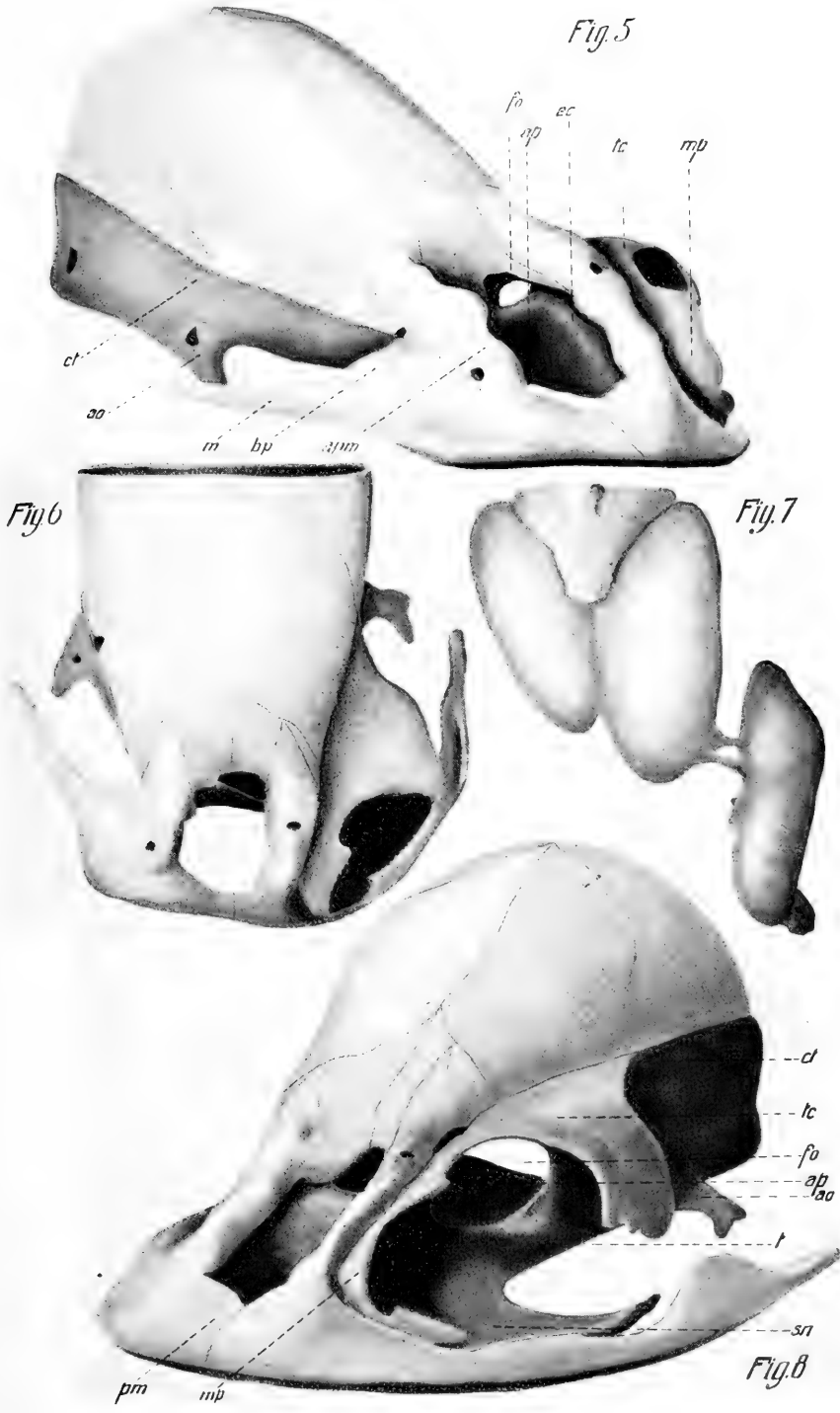


PLATE 2

EXPLANATION OF FIGURES

9 Cross section of head of a unilaterally operated larva showing reduction in the height of crista (*ct*) and in the size of the right telencephalon. $\times 100$.

10 Cross section 690 microns anterior to figure 9 showing bony plate connecting maxilla and premaxilla, the caudal end of the naso-lacrymal duct, the tectum and solum nasi and the ethmoid column. $\times 100$.

11 Cross section 410 microns anterior to figure 9 showing the left telencephalon with its glomeruli and no sign of the right, the distal end of the naso-lacrymal duct and on the operated side the much thickened trabecula. $\times 100$.

ABBREVIATIONS

<i>ap</i> , anterior trabecular plate	<i>nl</i> , naso-lacrymal duct
<i>apm</i> , ascending process of premaxilla	<i>ns</i> , nasal sac
<i>bp</i> , bony plate connecting premaxilla and maxilla	<i>sn</i> , solum nasi
<i>ct</i> , crista trabeculae	<i>t</i> , trabecula
<i>ec</i> , ethmoid column	<i>tc</i> , tectum nasi
<i>m</i> , maxilla	<i>tel</i> , telencephalon

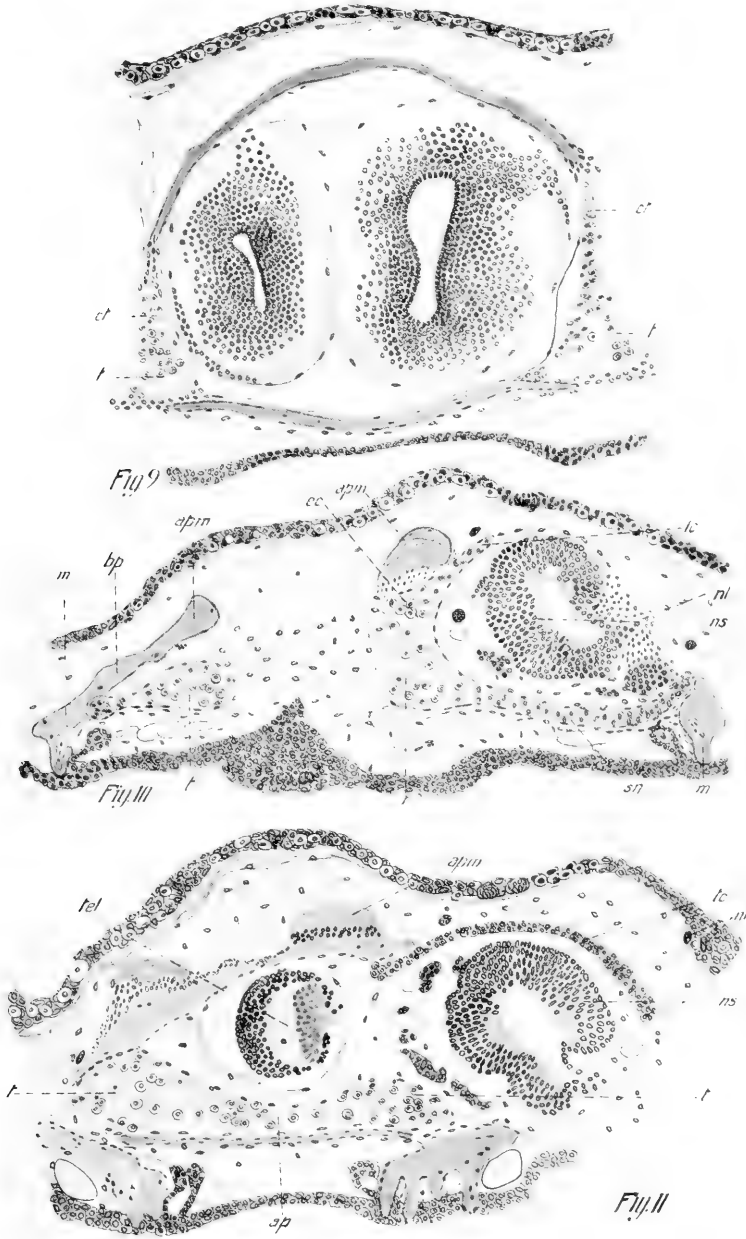


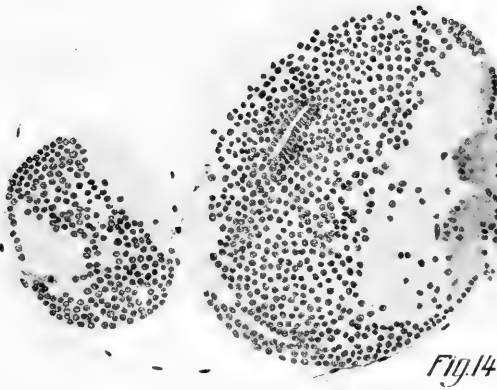
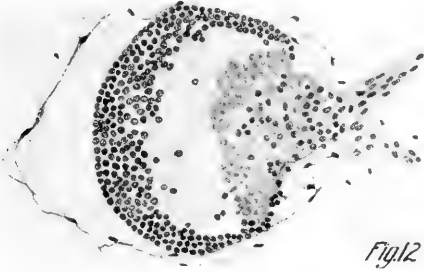
PLATE 3

EXPLANATION OF FIGURES

12 Cross section of the forebrain of a unilaterally operated larva through the olfactory bulb showing the glomeruli and the absence of the olfactory bulb on the operated side. $\times 160$.

13 Cross section of the forebrain of a normal larva at same level as figure 12 $\times 160$.

14 Cross section of the same forebrain that is figured in figure 12, 17 microns caudally showing the first signs of the right telencephalon. $\times 160$.



THE PERIODIC REORGANIZATION PROCESS IN PARAMAECIUM CAUDATUM

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THIRTY-FIVE FIGURES (SEVEN PLATES)

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I. INTRODUCTION

In previous papers¹ there have been presented the morphological and physiological details of a new reorganization process in the life of *Paramaecium aurelia*, and the statement that the phenomenon occurs also in *Paramaecium caudatum*. The present paper is a study of this reorganization process, which we term endomixis, in the latter species of *Paramaecium*. We

¹ Woodruff and Erdmann ('14, I): Complete periodic nuclear reorganization without cell fusion in a pedigreed race of *Paramaecium*. *Proc. Society for Experimental Biology and Medicine*, vol ii, Feb. 18. (Preliminary paper.)

Erdmann and Woodruff ('14, II): Vollständige periodische Erneuerung des Kernapparates ohne Zellverschmelzung bei reinlinigen *Paramaecien*. *Biol. Centr.* Bd. 34. (Preliminary paper).

Woodruff and Erdmann ('14, III): A normal periodic reorganization process without cell fusion in *Paramaecium*. *Journal of Exper. Zoölogy*, vol. 17, no. 4, Nov. (Complete paper.)

shall in this paper confine ourselves chiefly to a description of the facts observed and to a very brief discussion of the theoretical bearings, leaving for consideration at another time, when the details of this reorganization process in other species of Protozoa have been discovered, the more or less academic discussion as to the exact classification of this process among phenomena of *Entwicklungserregung*. However, we may state at once, in view of the somewhat premature criticism by R. Hertwig of our work as presented by us in an avowedly preliminary paper ('14, II), that we are convinced, after such a careful consideration as any paper by Hertwig demands, that not a single objection which he raises is well founded. We are also convinced that the theoretical interpretations which Calkins ('15) advances in regard to our results are not warranted by the facts so far at hand. We take, then, in their entirety the facts and discussion as presented in our complete paper ('14, III) as the starting point for our present communication.

II. MATERIAL AND METHODS

The material used in the present study has been derived from pedigreed races of *Paramaecium caudatum* which have been bred in exactly the same way as described in our work on *Paramaecium aurelia*, and the reader is again referred to that paper for details ('14, III, p. 432).

From our study of several races of *Paramaecium caudatum* it seems clear that this species is less well adapted than *Paramaecium aurelia* to withstand the conditions necessary for pedigreed culture work. Whereas all the races of *Paramaecium aurelia* which we have studied have survived indefinitely under the daily isolation slide method of pedigreed culture manipulation, most of the *Paramaecium caudatum* races have sooner or later refused to divide under these conditions. As will be discussed in detail later, the races on the slides can undergo the reorganization, perhaps two or three times and then at the next onset of the phenomenon, about 90 generations later, are unable to carry it to proper completion and die. If, however, the

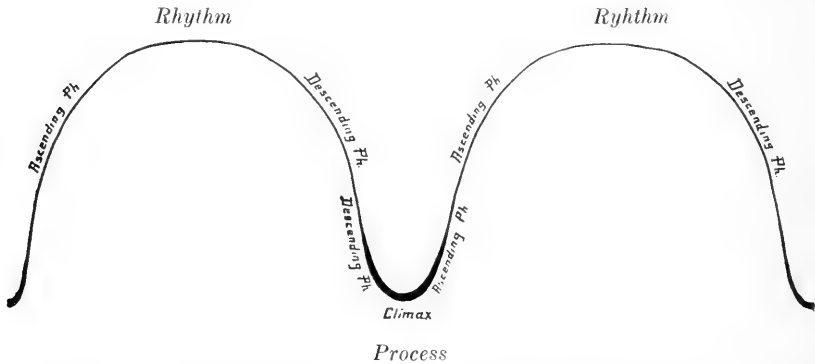
animals are placed in tiny tubes of culture medium, instead of on slides, so that the volume of culture medium is somewhat greater, but not large enough to render it impossible to control them daily and so detect conjugation if it occurs, then the animals apparently can live indefinitely. This result is, in general, in accord with the earlier observations of Woodruff ('11c, p. 64) though at that time he used somewhat larger volumes of medium to save the *Paramecium caudatum* and so was not able to positively prove that conjugation did not occur.

It has seemed best to consider this point in justification of our resorting to material from tiny tube cultures for certain stages of the reorganization process. But we would emphasize that many of the stages of this process in *Paramecium caudatum*, as all of the stages of the process in *Paramecium aurelia*, have been secured from daily isolated pedigreed animals so that it is absolutely positive that the phenomenon under discussion occurs in *caudatum* as in *aurelia* at clearly defined periods. To repeat—we have shown, by the isolation slide method, the physiological characteristics of the reorganization process in the recurrent rhythms of the division rate, and the accompanying cytological changes of this process to the climax at the low points of the rhythms. The completion of the process in isolated slide animals is evident from the completion of the rhythms in many cases. But owing to the longer rhythmic periods in *Paramecium caudatum* as compared with *Paramecium aurelia*, and owing to the lesser viability of the former species and the smaller number of generations over which its reorganization process extends, we did not secure all of the later cytological changes from isolated slide animals but resorted to carefully controlled 'tube' animals as already described. We will grant that the original discovery of the reorganization process in *Paramecium caudatum* would have been more difficult than it was in *Paramecium aurelia* because of the longer rhythmic period in the former species and its less marked viability under the artificial slide pedigreed method during the crucial stages of the phenomenon.

III. THE CYTOLOGICAL PHENOMENA OF THE REORGANIZATION PROCESS IN PARAMAECIUM CAUDATUM

The complete reorganization process without cell fusion which occurs in *Paramecium caudatum*, as in *Paramecium aurelia*, synchronously with the rhythms, presents essentially the same fundamental cytological features as those we have described in the latter species ('14, III, pp. 436-473).

We shall, therefore, present merely a general summary of the phenomenon in *Paramecium caudatum* and point out the differences which could be traced in the morphological features. In accordance with our previous investigation we term the three



Text fig. 1 Diagram illustrating the relation of the reorganization process to rhythms. (Woodruff and Erdmann, '14, III.)

phases into which the reorganization process (endomixis) naturally resolves itself as descending phase, climax and ascending phase (text figure 1).

A. The descending phase. The micronucleus of a typical *Paramecium caudatum* which is not undergoing the reorganization process or normal vegetative division is situated in a slight depression of the macronucleus. Text-figure 2 (p. 63) shows an animal shortly after a typical vegetative *division* in which the micronucleus has not yet reached its accustomed position. The structure of the resting micronucleus between two vegetative cell divisions is shown in figure 1, p. 85. The position of the micronucleus in figure 2 has not changed but its

morphological structure is characteristic of beginning reorganization. As this phenomenon approaches the micronucleus fails to take up its accustomed place, and remains free in the cytoplasm, while the chromatin of the micronucleus seems to assume the form of short bands. Figures 3 and 4 show two animals in the 203d generation from different sub-lines of Culture Y just when the process of reorganization begins—the formation of bands of chromatin, and the wandering of the



Text fig. 2 Culture Z, Line I, 38th generation, October 12, 1914

micronucleus in the cytoplasm, indicating the definitive onset of the process. This wandering of the micronucleus has been described by us in the reorganization of *Paramecium aurelia* ('14, III, figs. 3, 4, 5).

Projections of the macronucleus which extrude at a very early stage of the process of reorganization in *Paramecium aurelia* have not been seen in our races of *Paramecium caudatum*.

Here the macronuclear surface loses its smooth appearance while clefts and wrinkles appear in the stained preparations thus indicating that in the living cell the distribution of the chromatin is not homogeneous in the macronucleus (figs. 4 and 7).

The micronucleus begins to swell shortly after emergence from its depression in the macronucleus. Figure 5, shows the chromatin arranged at the equator of the micronucleus in a band of granules while one pole is nearly devoid of chromatin. (Compare Calkins and Cull '07, fig. 1). The swelling continues until the so-called sickle stage appears. Figure 8 shows a more elongated micronucleus than the one shown in figure 5. The formation of the 'sickle' is completed in an animal from the 202d generation, Culture Y, Line III g (fig. 7). The paucity of the chromatin in the portion of the micronucleus which protrudes from under the macronucleus is remarkable; no distinct granules or threads being recognizable as the chromatin is apparently homogeneously distributed throughout the dividing micronucleus at this stage. The formation of chromatin bodies has begun in the macronucleus. Maupas, Hertwig and Hamburger described, in the different species of *Paramecium* which they investigated, the occurrence of the 'sickle' stage before the onset of the reduction divisions preceding *conjugation*. The 'sickle' stage changes in conjugation into the first reduction spindle as described in detail by Calkins and Cull, '07, p. 383, in *Paramecium caudatum*. In the reorganization process of *Paramecium caudatum* we secured an animal from the 184th generation with the very characteristic dumb-bell form of the micronuclear division spindle (fig. 6). The 'dumb-bell' micronuclear division stage in the reorganization process resembles closely the stage which Calkins and Cull ('07, fig. 15) interpret as the telophase of the second maturation division in conjugation. In the reorganization process of *Paramecium caudatum* there is a slight difference in size of the arising micronuclei as is indicated in the cell shown in figure 6. The amount of chromatin at the two ends is apparently different. It is possible that the arising micronucleus which is nearly devoid of chromatin is the one whose products after the next division are doomed to degeneration.

Calkins and Cull do not describe a 'dumb-bell' formation in the first reduction division in conjugation, but we must interpret our figure as the first 'reduction' division of the reorganization process because no trace of other micronuclei are in the cell. An animal in the second 'reduction' division is shown in figure 10. Two micronuclei are dividing. In one the equatorial band of chromatin is intact while in the other it is divided. The macronucleus, fragmented into two parts, is rapidly losing its chromatin.

The breaking up of the macronucleus begins relatively late in *Paramecium caudatum* and generally does not extend through several generations as we found to occur in the descending phase of the process in *Paramecium aurelia* (Compare '14, III, pl. 1). The expulsion of chromatin bodies (figs. 14, 15 and 16) or the breaking of the macronucleus into two or more pieces (figs. 9, 10 and 11) seem to be a rapid process in the life of the caudatum cell. Culture Z, 91st generation (fig. 13) does not show the slightest trace of chromatin bodies, but in the 93d generation (fig. 14) there are many of them in the cytoplasm. These chromatin bodies, however, do not have the definite condensed appearance so characteristic of those in *Paramecium aurelia*, but are relatively pale and indistinct and seem to suddenly appear in nearly maximum numbers. This possibly may be accounted for by the fact already mentioned that in *Paramecium caudatum* finely divided chromatin material, in certain cases at least, streams from the macronucleus into the cytoplasm. This leaves a paucity of chromatin for the chromatin bodies and may change the tension within the macronuclear membrane so that the chromatin bodies are formed rapidly and thus appear suddenly. Figure 13 gives a good idea of this phenomenon, as does also figure 6 in which the macronucleus is partly devoid of chromatin while no chromatin bodies are visible.

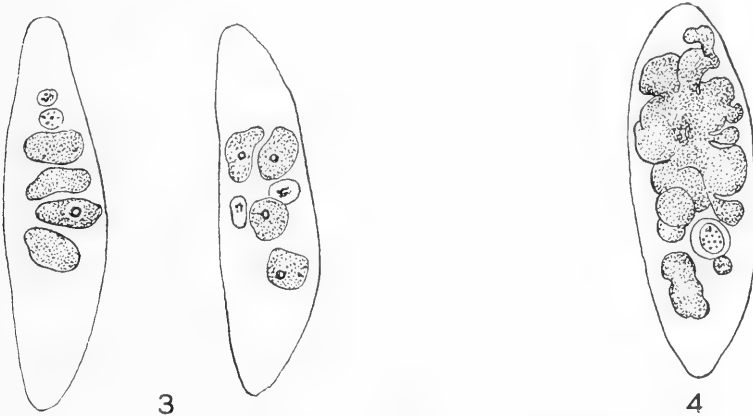
This breaking up of the macronucleus into large pieces was observed in animals from small tube cultures (fig. 10) as well as in isolated animals (figs. 9 and 11) as already described. These animals cannot be dividing specimens because in normal vegetative divisions the macronucleus is greatly elongated and breaks

only at one point when the cytoplasm itself has nearly completed division ('14, III, fig. 7).

To summarize: The loss of chromatin occurs either by the extrusion of chromatin bodies (*Paramecium caudatum* and *Paramecium aurelia*); by extrusion of small granules from the macronucleus (*Paramecium caudatum*); by breaking up of the old macronucleus into two or more large pieces (*Paramecium caudatum*; *Paramecium aurelia*, Hertwig '89, p. 74). The result—the total destruction of the individuality of the macronucleus—is the same in each case.

Extruded chromatin bodies were figured by Calkins '04 (fig. 16) in *Paramecium caudatum* and these we have compared with certain stages of *Paramecium aurelia* at the beginning of the ascending phase of the process of reorganization ('14, III, p. 484, text figs. 20 from our own preparations, and 19, copied from Calkins). Calkins does not mention how the nuclear fragmentation has been effected but this probably has occurred in the same way as we have described in our cultures of *Paramecium aurelia* (Woodruff's main culture I, and Erdmann's culture B) and in some animals from our culture Y of *Paramecium caudatum* ('14, III, fig. 34), because the morphological features are identical. Those cultures of *Paramecium caudatum*, in which the macronucleus breaks into large pieces, present in the further stages of macronuclear degeneration a different cytological appearance as is shown in an animal from about the 82d generation, culture Y (fig. 8). Here the chromatin is distributed in the cell without first being condensed into spherical chromatin bodies. The macronuclear membrane is torn and its contents are intermingling with the cytoplasm. This general type of fragmentation of the macronucleus has been described with different interpretations by several authors, for example, Kasanzeff '01, working under R. Hertwig; Popoff '07, figure 18, plate 4; Popoff '09, figure 26, plate 2; Calkins '04, figure 8, plate 1 and figure 11, right animal, plate 2; and Hertwig '14, p. 568. All these authors considered these changes as depression phenomena which, according to Popoff, have close resemblance to phenomena characteristic of the onset of conjugation.

Now Hertwig '14, reviewing and commenting on our preliminary paper ('14, II) on the reorganization process of *Paramecium aurelia*, in the light of his theoretical beliefs in regard to depression in Protozoa, shows in his text figure 3, animals 4 and 5 (copied as text fig. 3 in this paper) the breaking up of the macronucleus together with the formation of two micronuclei. These stages correspond somewhat to our stages (figs. 8, 9, 10 and 11) at the end of the descending phase of the reorganization process when the first or second 'reduction' division has occurred. But it is clear that Hertwig has confused two different series of phenomena: animals with hypertrophied macronucleus and not



Text figs. 3 and 4 *Paramecium caudatum*. From Hertwig, '14, after Kasanzeff.

clearly discernible changes in the micronucleus, with those of the reorganization process which we have described as endomixis (cf. text fig. 3, copied from Hertwig). We have never found in the thousands of isolated animals studied that the macronucleus was enlarged by any means to such a degree as Hertwig figures in his text figure 3, animals 1, 2, 3, from which we have copied the second animal as our text figure 4.

The crucial stage, i.e., the total reorganization of the nuclear apparatus with the formation of the macronuclear anlagen, could not have been determined as a phase of the reorganization

process either by Hertwig, Kasanzeff or Popoff in their *mass* cultures. However, Hertwig's suggestion that "Woodruff und Erdmann könnten somit die Stadien als Beweise für eine vorausgegangene Parthenogenese in Anspruch nehmen" is a partially right prediction, provided one substitutes for 'parthenogenesis,' reorganization process—endomixis! Since Kasanzeff did not find stages of the ascending phase, that is the formation of the macronuclear Anlagen, in *Paramecium caudatum* mass cultures, Hertwig still believes that the described phenomena are depression conditions and not stages of the reorganization process, though he himself described in *Paramecium aurelia* isolated stages of a process which he called 'parthenogenesis.'²

We wish to emphasize that in a mass culture it is impossible to know the ancestry of the individual cell. It is clear that from a mass culture so-called depression stages may be either animals which are about to begin the reorganization process, or animals which have just undergone conjugation, or abnormal animals due to some exigencies of the environment. From material of this sort, in view of the fact that the reorganization was not known previous to our work, there has been drawn into the literature on infusorian life histories the long series of atypical animals as evidence of depression. However, by pedigreed culture methods we have resolved this heterogeneous material into its component parts and have shown for both *Paramecium aurelia* and *Paramecium caudatum* that *many* of the so-called depression phases are normal stages in a complex reorganization phenomenon.

B. Climax. Culture Y of *Paramecium caudatum* we had under observation from February 18, 1914, to June 9, 1914, and

² We have not called the reorganization process 'parthenogenesis' and have not introduced even our new term 'endomixis' in our preliminary papers ('14, I and '14, II) because we wished to wait until our complete data was presented ('14, III) so that they could be discussed in their entirety. However, in our brief paper ('14, II) an error is present on page 495, owing to the fact that the printer misunderstood our directions and that we were unable to revise the proof on account of the war. The sentence "Diese Parthenogenese hier ist ein Sexualakt." is a remnant of a paragraph which appeared in the manuscript but was entirely deleted in the proof, but the printer in some way left this one isolated sentence for the complete paragraph!

during this time the culture attained the 207th generation. The process of reorganization occurred at about the 18th, 136th and 203d generation (table 1). Line III which had successfully undergone the process at the 136th generation lived to the 192d generation, just at the onset of the next reorganization period. That the phenomenon was imminent is evident also from Sub-line III fa, in which it appeared at the 202d generation. From

TABLE 1

Showing the occurrence of the reorganization process in Culture Y

PERIODS =	1-90 GEN.	90-180 GEN.	180-270 GEN.	REMARKS
I. 1-59.....				Died
II. 1-107.....	18			Died
III. 1-192.....		136	191	Died
III a.* 120-130.....		139		Killed in descending phase
III b. 143-163.....				Died
III c. 161-163.....				Killed
III d. 161-165.....				Killed
III e. 165-169.....				Died
III f. 167-184.....		137		Killed
III fa. 169-203.....			202	Killed in descending phase
III fb. 169-206.....			203	Died
III g. 192-207.....			204	Died
IV. 1-89.....	26			Killed
IV a. 85-115.....				Killed
IV aa. 93-143.....		139		Killed in ascending phase
IV ab. 108-114.....				Died
IV ac. 120-137.....		137		Killed in descending phase

* Designated Line 2 in our 1914 (III) paper.

a close analysis of the division rate, and of the longevity of the component lines of Culture Y (table 2) it is apparent that of 4 lines and 13 sublines, 13 did not undergo a division for at least twenty-four hours, and in some cases for forty-eight hours, before their death. Death occurred in these lines either 'naturally' or as a result of the animals being killed for study since they were dividing slowly. Two lines or sublines died naturally without a lowering of the division rate, ten were purposely killed and four died after a lowering of the division rate. Sub-line III a,

TABLE 2

Culture Y

LINE	GENERATIONS	DIVISIONS	FATE
I.....	59	None in 48 hours	Died
II.....	107	Two in 24 hours	Died
III.....	192	None in 24 hours	Died
III a.....	139	None in 24 hours	Killed
III b.....	161	One in 24 hours	Died
III c.....	163	None in 24 hours	Killed
III d.....	165	None in 24 hours	Killed
III e.....	169	Two in 24 hours	Died
III f.....	184	None in 24 hours	Killed
III fa.....	202	None in 24 hours	Killed
III fb.....	206	None in 24 hours	Died
III g.....	207	None in 24 hours	Died
IV.....	89	None in 24 hours	Killed
IV a.....	115	None in 24 hours	Killed
IV aa.....	143	One in 24 hours	Killed
IV ab.....	114	None in 24 hours	Killed
IV ac.....	137	None in 24 hours	Killed

III fa and IV ac, were killed in the descending phase of the process. Sub-line IV aa was killed after the climax.

Thus although cytological indications of the reorganization process were not observed in certain of the lines and sublines of Culture Y at the time of extinction, nevertheless this extinction was synchronous with the process as actually observed in other lines and sublines from the 136th to 143d generations, 184th to 191st and 202d to 209th generations. These periods clearly are critical ones in the life of this culture (table 2).

However our culture of *Paramaecium aurelia* was able to undergo the reorganization process frequently and with apparent facility under daily isolation methods, and therefore the number of lines which were eliminated by death during the phenomenon was relatively small (cf. '14, III, table 1, p. 462).

From all our work the conclusion evidently follows that before cytological signs of reorganization are discernible in the cell the physiological conditions for its onset are in evidence.

Culture Z was bred from September 20, 1914, and Culture M from January 7, 1915, to the end of our study. Culture Z under-

went reorganization successfully in the 11th, 91st, 184th, 282d and 398th generations, and Culture M at the 89th generation, that is at intervals of about 90 generations, thus confirming our observation on Culture Y that the reorganization process occurs in *Paramecium caudatum* at intervals of from 80 to 100 generations.

The difficulty of securing stages of the climax and of the ascending phase compelled us, as previously stated, to amplify our material of isolated pedigreed animals with small tube cultures seeded from our pedigreed cultures, just before reorganization was due to take place on the basis of our computation of the

TABLE 3

Showing the occurrence of the reorganization process in Culture Z

PERIODS=	1-90 GEN.	99-180 GEN.	180-270	270-360	360-450	REMARKS
I. 1-378	11	90	188	282		Killed
I a. 93-165						Killed
I b. 144-187			184			Killed
I c. 142-201			187			Killed
I ca. 171-260						Died
I d. 192-201						Killed
I e. 275-454						Discon'd
I f. 394-455					398	Discon'd
II. 1-92	91					Killed

number of generations since its last occurrence and by the division rate. The most critical stage for the vitality of the race is when the individuality of the macronucleus is lost and the chromatin bodies are undergoing degeneration. The results of Calkins' extended experience with *Paramecium caudatum* all indicate that the culture of *Paramecium caudatum* in isolated lines on depression slides is sooner or later fatal. Woodruff's comparison, ('11c, p. 60,) of pedigreed lines of *Paramecium caudatum* with those of his main culture of *Paramecium aurelia* showed clearly that it was impossible to breed indefinitely *Paramecium caudatum* by the daily isolation method which was so highly favorable for *Paramecium aurelia*.

The following tube cultures therefore were started from the respective isolated slide cultures as indicated above:

Culture Y	{	Tube culture a.....	March 26, 1914
		Tube culture b.....	April 1, 1914
		Tube culture c.....	April 3, 1914
		Tube culture d.....	April 20, 1914
		Tube culture e.....	April 22, 1914
Culture Z	{	Tube culture a.....	March 12, 1915
		Tube culture b.....	March 13, 1915

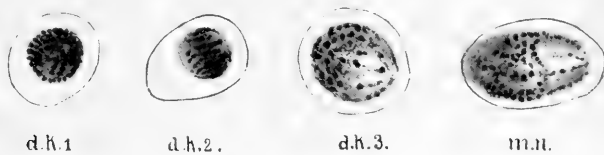
Continuing the discussion of the cytological changes of the reorganization process, fig. 18 shows an animal in the climax, from Culture Y about the 137th generation, without a complete macronucleus and with degenerating chromatin bodies as is indicated by their elongated form. This shape of the degenerating bodies we have frequently mentioned in our description of the climax and ascending phase of *Paramecium aurelia* ('14, III, pl. 2). The definitive micronucleus (fig. 18), which by three subsequent divisions will form eight micronuclei from which the new nuclear apparatus will be formed, is characterized by its lucid appearance and is lying above the main remnant of the macronucleus. This cell has a perfectly healthy appearance and should be contrasted with the animal shown in figure 17, which is an animal from Line III g, 202d generation, which several generations later died in the process of reorganization.

Paramecium caudatum cells with one micronucleus and no macronucleus have been observed by Calkins, Kasanzeff and Hertwig. Calkins '04, fig. 14, pl. 2, illustrates such an animal. Abnormal animals of this character must not be confused with animals undergoing the reorganization process. The cell without a complete macronucleus (fig. 18), which we figure, is an entirely normal animal. Its sister cells carried on the race for nearly 70 generations more, some of them showing the crucial formation of macronuclear anlagen as will be described in detail in the next section.

C. Ascending phase. Before the onset of the ascending phase the same general cytological changes are to be observed as those

we have described for *Paramecium aurelia*, i.e., animals with only one micronucleus and a completely destroyed macronucleus, the remnants of which are scattered in the cell. This one micronucleus, as in *Paramecium aurelia*, is the carrier of the life of the race through the new rhythm.

Maupas and Calkins in the conjugation of *Paramecium caudatum* figure eight micronuclei, the products of the syncaryon, which are formed immediately after the separation of the conjugants. This we have verified in conjugation (fig. 25). In the reorganization process we have discovered animals at a slightly later stage with four micronuclei and four macronuclear anlagen, the latter representing four transformed micronuclei. The micronucleus which persists and carries the life of the race there-



Text fig. 5

fore must have undergone three divisions producing the four micronuclei and four macronuclear anlagen in the stage under discussion. The micronuclei (fig. 19) are characterized by their granular structure and somewhat glistening appearance. In the preparations the few granules appear pure blue without any reddish tinge. These micronuclei resemble, as far as one may judge from figures, one of the four micronuclei which Klitzke ('14, text fig. C, copied in the present paper as text fig. 5) shows in the conjugation of *Paramecium caudatum*. This micronucleus (Klitzke's 'Micronucleusanlage,' d.k. 3) he believes undergoes degeneration together with two other micronuclei (d.k. 1, d.k. 2). The marked differences he points out are not observable in our specimen in the reorganization process, but this may be due to the fact that the animal has not yet reached this point. Or it may be that no micronuclear degeneration

occurs, as is the case in the description of this phase in the conjugation of *Paramecium caudatum* as worked out by Calkins and Cull ('07, p. 396). We summarized in our former paper ('14, III, pp. 453, 454) the various opinions in regard to the formation of the macronuclear anlagen in *Paramecium*, and showed that the problem is not settled since Maupas and Klitzke believe that four micronuclei and four macronuclear anlagen arise, three of the micronuclei undergoing degeneration. Maupas states definitely that four typical micronuclei are transformed into macronuclear anlagen. In the animal in the process of reorganization under discussion (fig. 19) this transformation of micronuclei into macronuclear anlagen has been completed. The anlagen are quite homogeneous, with the exception of a few granules frequently dispersed in a circle. In preparations stained with Delafield the anlagen show a diagnostic reddish tinge which cannot be expressed without colored plates. (Compare our present plates with those reproduced in colors in our paper on *Paramecium aurelia*). The chromatin bodies are scattered throughout the cell in various stages of disintegration.

The next two figures (20 and 21) show only two micronuclei and four macronuclear anlagen in each animal. This stage may be interpreted as an animal in which two micronuclei have degenerated, or as an animal after the degeneration of three micronuclei and after the first somatic micronuclear division. In our work we found it necessary to study the cytology of conjugation of our pedigreed races of *Paramecium caudatum* with particular reference to the fate of the four micronuclei which do not form macronuclear anlagen. In our races it appears clear that all four of the micronuclei do not persist and become distributed by the following two cell divisions as the definitive micronuclei of the four completely reconstructed *Paramecium* cells. Figure 26 shows an exconjugant with clearly four macronuclear anlagen and four micronuclei. Figure 27 gives a pedigreed animal, after the first cell division subsequent to conjugation, in which only one micronucleus is present. Figure 28 shows a pedigreed animal, after the second cell division subsequent to conjugation, with a macronucleus and micronucleus.

This is in harmony with the stages in the reorganization process in our races having four anlagen and two micronuclei (figs. 20 and 21) which follow without a cell division the stage with four macronuclear anlagen and four micronuclei (fig. 19).

The reconstruction in the reorganization process of the typical vegetative *Paramecium* cell is now effected by two cell divisions. Figure 22 shows an animal which has undergone both of these divisions. The cell has a single well developed anlage which has attained more typical macronuclear characteristics. The chromatin bodies are rapidly degenerating. The reorganized cell at the very beginning of the new rhythmical period is shown in figure 23.

A critical survey of the ascending phase shows that even in our small tube cultures, which might be compared with Kasanzeff's culture methods (except that he starved his animals while we attempted to supply ideal conditions), relatively few *Paramecium caudatum* were able to accomplish the reorganization process. This is in agreement with Popoff's results because he found, according to our interpretation, only the early stages of the reorganization process. Calkins, though studying carefully his pedigreed cells, figures but one animal which we would interpret as showing the completion of the reorganization process ('14, III, text figs. 19 and 20), Hertwig has never observed what he considered a reorganization process in *Paramecium caudatum* ('14, pp. 568, 569). We ourselves, using the same methods which proved successful in *Paramecium aurelia* and also new ones adapted to the peculiarities of *Paramecium caudatum* in culture, could not work out in such detail the uninterrupted sequence of endomictic events from pedigreed series of cells as in the case of *Paramecium aurelia*. Nevertheless we have proved that the process of reorganization can be successfully accomplished in certain cases under pedigreed slide conditions though it is clear that these artificial slide cultures afford obstacles which the average *caudatum* cell finds it difficult to overcome when in the critical climax of the reorganization process or of conjugation. We believe the data presented establish beyond doubt that the reorganization process is a normal periodic event in the life history of *Paramecium caudatum*.

IV. COMPARISON OF THE CYTOLOGICAL PHENOMENA OF THE
REORGANIZATION PROCESS IN PARAMAECIUM CAUDATUM
AND PARAMAECIUM AURELIA

This short outline of the cytological changes of the reorganization process in *Paramaecium caudatum* makes it clear, we believe, that there is no fundamental difference between the morphological features of this process in *Paramaecium caudatum* and *Paramaecium aurelia*, further than that incidental to the fact that the former species has one micronucleus and the latter two micronuclei in its typical vegetative stages.³ The destruction of the old macronucleus and the formation of a new macronuclear apparatus of micronuclear origin is effected in both species.

However, there are some interesting minor variations which it may be well to contrast. The 'reduction' division in the reorganization process of *Paramaecium caudatum* with its 'dumb-bell' formation resembles more closely the phenomenon in the conjugation of this species than do the features of the 'reduction' micronuclear phenomena in the reorganization process of *Paramaecium aurelia* resembles those of conjugation in that species. We were unable to discover the same features in the 'reduction' division in *Paramaecium aurelia* during the reorganization process that were described by Hertwig for the comparable stages in conjugation. Hertwig ('14) figures a stage (text fig. 2, animal 3) which he interprets, together with the condition in the two previous animals (animals 1 and 2), as "Depressionserscheinungen von *Paramaecium aurelia*." We would interpret Hertwig's animal 3, on the basis of the animals figured in our earlier paper (fig. 12, pl. 1, etc.) as a cell in a stage of the reorganization process after the formation of four 'reduction' micronuclei two of which are already preparing for the second 'reduction' division. Animals with this morphological structure will actually complete the reorganization process, as we have proved ('14, III), and therefore such animals as figured by Hertwig cannot be interpreted as depression stages. Thus Hertwig indirectly and we

³ For a discussion of the specific characters of *Paramaecium aurelia* and *Paramaecium caudatum* and the literature on the subject, cf. Woodruff, *Journal of Morphology*, vol. 22, p. 223, 1911.

directly prove that the 'reduction' micronuclei in *Paramecium aurelia* in the reorganization process have somewhat different morphological characteristics from those of the same species in conjugation.

The destruction of the macronucleus before the formation of macronuclear anlagen in *Paramecium aurelia* occurs, according to our observations, only by the extrusion of chromatin bodies from the macronucleus. But in *Paramecium caudatum* there are clearly two methods of macronuclear dissolution in the descending phase of the reorganization process. One of these involves the breaking up of the macronucleus into one or more large pieces which finally degenerate in the cell (figs. 8, 9 and 10); the other involves the extrusion of chromatin bodies from the more or less intact macronuclear membrane (figs. 14, 15 and 16). This would seem to indicate that there may be also two distinct methods of accomplishing the ascending phase of the reorganization process in *Paramecium caudatum*. One closely resembles the process in *Paramecium aurelia*, being characterized by the presence of chromatin bodies, the absence of the old macronucleus in a cell with one micronucleus, this single micronucleus being the one which is to form the new nuclear apparatus. The other method is characterized by the breaking of the macronucleus into relatively large pieces and the streaming of chromatin out into the cytoplasm (figs. 10 and 13). Thus when the macronuclear destruction takes this form, there are few, if any, chromatin bodies to be found in the ascending phase. It may be mentioned incidentally that we have some data which suggest that under certain conditions merely a partial reorganization, not involving the formation of macronuclear anlagen, may lead, at least temporarily, to the continuance of the life of the line.

Our material did not allow us to prove whether the third so-called reduction division occurs or not, but we lean to the view, on the basis of our detailed study of this point in *Paramecium aurelia* ('14, III, pp. 446-450 and p. 495) that this division actually is absent in the reorganization process in *Paramecium caudatum* as in *Paramecium aurelia*. Further, we have no indication

of a cell division in the climax, and this point must be definitely settled for *Paramaecium caudatum* by further investigation.

Thus, although we have presented sufficient data to establish the occurrence of the reorganization process—endomixis—in *Paramaecium caudatum*, we have found no new fundamental facts to modify our brief theoretical suggestions as given in our earlier study of *Paramaecium aurelia*. We believe the suggestions—as there stated—must stand or fall on the basis of further study of endomictic phenomena in other Protista.

V. DISCUSSION AND CONCLUSIONS

We proved in our previous paper that a periodic reorganization process, to which we gave the name endomixis, occurred periodically throughout the seven years of the life of the main culture of *Paramaecium aurelia*. We showed in subcultures, from this main culture, in which conjugation was allowed to occur that lines derived from exconjugants underwent endomixis at the regular intervals. We thus proved that endomixis and conjugation are phenomena common to the same race of *Paramaecium aurelia*.

We showed further that endomixis occurred in a race of *Paramaecium aurelia* (culture B of our former paper) isolated in Germany. On the basis of this we stated ('14, II, p. 494, and '14, III, p. 474): "Therefore, the data justify the conclusion that this reorganization process is a normal phenomenon and probably occurs in all races of the species *Paramaecium aurelia*."

But since Hertwig intimates that endomixis is probably a peculiarity of Woodruff's main culture, we may cite further evidence to substantiate our former conclusion. We have had occasion, for certain experiments, to secure other races of *Paramaecium aurelia*. One of these was obtained from material sent to us by Prof. R. A. Budington of Oberlin, Ohio, and the other from material sent by Dr. Florence Peebles from Bryn Mawr, Pennsylvania. These two races, taken at random from material collected at widely separated localities, immediately showed endomixis at periods similar to those of the races already studied.

Thus endomixis has now been demonstrated in each of the four races which we have studied.

Further, the idea of Hertwig that endomixis occurs only after long cultivation of a race of *Paramaecium* was shown not to be true in Woodruff's main culture. We stated ('14, II, p. 492) that animals preserved during the first year of its cultivation showed stages of the process, and further we stated that the race from Berlin showed stages of endomixis very early in its history ('14, II, p. 493). Now, with this point in mind, we have found endomixis in each of the new aurelia races within the first thirty days of their life in culture.

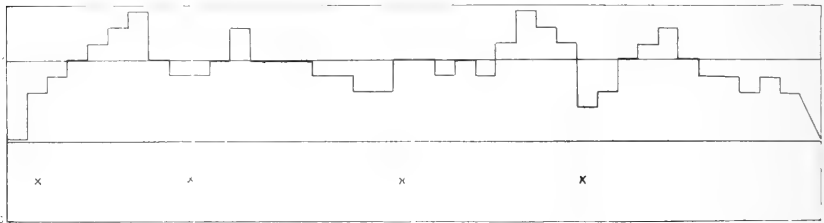
Therefore, we have proved that endomixis is a phenomenon common to all four races of *Paramaecium aurelia* which we have studied and thus it is highly probable that it occurs in all races of this species. Further, we have proved that endomixis is not a phenomenon which is gradually acquired after long pedigreed culture but is completely developed in animals at the time of isolation from wild cultures, and still further we have proved that endomixis is a potentiality of lines which have the power of conjugation ('14, III, p. 473).

Having, we believe, disposed of these questions which have been raised in regard to our work on *Paramaecium aurelia*, we are in a position to return to endomixis in *Paramaecium caudatum*. The cytological phenomena of this process have been presented in the previous sections, and we believe that we established beyond peradventure the truth of our statement ('14, III, p. 475) that endomixis "occurs at least with essentially similar features in *Paramaecium caudatum* also" and therefore that endomixis is a regular normal periodic process in the life of *Paramaecium caudatum* (text fig. 6).

Early work on Woodruff's main culture of *Paramaecium aurelia* ('09, '11) showed that there are periodic fluctuations (rhythms) in the rate of reproduction which are not the results of environmental variation, but which are due to some periodic internal phenomena of unknown character (Woodruff and Baitsell, '11). We have shown ('14) that endomixis is the underlying internal process whose physiological effect had been observed but whose

nature had only been suspected ('14, III, p. 430), and stated ('14, III, p. 481) "Therefore, it is evident not only that the reorganization process is coincident with the low points between two rhythms, but also that there is a causal relation between the reorganization process and the rhythms."

We have now established this same conclusion for *Paramecium caudatum*. In our races of this species, however, the endomictic periods appear at intervals of about 50 to 60 days, or from 80 to 100 generations, instead of from 25 to 30 days, or 40 to 50 generations as in *Paramecium aurelia*. But they are fundamentally the same morphologically and physiologically in both species.



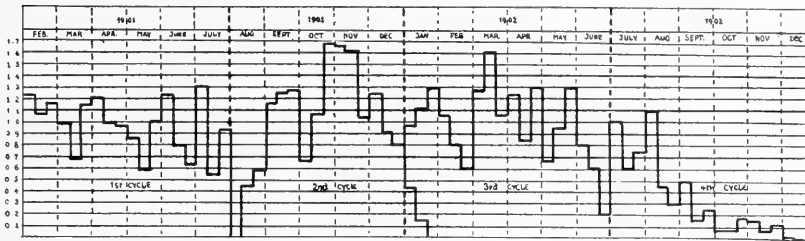
Text fig. 6 Graph of the rate of division of Culture Z, Line I, averaged for five-day periods. The periods during which the reorganization occurred are indicated by a \times . Cf. page 71, table 3; and also Woodruff and Erdmann, '14, III, text figures 16 and 17.

Now a critical examination, in the light of our present knowledge of endomixis, of the division rate of Calkins' pedigreed culture of *Paramecium caudatum*, the study of which led him to his well known conception of protoplasmic old age in Protozoa, shows clearly that his periods of degeneration can be perfectly interpreted as endomixis (text fig. 7, copied from Calkins). Woodruff in an early paper ('09, p. 300) wrote:

I have previously interpreted as rhythms the tri-monthly depressions in vitality, which Calkins and earlier workers on *Paramecium* have noted, and the results obtained from my culture of *Paramecium* seem to indicate that the semi-annual cycles of Calkins are also actually rhythms, recovery from which was not autonomous under the conditions of a constant environment. The general occurrence of rhythms in the life history of infusoria is established, I believe, but to what they are due is still awaiting discovery.

In other words the present studies on *Paramaecium caudatum* show that most if not all the depression periods of Calkins are undoubtedly rhythms and the 'cycle' is non-existent—it is merely, as stated above, a rhythm at which the organisms are unable to recover autonomously by endomixis owing to the more or less artificial culture methods imposed in daily isolation pedigreed cultivation (text fig. 7, copied from Calkins, '04).

It may be well, in view of the recent comments by Hertwig ('14) and by Calkins ('15) in their discussion of our paper on endomixis, to state the position of the problem of depression and



Text fig. 7 "History of the A Series from start (Feb. 1, 1901) to finish (Dec. 19, 1902) by ten-day periods (three periods to each month). The ordinates represent the average daily rate of division. The heavy dotted lines indicate the limits of the several cycles, and the lines of the curve carried to the base indicate that the individuals that were not stimulated by change of diet died out. The points at which such lines leave the curve indicate the time of the successfully changed diet." (Calkins, '04, p. 426.)

the significance of conjugation in *Paramaecium* in 1907 when Woodruff began his work on *Paramaecium*.

The consensus of opinion of biologists, chiefly on the basis of the work of Maupas, Calkins and Hertwig, was that infusoria are able to reproduce by division for only a limited number of generations, after which protoplasmic old age, depression, and physiological death ensue. For this the sole panacea was conjugation. But Woodruff found that by supplying proper environmental conditions it was possible to breed a pedigreed race of *Paramaecium aurelia* indefinitely (so far, April 1915, more than 5000 generations) without recourse to conjugation. Therefore, he concluded, in direct opposition to Maupas, Calkins

and Hertwig, that conjugation is unnecessary for the indefinite life of *Paramaecium* under favorable environmental conditions.

To Hertwig's and Calkins' recent contentions that their conclusion was correct and Woodruff's was wrong, we would reply, that the reorganization process (endomixis) is not conjugation and no one had any other phenomenon than conjugation—involving syncaryon formation—in mind until Woodruff and Erdmann discovered endomixis—in which a syncaryon is not formed. To say that endomixis fills essentially the same rôle in the life history of the infusorian as conjugation is to beg the entire question. Had Hertwig worked out the stages which he found over twenty-five years ago, or realized their general significance, and had he related these with rhythms ten years ago when they were discovered, the problem would have been cleared up then. The aspect of the problem has changed with the discovery of endomixis. The question is now not whether conjugation is necessary—for Woodruff has shown that it is not—but whether endomixis, a new phenomenon which Woodruff and Erdmann have shown to exist, is necessary.

Whatever the answer afforded by future work to this new question may be, it is clear that conjugation—and by this always was meant, and always is meant, the formation of a syncaryon—is not necessary, since an individual *Paramaecium* is self-sufficient to reproduce indefinitely without recourse to conjugation.

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VII. EXPLANATION OF PLATES

All the figures represent specimens of *Paramaecium caudatum*. The animals were fixed in Schaudinn's sublimat-alcohol and stained with Delafield's hematoxylin. The drawings were made from total preparations, with Abbe camera lucida, Zeiss homogeneous immersion 2 mm. and compensating ocular 12, with drawing board level with stage of microscope. Magnification, about 1500 diameters. Reduction in reproducing plates 1, 2, 3, 4, 5 is one third; plates 6 and 7 is one half.

PLATE 1

EXPLANATION OF FIGURES

1 Culture Y, Line IV, 85th generation, April 3, 1914. Normal animal in a period when endomixis is not in progress.

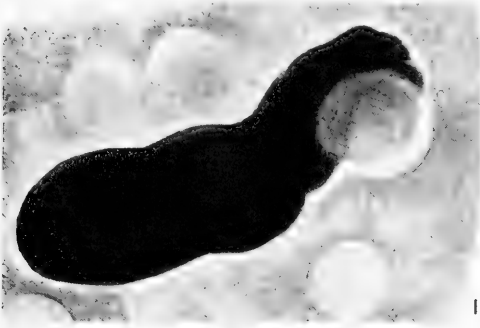
2 Culture Y, Line IV ae, 136th generation, April 28, 1914. Typical animal just at the start of endomixis.

3 Culture Y, Line III fa, 203d generation, June 8, 1914. Animal just in a very early stage of endomixis. Macronucleus wrinkled. Micronucleus has shifted from its typical position close to the macronucleus.

4 Culture Y, Line III fb, 203d generation, June 8, 1914. Animal in a slightly more advanced stage than the one shown in figure 3.

5 Culture Y, small tube culture, about 137th generation, April 22, 1914. Start of the first 'reduction' division.

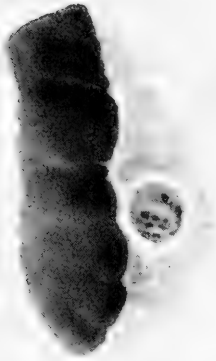
6 Culture Z, Line IV, 184th generation, December 24, 1914. Macronucleus partly devoid of chromatin. First 'reduction' division nearly completed.



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PLATE 2

EXPLANATION OF FIGURES

7 Culture Y, Line III g, 202d generation, June 7, 1914. Markedly wrinkled macronucleus and one chromatin body. Part of crescentic stage of micronucleus shown protruding from under the macronucleus.

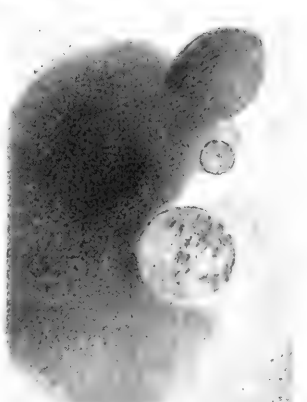
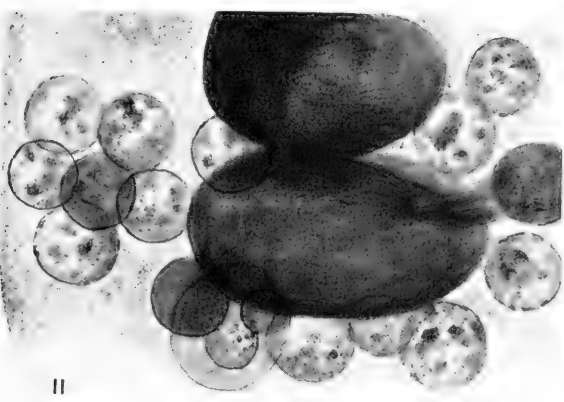
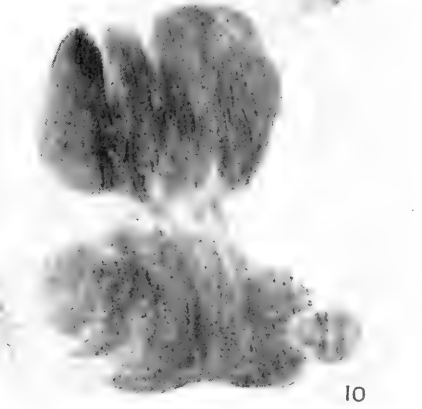
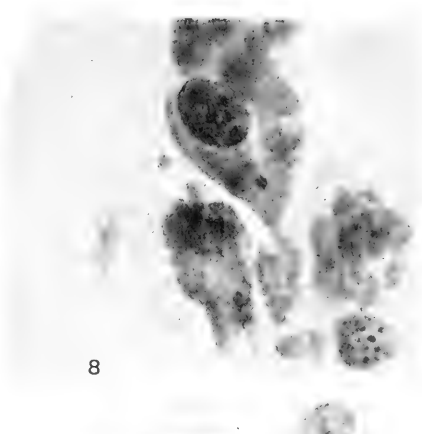
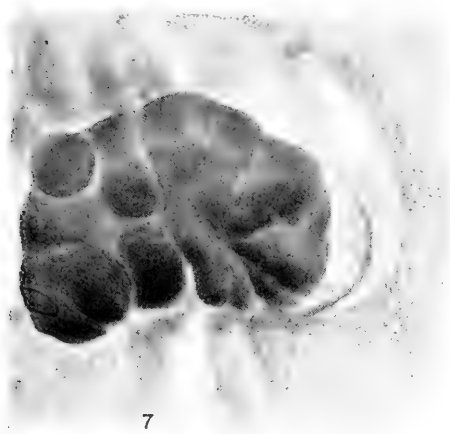
8 Culture Y, small tube culture, about the 82d generation, April 1, 1914. Macronucleus broken into several large pieces. Elongated micronucleus, one pole partly devoid of chromatin.

9 Culture M, Line II, 89th generation, February 19, 1915. Completed fragmentation of macronucleus into two parts.

10 Culture Y, small tube culture, about the 137th generation, April 22, 1914. Animal with macronucleus breaking into two parts. First 'reduction' micronuclear division completed.

11 Culture M, Line I, 90th generation, February 19, 1915. Macronucleus broken into two pieces from which chromatin is streaming and forming chromatin bodies. Two micronuclei are present, one of which is drawn. The presence of numerous food vacuoles is due to transference of this animal to rich hay infusion medium previous to killing.

12 Culture Y, Line IV, 26th generation, March 2, 1914. Two 'reduction' micronuclei are present.



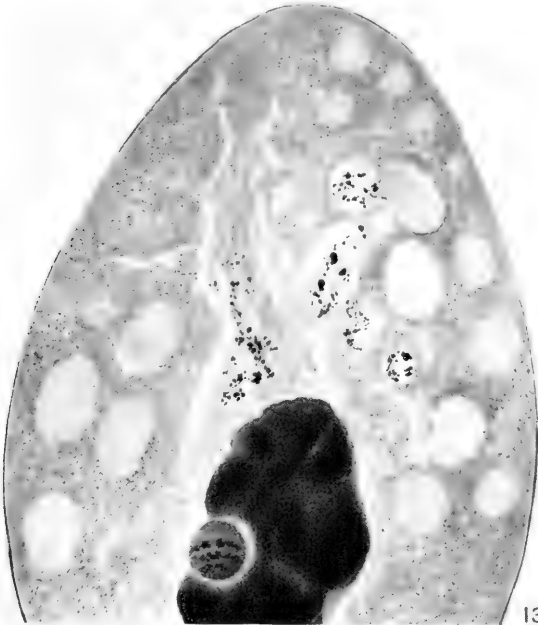
11
L. Krause del.

PLATE 3

EXPLANATION OF FIGURES

13 Culture Z, Line I, 91st generation, November 5, 1914. Illustrating the method of macronuclear dissolution by streaming out of chromatin. Micronucleus shows the characteristic band formation of early endomixis.

14 Culture Z, Line I, 93d generation, November 6, 1914. Macronuclear dissolution by elimination of chromatin bodies. Single micronucleus close to macronucleus. Five food vacuoles shown in the cell.



13



14

L. Krause del.

PLATE 4

EXPLANATION OF FIGURES

15 Culture Y, Line IV, 18th generation, February 26, 1914. Descending phase. One micronucleus and some chromatin bodies visible.

16 Culture Y, small tube culture, about 137th generation, April 22, 1914. Descending phase. One chromatin body is in the macronucleus, others have left it.



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L. Krause del.

PLATE 5

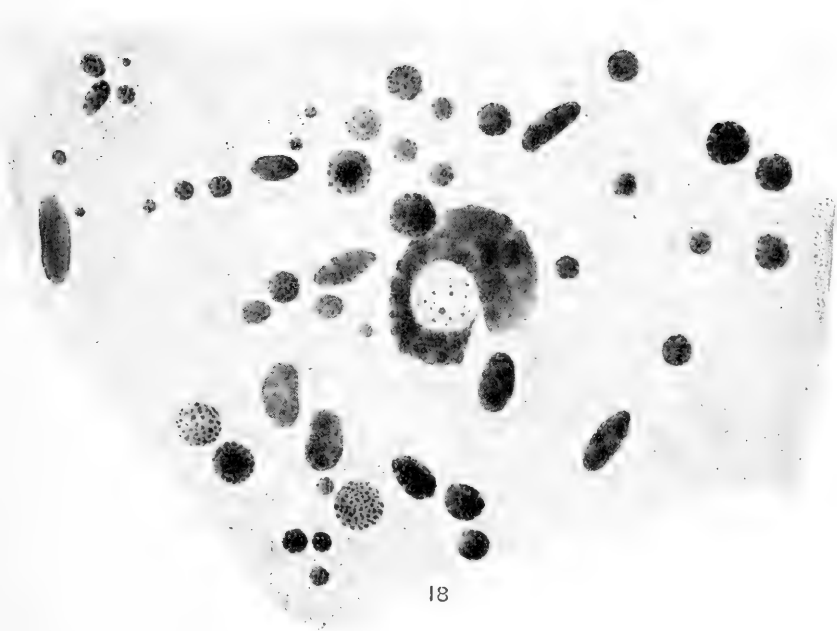
EXPLANATION OF FIGURES

17 Culture Y, Line III g, 202d generation, June 6, 1914. To illustrate that essentially the same stage as that shown in figure 14 from Culture Z occurred in another Culture Y in isolated animals.

18 Culture Y, small tube culture, about 137th generation, April 22, 1914. Animals in the climax of endomixis. Macronucleus partially resolved into degenerating chromatin bodies. Micronucleus after 'reduction.'



17



18

PLATE 6

EXPLANATION OF FIGURES

19 Culture Y, small tube culture, about 137th generation, April 22, 1914. Animal in ascending phase of endomixis. Four micronuclei and four macronuclear anlagen present. Numerous chromatin bodies in all stages of degeneration.

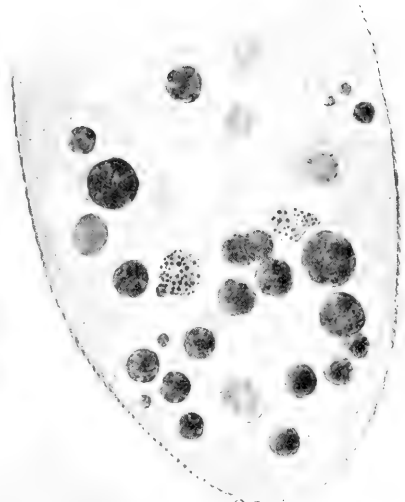
20 and 21 Culture Y, small tube culture, about 82d generation, April 1, 1914. Animals in ascending phase. Two micronuclei and four anlagen are present.

22 Culture Y, small tube culture, about 82d generation, April 1, 1914. Animal in the ascending phase, showing one anlage.

23 Culture Y, small tube culture, about 82d generation, April 1, 1914. Paramecium cell immediately after undergoing endomixis.



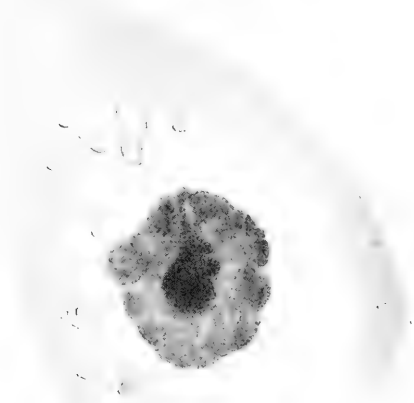
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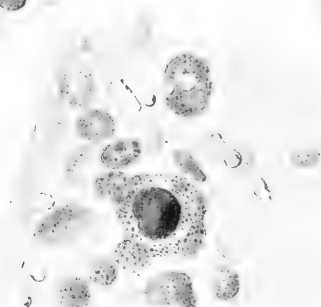
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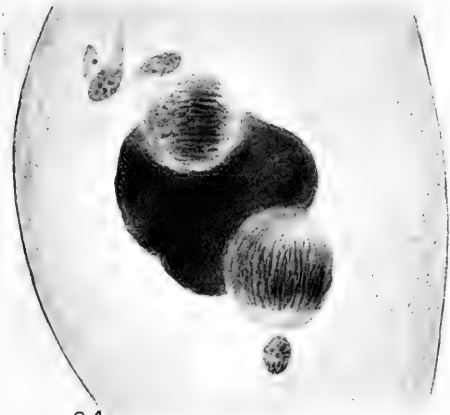


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PLATE 7

EXPLANATION OF FIGURES

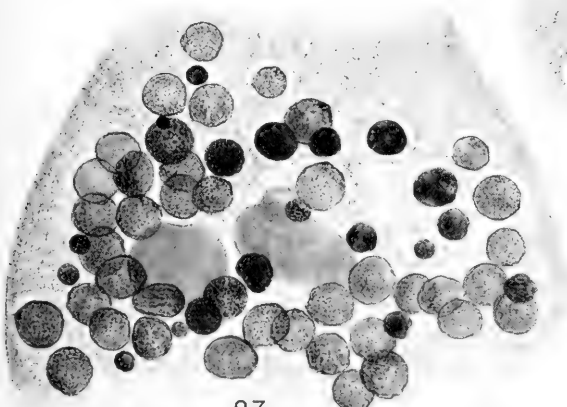
- 24 Culture Z, March 27, 1915. Exconjugant. Division of syncaryon.
- 25 Culture Z, March 27, 1915. Exconjugant. Macronucleus show typical ribbon formation. Miconuclear divisions preliminary to formation of macronuclear anlagen.
- 26 Culture Z, March 27, 1915. Exconjugant. Four macronuclear anlagen and four micronuclei.
- 27 Culture Z, April 4, 1915. Individual from the first division of exconjugant.
- 28 Culture Z, April 5, 1915. Individual from the second division after conjugation.



24



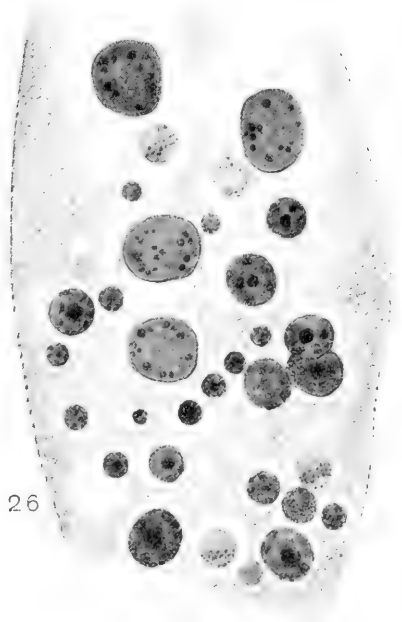
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26

AN ANALYSIS OF THE PROCESS OF REGENERATION IN CERTAIN MICRODRILOUS OLIGOCHAETES

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TWENTY-FOUR FIGURES

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The importance of regeneration as an experimental method has not been sufficiently recognized. The numerous investigations which have been carried on in this field have given us a vast amount of data regarding the capacity for regeneration of all groups of animals, the axial differences in rate and amount of regeneration, the histology of the process, and so on, but little progress has been made in the interpretation and analysis of the facts observed. Child ('11 d) has pointed out the fundamental biological significance of regulatory phenomena, and has shown

that important problems may be attacked by means of this relatively simple experimental method.

Under Professor Child's direction, I have been carrying out experiments along similar lines on several species of microdrilous oligochaetes; this paper is a partial report of the results of these investigations.

I. MATERIAL

The following species have been used in these experiments:

Aeolosomatidae

Aeolosoma hemprichii Ehrenberg.

Naididae

Dero limosa Leidy.

Dero furcata Oken.

Stylaria lacustris Linnaeus.

Chaetogaster diaphanus Gruithuisen.

Nais elinguis Müller.

Lumbriculidae

Lumbriculus inconstans Smith.

Tubificidae

Tubifex tubifex Lamarck.

Limnodrilus claparedianus Ratzel.

Aeolosoma was obtained from ordinary Protozoan cultures such as are made up for class use. The naids were collected from old ponds and streams, especially Wolf Lake, Indiana, and the Des Plaines River near Lyons, Illinois; quantities of mud and vegetation were brought in from such places, and put into large crystallizing dishes. I have not found it practicable to keep any naids but *Dero* for any length of time in the laboratory, but this form is readily cultivated, and thrives and reproduces rapidly if a small amount of fermenting grain is added to the culture. The tubificids were collected in the same places and in the same way as the naids; they can be kept for months under laboratory conditions, and are also benefited by the addition of grain to the culture. *Lumbriculus inconstans*, unlike the other fresh-water oligochaetes, is very restricted as to habitat, occurring, in the Chicago vicinity at least, only in temporary

forest pools of a characteristic ecological type (see Shelford, '14, pp. 179, 185). This species is active, therefore, only during the spring months when the pools contain water, since it passes into an encysted condition when the water evaporates with the approach of summer.¹ Mrazek ('13) states that such pools form also the typical habitat of *Lumbriculus variegatus*, and his further remarks on the ecology of this species apply equally well to *Lumbriculus inconstans*.

II. THE AXIAL GRADIENT

1. *The cyanide method*

The most important general fact which has come out of all the work on regeneration is this;—that the result differs according to the level along the antero-posterior axis at which section is made. This difference may be one of rate or of amount of new tissue formed, or, in the most interesting cases, the structure which appears at the cut surface varies according to the level of section. Such an axial difference is obviously the expression of a preexisting internal gradient of some sort,—a protoplasmic gradient independent of the more obvious morphological features of the organism. This gradient must undoubtedly have both structural and functional components, for structure and function always interact; in the living organism they can have no separate existence. But it is the functional component alone with which I propose to deal in this discussion of the axial gradient.

What means have we for demonstrating a functional, dynamic gradient—or, to put it more simply, a gradient in metabolic processes—along the axis of the living organism? Child ('13 a) has devised and extensively employed a simple method depending on the use of lethal concentrations of anaesthetics and cyanides. In the presence of such substances—and of many other poisons also—metabolism cannot continue, and if there exists a metabolic gradient in the organism, its parts will show a different

¹ A complete account of the life cycle of *Lumbriculus inconstans* in relation to its peculiar habitat will be published elsewhere.

degree of susceptibility, or, conversely, a different degree of resistance to the action of these substances. This will be especially true if the metabolic difference is one of rate, for those parts in which metabolism is going on most rapidly must be most susceptible to substances which stop metabolism; moreover, we have no reason to assume that the fundamental metabolic processes are different in kind along the axis of a simple organism. The method is, therefore, within certain limits, specifically a method for demonstrating differences in metabolic rate between the parts of an organism, or between comparable organisms.

Regarding the exact way in which substances containing the cyanogen radical and anaesthetics act on protoplasm, there is at present much difference of opinion. It would be fruitless to review here the numerous theories which have been advanced with regard to the action of anaesthetics (Gwathmey, '14, Chap. II) among them probably the Verworn conception of anaesthesia as an asphyxiation has been received with the most favor. The most recent evidence does not, however, support this view. Thus Loeb and Wasteneys ('13 a, '13 b) and Winterstein ('14) have measured the oxygen consumption under anaesthesia, and have found that narcosis may occur without any decrease in oxygen consumption, or usually only a slight one, or even a slight increase. Winterstein ('13, '14) also calls attention to many other data leading to the conclusion that the depression of oxidation is not the primary factor in the production of anaesthesia, as Verworn maintains. On the other hand, Tashiro and Adams ('14) have reported a decided decrease in CO_2 production in the anaesthetized nerve of the spider crab. Further experimental work is much needed before any conclusion regarding the nature of anaesthesia can be drawn.

More agreement exists as to the effect of the cyanides on living matter. Since the work of Geppert, it has been generally accepted that the cyanides act by diminishing or inhibiting the oxidation processes. Geppert ('89) showed that an animal poisoned by hydrocyanic acid was consuming less than the usual amount of oxygen, even though such an animal goes into violent convulsions; and further, that the oxygen content of the venous

blood in cyanide poisoning is abnormally high. Geppert therefore concluded that cyanogen reduces the capacity of the cells to use oxygen. This conclusion is supported by the experiments of Warburg ('10), of Loeb ('06 a, '06 b, '10 a, '10 b), and of Loeb and Wasteneys ('10, '11, '13 a, '13 b), who found that the oxygen consumption of eggs and other cells is decreased in the presence of cyanides; of Schroeder ('07) who obtained the same result with *Aspergillus*; and of Vernon ('06) who showed that the oxygen consumption of a perfused kidney is diminished when cyanides are present in the perfused fluid; and I myself, working with sponges, have recently demonstrated to my entire satisfaction that the oxygen consumption of these animals is invariably lowered when potassium cyanide is present in the sea water, even in concentrations as low as $\frac{N}{25000}$.² The cyanides also have a general depressing effect on enzymatic processes, and on many chemical actions, oxidative and otherwise. On the other hand, there have not been wanting objections to the idea that the cyanides inhibit oxidations; principal among these is the statement that the cyanides are equally poisonous to tissues and organisms which are not affected by the absence of atmospheric oxygen, as the nerve cord of the *Limulus* heart (Carlson, '07), and anaerobic bacteria. In reply to this, it may be suggested that the series of chemical processes which we call oxidation is probably much the same up to a certain point in both aerobic and anaerobic organisms, and it is these initial reactions which the cyanide affects (Matthews, '05).

Direct evidence that the susceptibility to cyanide runs parallel with the rate of metabolism has been furnished by Child ('13 a), who has experimentally determined the following facts:

1. Animals stimulated to motor activity are more susceptible than quiescent ones.
2. The susceptibility increases with rising temperature; and the temperature coefficient of susceptibility is the same as the usual temperature coefficient for chemical reactions in general.
3. Other forms of stimulation (as injury, cutting the animal into pieces) increase the susceptibility to cyanide.

² These results will be published shortly.

4. Young animals are invariably more susceptible to cyanide than old ones.

5. The CO_2 production of pieces or animals runs parallel with their susceptibility to cyanide, *i.e.*, those more susceptible to cyanide show also a more rapid CO_2 production. The CO_2 production was determined by Dr. Tashiro with his very ingenious apparatus for measuring minute amounts of carbon dioxide (Tashiro, '13).

According, then, to the facts here presented, the time of death in cyanide bears a direct relation to the previous rate of metabolism. Individuals or parts with the highest rate of metabolism die first, those with the lowest rate last, and the others at intermediate times; the cause of this, as already stated, is to be sought in the asphyxiating action of the cyanides, as a result of which the time of death of each part is proportional to its rate of oxygen consumption. It is only necessary that the death point should be clearly indicated to the observer; this is brought about in the lower invertebrates through the disintegration which promptly follows death. As a check on one's observations, one may remove the animal or piece at any stage of the disintegration to water, whereupon recovery of the intact parts takes place. In this way, I have satisfied myself that disintegration follows death almost instantly. The cyanide method is, of course, not applicable to forms in which, owing to resistant outer structures, disintegration cannot occur; but even in such forms, the death of the animal as a whole may usually be determined by employing some other criterion of the death point.

The technique of the cyanide method is simple. A concentration of potassium cyanide sufficient to kill the animals within one to three hours is used. This concentration must be determined for each species by preliminary experiments. For the oligochaetes it varies from $\frac{1}{50}$ to $\frac{1}{1000}$ normal. The cyanide solution is made up fresh by weight for each experiment. Animals or pieces which are to be compared as to susceptibility must have been kept under the same conditions of food, temperature, etc., previous to the experiment, and must be of approximately the same size, unless size differences are the object of the experiment.

Wherever susceptibilities are compared, I have always done so at the same time, and with the same cyanide solution, thus avoiding sources of error arising from differences in the solution, external conditions, etc. I have found it most convenient to carry out the experiments in watch glasses. The animals are placed in these, freed as much as possible from water, and a cover put on in such a way as to exclude all air bubbles. In such covered watch glasses evaporation of the cyanide is reduced to a minimum, and the whole can be placed under the low power of the compound microscope, and the progress of the disintegration followed very exactly.

The following changes take place in cyanide. The worms at first move about vigorously but eventually pass into a state of anaesthesia. The peristalsis of the intestine and especially of the dorsal blood vessel keeps up as a rule until the time of death. Frequently there is a swelling of the animal, due to the intake of fluid into the coelomic spaces, so that the body wall is distended, except at the septa, and the animal then resembles a string of beads. This condition appears to be a regular ante-mortem change since it also occurs in pieces dying in water. The death point is characterized by an abrupt change from the normal yellowish-red color of the oligochaetes to an opaque white; this change of color is quite apparent to the naked eye, and under the microscope can be followed from segment to segment. Simultaneously with or immediately following this alteration of color, the body wall breaks (it may previously have shown blister-like elevations), and all the structures disintegrate into a shapeless mass of granules. This disintegration, as already indicated, does not occur simultaneously throughout the animal, but proceeds in a perfectly definite manner along the antero-posterior axis. This disintegration gradient of the various oligochaetes will now be described in detail.

2. The primary gradient

The kind of gradient which Child has described for Protozoa, Coelenterates and flatworms (Child, '13 c, '14 a, '14 b) constitutes what I call here the primary gradient. In these forms, disinte-

gration begins at the anterior end, and proceeds backwards along the axis to the posterior end. This is interpreted to mean that the head, or what represents the head, of the organism is carrying on metabolic processes at the highest rate, and that the rate of metabolism decreases along the antero-posterior axis. This kind of gradient exists in the egg, and Child ('13 c) has suggested that it is the physiological basis for the law of antero-posterior development. Child further suggests that this gradient, existing in the protoplasm of the egg is carried over to the nervous system when the latter develops. The cephalic end of the nervous system thus exercises from the very first physiological dominance over the rest of the organism, and retains this dominance by virtue of its functional relations to other parts, even though its actual metabolic rate may, and does, fall throughout ontogeny. The gradient of the adult animal may therefore vary markedly from the original gradient; various parts may attain a higher rate of metabolism than the head itself; but the nervous system, owing to long-established antero-posterior conduction paths, can maintain control, for some time at least, over regions of higher metabolic activity than its own.

Is the gradient of the adult oligochaete of the primary type? I have found it so in but one species examined, namely, *Aeolosoma hemprichii*, a member of the most primitive family of oligochaetes. This worm is very small, only 1-2 mm. in length, has a rounded prostomium, a ciliated funnel-shaped pharynx leading into the intestine, and numerous red oil globules in the body wall. Each animal nearly always consists of two or more zooids which arise in connection with fission planes in the typical annelid manner; before they appear morphologically, they are present physiologically, as is readily demonstrated by the cyanide method.

For the disintegration experiments, a concentration of $\frac{N}{100}$ KCN is used, and the animals are placed in covered watch glasses as already described. In an individual without zooids, the disintegration begins at the tip of the prostomium and proceeds at first slowly, then more rapidly along the axis. The integumental oil globules remain intact longer than the parts in which they were imbedded, but eventually they vanish by sudden extrusion

of their colored contents. The gradient of such an individual is graphically depicted in text figure 2. In these graphs the abscissae represent the number of segments and the ordinates, the time of death in minutes. The dots along the curve are the points actually determined experimentally. In text figure 2, the flatness of the curve between the third and the fourth segments indicates that the head of a zooid is forming there; this graph should therefore be compared with figure 1, which is the disintegration gradient of a fully developed posterior zooid of *Aeolosoma*, and which illustrates a typical primary gradient—

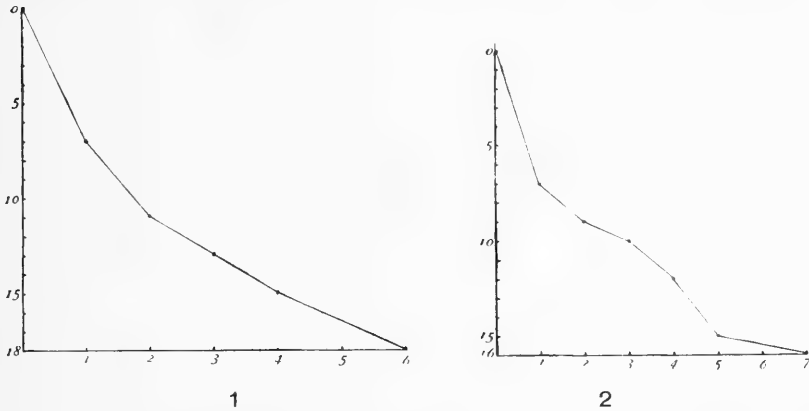


Fig. 1 The axial gradient of a mature zooid of *Aeolosoma hemprichii*, illustrating an ideal primary gradient.

Fig. 2 The axial gradient of an individual *Aeolosoma* in which physiological isolation of a zooid is beginning.

i.e., one that is steepest at the anterior end, and gradually falls off posteriorly.

As the individual *Aeolosoma* grows, the posterior zooid continues to differentiate physiologically. When such an individual is allowed to disintegrate in cyanide, the first change that occurs is the appearance of a constriction near the posterior end. This constriction marks the position of the head of the posterior zooid. Disintegration then proceeds independently in both zooids, from the anterior to the posterior end of each. Four stages in the disintegration of such an individual are illustrated in figure 3,

and a graph of the same in figure 4. The relative time of disintegration of the second zooid as compared with the first depends

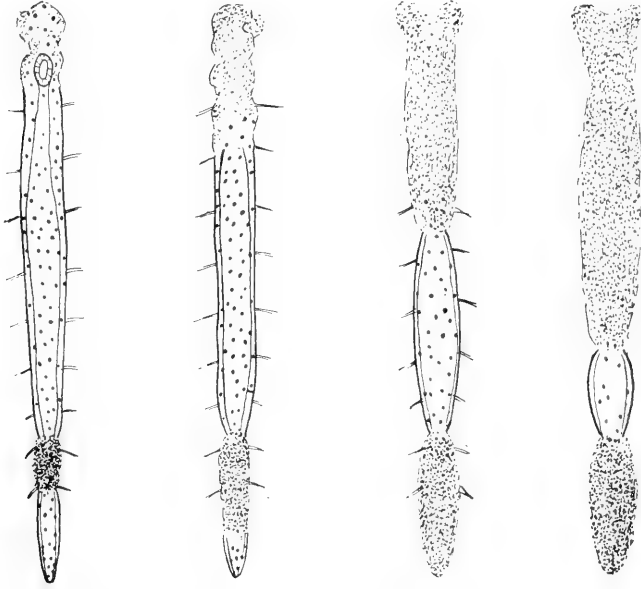


Fig. 3 Three stages in the disintegration of an *Aeolosoma* in which physiological isolation of a zooid is well advanced.

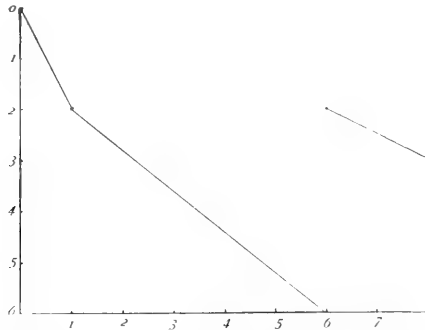


Fig. 4 Graph of the axial gradient of an individual similar to figure 3.

on the degree of development of the former; at the beginning of its existence, when it is present physiologically only, its time

of death about coincides with that of the principal zooid; but, owing to the processes of dedifferentiation and growth involved in its formation, its susceptibility to cyanide continually increases so that its death comes to be preceded by a considerable time interval that of the principal zooid. The rate of metabolism of the zooid thus continuously rises during its development.

Eventually the presence of the posterior zooid is made known through the appearance of a fission plane, and its structural

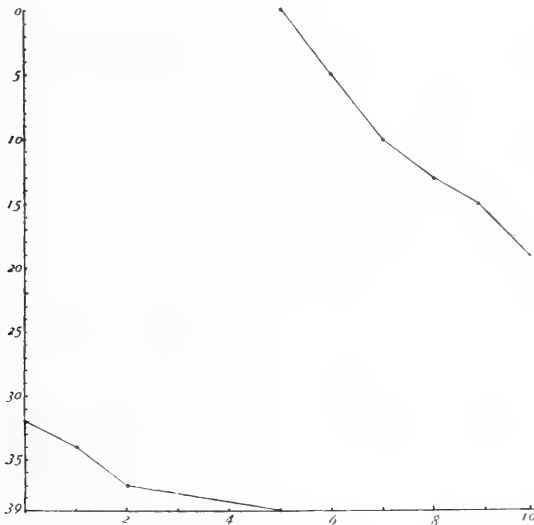


Fig. 5 Graph of an *Aeolosoma* with a zooid well differentiated morphologically, showing the two independent gradients, that of the zooid being at a higher level.

differentiation proceeds. If examined in cyanide at this time, the two independent gradients show very clearly, that of the posterior zooid being at a higher level (fig. 5). The axial gradient of the posterior zooid is of the primary type, and remains so, indeed, until it has separated from the parent animal and begun to produce zooids of its own. The gradient of the anterior zooid is at the beginning of the development of the posterior zooid also of the primary kind but later becomes altered. Disintegration starts at its head and proceeds posteriorly, but soon the

posterior end begins to disintegrate, and this disintegration proceeds anteriorly. The two waves of disintegration meet somewhere about the middle of the zoid. The interpretation of this sort of gradient is simple. Annelids grow characteristically by the formation of new segments in front of the anal segment;

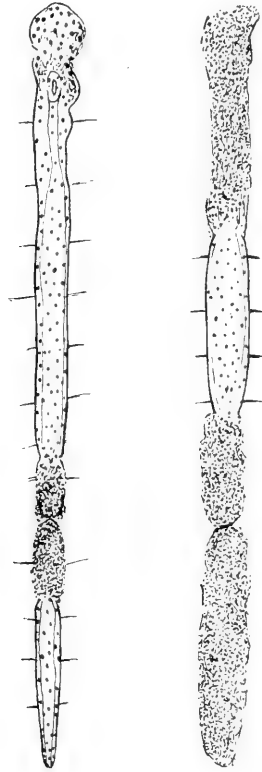


Fig. 6 Two stages in the disintegration of an Acolosoma with a zoid, showing the secondary posterior rise in the principal zoid.

the new segments thus formed would be expected to have a high rate of metabolism, and therefore an increased susceptibility to cyanide. As soon as the Acolosoma individual has formed a posterior zoid, it begins to grow in this fashion at its new posterior end, and this growing region shows increased susceptibility to cyanide (fig. 6). In fact, this process may go to such an

extent that the axial gradient of the anterior zooid is reversed (fig. 7); here disintegration begins at the posterior end and proceeds to the anterior end. This condition is found in individuals in which the posterior end has grown considerably without becoming physiologically isolated as a zooid; in such cases, the increasing youth of the segments posteriorly, as well as the fall in rate of metabolism of the anterior end through senescence contribute to cause the reversal of the gradient. As soon as the posterior end of such an individual becomes isolated as a zooid, the primary gradient reappears in it.

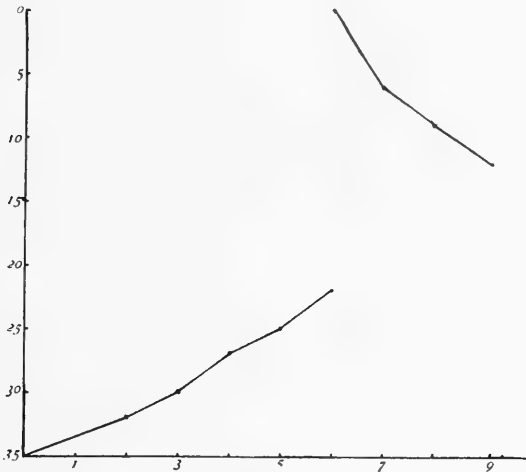


Fig. 7 Graph of an *Aeolosoma* with a zooid, showing reversal of the gradient in the principal zooid.

As a further interesting detail, I may add that although the disintegration of the head usually begins at the tip of the prostomium, yet sometimes, and especially in old individuals, the first region of the head to disintegrate is the ciliated pharynx. Such a specialized organ by virtue of its sensory functions, ciliary activity, etc, must possess a relatively high rate of metabolism which it retains when the rate of surrounding parts has diminished through senescence. Such specialized parts with high susceptibility to cyanide are frequently met with in disintegration

experiments; as examples may be mentioned the sensory auricles of *Planaria*, and the lower lip of the oligochaetes.

Summarizing these results, it is shown that in *Aeolosoma* the primary gradient is present, i.e., the rate of metabolism—as measured by degree of susceptibility to cyanide—is highest at the head, and decreases along the antero-posterior axis. This gradient is present in the zooid from the very beginning of its existence, and continues during its differentiation, and after it has separated. Its rate of metabolism continually increases during this period, but after it has separated and begun to form zooids of its own, the rate falls, and may eventually be exceeded by that of its growing posterior end.

3. *The gradient of the naids*

The primary gradient which exists in *Aeolosoma* is not retained in any other oligochaetes which I have examined except in the zooid stages. I have already spoken in the case of *Aeolosoma* of the rise in susceptibility at the posterior end owing to the formation of new segments there. *Aeolosoma*, however, gives rise to zooids so rapidly that this growing posterior region does not attain to any considerable size because it is always being cut off by zooid formation. But in other Oligochaetes, especially those which do not reproduce asexually at all, this posterior growth is extensive, and has a marked effect on the axial gradient.

Among the naids, I have worked for the most part with *Dero limosa*. A concentration of KCN of $\frac{N}{400}$ or $\frac{N}{500}$ was used. In individuals without fission planes the process of disintegration is as follows. Disintegration begins at the anterior end, involving the tip of the prostomium and the sensory region about the mouth first, and passes posteriorly along the axis; after it has progressed some distance, which varies with individuals, disintegration begins at the posterior end and proceeds forwards; the two waves of disintegration meet about the middle or behind the middle of the worm, the exact point also varying with individuals.

This is the typical annelid gradient,—i.e., one in which the rate of metabolism decreases from the head backwards and rises again at the posterior end.³ This gradient, as already stated, is due to the characteristic method of growth of annelids by formation of new segments posteriorly. If the posterior end has been growing very rapidly, as it does when there is abundant food supply, its rate of metabolism may be higher than that of the head, and it may, therefore, disintegrate first, but the disintegration of the head always follows shortly after.

Three stages in the disintegration of *Dero limosa* are illustrated in figure 8, and a graph of the same in figure 9. In this graph, and in the succeeding ones, I have attempted to compensate for the continual decrease in size of the posterior segments by gradually increasing the number of segments per unit of the cross-section paper. In this way, a truer picture of the gradient is obtained.

The posterior disintegration may begin with the anal segment, or in the region just anterior to this where the youngest segments are forming. In the latter case, the anal segment, which in the

³ As a matter of fact, Morgan ('04), if he had only known it, long ago demonstrated the typical annelid gradient in the earthworm by means of a galvanometer. He found that in the earthworm the anterior and posterior ends are electronegative to the middle part. It is a familiar physiological fact that stimulated regions (i.e., regions of increased metabolism) are electronegative to non-stimulated ones (current of injury, negative variation, electrical variation during the heart beat, etc). The fact that cutting is a stimulation also accounts for the general result of Morgan that cut surfaces of the earth worm are electronegative to intact ones. The results of Morgan also indicate that the clitellum and the fifteenth segment are local regions of high metabolic activity, probably owing to their secretory nature. The data which seemed inexplicable to Morgan therefore find easy explanation when the nature of the annelid gradient and the stimulating effect of cutting are known. There can be no possible doubt that the difference of potential between the intact and the cut surfaces has a different value when the cut surface is to form a head than when it is to form a tail, but there is no reason whatever for assuming that a reversal of potential should occur in the tail forming region of the earthworm. In experiments of this kind there are many important factors to be considered, such as the length of time after cutting, length of the piece, part of the animal from which the pieces are cut, part of the intact surface to which the other electrode is applied, etc. The observations of Czwiklitzner (Arch. f. Entw'mech, Bd. 19) on the disintegration and death of the polychaete *Ophyotrocha* also constitute a demonstration of the annelid gradient.

genus *Dero* is enlarged to form a gill pavilion, remains alive longer than any other part of the body. The anal segment is, of course, one of the oldest parts of the body in Annelids, but in forms which divide by fission, each anterior zooid has to form a new anal segment, while the posterior zooid retains the old anal

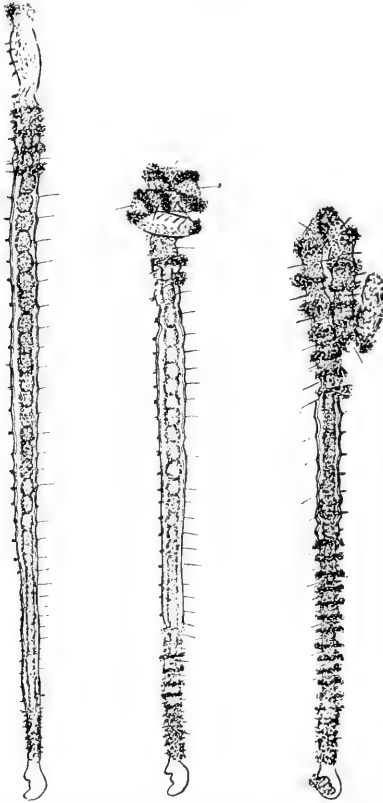


Fig. 8 Three stages in the disintegration of *Dero limosa*.

segment. It thus happens that in any individual *Dero*, the anal segment may be very old, or it may be relatively young, and this accounts for differences in the time of disintegration of this part as compared with the rest of the body.

The axial gradient of *Dero* is modified by the presence of zooids. When the worms have reached a length of 10–15 mm. with 50–80

segments, a fission plane in the form of a constriction appears posterior to the middle of the body. This constriction is coincident with a septum; the anterior end of the new zooid arises between this septum and the succeeding bundles of setae, and the region between the septum and the preceding bundles of setae develops a new posterior end for the old animal. All structures develop completely before separation of the zooids takes place. Usually but two zooids are present. In *Dero*, the region where the fission plane is to appear is not detectable by an increased

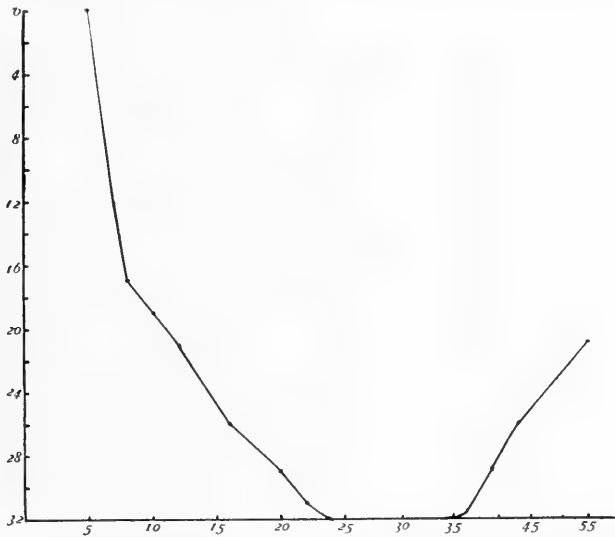


Fig. 9 Graph of the axial gradient of *Dero limosa*, showing the secondary posterior rise typical of oligochaetes.

susceptibility to cyanide, as is the case in *Aeolosoma*. In fact, even after the fission plane is visible, there is no increased susceptibility at that point. It is only after differentiation has begun on the two sides of the fission plane that any alteration of susceptibility is noticeable. At first this consists in a slight increase in susceptibility at the anterior end of the second zooid—that is, disintegration begins at the anterior end of the first zooid, proceeds back some distance, then attacks the anterior end of the second zooid, then the posterior end, and finally the remaining

parts of both. In terms of metabolism, the metabolic rate decreases from the head of the anterior zooid backwards, rises at the head of the second zooid, falls again, and finally rises steadily to the posterior end. Figure 10 illustrates a stage in the disinte-

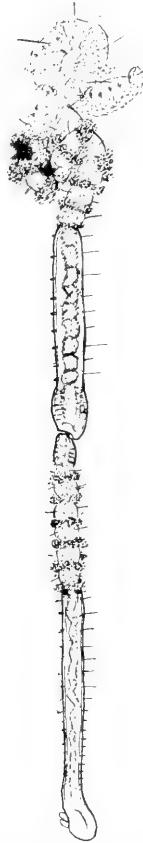


Fig. 10 A stage in the disintegration of a *Dero* with a well developed posterior zooid.

gration of the two zooids, and figure 11 is the corresponding graph. As the development of the head of the posterior zooid proceeds, its susceptibility to cyanide continually increases, approaches that of the head of the first zooid, and eventually exceeds it. Meantime, processes of reorganization have been going on

in the posterior zooid, so that a new gradient is established; this gradient is of the primary type. If, therefore, a worm with a well-developed posterior zooid is allowed to disintegrate in cyanide, it is found to consist of two independent gradients; disintegration begins at the anterior end of each zooid, and proceeds to the posterior end of each (fig. 12). There may be a slight increased susceptibility at the posterior end of the first zooid. After separation of the zooids, posterior growth sets in, producing

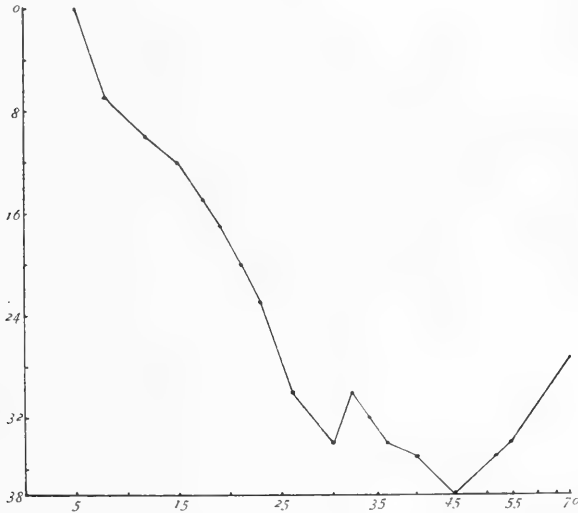


Fig 11 Graph of the axial gradient of *Dero limosa* with a young zooid.

again the rise in rate at the posterior end, which is characteristic of Annelids.

It is to be noted that it is the new anterior end and not the new posterior end in which the marked rise in the rate of metabolism occurs. This change in rate is connected, first, with the formation of a new head, and, secondly, with the reorganization of the parts behind this head to form a new individual. The visible changes in this latter process consist in the working over of the anterior part of the intestine to form an oesophagus, and of the circulatory system to form the 'hearts.' As a result of these changes, the primary gradient is restored.

The axial gradient of other naids is similar in all respects to that of *Dero limosa*, and the changes connected with zooid formation are also the same. I have examined *Stylaria lacustris* in some detail with regard to the gradient, and *Dero furcata*, and *Nais elinguis* less thoroughly, and these forms agree completely with *Dero limosa*. I have also performed some disintegration experiments with the marine syllid, *Autolytus cornutus*, which also reproduces asexually, and whose gradient is quite like that

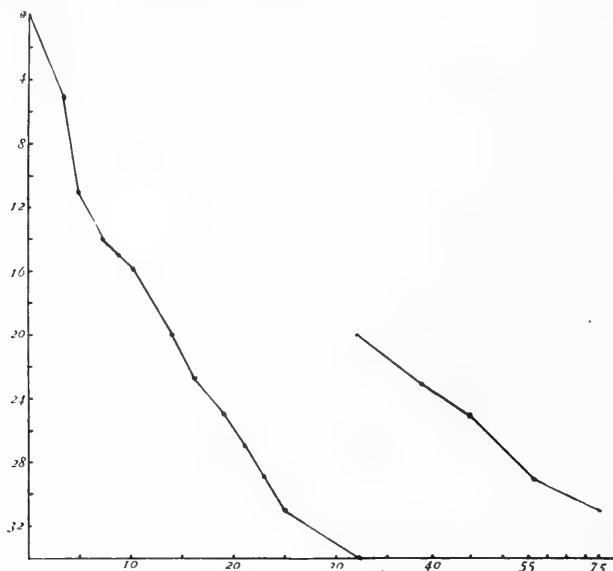


Fig. 12 Graph of the axial gradient of *Dero limosa* with a well-developed zooid, showing the two independent gradients.

of the naids. The polychaetes, are however, unfavorable for this kind of experiment because they do not disintegrate readily. The gradient of *Chaetogaster diaphanus*, however, is unlike that of other naids, and is so peculiar that it would be profitless to describe it. The very young zooids of *Chaetogaster* have, nevertheless, the primary gradient, but as soon as morphological differentiation begins in the zooids, the primary gradient is replaced by the adult gradient, whose chief peculiarity is that the head is least susceptible to cyanide, while a small region at the posterior

end of the stomach is most susceptible. *Chaetogaster* differs from other naids in a much lower degree of cephalization, and greater differentiation in the alimentary tract, and these differences probably account for its gradient.

In the naids, then, the primary gradient is present only in the zooid stage; in the adult individual there is always present a posterior growing region which has a high rate of metabolism. This region varies in length, but usually includes about the posterior third of the worm. It does not become independent by virtue of its increased rate of metabolism, but continues to be dominated by the cephalic nervous system as shown by the simple fact that it is stimulated on being isolated from the rest of the worm. This stimulation is demonstrable in two ways,—first, by the increased motor activity after isolation, and secondly, by increased susceptibility of such isolated posterior ends to cyanide. It is obvious that an independent part would not be stimulated by physical isolation from the rest of the organism, for it is already physiologically isolated; thus when the head is cut off, it exhibits no increased activity, and no increased susceptibility to cyanide.

With the formation of a zooid in the posterior region, its gradient returns to the primary condition. The head of the zooid becomes dominant over this region; its rate of metabolism grows higher and higher as differentiation proceeds; and with the reorganization of the remaining part to form the new animal, a new independent gradient of the primary kind becomes established.

4. *The gradient of Lumbriculus*

In this oligochaete, the posterior rise in metabolic rate is very marked, owing to the greater length which this worm attains (50 mm. or more). A concentration of $\frac{N}{200}$ to $\frac{N}{400}$ KCN is used for this species. Disintegration begins about simultaneously at both ends, or either end may precede, generally the posterior end. The waves of disintegration then sweep rapidly forward from the posterior end and slowly backward from the anterior end, meeting somewhat anterior to the middle of the worm (fig.

13). The gradient of *Lumbriculus* is not very steep, except at the anterior end, and slight variations, which seem to be related to temporarily increased muscular activity, often occur. Thus if the worm happens to coil itself, the coiled part may show slightly increased susceptibility as compared with surrounding parts, which may be explained as due to contraction of muscles in coiling. Such variations become apparent in a slightly sloping gradient but are concealed in steep gradients such as occur in the naids. The slight slope of the gradient of *Lumbriculus* indicates a low degree of correlation.

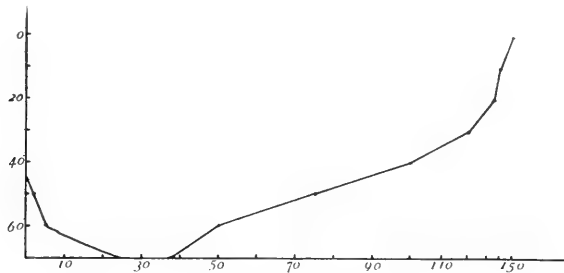


Fig. 13 Graph of the axial gradient of *Lumbriculus inconstans*.

5. *The gradient of the tubificids*

In the tubificids, the posterior rise in metabolic rate has proceeded to a maximum extent. The forms used were *Tubifex tubifex* and *Limnodrilus claparedianus*, and the concentration of cyanide about $\frac{N}{5000}$. Disintegration always begins at the posterior end and proceeds far forward before the head begins to disintegrate. The disintegration proceeds very slowly back from the anterior end and meets the posterior disintegration somewhere between the fifth and the fifteenth segments of the body (figs. 14 and 15).

6. *The gradient in embryos*

Before concluding this section, I should like to speak briefly of the gradient of the annelid egg and embryo. Child ('14 a) has worked with the eggs of *Chaetoperus* and *Nereis*, and found

that in the early stages the animal pole shows the highest susceptibility, but that later a second region of high susceptibility appears in the somatic plate from which the three larval segments developed. Among the *microdrilus oligochaetes*, I have been able to obtain young of *Tubifex tubifex* only. In the spring of 1914, nearly all of the individuals of a *Tubifex* culture which had been kept in the laboratory since the preceding autumn became sexually mature and produced a large number of egg capsules. From these capsules, embryos were removed during

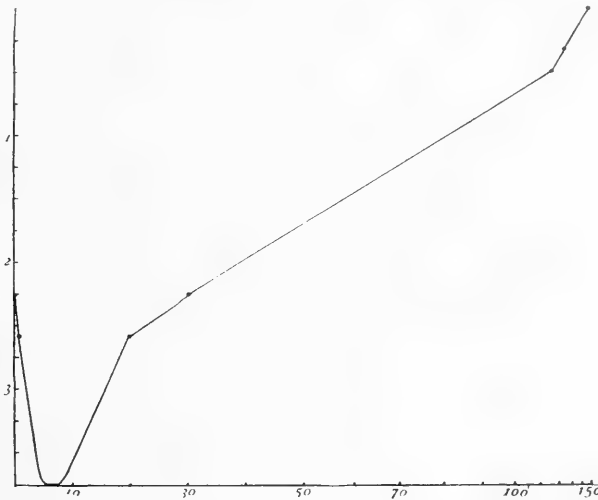


Fig. 14 Graph of the axial gradient of *Tubifex rivulorum*, showing the great extent of the secondary rise. Time in hours.

the later stages of development, and examined in cyanide. It was found that in the stage when the embryo has begun to elongate, its posterior region is most susceptible to cyanide, and the susceptibility decreases anteriorly. Four stages in the disintegration of an embryo at this stage are shown in figure 16. Later, the head becomes a second region of high susceptibility (fig. 17), and the susceptibility of the head increases until it surpasses that of the posterior end. At hatching, therefore, the head is the most susceptible region. The young worms just after hatching consist of about thirty segments. When placed in

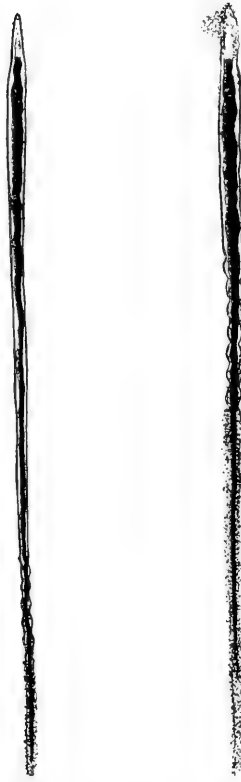


Fig. 15 Two stages in the disintegration of *Limnodrilus clapaedeanus*.



Fig. 16 Four stages in the disintegration of an embryo of *Tubifex rivulorum*. (The vitelline membrane which surrounds the embryo at this stage is omitted.)

cyanide, disintegration begins at the anterior end, then at the posterior end, and the two waves of disintegration meet at about the middle (fig. 18). As growth proceeds, the susceptibility of the head continually falls, but that of the posterior end remains high because of its unceasing growth which keeps it young. These experiments of Child and myself show that the posterior region of high metabolic rate which is characteristic of the adult annelid arises very early in the embryonic development.

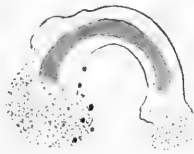


Fig. 17 Stage in the disintegration of a later embryo than figure 16, showing two regions of high susceptibility.

7. Summary

These various species of oligochaetes which have been examined form a series, then, with respect to the axial gradient and also, as I shall show later, with regard to regeneration. In *Aeolesoma* the gradient is of the primary type with some indications of a posterior region of high metabolic rate. In the naids, the primary gradient exists in the zooids only, and is modified in the adult by the development of a posterior region of high metabolic rate, which occupies about one-third of the body. In this region the gradient runs in the reverse direction from that of the primary gradient; it may be regarded as a secondary gradient imposed upon the primary gradient as a result of the annelid method of growth. In *Lumbriculus*, the secondary gradient is more extensive than in the naids, including more than the posterior half of the body; and in the tubificids it comprises all of the body except the first few segments.

III. THE DATA OF REGENERATION

1. *The head region of oligochaetes*

I have frequently used the term head region or head in speaking of these organisms and I wish now to explain what is meant by this expression. The head of oligochaetes comprises a



Fig. 18 Four stages in the disintegration of *Tubifex rivulorum* at hatching.

definite number of the most anterior segments, which are generally sharply marked off from the rest of the body; the exact number of segments involved varies with the species. The chief distinguishing characteristic of the head is that it contains the

pharynx, which is completely lacking in chloragogue cells. The outer wall of the remainder of the alimentary tract is composed of these chloragogue cells, which contain numerous brown droplets, apparently metabolic products (since they increase with age), which give to the intestine a characteristic dark brown color. There is thus a sharp line between the white pharynx and the dark intestine, and this line is the boundary between head and trunk. Other less obvious characteristics of the head, which are mentioned by Beddard ('95, p. 11) in his discussion of cephalization in oligochaetes, are: the absence of nephridia, irregularity of the septa, absence of the circum-intestinal loops of the circulatory system, and, in the naids, absence of the dorsal bundles of setae.

That the head of the oligochaetes is a real unit is shown not only by its morphology but by the fact that usually the head segments only are regenerated, no matter how many anterior segments are removed. This statement, although true in general, requires modification, for the regeneration of the anterior end varies according to the size of the piece and the region from which it is taken. Thus *Lumbriculus* regenerates the typical number of head segments only when the piece is of sufficient size. An insufficient investigation of cases such as this has led some investigators to question the existence of a definite head region in oligochaetes. Thus von Wagner ('00) working on *Lumbriculus variegatus* says that the number of anterior segments regenerated in this form is not definite but varies from five to nine. Semper ('76) seems to have been the first investigator to call attention to the sharp differentiation of the head from the trunk in oligochaetes; he observed it in the naids, in which forms it is most obvious. Later Bülow ('83) stated that although there is indeed variation in the number of anterior segments regenerated by *Lumbriculus variegatus*, yet one number—namely nine—predominates. Iwanoff ('03) supports Bülow's conclusion in regard to *Lumbriculus*—although he finds the number to be seven—and has maintained positively that there exists in oligochaetes a head region comprising a fixed number of segments. He uses the same criterion for distinguishing the head as I do, namely, the absence of chloragogue cells.

My own observations on oligochaetes have convinced me that in this group a definite head region is present. It is most sharply differentiated in the naids, where it generally consists of five segments (Dero, Stylaria, Nais). The statement of some authors (Iwanoff, '03) that these forms have but four head segments is probably due to a failure to count the first segment, which, as in all oligochaetes, bears no setae. In *Tubifex tubifex*, there are five head segments; in another species of *Tubifex* which lives in the temporary ponds along with *Lumbriculus* (probably *Tubifex multisetosus*) there are ten head segments. *Limnodrilus* has four, and *Lumbriculus inconstans* seven head segments, although in the latter they are not very sharply defined because the heavy pigmentation of the anterior part of the body conceals the division between head and trunk. In the Lumbricidae, also, there appears to be a definite number of cephalized segments; thus in *Allolobophora foetida* according to the account of Morgan ('97), the number is four or five, and in *A. terrestris* (Hescheler, '98), four. I have been able to find but one exception to this general rule; this is the case of *Criodrilus*, in which form there is apparently no definite cephalization since, according to Tirala ('12), it regenerates up to 28 segments anteriorly, replacing, to a considerable extent, the number removed. However, from the data presented by Tirala, it appears that even in *Criodrilus* there is a tendency to regenerate a certain number of segments, namely, about fifteen, and that with more detailed work on a larger number of individuals this would appear more clearly. I am therefore convinced from any own observations and those of others that the differentiation of a certain number of anterior segments as head segments is practically universal among oligochaetes.

2. General considerations on regeneration in oligochaetes

Regeneration in oligochaetes is always by outgrowth; the head is never replaced within the old tissue, as often occurs in Protozoa, Coelenterates, and flatworms. The process of regeneration, as it occurs in *Dero limosa*, for example, is as follows.

Within a day or so, new tissue begins to grow out from both cut surfaces of the piece. The anterior outgrowth develops faster than the posterior one, but when it has reached a certain length, its growth ceases. Its tip forms a prostomium; mouth, brain and pharynx differentiate within it, and lastly the bundles of setae appear in order, according to the usual law of antero-posterior development. Meantime, the posterior outgrowth has continued its development; within a few days an anus is established, and in front of this anal segment new segments continue to be formed indefinitely but with gradually decreasing rate if no food is given.

Anterior regeneration occurs in a very definite manner in oligochaetes. If the number of segments removed is less than the number of head segments, then only those removed are replaced. If more than the head segments are removed, then the head segments only are regenerated. This fact is not at all remarkable, although it seems to have been so regarded, since it is true of all forms in which regeneration of the anterior end occurs. If pieces are taken from the body of a flatworm, or the stem of a hydroid, the head regenerates directly at the cut anterior surface, and the intermediate parts are not replaced. What really happens is the reorganization of the rest of the body to fit the new head. This reorganization is more extensive in the oligochaetes than in the lower forms, owing to their higher degree of morphological differentiation. The most noticeable changes in these animals are the transformation of the old intestine, which adjoins the regenerated pharynx, into an oesophagus, and the development of the 'hearts' characteristic of the oesophageal region. Histological examination would doubtless reveal many other changes, but I have not attempted to follow the regenerative processes histologically. The normal morphological features of the species are thus restored, even to the formation of the sex organs in parts of the body where they would never have appeared ordinarily (Janda, '12, on *Criodrilus*, and Mrazek, '06, on *Lumbriculus*). Parts anterior to the level of section are never restored unless a head, or an approach to a head is regenerated at the cut surface.

Another general feature of oligochaete regeneration is the inability of the extreme anterior and posterior ends to regenerate. This was noted by Bonnet, and by most investigators since. The head, using the term in its strict sense, when isolated, never regenerates a posterior end; it either dies very soon or else gives rise to 'tailless' heads. The head must have a certain minimum number of body segments behind it before posterior regeneration can occur. This fact, which has long puzzled workers on regeneration, is readily explained on the basis of Child's investigations (a preliminary discussion of these matters appears in the second of the Studies, '11 e). He has pointed out that a large number of data from plants and lower invertebrates show that anterior regions are physiologically dominant over posterior, and the most anterior region, the apical end in plants, and the head in animals, dominates over and controls all other parts of the organism. In regulation, the first step is the formation of a dominant region, and the development of other parts then follows in correlation with this. For the formation of a dominant part, the cells which are to form it must have a rate of metabolism sufficiently high to enable them to become physiologically isolated and independent of the rest of the piece. If the piece does not contain sufficient material for both dominant and subordinate parts, then the latter cannot arise, as in the case under discussion. This production of dominant parts and nothing else from short pieces is a familiar fact in the case of many coelenterates and flatworms, and occurs also in *Lumbriculus*. Such pieces may also give rise to biaxial dominant structures, if the cells at the posterior end can attain a rate of metabolism sufficiently high to enable them also to become physiologically isolated. Under such conditions, a dominant structure will arise in each of the physiologically isolated regions, namely, at each end of the piece. A piece which does not contain enough material for both dominant and subordinate parts, or which consists to begin with of a dominant part only, can therefore do one of two things,—form a tailless structure, or give rise to biaxial dominant parts.

The posterior end is incapable of regenerating an anterior end unless it be of a certain minimal length. The reason for this is again to be sought in the metabolic relations between the piece and the new cells at its cut surface. The posterior end of oligochaetes, as I have shown, has a high rate of metabolism; unless the new cells at the cut surface can attain a rate of metabolism sufficiently high to enable them to grow at the expense of the old piece, a head cannot form.

Head formation exhibits a progressive decrease along the antero-posterior axis. Posterior regeneration also decreases along the axis; anterior pieces produce more segments posteriorly than do posterior pieces. As the head itself does not regenerate readily posteriorly, as already explained, it is the region behind the head which produces the greatest number of posterior segments. The reason for this appears to be that the more anterior the piece, the higher its level in the primary gradient, and the greater the vigor and intensity of its metabolic processes. As the posterior end develops wholly in correlation with the piece, it follows that the more vigorous the piece, the greater the amount of posterior regeneration, i.e., the more nearly does it approach the normal. Morgulis ('07) has given fully the data regarding regeneration of the posterior end in *Lumbriculus limosus*, and as my own observations regarding number of segments regenerated posteriorly at the different levels of the body agree with his account, I shall not discuss this point any further. On the contrary, the differences in head formation along the axis are of great importance, and will form the subject matter of most of the remainder of this paper.

With these remarks on the general features of regeneration, common to all oligochaetes, I shall now take up the details of regeneration in the four species with which I have worked,—*Dero limosa*, *Lumbriculus inconstans*, *Tubifex tubifex*, and *Limnodrilus claperedianus*.

3. *Regeneration in Dero limosa*

While many investigators have stated that the naids possess high capacity for regeneration, yet I have been unable to find any very definite data on the physiological aspects of regeneration in these forms, and no data at all on *Dero limosa*. Most of the regenerative studies on naids have been histological, and have concerned themselves with the germ layer hypothesis, and are, therefore, of little value from the present physiological point of view.

In working on regeneration, I have usually followed the simple method of cutting the worms up into a series of equal pieces along the axis, as fourths, eighths, sixteenths, etc. In this way, one discovers at once the effect of difference in size, and regional differences. A number of worms are cut up at once, and all the pieces of the same level put together. The pieces are kept in large stender dishes or finger bowls, in filtered water, which is changed occasionally. It is not necessary to add any débris except in the case of the naids, where the presence of a small amount of the cultural material seems to reduce the mortality, although it makes the finding of the pieces difficult.

Dero limosa possesses high regenerative capacity. If these worms are cut up into two, three, or four equal pieces along the axis, each piece regenerates a complete anterior and posterior end, with practically no mortality. A complete anterior end is the head of five segments with brain, mouth, prostomium, pharynx, and four sets of setae; a complete posterior end consists of the anal segment with its expanded gill and pavilion, four ciliated gills, and in front of this a variable number of new segments. The new tissue is always readily distinguishable from the old for a considerable length of time, by its lighter color, for it takes some time for the dark granules to accumulate in the chloragogue cells.

With shorter pieces, sixths or eighths—it is not practicable to cut pieces shorter than this in *Dero*,—there is considerable mortality, so that it is difficult to secure constant results. The anterior piece, containing the head, does not regenerate poste-

riorly unless it includes a certain minimum number of body segments. In *Dero*, this number is three, i.e., the anterior piece must be eight segments long before it will regenerate posteriorly. Anterior pieces shorter than this almost invariably die within a few hours after cutting. In only one case did such a piece survive; it consisted of six segments, and did not regenerate posteriorly, forming, in fact, a 'tailless' head. Similarly in short pieces, the last piece, containing the posterior end will not regenerate anteriorly unless it contains 12-15 segments; pieces shorter than this invariably die. The reason for the failure of these anterior and posterior ends to regenerate has been discussed in the preceding section.

As regards the rest of the body, pieces three or four segments long from any part will regenerate complete anterior and posterior ends. Is there then in *Dero* no difference in regenerative capacity along this axis? An axial difference does exist and reveals itself in the following ways; anterior pieces regenerate faster than posterior ones; the regenerated head is larger on anterior pieces than on posterior pieces; the number of segments regenerated posteriorly is greater in pieces from the anterior regions of the body, and decreases along the axis; smaller pieces from the anterior regions will regenerate completely than from the posterior regions. In regard to this last point, pieces from the anterior half of the body, containing only one or two segments will regenerate into complete worms; while from the posterior half of the body, pieces must be at least three or four segments long before this can occur. These facts indicate an axial difference, which is, however, relatively slight as compared with other forms.

Biaxial heads arise in short pieces of *Dero*; two such were observed, and undoubtedly more could be readily produced. One of the observed pieces consisted of about three segments; it regenerated a complete head at each end (fig. 19). The other piece consisted of little more than one segment; in this case, the anterior head was complete, but the posterior one regenerated only three setigerous segments, instead of the normal four. This piece was cut in two, but both portions died. An adequate

explanation of the formation of biaxial heads has been given by Child ('13 b). The original gradient must be largely eliminated, and this is accomplished by cutting short pieces,—which include such a small portion of the original gradient that there will be little difference in rate of metabolism between the two ends of the piece,—or by depressing the rate of metabolism of the pieces (low temperature, cyanide, anaesthetics, etc.). If, then, the growing cells at the two cut surfaces of these pieces in which the gradient has been eliminated attain a sufficiently high rate of metabolism, each will develop an independent part, that is to say, a head. If, on the contrary, the rate of metabolism of the old piece remains higher than that of the growing cells, it will dominate over them, and they will be able to give rise to subordinate structures only, namely, tails. Child has been able to



Fig. 19 Biaxial heads in *Dero limosa*.

demonstrate the correctness of this explanation as follows. *Planaria dorotocephala* seldom gives rise to biaxial heads, but if short pieces are put into depressing agents to eliminate the gradient, and then allowed to regenerate in water, a considerable percentage of biaxial heads is obtained. As far as I am aware, biaxial heads have not previously been observed in annelids although a reversal of polarity of a similar character has been produced in lumbricids by grafting short pieces in the reverse position upon the anterior end of long pieces (Hazen, '99 and Ruttloff, '08).

Regeneration in *Dero* is nearly always complete and 'normal.' In anterior regeneration, the only variation from the normal is the occasional appearance of heads containing fewer or more than the five typical head segments. Such heads may be designated as hypomeric, when the number of regenerated segments is less than normal, or hypermeric, when it is more. Hypo-

meric heads, consisting of only four segments, of which three are setigerous, but normal in all other respects, occur occasionally; but heads containing less than four segments have never been observed. Hypermeric heads are rare, but I have observed a few, consisting of six, and, in one case, seven segments; in such heads, the setae tend to be irregularly disposed, so that one is uncertain of the boundaries of the segments. The hypomeric and hypermeric heads do not regulate to normal, at least in the time which I observed them, nor is the abnormal number of head segments inherited in asexual reproduction. In posterior regeneration, there arise, not infrequently, abnormalities in the shape of the gill pavilion, and in the size, and number of the gills—one, two, or three appearing instead of the normal four; but in the case of posterior structures, regulation to normal usually occurs.

In regard to the regeneration of *Dero*, then, the important fact is that complete anterior regeneration occurs everywhere along the axis (with the exception of the extreme posterior end).

4. *Regeneration in Lumbriculus inconstans*

Lumbriculus inconstans is undoubtedly the most favorable oligochaete for experimental work on account of its large size, high capacity for regeneration, low mortality, and, most important of all, because it exhibits *qualitative* axial differences.

It has this disadvantage, however,—that, owing to its peculiar habitat, it can be collected only in the spring months; freshly collected material is necessary for experimental work because the vitality of the worms decreases when they are kept for any length of time in the laboratory. Owing, therefore, to the relatively short time during which material is available, my experiments on this most instructive species are as yet incomplete in many details.

The genus *Lumbriculus* has long been noted for its high regenerative capacity, and the species *L. inconstans* is no less remarkable in this respect than are its relatives, *L. variegatus*, a favorite object of research with European investigators, and

L. limosus, on which Morgulis ('07) has experimented. *Lumbriculus inconstans*, however, differs from both of these forms in a very important way as will appear shortly.

If *Lumbriculus inconstans* is cut up along the axis into pieces of moderate length,—that is, pieces including more than ten segments—then each of these regenerates completely. A complete anterior end is the head of seven segments, of which six are setigerous, a large prostomium, brain, pharynx, etc.; but the transverse green pigment stripes do not appear on the regenerated head for some time. In long pieces, the regeneration of seven head segments is almost invariable, demonstrating that a definite number of segments is differentiated into a 'head' in *Lumbriculus*; but in short pieces, the number of head segments regenerated is very variable, and this variability has led to much controversy over the question of cephalization in the oligochaetes. The regeneration of the posterior end consists simply of the formation of an anal segment, and of an indefinite number of new segments in front of this. As is general in oligochaetes, neither the extreme anterior or posterior ends are capable of regeneration. The head piece must include at least four trunk segments, or eleven in all, before it will regenerate posteriorly; isolated heads shorter than this remain tailless. The posterior end must be twenty or thirty segments long before it will regenerate anteriorly, otherwise it dies without regeneration.

With shorter pieces, less than ten segments long, an axial difference becomes apparent. Disregarding now the extreme anterior and posterior piece, it is found that the capacity for both anterior and posterior regeneration decreases along the axis. Posterior regeneration may be dismissed briefly. Pieces from the anterior regions, although they may remain tailless in a small percent of cases, in which the pieces are too short to contain enough of the axial gradient for formation of both dominant and subordinate parts (see above, and Child, '13 b), in general produce very long posterior ends. As one passes back along the axis, the number of posterior segments regenerated gradually decreases, and in pieces from posterior regions, frequently only

the anal segment is regenerated, although such pieces are never tailless. With the exception, then, that extreme anterior pieces may remain tailless, all levels of the Lumbriculus body regenerate normal posterior ends, but the amount regenerated decreases along the axis.

In anterior regeneration, the character of the anterior outgrowth depends upon the level of section, and shows all gradations from a normal head to a normal tail. In an axial series of short pieces, the most anterior ones regenerate normal heads, usually of seven segments, but not infrequently hypo- or hypermeric. Pieces farther back tend to produce structures which depart more and more from the normal head; and pieces from posterior regions give rise to various types of abnormal, or, as I prefer to call them, inhibited structures, among which are represented all gradations from a normal head to a normal tail. I have attempted to classify these various kinds of anterior structures into several categories, although it must be understood that they grade into each other perfectly. The following description together with the accompanying figures will give some idea of their appearance.

1. *Normal heads.* This head has a large well-developed prostomium, mouth, pharynx, supraoesophageal ganglia with circumoesophageal connectives, and usually consists of seven segments, of which six are setigerous (fig. 20 b). It resembles in all respects the head of the animal as it occurs in nature (fig. 20 a), except that the transverse pigment stripes are lacking on the regenerated head. The typical number of segments in the normal head is seven, but in short pieces, the number of regenerated segments varies from four to nine. Normal heads are regenerated at any level of the Lumbriculus body, but in short pieces, the frequency of their occurrence decreases, and the tendency to hypomerism increases along the antero-posterior axis.

2. *Hypoprostomic heads.* These differ from the normal in that the prostomium is reduced in size, and frequently abnormal in shape (fig. 20 c). The hypoprostomic head has a brain (which has not been investigated histologically) mouth, and pharynx,

but is usually hypomeric. The percentage of such heads is small in pieces from anterior regions, but increases along the axis.

3. *Aprostomic heads.* This type of inhibited head consists of a rounded outgrowth, in which the prostomium is entirely

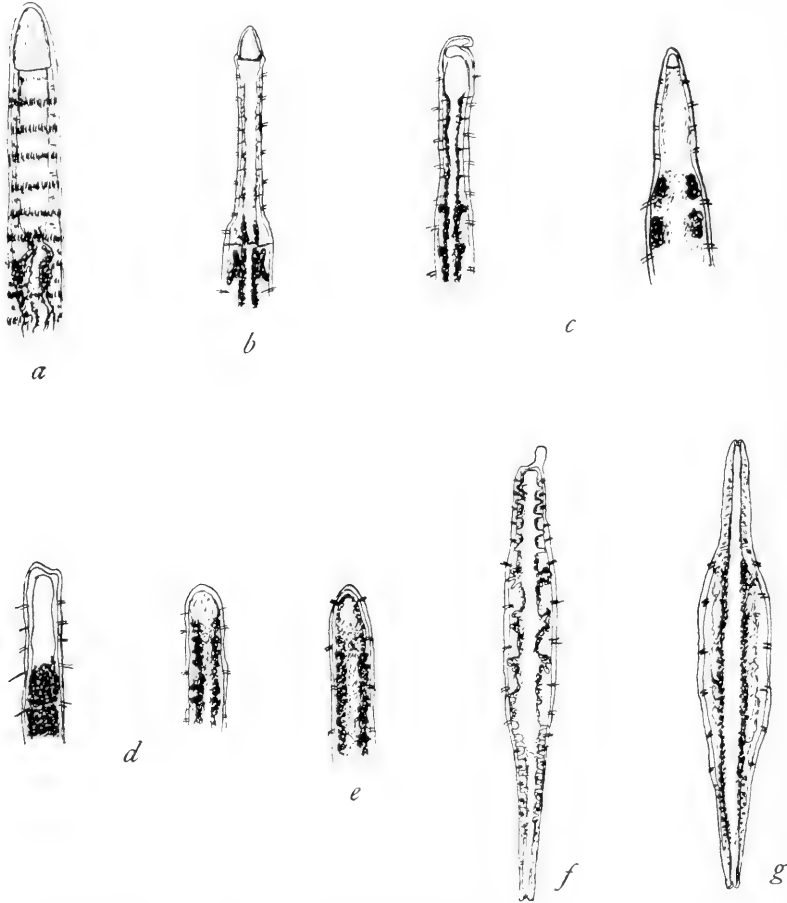


Fig. 20 Types of anterior outgrowths in *Lumbriculus*. *a*, normal head in nature; *b*, normal regenerated head; *c*, two types of hypoprostomic heads; *d*, two aprostomic heads, which are also markedly hypomeric; *e*, an acephalic condition; *f*, cephaluran outgrowth; *g*, biaxial tails.

lacking, (fig. 20 d). There is no mouth, the pharynx ending blindly. Histological examination shows that the supraoesoph-

ageal ganglia, and circumoesophageal commissures are absent, and that the ventral nerve cord terminates in a mass lying beneath or in front of the blind end of the pharynx. It seems very probable to me that the brain and commissures do not develop as distinct structures because the mouth is absent. The aplostomic head is always hypomeric, usually markedly so, and like the preceding type is of most frequent occurrence in pieces from posterior regions.

4. *Acephalic pieces.* In these pieces, the cut end simply heals over and no outgrowth takes place (fig. 20 e). These headless pieces are rare in *Lumbriculus*, and are confined to posterior levels of the body.

In these four classes of structures, we have, as in *Planaria dorotocephala* (Child '11 b) all gradations from the normal head to the acephalic condition. There is a gradual reduction of certain cephalic structures,—the prostomium, cephalic nervous system, mouth, and number of head segments, ending with complete inhibition of head formation. The various types of heads, are not, however, so definitely localized along the axis, as is the case in *Planaria*, although in general, the percentage of normal heads decreases along the axis, and the percentage of inhibited heads increases. *Lumbriculus* further differs from *Planaria* in that in place of acephalic pieces, posterior structures may arise. I have distinguished two kinds of these.

5. *Cephaluran outgrowths.* This interesting type of anterior outgrowth appears to have, as the name adopted for it implies, characteristics of both head and tail (fig. 20 f). In my opinion, such an outgrowth starts as an inhibited head, an aplostomic head, but is unable to maintain sufficient dominance over the old piece to continue to develop as a head; its further development is therefore that of a subordinate part, a tail. The cephaluran outgrowths have a rounded or pointed end in which there is a terminal nerve mass like that found in the aplostomic heads; like the latter, also, there is no opening to the exterior, neither mouth nor anus. The tail characteristics of these structures are the following: large number of segments, for they are nearly always hypermeric, decrease in the size of the segments ante-

riorly, presence of a pair of transverse blood vessels in each segment, and, in some cases, reversal of the direction of blood flow, such as occurs in the biaxial tails. Tail characteristics predominate but the cephaluran structure differs from a tail in the absence of the anus, and the presence of the nerve mass. Cephaluran outgrowths arise in pieces from posterior levels only.

6. *Biaxial tails.* The regeneration of tails at both ends of pieces has long been known in *Lumbriculus*; like the preceding type of structure, biaxial tails arise in pieces from posterior regions only. Both tails are identical in structure, the anterior tail being however usually shorter, (fig. 20 g); and the blood always flows from the tip of each towards the middle of the piece.

7. *Multiple outgrowths.* In this category I have grouped all those cases in which multiple structures arise at the cut surface. Such multiplication of parts is limited to anterior regeneration. The commonest case of this kind is the duplication of the head; and all gradations are found from two completely separate heads to cases where only the prostomia are separate, as in figure 21. The double structure may consist, however, not of two heads, but of head and a tail (fig. 22); this is not a case of axial heteromorphosis, for here the tail grows out in correlation with the new head, while the biaxial tail develops in correlation with the old piece. If both parts of a double outgrowth develop with equal rates of metabolism, then neither one will be able to control the other, and both will give rise to heads; but if one of the outgrowths attains higher metabolic activity than the other, it will become a dominant part, a head, compelling the other outgrowth to develop as a subordinate part, a tail. The result is a head with two tails. Pieces of *Lumbriculus* may give rise to three or four such outgrowths at their anterior ends, some of which are heads of various kinds, and some of which are tails.

To illustrate now the distribution of these different types of anterior outgrowth along the axis, the results of a series of twenty-five worms cut up into sixteenths are given in table 1. As the first sixteenth pieces include the original head, and the last sixteenth pieces all died, they are omitted from the table.

This table shows that the different kinds of anterior structures are not very sharply localized along the axis, but that in general, the percentage of inhibited forms is greater in posterior regions. The increase in percentage of normal heads in extreme posterior pieces (fourteenth and fifteenth pieces in the table) appears to be a general result, and is probably due to two factors: first, owing to the decrease in size of segments posteriorly, these pieces

TABLE 1

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Normal.....	21	17	15	18	17	16	18	14	15	7	4	6	11	14
Hypoprostomic.....	3	4	3	4	1	2	2	1	3	3	6	5	1	1
Aprostomic.....		2	3	3	5	3	2	1	3	7	9	6	7	1
Acephalic.....												2		
Cephaluran outgrowths.....									1	2	4	2	2	
Biaxial tails.....										1	1	2		
Multiple outgrowths.....										3	1		1	
Dead.....	1	2	4	0	2	4	3	9	3	2	0	2	3	9

TABLE 2

9 segments.....									1	1		1		
8.....	1		2						2		1	2		
7.....	21	16	11	16	18	11	18	12	14	7	4	2	1	1
6.....	2	6	5	4	2	7	3	3	3	4	5	5	5	3
5.....			1	4	2			2	2	4	7	2	8	3
4.....		1	2	1	2	3	1			1	1	3	2	6
3.....										1			1	1

contain more segments than anterior pieces of equal length, and, secondly, these pieces are in the region where autotomy occurs,⁴ and where, therefore, new individuals already exist to some extent physiologically. Similarly, in *Planaria dorotocephala* (Child, '11 b), pieces from the levels where the zooids are situated generally produce normal wholes.

In table 2, the number of head segments regenerated by the same sixteenth pieces is given (cephaluran outgrowths and biaxial

⁴ *Lumbriculus inconstans* reproduces asexually by autotomy. Upon stimulation, this worm breaks in two at a rather definite level, near the posterior end of the body.

tails being omitted). It is seen that anterior pieces usually regenerate seven head segments, while posterior pieces show a marked hypomerism.

The most striking fact about the regeneration of *Lumbriculus inconstans* is, then, that there exists an axial difference in head



21



22

Fig. 21 Multiple outgrowths; double prostomia.
 Fig. 22 Multiple outgrowths; head and tail.

formation. In short pieces normal heads tend to be formed from anterior levels, but as one passes posteriorly along the axis, the process of head formation is inhibited to a continually increasing degree.

5. Regeneration in the tubificids

In these forms, the inhibition of head formation has proceeded to such an extent that all pieces back of a certain anterior level remain headless. By removing definite numbers of anterior segments, one may determine the level at which head formation ceases. In the case of *Tubifex*, five, ten, fifteen, and twenty anterior segments were removed, with the following results. At the level of the fifth segment, a normal head is usually regenerated. The normal head of *Tubifex* consists of five segments, four setigerous, and contains mouth, brain, pharynx, etc. When ten anterior segments are removed, the result is the same. When fifteen are cut off, some normal heads are regenerated, but most of the pieces give rise to inhibited heads, and some remain headless. I find in my notes unfortunately, no description of the appearance of these inhibited heads, nor any statement as to whether various types could be distinguished. At the level of the twentieth segment, all pieces remain headless. Between the fifteenth and the twentieth segments, therefore, head formation ceases rather abruptly, although inhibited heads occur as stages between the normal and the acephalic condition. These results agree with those of European investigators working on the same species, *Tubifex tubifex*, except that they found head formation to cease at a more anterior level—tenth to twelfth segment (Abel '02, Haase '98). The regeneration of the head is slow in *Tubifex*, and the greater the number of anterior segments removed, the more slowly does regeneration take place.

In *Limnodrilus claperedianus*, the capacity for head formation is even more limited than in *Tubifex*. As this species has already been thoroughly investigated by Kreckler ('10), it was not necessary for me to perform any very extensive experiments. The head of *Limnodrilus* consists of four segments, but two is the maximum number regenerated; of these, the first bears prostomium, mouth and brain, all of normal appearance, and the second is setigerous. From this condition, one finds the following gradations to the acephalic state; regeneration of one segment only with normal prostomium, mouth, and brain, reduction of

prostomium, aprotomic condition ('rounded ends' of Kreeker), and finally the acephalic condition, where no tissue grows out at all. All pieces from which more than seven anterior segments are removed remain acephalic.

As regards posterior regeneration, all levels of the body regenerate normal posterior ends, with the usual exception that the head itself remains tailless unless it includes a certain minimum number of body segments.

6. *Summary*

It thus appears from these data that the oligochaetes investigated form a series with regard to head formation. In *Dero*, normal heads are formed at all levels of the body, in both short and long pieces; in *Lumbriculus*, normal heads occur at all levels in long pieces, but in short pieces, head formation exhibits progressive inhibition along the axis; and in the tubificids, head formation is limited to extreme anterior levels, regardless of size of piece. It seems to me that the earthworm and its relatives occupy an intermediate position between *Tubifex* and *Lumbriculus*. Thus in *Allolobophora foetida* described by Morgan ('97), regeneration of the head occurs with increasing difficulty back to the level of the twelfth to fifteenth segments; behind this level is a region where pieces either remain headless or produce bi-axial tails, and posterior to this all pieces remain headless. In the tubificids, head formation ceases at anterior levels without any intermediate production of biaxial tails.

The regeneration of the anterior end exhibits, then, these striking axial differences which are of a qualitative nature. On the other hand, the regeneration of the posterior end occurs at all levels of the body in all of these oligochaetes, and with all sizes of pieces (with the exception of the head piece, which remains tailless unless of a certain size). The axial differences in posterior regeneration are quantitative merely.

IV. ANALYSIS OF THE REGENERATIVE PROCESS

A consideration of the data presented above leads us to the conclusion that the formation of an anterior end is a different process from the formation of a posterior end. The fact that in a set of pieces of *Lumbriculus* of equal size, and taken from the same level of the body, any type of anterior outgrowth from a normal head to a normal tail may be obtained, proves that the kind of anterior structure which is to arise is not predetermined in the piece, but is due to dynamic conditions arising within the piece after its isolation from the whole. The previous position of the piece as part of the whole is only an indirect factor in head determination.

The consideration of a similar set of facts obtained from his experiments with *Planaria dorotocephala* led Child ('13 b, '14 e) to an analysis of anterior regeneration in this animal in metabolic terms. In the following pages a similar analysis is made for *Lumbriculus inconstans*.

1. The time of head determination

It is necessary to know, as the first step towards the understanding of the process of anterior regeneration, at what time the head is determined in a regenerating piece. This can be readily discovered in the case of organisms which show progressive inhibition of head formation along the axis by means of the method devised by Child ('14 e). The piece *ac* in figure 23 gives rise to normal heads in 90 to 95 per cent of cases (the remaining forming mostly multiple structures). The piece *ab* with the same anterior level will, however, produce under ordinary conditions only 20 to 30 per cent of normal heads, and 70 to 80 per cent of inhibited heads and other types of anterior outgrowths. It is therefore obvious that whether or not the cells shall give rise to a normal head is dependent upon their forming part of the long piece *ac*; and if they remain a part of *ac* for a sufficient length of time, then they should, when isolated, give rise to nothing but normal heads. To discover the period of time required for the determination of the head as normal, it is only necessary

to cut a large number of pieces *ac*, and at different intervals after this operation to cut off pieces *ab* from the anterior ends of



Fig. 23 Semi-diagrammatic figure of *Lumbriculus inconstans*, to show levels of section.

the *ac* pieces. These *ab* pieces are then allowed to regenerate, and it is found that the percentage of normal heads produced

by them runs parallel to the length of time that they were allowed to remain part of the long pieces.

Experiments of this kind carried out on *Planaria dorotocephala* (Child, '14 e) yielded the unexpected result that it is determined within six to eight hours after section whether or not a piece of *Planaria* shall produce a head or remain headless, and within eighteen hours whether the head shall be normal. In the case of *Lumbriculus*, acephalic pieces are so rare that one cannot discover the time at which head determination occurs; I have therefore merely attempted to find out when the head is determined as normal. As preliminary experiments showed that at least ten hours are required for this, I began my later experiments with a fifteen hour interval. The result of such an experiment are given in table 3. Two hundred long pieces *ac* were cut from

TABLE 3

TIME BETWEEN TWO OPERATIONS	NORMAL	HYPOPROSTOMIC	APROSTOMIC	BIAXIAL TAILS	DEAD
No hours.....	20	42	12		26
15 hours.....	60	30	6	2	2
20 hours.....	70	26	2		4
30 hours.....	88	4			8

the posterior ends of worms of the same size and from the same stock; fifty short pieces *ab* were cut from the anterior ends of these immediately; and fifty more after elapse of fifteen, twenty, and thirty hours respectively. The results are given in percentages; multiple outgrowths are classified under the types of heads. While I regret that I have not a closer series of time intervals, yet I think that it is evident that the head of *Lumbriculus* is determined as normal in the majority of cases within twenty hours after section, and in practically all cases within thirty hours.

2. Stimulation by section

As a second step in the analysis of the process of anterior regeneration, one must know what the metabolic condition of the pieces is at the time when the head is determined as normal.

The rate of metabolism of pieces is not the same as that of corresponding parts of the intact worm because, as Child has shown ('14 d), the rate of metabolism is increased by the operation of cutting; this increase is greater the shorter the piece, and the lower its previous rate as part of the axial gradient. These facts have been demonstrated not only by the increased susceptibility of the pieces to cyanide, but also by their increased CO_2 production in Tashiro's biometer. Similarly, in all the oligochaetes which I have tested *Dero*, *Lumbriculus*, *Tubifex*, and *Limnodrilus*—stimulation results from section. In long pieces there is little stimulation, and the disintegration gradient of the intact worm is preserved in the pieces, except for increased susceptibility at the cut surfaces. Short pieces are stimulated to a much greater extent, and their disintegration in cyanide takes place without regard to the previous gradient; it begins at the cut surfaces and proceeds towards the middle. It is therefore the region of the wound which is the seat of the stimulation, and in short pieces the wound regions practically include all of the piece. Stimulation as a result of injury is undoubtedly a general phenomenon exhibited by living matter (Tashiro, '13), and gives us a simple explanation of such facts as the current of injury of nerve and muscle.

The degree of stimulation of pieces is a function of their previous axial position, and this is particularly noticeable in short pieces. The isolated head shows no stimulation; pieces from anterior regions where the metabolic rate is high are stimulated to some extent; pieces from middle regions where the rate is lowest are stimulated most; pieces from the posterior region of high rate are stimulated to a considerable extent, and since they already possessed a high rate before section, their rate after section is usually higher than that of the middle pieces, although the rate of the latter has been increased more relative to their previous rate. The metabolic condition of the axial series of pieces is not, therefore, the reverse of the metabolic gradient of the intact animal, as is the case in *Planaria*, because of the existence in oligochaetes of the posterior region of high rate. That this posterior region is stimulated by section indicates, as I have al-

ready pointed out, that it is not a truly independent part. The reversal of the gradient after section appears most clearly in a form like *Dero*, where the posterior region is of small extent. Table 4 is the record of such an experiment on *Dero limosa*; ten worms were cut up into sixth pieces, and put immediately into $\frac{N}{500}$ KCN. The experiment was begun at 12.45, and the number of pieces completely disintegrated recorded at fifteen minute intervals.

In general, then, the regions which in the intact animal have lowest susceptibility have highest susceptibility after section, while the regions having high susceptibility in the whole are not much altered by section, with the exception of the posterior end

TABLE 4

TIME	1	2	3	4	5	6
1.00.....	0	0	0	0	0	0
1.15.....	0	1	2	2	2	1
1.30.....	0	4	5	3	4	2
1.45.....	0	7	8	5	4	6
2.00.....	0	8	8	8	6	8
2.15.....	1	8	8	9	7	9
2.30.....	4	9	10	9	7	9
2.45.....	10	10		9	8	10
3.00.....				10	9	
3.15.....					10	

which although already having a high rate is somewhat simulated by section. In the case of *Lumbriculus* and the tubificids, where over half of the body is involved in the posterior rise of metabolic rate, the susceptibility of all pieces except the most anterior is increased by section, that of the middle pieces relatively most but not enough to raise it above that of posterior pieces which had a much higher rate before section. The gradient is therefore not reversed in these forms; it simply is raised to a higher level, and becomes less steep.

The stimulation following section may be explained as due to the severance of conduction and correlation paths. The fact that the head and anterior regions in general are little stimulated by isolation indicates that they are relatively independent of

other parts; posterior regions are, on the other hand, dependent on anterior parts and subordinate to them, since the severance of conduction paths between them results in stimulation.

The increase of metabolism after section is only temporary. If the pieces are tested in cyanide at various intervals after cutting, it is found that the susceptibility to cyanide gradually decreases to far below normal, and then begins to rise again as regeneration sets in. For an analysis of the process of head formation, it is necessary to know what the metabolic condition

TABLE 5

Susceptibility of short anterior piece de to KCN $\frac{N}{500}$ at various times after cutting

TIME	SAME REGION OF CONTROL	IMMEDIATELY AFTER SECTION	5 HRS.	17 HRS.	24 HRS.	48 HRS.	4 DAYS
1.45.....	0	0	0	0	0	0	0
2.00.....	0	1	1	0	0	0	0
2.15.....	0	2	1	0	0	0	0
2.30.....	0	5	1	0	0	0	0
2.45.....	1	8	2	0	0	0	0
3.00.....	4	10	3	0	0	1	0
3.15.....	8		3	0	0	1	1
3.30.....	9		7	0	1	1	3
3.45.....	9		7	0	1	1	3
4.00.....	9		9	0	3	4	8
4.15.....	10		9	1	4	6	10
4.30.....			10	4	6	7	
4.45.....				6	7	10	
5.00.....				9	9		
5.15.....				9	9		
5.30.....				10	10		

of the pieces is during the period in which the head is determined. I have therefore tested the susceptibility to cyanide of short anterior and posterior pieces of Lumbriculus. In table 5 is given the susceptibility to cyanide of the anterior piece *de* in figure 23, immediately, 5, 17, 24, 48, and 96 hours after section; and in table 6, the susceptibility of the posterior piece *ab* in figure 23 immediately, 5, 14, 19, 24, 48, and 96 hours after cutting. Ten pieces are used in each case, and the time of death of corresponding regions of whole worms also noted. The concentration of cyanide used was $\frac{N}{500}$; observations were taken every

fifteen minutes, and the number of pieces completely disintegrated recorded.

The general result of experiments of this kind is that the rate of metabolism of anterior pieces falls rapidly after cutting, and rises again, so that within four days, it has reached the normal rate. In posterior pieces, the rate stays up longer after cutting, then falls to a greater extent, and remains low for a longer period of time. Further experiments show that it begins to rise by the

TABLE 6

Susceptibility of short posterior piece ab to KCN $\frac{N}{506}$ at various times after section

TIME	SAME REGION OF CONTROL	IMMEDIATELY AFTER SECTION	5 HRS.	14 HRS.	19 HRS.	24 HRS.	48 HRS.	4 DAYS
1.45.....	0	0	0	0	0	0	0	0
2.00.....	0	0	1	0	0	0	0	0
2.15.....	0	1	1	0	0	0	0	0
2.30.....	0	4	2	1	0	0	0	0
2.45.....	0	5	3	1	0	0	0	0
3.00.....	0	7	4	2	0	0	0	0
3.15.....	0	9	6	4	2	1	1	0
3.30.....	1	9	7	7	4	3	1	0
3.45.....	3	10	9	8	5	3	1	0
4.00.....	4		9	10	6	3	1	0
4.15.....	5		9		6	4	1	0
4.30.....	10		10		7	5	3	1
4.45.....					9	6	5	1
5.00.....					10	7	7	3
5.15.....						8	8	4
5.30.....						10	10	4
5.45.....								6
6.00.....								7

sixth day. Eventually the rate of metabolism of all regenerating pieces becomes much higher than their original rate as parts of the organism; rejuvenation thus occurs as a result of regeneration.

The rate of metabolism, then, of anterior pieces of *Lumbriculus* is low during the period that the head is determined as normal; and these pieces give rise to a high percentage of normal heads. On the contrary, the rate of metabolism of posterior pieces is

high during the time of head determination; and it is these pieces which produce inhibited structures.

3. *General conception of the process of regeneration*

This relation between frequency of normal head formation, and rate of metabolism at the critical period of head determination in *Lumbriculus* has lead me to accept the conclusions to which Child has come from the consideration of similar facts in *Planaria dorotocephala*. Since he has already presented his conclusions (Child, '14e), it is not necessary for me to discuss them in any very great detail. For the following brief presentation, it will be convenient to employ a figure similar to that frequently used by Child (fig. 24). The cells at the cut surface x

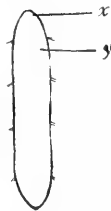


Fig. 24 Diagram for theory of head formation.

as a result of the wound and altered conditions begin to produce new tissue from which the new head is to arise. These cells grow out with a certain rate of metabolism which is relatively high as shown by disintegration experiments. Now if the rate of metabolism of the old tissue y is low, there is nothing to hinder x from continuing its development; it becomes dominant over y , uses up the material of y for regeneration, and produces a normal head. This is the case in anterior pieces of *Lumbriculus* where, as was shown in the preceding section, the rate of y is low during the time that the head is determined as normal. If, on the contrary, the rate of y is high, then x will not be able to dominate over y , nor to attain sufficient independence to produce a normal head; the development of x will be inhibited, and in proportion to the metabolism of y . Thus are produced the various types of inhib-

ited heads; the higher the rate of y the more will it inhibit the anterior structure which arises, and if the rate of y is sufficiently high, then region x will not be able to become independent at all but will be dominated by y , and give rise to a subordinate part, a tail. Under such conditions biaxial tails result. It is also conceivable that x might begin head development but later be dominated by y to such an extent that head formation can no longer continue but tail formation sets in; this in my opinion is the explanation of the cephaluran outgrowths. In the case of multiple outgrowths, the outgrowth or outgrowths which have the highest rate become heads, and dominate over the others which then give rise to tails. In Planaria, when the region y dominates the region x , the acephalic condition results; but this is not the case in Lumbriculus, probably because the new tissue begins to grow out before its fate has been decided, and it then forms a head or a tail according as x or y dominate.

It is not necessary that the region y should have a metabolic rate which is actually higher than that of x ; in fact it probably never has, for disintegration experiments show that x usually disintegrates before y . But it is the ratio of the rate of x to the rate of y which is important, and in all probability the rate of x must exceed the rate of y by a considerable amount before normal head formation can occur. Relatively slight alterations of the ratio $\frac{\text{rate of } x}{\text{rate of } y}$ are sufficient to affect the process of morphogenesis. Thus is a set of pieces of the same size, and from the same level of the body, the value of y cannot be very different in the different individual pieces; the rate of x probably differs even less; yet from such a set of pieces all types of anterior structures are obtained.

If this conception of the process of head formation is correct, an experimental control of morphogenesis should be possible. By depressing the rate of y as compared with x , one ought to be able to increase the percentage of normal heads; and, conversely, by increasing the rate of y as compared with x , a decrease in the percentage of normal heads should result. The first possibility is readily realized experimentally; the rate of y can be easily

lowered by placing the pieces in dilute cyanide solutions immediately after section. While this treatment must depress the rate of x also, yet the depression is less than in the case of y , because x is young, growing tissue, and regulates better to the depressing condition. The desired increase in the value of the ratio $\frac{\text{rate of } x}{\text{rate of } y}$ is thus attained, and as postulated, the percentage of normal heads is decidedly increased. This beautiful experiment was first performed by Child on *Planaria dorotocephala*, and is readily repeated in the case of *Lumbriculus inconstans*. In Table 7 are given the results of such an experiment. One hundred short posterior pieces (ab in fig. 23) were cut; fifty were put into water as control and fifty put immediately into cyanide solution of concentration $\frac{N}{25,000}$ for four days, then removed to water.

TABLE 7

	NORMAL	HYPOPROS- TOMIC	APROSTOMIC	ACEPHALIC	BIAXIAL TAIL	DEAD
Control.....	18	30	24	6		22
Experiment....	46	16	22		2	14

As the ab pieces never in my experience yield more than 30 per cent of normal heads, the increase here is due to the depressing action of the cyanide.

The converse experiment, that of decreasing the ratio $\frac{\text{rate of } x}{\text{rate of } y}$ is very difficult to carry out, for it is almost impossible to increase the rate of y , without at the same time increasing the rate of x more. Therefore stimulating conditions, such as increased temperature, increased motor activity produce the same effect as in the preceding experiment, as Child has shown; the rate of x is increased more than that of y , and a higher percentage of normal heads results. In *Lumbriculus inconstans*, however, the desired condition is realized in an unexpected way; in my earliest experiments upon this species I soon found that the 'vitality' of the worms decreases greatly if they are kept for any length of time in the laboratory. The capacity for regeneration diminishes to such an extent that the worms can no longer be used for

experimental purposes. The cause of the decrease in regenerative power appears to be that the cells at the cut surface fail to grow out with their usual vigor. The rate of x is thus lowered, as required in the experiment, and the expected decrease in the percentage of normal heads occurs. In table 8, a comparison is made between the regenerative capacity of *ab* pieces cut on April 24, and two lots cut on May 30, from the same stock. Fifty pieces were taken in each case, and the results are expressed in percentages.

TABLE 8

TIME OF CUTTING	NORMAL	HYPOPROS- TOMIC	APROSTOMIC	ACEPHALIC	BIAXIAL TAILS	DEAD
April 24.....	18	30	24	6		22
May 30.....	6	34	20	2	6	32
May 30.....	4	20	28	6	6	36

The decrease in normal heads is very characteristic, also the appearance of biaxial tails. The number of normal heads may be considerably increased in these pieces by putting them in dilute cyanide. The mortality in these pieces cut late in the season is unfortunately always high, but as the decrease in normal heads has been noticed in every experiment performed with worms kept some time in the laboratory, it cannot be accounted for on the basis of the increased mortality.

The rôle of the axial gradient in morphogenesis has not perhaps been sufficiently emphasized. In the case of short pieces, the dynamic conditions developed after section are the principal factors, yet it should be obvious from what has been said that these conditions are dependent on the previous position occupied by the pieces in the axial gradient of the intact animal. In long pieces, the axial gradient is more directly concerned; these pieces are only slightly stimulated by section, and the axial gradient is preserved in them in practically its original state. Therefore the anterior end of long pieces always has the highest rate in the piece, and the region x is never threatened with inhibition from the parts behind it. For this reason, long pieces of *Planaria* and *Lumbriculus* always give rise to normal heads. It is the

axial gradient also which determines that the head shall arise at the anterior end of the piece (Child, '14 e, p. 73).

The objection may be raised to these statements that in some of the oligochaetes which I have been considering, the axial gradient is such as to be higher at the posterior end than at the anterior end of long posterior pieces. Now there appears to be a correlation between the extent of the characteristic posterior rise in metabolic rate, and the capacity for head formation. Thus in *Dero*, where the posterior rise is of small extent, head formation occurs at all levels; in *Lumbriculus*, where the region of high rate occupies more than the posterior half of the body, head formation tends to be inhibited in this region; and in the tubificids where nearly all of the body is involved in the ascending gradient, head formation is impossible except at extreme anterior levels. In my opinion, however, the ascending gradient plays only a minor rôle in morphogenesis. I wish to point out that a similar series with regard to head formation can be found in the Turbellaria; *Planaria maculata* regenerates normal heads at all levels in reasonably short pieces; *P. dorotocephala* in pieces of similar size forms normal heads at anterior levels only; *Dendrocoelum lacteum* will not produce heads at all behind the anterior third of the body; while in many polyclads head formation ceases posterior to the cephalic ganglia. In these forms, there is no posterior region of high rate to account for the facts, but the explanation lies in all probability in the character of the primary gradient, and in the rate at which new tissue grows out. The posterior rise in rate in oligochaetes is the expression of the increasing youth of cells in the posterior direction; it must be regarded as a secondary gradient superposed on the primary integrative gradient in the nervous system. Evidence for this point of view is found in the experiments on stimulation after section, where it was shown that these posterior regions of high rate are affected in the same way although not to the same extent as regions of low metabolic rate, and are, therefore, like the latter, subordinate parts dependent on correlation with more anterior regions. The fact, however, that they are stimulated to a less degree than the regions of low rate indicates that they possess a certain slight

amount of independence. One may say, then, that there is an antagonism between the primary and the secondary gradient, the one making for subordination of the posterior region, the other for independence. The primary gradient, however, always has the upper hand, for it is there from the very beginning, and each new segment which arises is forced, despite its high metabolic rate, to become part of the system of conduction, which has already been long established in the antero-posterior direction. The secondary gradient probably aids in the inhibition of head formation, but is unable otherwise to influence the morphogenetic effect of the primary gradient, unless the latter is eliminated by other factors. If this were not so, it would be impossible to understand why heads do not arise at the posterior end of long pieces of *Lumbriculus* and *Tubifex*. I might further point out that the zooids of the naids arise without regard to the secondary gradient, and often within it, and that the 'breaking' region of *Lumbriculus* is always within the secondary rise; both of which facts indicate that the secondary gradient offers very ineffective opposition to the primary gradient. For zooid formation is, as Child has shown ('11 a), a matter of physiological isolation from the primary gradient, usually as a result of decreased intensity of correlative stimulation, and would be inhibited by the presence of a gradient running in the other direction.

In how far do these conceptions, developed from the data on *Lumbriculus*, explain the facts of regeneration in the other forms? Why does *Dero* form normal heads at all levels? Experiments on the rate of metabolism of pieces of *Dero* at various times after section have shown that the rate is very high immediately after section, but begins to fall at once. Within three hours it has fallen below that of the corresponding part of the whole worm, in five hours it is still lower, and continues to fall until about twenty hours, after which a permanent and increasing rise in rate sets in. While I have not been able to devise any method for determining the time of head determination in *Dero*, yet it certainly is most improbable that it could occur during the very brief period of stimulation. One may therefore safely say that

the head of *Dero* is determined at a time when the rate of metabolism of the pieces is low, and that therefore normal heads are always produced.

Why does head formation cease at an extreme anterior level in the tubificids? To answer this question is one of the most difficult problems with which we have to deal in the field of morphogenesis. The dynamic factors which have thus far sufficed to explain the experimental results cannot be called upon here, for this result is independent of size of piece. Numerous attempts to alter the regenerative capacity of *Tubifex* and *Limnodrilus* have yielded negative results. The following suggestions are offered as to the cause of failure of head formation in these forms. In the first place, the new tissue grows out very slowly, and with a relatively low rate of metabolism. It therefore is very easily inhibited. Secondly, the secondary gradient runs far forward in these forms, and may serve as the inhibiting factor. The cells at the cut surface of a piece of *Tubifex* taken back of the fifteenth segment are in contact with a region of high rate, which is itself in contact with regions of higher rate, and so on. Unless, therefore, the cells at the surface grow out rapidly and with a very high rate, and this is contrary to fact, they could not dominate the regions behind. Heteromorphic tails would be expected under such conditions; they actually occur in the earthworms, but here the cells do not grow out fast enough, and they are inhibited before they have an opportunity to produce anything. It is obvious that this explanation can be tested experimentally, and I intend to continue my experiments with *Tubifex* in the hope of obtaining positive results. As to why the posterior end of such pieces does not give rise to heads has already been discussed; the secondary gradient cannot eliminate the primary gradient to that extent.

Regarding the formation of the posterior end, a word should be said. The cells at the posterior end grow out with a high rate of metabolism, but cannot become independent as long as the primary gradient persists. Owing to this gradient they are in contact with subordinate parts, and must develop in correlation with more anterior regions. If, however, the gradient is elimi-

nated, as can be done by taking very short pieces, or putting the pieces in depressing agents, then the cells at the posterior end can become independent, and develop a head. Biaxial heads result under these conditions. On the other hand, if the old piece has a high rate of metabolism, it may be able to dominate the new cells growing out at the cut surfaces to such an extent that neither one can produce a head but each develops a subordinate part, resulting in biaxial tails. In most organisms these occur in short pieces only, where the piece attains a high rate of metabolism through the stimulation from section. But in some oligochaetes, particularly the earthworm, biaxial tails are produced by long pieces. Dynamic factors alone cannot account for this result, but it is probably due to the presence of the secondary gradient.

The conception of the process of form regulation to which Child has come as the result of his extensive experiments with Coelenterates and flatworms can then be satisfactorily applied to the microdrilous oligochaetes. This conception may be briefly summarized as follows. The head can only arise if the cells which are to form it attain physiological isolation and independence from the rest of the piece. The head is a self-differentiating system; it does not develop in correlation with other parts but is the starting point for a new system of correlations, in other words, a new individual. In long pieces the head forms at the anterior end of the piece because the axial gradient determines that at this end alone can sufficient physiological isolation be attained. In short pieces, head formation depends on certain dynamic relations between the head-forming cells, and the old piece, and these dynamic relations are, in turn, dependent, although indirectly, on the axial gradient. All other parts are subordinate, and arise in correlation with the head, or with anterior regions, which themselves develop in correlation with the head. This statement is proven conclusively by the fact that no piece ever regenerates structures anterior to its level unless a head is formed first at its anterior end. The response of cells or groups of cells to isolation, physical or physiological, is the production of an apical region or head; this response is the

“fundamental reaction of the species,” to use the phraseology of Child.

The apical region, or head region, or, in animals which develop a morphologically differentiated nervous system, the cephalic region of the nervous system which is the dominant part of the head is a closer approach than any other part of the organism to a morphological expression of this fundamental reaction system. The fundamental reaction system, dominance of the apical region and the axial gradient are all merely different aspects of the same general idea, viz., that the specific protoplasm of any organism consists fundamentally of a single physico-chemical reaction system. This system is the basis of inheritance and its dynamic capacities, the foundation of hereditary characters. The first step in organization and in embryonic development results from the establishment in one way or another, of some region or portion of this protoplasmic reaction system as a region of higher rate of dynamic activity. This region dominates development, becomes the apical or head region and determines the axial gradient or gradients which constitute the dynamic basis of polarity and of individuation. The organization and development of various parts of the organism rests upon a similar basis of fundamental reaction system and dominance and subordination of parts resulting from differences in rate of reaction (Child, '14 e).

V. SUMMARY

1. A gradient in rate of metabolism is demonstrated in the oligochaetes.

2. In the primary form of the gradient, the rate of metabolism is highest at the head and decreases along the antero-posterior axis. Among the oligochaetes this primary gradient is found only in *Aeolosoma*, and the zooids of the naids. The primary gradient is an integrative gradient.

3. In the other oligochaetes examined a posterior region of increased metabolic rate exists, and constitutes a secondary gradient superposed upon the primary gradient. The secondary gradient runs in the reversed direction from the primary; it results from the characteristic method of growth of annelids by continuous formation of new segments posteriorly, and is not integrative in character.

4. In *Dero limosa*, the secondary gradient involves the posterior third of the body; in *Lumbriculus inconstans*, it includes the posterior half of the body or more; and in the tubificids, it includes all of the body except the first five to fifteen segments.

5. In zooid formation, the gradient of the zooid gradually becomes independent of the gradient of the parent animal and is of the primary form. Owing to the processes of growth and dedifferentiation involved in zooid formation, the rate of metabolism of the fully developed zooid is higher than that of the parent; i.e., rejuvenescence results from asexual reproduction.

6. In oligochaetes a certain number of the most anterior segments are differentiated as a head.

7. In regeneration, the head and tail are replaced by outgrowth, the other parts by reorganization of the old tissue. No matter how many anterior segments are removed, only the typical number of head segments is, in general, replaced.

8. The head of oligochaetes will not regenerate a tail unless a certain number of trunk segments are included with it; nor will the end of the tail regenerate a head unless of a certain minimum size. Explanations of these facts are suggested.

9. In *Dero limosa*, any part of the body, whether long or short, regenerates a normal worm (with exceptions noted in 8).

10. In *Lumbriculus inconstans*, any part of the body, if of sufficient length, regenerates a normal worm. Short pieces show progressive inhibition of head formation along the axis; they give rise to anterior structures showing all gradations between a normal head and a normal tail. Normal posterior regeneration occurs at any level, and with any size of piece, but the number of segments regenerated decreases along the antero-posterior axis.

11. In *Tubifex*, head formation ceases at about the level of the fifteenth anterior segment, and, in *Limnodrilus*, at the level of the seventh segment, regardless of size of piece. Tail formation occurs at any level.

12. In *Lumbriculus inconstans*, it is determined, whether or not the head shall be normal within twenty to twenty-five hours after the pieces are cut.

13. The gradient of an axial series of pieces is not the same as that of a whole worm, because cutting stimulates. This stimulation is greater the shorter the piece and the lower its previous rate of metabolism. This stimulation is temporary, the time of

its duration varying with the different species, and is followed by a depression.

15. In *Lumbriculus inconstans*, short pieces from anterior regions are not much stimulated by section, and depression sets in within the time required for head determination. These pieces produce a high percentage of normal heads. Short posterior pieces are stimulated much more, and the stimulation lasts for a longer period of time, as long as the time required for head determination; these pieces produce a low percentage of normal heads, and a high percentage of inhibited structures.

16. The rate of metabolism of the piece during the time when the head is determined is, therefore, the important factor in anterior regeneration in short pieces. If the rate be high, then the region of new tissue which is to form the head is prevented from attaining the degree of independence and isolation necessary for normal head formation. Head formation will be inhibited in proportion to the metabolic rate of the old piece. On the other hand, if the metabolic rate of the old piece is low, then the new tissue suffers no inhibition and gives rise to a normal head. The dynamic relations set up between the old and the new tissue after cutting determine the character of the head, or whether a head shall form at all, or whether a tail shall form.

17. In long pieces, the dynamic factors are unimportant as the primary gradient determines that the cells at any anterior level are always more independent than those of a more posterior level. Therefore, normal heads are always formed on long pieces.

18. Experimental proof of the above conception is presented. If the rate of metabolism of the piece be depressed by means of cyanide, then the percentage of normal heads is increased; if the rate of the new tissue be decreased, then the percentage of normal heads decreases.

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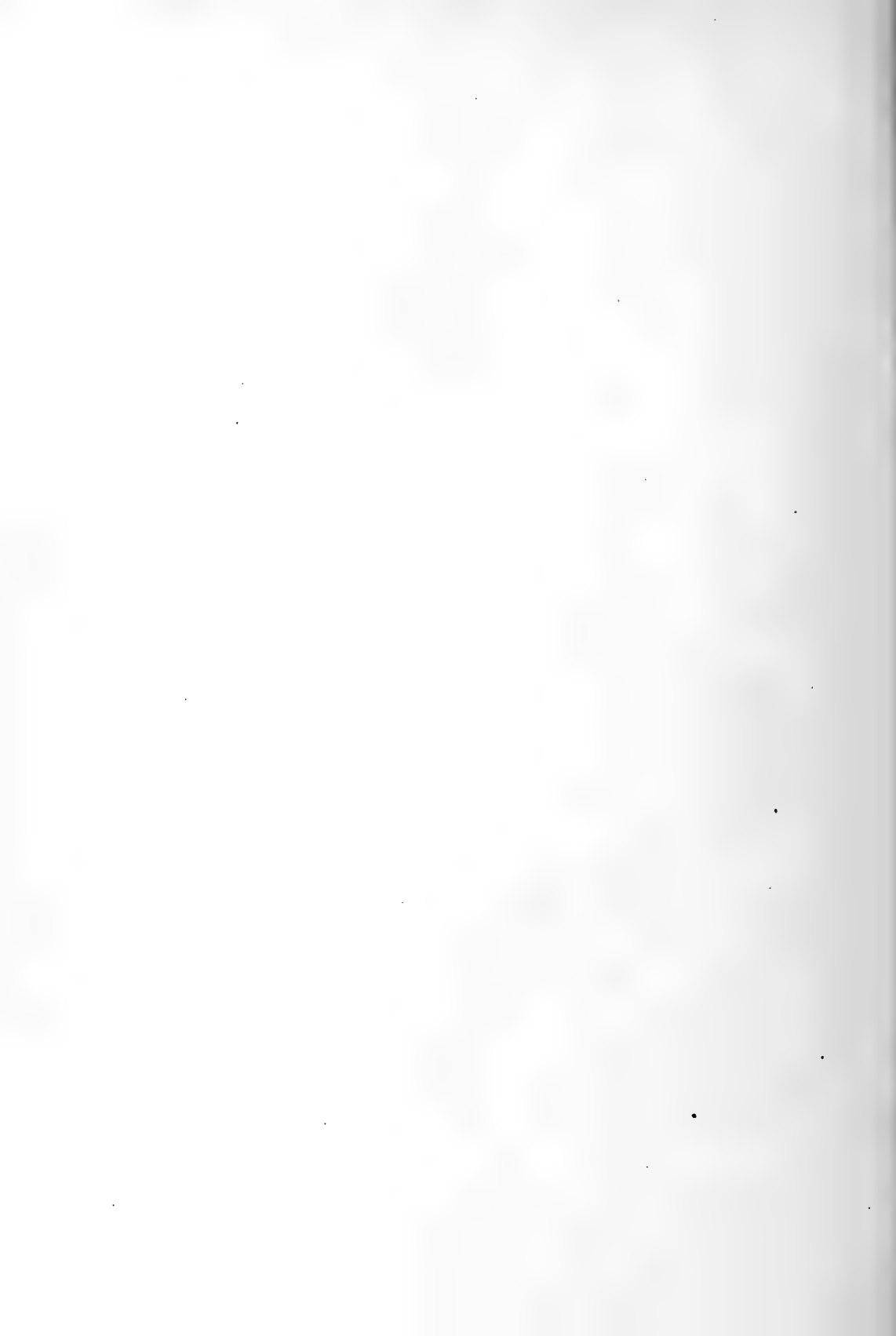
March 20, 1915.

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THE EFFECT OF LIGHT ON THE RETINA OF THE TORTOISE AND THE LIZARD

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ELEVEN FIGURES

INTRODUCTION

Since the discovery of the migration of pigment by Boll ('77) and by Kühne ('77) and of the contraction of the cones by van Genderen Stort ('87,) (see Engelmann, '85) in the retina of the frog, this subject has been carefully investigated by many authors in many animals. Garten ('07) has brought together the main and important results of this work on the changes induced in the retina by light. From this we see that light produces a variety of effects on the form and staining reactions of the different parts of the retina. Of these effects three are particularly interesting to us. 1) Migration of the pigment in the epithelial cells of the retina. 2) Changes in form and position of the visual cells. 3) Changes in form, position and ability to stain of the ganglion cells and of the nuclei of the inner and outer granular layers.

As far as the reptiles are concerned these questions seem far from settled, and therefore worthy of further investigation. In the first place concerning the migration of pigment, Angelucci ('78, p. 372) was not able to say from the results of a few experiments on the turtle, *Testudo graeca*, and on lizards (*L. agilis*, *L. muralis* and *L. viridis*), which have no rods, whether pigment migration took place or not. If it does, he remarks, it is much less marked than in the amphibian eye. Boll ('81, pp. 20 and 21) in an incompleated work, also considered this matter and in a theoretical consideration of the physiological properties of the

pigment epithelium, in which he regards the migration of the pigment to be bound up with the using up and regeneration of visual red in the rods, he concludes that pigment migration should not take place in a rodless retina, such as the lizards have. Boll, however, cannot say whether it does or not.

Angelucci ('94) (see Garten, p. 68), however, found that in *Testudo marina* the pigment does migrate, though less strongly than in the frog. And Chiarini ('06) was also able to clearly demonstrate pigment migration in the retina of the lizard (*L. agilis*). He figures, rather diagrammatically, side by side a dark and light retina, and, although he gives no measurements of the extent of the migration, it is clear that the pigment in the illuminated eye is nearer the external limiting membrane than it is in the dark eye, covering the paraboloids and drawing away from the bases of the pigment cells so that their nuclei are entirely uncovered.

Garten ('07, p. 68), however, was unable to obtain preparations of the retina of *Emys*, Chameleon or of *Lacerta* which showed constant differences in the position of the pigment according as to whether the animal had been kept in darkness or in bright light. His results will be referred to again. And finally Hess, ('10, p. 281), was no more successful than Garten with *Emys europaea*, the position of the pigment in eyes of individuals that had been kept for 22 hours in darkness, 2 hours in sunlight and several hours in light of weaker intensity being in all not markedly different, the outer segments being always covered by a mantle of pigment.

Concerning the contraction of the cones in light, Engelmann ('85, p. 500) found that in the eye of the snake *Tropidonotus natrix*, which contains no rods in the retina, the cones contracted but little. Also that in *Testudo graeca* it is doubtful whether any contraction takes place. Angelucci ('94) (see Garten '07, p. 25) however, claims that in *Testudo marina* contraction of the cones does take place, though less in extent than in the frog. Chiarini ('06) also reports that in the eye of *L. agilis* the cones shorten when the eye is brightly illuminated, but only slightly, for the cones measure in dark eyes 25–35 μ , in light eyes

23–30 μ . Finally Garten ('07, p. 25) found also a very slight contraction (not more than 1.1 μ) in the eye of Chameleon.

From this brief review of the few papers on the subject concerning reptiles we see that pigment migration and cone contraction are very slight if they occur at all. No work of this nature has been carried out on American species, and since it is desired to carry out a series of further experiments on tortoises and lizards with particular reference to vision, it was thought that something should be known concerning the reactions of the various parts of their retinæ to light. Three species of tortoises and one of lizards were used in the present investigation, viz., *Chelopus guttatus*, *Chelopus inculptus*, *Chrysemys picta*, and the common southern fence lizard, *Sceloporus undulatus*. Most of the work was carried out on *Chrysemys* and *Sceloporus*.

This investigation was taken up at the suggestion of Dr. Henry Laurens. It gives me pleasure to express here my thanks to Dr. Laurens for the assistance that he has given me during its completion.

METHODS

The methods of exposing the animals to light and to darkness were as follows: Two active animals were selected and placed in darkness for 24 hours. At the end of that time one of them was taken from the dark room and placed in direct sunlight for at least 6 hours after which it was killed. The other animal was either killed after it had been in darkness for 24 hours or after it had remained there at least 6 hours more. The eyes were removed as quickly as possible after the animals had been killed by decapitation—the dark eyes under red light, the light eyes in sunlight—and immediately dropped into the fixing fluid. The time consumed between decapitation and fixation was 5 minutes or less.

The fixation and subsequent procedure which gave the best results was the following: Fixation in Kleinenberg's strong picrosulphuric for 4 to 5 hours, followed by 70 per cent alcohol, which was frequently changed and in which the eyes were allowed to

remain for several days. Further dehydration, consuming at least 2 days, the lens being removed after the eyes had been in 95 per cent alcohol for several hours. For infiltration the chloroform paraffin method was employed, paraffin melting at 52° C. being used for imbedding. Sections were cut 8 to 10 μ thick, stained in Ehrlich's haematoxylin, followed by eosin. In a few cases, in an attempt to secure a quicker and perhaps more perfect fixation, the lens was removed before the eyes were dropped into the fixing fluid. It was found, however, that this method caused shrinkage and folding of the retina to such an extent, at the same time producing no better fixation, that it had to be given up.

ANATOMICAL

Before we proceed to consider the results of the comparison of light and dark eyes it will be best to give a brief account of the anatomical relations of the species with which we are working. Considerably more has been done by previous investigators on the morphology of the reptile retina than on its physiology, and it will be well to review briefly the results of this work.

Schultze ('66 and '67) noted that in *Emys europaea*, *L. viridis*, *L. agilis* and *L. muralis* there were no rods. From Hulke's ('67, p. 94) incomplete description one might assume that rods were to be found in the retinae of, among others, *Testudo graeca*, *Emys europaea*, *Chelone midas*, *Lacerta viridis* and *Anguis fragilis*, though, as Krause pointed out later, these 'rods' are more correctly to be considered as cones without oil drops. Heinemann ('77, p. 423) who examined the retinae of several Mexican species of tortoises concluded that, if the form of the outer segment be taken as the criterion, then rods as well as cones can be distinguished in the retina of Chelonians. That in lizards, however (p. 431) no elements with rod-like outer segments can be distinguished. In the Geckos, however, (p. 434) it is doubtful whether the visual elements are rods or cones.

Angelucci ('78, p. 371) found that rods are entirely lacking in *Testudo graeca*, *L. agilis*, *L. muralis* and in *L. viridis*. Boll

('81) briefly states (pp. 21 and 35) that there are no rods in the retina of *Testudo* or of *Lacerta*, while Cheivitz ('89, p. 143) adds that in *Emys europaea* and *L. viridis* there is but one form of visual cell which, from its form, is a cone.

Krause ('93) finds that in Chelonians there are no rods, and that the elements which had been earlier described as rods were nothing more than cones without oil drops. In *L. agilis*, however ('76 and '93), he describes rods as being present, but scarce, though in certain places they are found thick together. *Anguis fragilis* and *L. viridis* have only cones.

According to Angelucci, ('94, see Garten, p. 25) *Testudo marina* has no rods and Greeff ('00, p. 123) makes the brief statement that the reptilian retina (lizards, snakes and tortoises) has only cones. Chiarini ('06) says that the neuro-epithelium of lizards is formed exclusively of cones of various sizes. Garten ('07, p. 24) points out that in *Tropidonotus* there are no rods, that in *Testudo graeca* there are probably none, and that in the Chameleon there are certainly no rods.

Pütter ('09, p. 103), concludes from the mode of centripetal connection (dendritic) of the visual cells of the reptiles that all of them must be cones, although the form of the single elements can be very different, e.g., in *Anguis fragilis* and in the Gecko there are found cylindrical or rod-like outer segments. But he adds that all Chelonians have conical outer segments.

Hess ('10, p. 281) gives a review of the literature and states, that, in addition to the anatomical features of the retina indicating that there are no rods, the futile endeavors of several investigators to obtain evidences of visual purple in the tortoise retina indicates that there can be no rods present. And finally Franz ('13, p. 52) in a very incomplete and, in some particulars, incorrect review makes the statement that tortoises possess both rods and cones.

From these papers the conclusion may be drawn that cones are by far the principal visual element of the retina of tortoises and of lizards. Further that rods may occur in a few, but that they are scarce. It is to be regretted that good figures of the visual cells are not given in any of the articles reviewed. More-

over except in the work of Heinemann and Krause, American species have not been investigated. Of those mentioned by these authors only one genus is represented, viz., *Sceloporus*, that has been studied by me, and this is a Mexican species. The need therefore for some account of the retina of the ordinary American species of tortoises and lizards is still urgent, and since the anatomical features of the visual cells have not yet been described, a short account of these will not be out of place.

To begin with the tortoises we find that in the retina of the three species examined there are no rods. The cones are of two sorts, single and double. The single cone is the more numerous type of visual cell, and they are all similar in the possession of an outer and inner segment, in the latter of which is found in all cases an oil drop, an ellipsoid and a paraboloid. In form and size, however, these single cones present individual variations, on the basis of which we may say that there are two kinds, the first of which is considerably broader than the other, but only a little longer. With this increase in size there is found a slightly larger paraboloid and oil drop (fig. 6).

The double cones, of which there is only one kind, are much fewer in number than are the single cones. They are composed of a principal and of an accessory part, there being no twin cones. The principal cone has a very long narrow myoid, a long ellipsoid and an oil drop, there being no paraboloid. The accessory, which is much broader and shorter than the principal cone, has the typical short myoid of a single cone, a paraboloid, a granular ellipsoid but no oil drop (fig. 6).

From preparations of fresh retinae it was found that the colors of the oil drops were those which have been usually described for the tortoise retina, namely red, orange, pale yellow and blue green. The red are the largest and the most numerous.

Krause ('93) describes single and double cones in the retina of *Emys europaea*, there being two varieties of each. The first kind of single cone is similar to that which we have just described, the second is much broader and has only a coarse granular ellipsoid, but with no oil drop or paraboloid. Concerning the double cones of *Emys* the first variety, which is extremely

numerous, is similar to those just described, differing only in that both the principal and the accessory cones may have oil drops. The principal cone has a plano-convex ellipsoid, while the accessory possesses a plano-concave ellipsoid and a homogeneous paraboloid. In the second variety of double cone the principal cone is similar to the first, but the accessory is similar to the second variety of single cone, that is, thicker, with no oil drop but with an ellipsoid.

Heinemann ('77, p. 423) from his study of the retinae of several Chelonians distinguishes two kinds of cones. 1) those with an oil drop, and 2) those without. Of the first of these the inner segments are much thinner than the others, so that they approximate the form of rods, containing an ellipsoid and a paraboloid. Heinemann subdivides these cones with oil drops into four varieties. a) those with bellied out inner segments and large lens-shaped bodies, b) those which are narrower and with a smaller body, c) those which are pointed on the inside and contain here either a body of appropriate size or none at all, d) cones with strongly bellied out outer portion of the inner segment and with irregularly formed, and always much narrower, inner portion of the same. Seldom there is to be found here a small lens-shaped body, but usually this part is structureless or filled with a finely granular mass. The last two kinds, c) and d), of cones with oil drops unite with the cones without oil drops to form double cones. But in *Testudo* gray, double cones, both parts of which contain an oil drop are also to be found.

Concerning the lizard retina here again it may be said that there are no rods. The cones, however, in addition to showing several varieties among themselves are different from those of the tortoises examined (compare figs. 6 and 7). We again find in the first place that there are single cones and double cones. The single cones are of two varieties, a) with a very long myoid, no paraboloid, but with an ellipsoid and an oil drop. And b) much thicker than the first, with a shorter myoid and with an ellipsoid, paraboloid and oil drop (fig. 7). Similar to the tortoise retina there is one kind of double cone. The principal cone is similar to the long narrow single cone while the accessory

is much thicker, with a large paraboloid, a granular ellipsoid, and no oil drop (fig. 7).

Krause ('93) holds that *Lacerta agilis* has four kinds of visual elements, one of which is a rod, the other three being cones. The second kind of single cone is distinguished from the first by having a thicker inner segment and lacking an oil drop, although there is present an ellipsoid and a paraboloid. Double cones come principally from a combination of a principal cone with an oil drop and an accessory cone without an oil drop, though he also found double cones, both parts of which contained an oil drop. Heinemann ('77) finds two kinds of cones, those with oil drops and those without. The cones with oil drops show all gradations from voluminous cones with large lens-shaped bodies to very thin ones with very narrow inner segments in which there is no lens-shaped body. These latter unite with cones without oil drops to form double cones.

Greeff ('00, p. 117) figures a single and a double cone for the retina of a lizard. The single cone is quite similar to the narrow type found in *Sceloporus* (fig. 7), the only difference being that the myoid is comparatively shorter and thicker. The second or broad type of single cone, which is the predominating type of single cone in *Sceloporus*, he does not figure at all. The double cone is entirely similar to the one found by me.

In passing, it is interesting to note that in the tortoise retina the external nuclear layer consists of two rows of nuclei. Of these only those nuclei in the row immediately internal to the external limiting membrane are cone nuclei, the other row being bipolar nuclei which connect the cone nuclei with those of the inner nuclear layer. The cone nuclei are in general larger and more oval, with the long axis in the same line as that of the cones. In *Sceloporus*, however and *Cnemidophorus* (the sand lizard) the external nuclear layer consists of only one row. Chievitz ('89, p. 146) considers the second row, which he found in both *Emys* and *Lacerta*, to be the nuclei of supporting cells. Another point of interest is the presence of a fovea and large papilla in the lizard retina, but neither of these in the tortoises, though there is a small area centralis. Heinemann ('77, p. 425)

could find no fovea in the retinae of any of the Chelonians which he examined, although in several there was a small papilla. Chievitz ('89, p. 143) describes an area for *Emys* but no fovea, while in *Lacerta viridis* (p. 147) he finds a fovea.

EXPERIMENTAL

When sections of eyes taken from animals which had been placed in sunlight are compared with sections of eyes from animals kept in darkness it is seen, in the first place, that pigment migration does take place (figs. 1 and 2). In the dark eye (fig. 1) the pigment occupies more of the body of the epithelial cell, so that the nuclei are for the most part covered, and extends forward just beyond the oil drop, which in a few cases can be seen through the pigment. In the light eye (fig. 2) it is seen that the pigment has migrated forward so that the pigment cell nuclei are almost entirely uncovered while the pigment extends further toward the external limiting membrane, in many cases as far as the paraboloid. It was also noted that in the light eye the pigment epithelium adheres more closely than in the dark eye and is not so easily torn away.

The results of a series of measurements to determine the extent of migration are shown in table 1 (column 3). It will be seen that the average distance from the external limiting membrane to the nearest pigment needle in the dark eye is 8.8μ while that for the light eye is 5.2μ . The difference of 3.6μ represents the extent of the migration.

TABLE 1¹

ANIMAL	DIST. FROM EXT. LIMIT. MEMB. TO CHOROIDAL EDGE OF PIGMENT EPITHELIUM	DIST. FROM EXT. LIM. MEMB. TO OUTER SEG. OF CONE	DIST. FROM EXT. LIM. MEMB. TO NEAREST PIGMENT NEEDLE
Chrysemys			
Dark.....	26.0 μ	20.7 μ	8.8 μ
Light.....	21.0 μ	18.4 μ	5.2 μ

¹ Based on 10 measurements taken about 1 mm. from the entrance of the optic nerve.

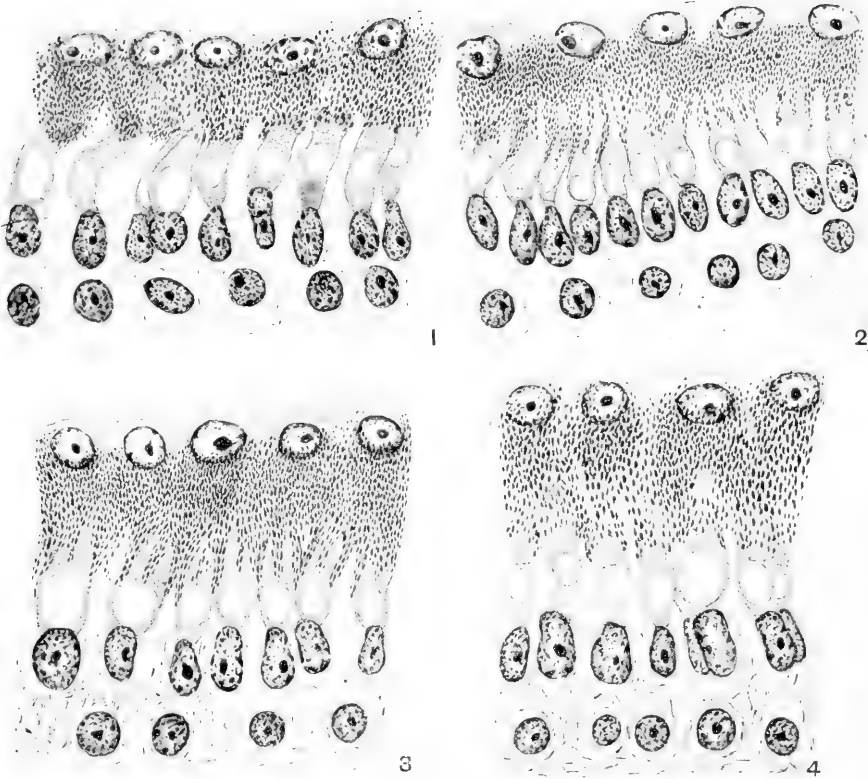


Fig. 1 A portion of the retina of *Chrysemys picta*. Animal kept in darkness for 24 hours.

Fig. 2 A portion of the retina of *Chrysemys picta*. Animal, after having been kept in darkness for 24 hours, placed in sunlight for 6 hours.

Fig. 3 A portion of the retina of *Chrysemys*. From an individual with cut optic nerve, kept in darkness for 24 hours, followed by a 7 hour exposure to sunlight.

Fig. 4 A portion of the retina of *Chrysemys*. From an individual with cut optic nerve and kept in darkness for 24 hours.

All of the figures were drawn with the aid of the camera lucida, and with Leitz oil immersion $\frac{1}{2}$ objective and ocular 4, giving an approximate magnification of 1340 diameters. The figures were then reduced $\frac{1}{3}$ for publication so that as they appear they have a magnification of about 890 diameters.

All of the drawings were made at about 1 mm. distance from the entrance of the optic nerve.

The comparisons of light and dark eyes of *Sceloporus* show that a pigment migration also takes place here. The average of measurements indicate that the extent of this migration is 3.1μ (table 2).

TABLE 2¹

ANIMAL	DIST. FROM EXT. LIMIT. MEMB. TO CHOROIDDAL EDGE OF PIGMENT EPITHELIUM	DIST. FROM EXT. LIM. MEMB. TO OUTER SEG. OF CONE	DIST. FROM EXT. LIM. MEMB. TO NEAREST PIGMENT NEEDLE
<i>Sceloporus</i>			
Dark.....	22.5 μ	14.0 μ	9.0 μ
Light.....	19.1 μ	12.5 μ	5.9 μ

¹Based on 10 measurements as in table 1.

Not only does the pigment migrate but the cones also show a contraction in light. In table 1 the results of measurements are shown. In the first place in the dark eye the distance between the external limiting membrane and the choroidal edge of the pigment epithelium is 5μ more than in the light eye. But this does not represent the amount of contraction of the cones, for as will be seen (column 2) the average length of the cones in the dark eye is only 2.3μ longer than in the light eye. From this it is evident that the light not only causes a shortening of the cones but a flattening of the pigment epithelial cells of, on the average, 2.7μ .

This flattening of the epithelial cells in the tortoise retina is in line with the results of others. Angelucci ('84 and '94) pointed out that in the illuminated eye of the frog a shortening of the epithelial cells in the direction of the axis of the rods was to be observed. Chiarini ('04 and '06) also saw this change in the retinal epithelial cells of the representatives of the five classes of Vertebrates which he examined. And Pergens ('96) noted the same thing in *Leuciscus*.

In addition to this change in form of the epithelial cell there has also been observed a change in the position of the nuclei of these cells. Pergens ('96) found that after illumination the nuclei of the pigment cells of *Leuciscus* were further forward than in dark eyes. Angelucci ('94) also observed this change of position in the frog. Garten ('07 b) on the other hand, al-

though he observed the differences in position of the nuclei of the epithelial cells of Abramis could find no constant differences due to the effect of light and darkness.

Concerning the third of the effects of light on the retina in which we are interested, namely changes in form, position and the ability of the ganglion cells and of the nuclei of the inner and outer granular layers to stain there has grown up a comparatively large literature. This has been excellently reviewed by Garten, who has in addition given a table in which the results of different investigators are shown.

We will first take up the effects of light on the nuclei of the cones. In Garten ('07 b, pp. 18-23) will be found a very good summary of what has been done. Czerny ('67), Gradenigo ('85), Angelucci ('94), and Chiarini ('04) in the frog; Pergens ('96) in *Leuciscus rutilus*; and Chiarini ('06) in the lizard found that light caused the cone nuclei to become longer and narrower. In addition, Birch-Hirschfeld ('06) found that light caused a difference in the volume of the cone nuclei of the pigeon, in that they are smaller and narrower. Birch-Hirschfeld further found in the pigeon that light caused the cone nuclei to approach nearer to the external limiting membrane.

Light was found to decrease the power of the outer granules to stain by Pergens ('96, '97, and '99) in *Leuciscus rutilus*, Mann ('94) in the dog; Birch-Hirschfeld ('00) in the dog and cat, and ('06) in the pigeon (very slight), Sgrosso ('05) in the frog, and Garten ('07, p. 23) in *Cercopithecus*, *Macacus rhesus*, fishes (*Abramis* and *Leuciscus*), *Salamandra*, frog and the owl. Chiarini ('04 and '06), however, found that in *Leuciscus* any difference between the ability of the dark and the light eye to stain was very uncertain, and that in the frog, lizard, crow and dog there was absolutely no difference.

We may pass now in the same way to a brief review of the results that have been obtained concerning the effect of light on the form and stainability of the inner granules and of the ganglion cells. Mann ('95) found that in the dog, illumination of the eye for 12 hours caused a decrease in the stainability of the inner nuclear layer and a decrease in the Nissl substance of the

protoplasm of the ganglion cells. Bach ('95) however, in the rabbit could find no differences in the stainability of the inner nuclear layer or of the ganglion cells in light and dark eyes. Birch-Hirschfeld ('00) however, did find differences between the light and dark eyes of rabbits and of dogs. The nuclei of the inner layer in the dark eye are rounder, in the light eye more oval. In the ganglion cells the Nissl bodies in the light eye have indistinct boundaries and with the protoplasmic background very diffuse. In the dark eye, on the other hand, the Nissl bodies possess sharp, distinct outlines. Chiarini ('04) found in *Leuciscus*, no decrease in chromatin in the inner nuclear layer after illumination, and in the ganglion cells hardly noticeable changes. Also later ('06) in the inner layer of reptiles, birds and mammals he found no differences between light and dark retinae. In *Lacerta*, however, he observed a slight decrease in the Nissl bodies of the ganglion cells, in *Corvus* a decided decrease, and in the dog again a slight chromatolysis.

Schüpbach ('05) found no differences, neither in the inner layer nor in the ganglion cells, between light and dark eyes of pigeons. But Birch-Hirschfeld ('06) was able to demonstrate clearly that the ganglion cells showed a distinct decrease in the number of the Nissl bodies and an indistinctness of their boundaries in the light eye. Carlson ('04) has shown the same for another bird—*Phalacrocorax penecillatus*, and finally Sgrosso ('05) found in the frog that the inner nuclear layer showed differences in stainability in the light and dark eye.

In order to make observations on these matters, particularly on the stainability of the nuclei, it was necessary to use some other methods of fixing, etc., than had been employed for the study of pigment migration, and of cone contraction. For this purpose the following method was finally decided upon, and gave excellent results. Fixation in warm concentrated sublimate for 5 hours. Removal of sublimate with iodine in 70 per cent alcohol, further dehydration, and infiltration by the chloroform paraffin method. Sections were cut 8μ thick, stained in eosin and toluidin blue, rapidly dehydrated, cleared in xylol and mounted in damar. In order to insure the same amount

of fixation, staining, decolorizing, etc., light and dark eyes were fixed in the same vessel, care being taken that they were distinguishable. Further, after sectioning, alternate rows of dark and light eyes (two of each) were placed on the same slide so that there could be no doubt of their similarity of treatment.

In the first place, the cone nuclei of both the tortoise and of the lizard are, for the most part, lengthened and narrowed by illumination. This change is, however, not very great and there is room for doubt owing to the great variability in the shape of these nuclei both in the light and in the dark eye. As far as their ability to stain is concerned the nuclei of the outer granular layer in the dark eye seem to be slightly more deeply stained than those of the light eye, but as far as the nuclei of the inner granular layer is concerned, no differences can be found. Neither could any changes in form of the inner nuclei be noticed after long illumination nor could any changes in the form and volume of the ganglion cells be observed. These latter cells, however, do show marked and clear differences between the light and dark eyes (figs. 8 and 9). Figure 8 represents three ganglion cells from a dark eye. By comparing it with figure 9 which is from a light eye, the differences can be observed. Not only is the amount of chromatin reduced but the Nissl substance has decreased in amount. Under the microscope these differences can be seen very distinctly, and it is possible to pick out the light and dark eyes by the comparative amount of chromatin and of Nissl substance which they contain.

These results show that light causes a migration of pigment and a contraction of the cones, as slight as these may be, in the retina of the tortoises and lizard. Moreover, that the cone nuclei of the tortoises are probably narrowed and lengthened and that furthermore a diminution in the amount of chromatin and Nissl substance in the ganglion cells is brought about, so that they stain less darkly and more diffusely than in dark eyes. It was considered sufficiently interesting to attempt to find out whether some or all of these changes could be brought about in eyes which had either been enucleated or had had the optic nerve cut.

Angelucci ('78, p. 367) observed that when the optic nerve of a frog is cut the physiological changes of the pigment took place, thirty days after the operation, as in normal eyes. Hamburger ('88), Arcoleo ('90) and Fick ('01, p. 4) also found that when the optic nerve is cut the pigment changes induced by light and darkness took place as in the normal eye. In addition Engelmann ('85, p. 505) observed that when the brain of a frog is destroyed that the effect of light on the migration of pigment is still present. Movements of the cones have also been observed in eyes, the optic nerves of which have been cut, or which have been removed from the body. Hamburger ('89) found that, when he cut the optic nerve of a frog or removed the eye, contraction of the cones will take place when the eye is illuminated. And Dittler ('07) found in the isolated frog retina placed in salt solution that upon illumination the contraction of the cones takes place.

Experiments on the enucleated bulbus of the tortoise were without results. The same is true of retinæ which were isolated in the manner described by Dittler for the frog. The experiments on eyes with the optic nerve cut, however, did yield rather interesting results. All the experiments were carried out on *Chrysemys picta*. The method of cutting the optic nerve is briefly as follows: Under deep ether anaesthesia a small wedge of bone was removed from the left side of the mouth beneath the eye. This was done by means of a long, very narrow saw. The small amount of bleeding consequent to the removal of the bone being stopped, the muscles were pulled to one side and cut, until the optic nerve could be seen. This was then cut by means of a fine pair of scissors. The total loss of blood was small and the animals quickly recovered. Out of eleven individuals operated upon only the first three died. The others after a few hours were active and seemed quite as fit for experimentation as normal animals. One effect of cutting the optic nerve, which was not always observed, was a slight enlargement of the pupil. Fick ('91, p. 3) states that after cutting the optic nerve of the frog the pupil is temporarily somewhat narrowed.

The tortoises were allowed sufficient time to recover from the effects of the operation before they were placed in light or darkness. Two operated animals were always used together for experiments and as controls two normal animals. All were placed in darkness for 24 hours, at the end of which time 1 operated and 1 normal individual were selected and placed in sunlight for 7 hours after which the eyes of all were removed and fixed as above described.

Upon examination and comparison of sections, it was seen that the pigment of the operated light eye had migrated forward and indeed further than it had in the normal eye of the same animal and in the eyes of the controls (figs. 2 and 3, and tables 1 and 3). Moreover that the cones had contracted about as much as in the normal eyes (see tables 1 and 3).

TABLE 3¹

ANIMAL	DIST. FROM EXT. LIMIT. MEMB. TO CHOROIDAL EDGE OF PIGMENT EPITHELIUM	DIST. FROM EXT. LIM. MEMB. TO OUTER SEG. OF CONE	DIST. FROM EXT. LIM. MEMB. TO NEAREST PIGMENT NEEDLE
Chrysemys			
Operated dark	29.6 μ	24.8 μ	4.7 μ
Operated light	23.5 μ	22.3 μ	2.7 μ

¹ Based on 10 measurements as in tables 1 and 2.

Now when the dark eyes of the operated and the control animals are compared, it is found that the pigment of the operated eyes is further forward than in the control eye of the same animal and in the eyes of the control animals. In fact the position of the pigment in the operated dark eye is about as far forward as it is in the normal light eyes (figs. 2 and 4, and tables 1 and 3). These results also show that the cones contract in light when the optic nerve is cut (table 3). Moreover, by comparing tables 1 and 3, it will be seen that the cones are longer in the operated dark eye than in the normal dark eye. These results would seem to be in line with those of Herzog ('05) who found that destroying the central nervous system of the frog brought about in the dark eye examined immediately or 24 hours later, a maximal forward migration (light position) of the

pigment and a maximal stretching (dark position) of the cones. It would seem as if a tonus, not only of the cones, but of the pigment epithelial cells, was removed or destroyed and that they both relaxed. Dittler ('07) and Garten ('07) were not able to corroborate Herzog's results. Garten, on the other hand, found that when the optic nerve of a frog is cut a marked stretching of the cones may take place, but does not mention any effect on the pigment, while Dittler (p. 306) says that destruction of the central nervous system, cutting of the optic nerve and enucleation of the bulbus has not the slightest effect upon the length of the cones.

Since the migration of the pigment and the contraction of the cones is relatively so small in amount, it was considered interesting to find out whether a greater amount of migration and of contraction could not be induced by means of other stimuli. The most natural stimulus to try was an electric current and therefore this was done, both induced and constant currents being used.

The experiments and results of stimulating the retina with an induced current will first be considered. The eyes, both dark and light, were removed as quickly as possible and placed in a watch glass containing a little 0.7 per cent salt solution. In one series of experiments both electrodes were placed on the optic nerve, in another, one was placed on the nerve and the other on the cornea. Stimuli of moderate strength were used and for a period of from 10 to 15 minutes. Upon examination of sections of such eyes it is seen that the pigment undergoes migration, but also a pronounced bunching and massing up in various places. The cones are also affected by the induced current being broader and shorter than normally the myoid being considerably contracted (figs. 6 and 10 and table 4). All of the cones did not show this effect, some of them appearing like normal dark cones and similar in measurements.

Engelmann ('85, p. 508) found that stimulation of the intact or enucleated dark eye of the frog with an induced current of moderate strength, produced the light condition of both cones and pigment.

When a constant current is passed through the tortoise eye much more definite and pronounced effects are obtained than with an induced current. The eyes, after being removed, were placed in a long trough containing tap-water to which a little salt solution had been added. The current (15–20 M. A.) was then passed from one end to the other and for from 15 to 30 minutes. Both light and dark eyes were again used and the current in some was passed from the posterior to the anterior of the eye (centrifugal), and in others, in the opposite direction (centripetal).

The examination of sections of eyes treated in this way show that in all cases a migration of the pigment takes place (fig. 5). This represents a typical case and in table 4 are given the average of a series of measurements.

TABLE 4¹

ANIMAL	DIST. FROM EXT. LIMIT. MEMB. TO CHOROIDAL EDGE OF PIGMENT EPITHELIUM	DIST. FROM EXT. LIM. MEMB. TO OUTER SEG. OF CONE	DIST. FROM EXT. LIM. MEMB. TO NEAREST PIGMENT NEEDLE
Chelopus in- sculptus			
Induced cur- rent eye.....	30 μ	19.4 μ	5.0 μ
Constant cur- rent eye.....	33.7 μ	25.0 μ	3.4 μ

¹ Based on 10 measurements.

A current which has been passed through the eye for 15 minutes is as effective as one which was allowed to pass for 30 minutes and presumably weaker currents of shorter duration would give the same results. In a few cases the retinae through which the currents had been passed for thirty minutes showed a partial breaking and tearing apart, particularly of the inner and outer nuclear layers.

The effects of a constant current on the cones is also very striking. Again the cones are much broader than normally, but instead of being contracted as after stimulation with the induced current they are much elongated (fig. 11, and table 4). The cone nuclei are not usually affected in any constant way

and are either round or oval as is ordinarily the case. In a few cases however they seem to be broken down and drawn out and up into the myoid (fig. 5).

It has been known for some time that the melanophores of the skin in many animals are caused to contract when stimulated with an induced current and to expand when stimulated with a constant current (Laurens '15, p. 609). It would seem that the same is true of the pigment cells of the retinal epithelium.

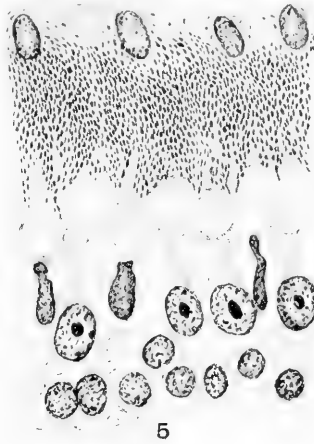


Fig. 5 A portion of the retina of *Chelopus inesculptus* showing the effects of passing a constant current of 18 M. A. for a period of 15 minutes through the eye. (Current passing from the cornea towards the optic nerve).

In all four cases in which the current was passed from the cornea to the optic nerve the pigment cell nuclei were changed in shape and position (fig. 5). Instead of being roundly oval with the long axis parallel to the choroidal limit of the cell, they are narrowed and lengthened and with the long axis at right angles to the usual position. This condition of affairs was never found when the current was passed in the opposite direction and would seem to indicate a polar effect of the current.

DISCUSSION AND CONCLUSIONS

In the retina of the tortoises (*Chelopus inculptus*, *Chelopus guttatus* and *Chrysemys picta*) and of the lizard (*Sceloporus undulatus*) pigment migration and cone contraction undoubtedly take place, however slight they may be as compared with the conditions observed in other groups of animals. Garten ('07 a and '07 b) has advanced a theory to explain the migration of the pigment and the contraction of the cones. His theory necessitates the assumption that there is no migration of pigment in the eyes of animals possessing a purely rod retina (skates and sharks) or a purely cone one (reptiles in general). Garten's theory in brief is as follows: one must assume that, were it not for the optical isolation of the visual elements by the pigment which migrates forward in the eyes of most of the lower vertebrates, a great deal of light would be scattered in all directions, on account of the large ellipsoids (fishes) and the strongly refractive oil-drops (many Amphibians, Reptiles and Birds) which would stimulate the neighboring rods and cones. In the narrow slender rods, however, total internal reflection prevents this dispersion of light, which is borne out by the fact that in pure rod retinæ the pigment is entirely lacking from the pigment epithelium. On the other hand in a pure cone retina with strongly refractive oil drops, the pigment in both light and dark eyes must be forward in position (that is, covering the outer segments and the oil drops). By means of the stretching of the cones in darkness or in faint light, which in many of the lower animals is accompanied by a contraction of the inner segments of the rods, there is presented for faint light stimulation, a cone-free purely rod layer, which does not need the pigment, which therefore moves backward thus enabling oblique light to enter the rods. On the other hand the contraction of the cones produced by bright light, which is accompanied by stretching of the rods, similarly leads to a forward migration of the pigment which surrounds the cone outer segments.

This is the part of Garten's theory which is of interest to us. It cannot be denied that the tortoise and lizard retina con-

tains only cones and no rods. This has been shown, not only morphologically but physiologically as well, in that it is impossible to demonstrate the presence of visual purple (see Garten '07 b; p. 148 and Hess '10, p. 281). Nevertheless, pigment migration and cone contraction do take place, however slight in amount. It is perfectly true that the outer segments and the oil drops are always covered with pigment, but when bright light (sun-light) is thrown on the retina the cones contract and the pigment migrates forward.

It cannot be argued, if we accept Garten's theory, that there is any physiological need for a moving backward of the pigment and a stretching of the cones in faint light since there is only one kind of visual cell. But, on the other hand, it must be assumed that the pigment and the cones are sensitive to light and respond to its stimulus, the one moving forward, the other contracting. Dittler ('07 a and '07 b) thinks that the cone myoid of the frog is not itself sensitive to light but that its contraction is brought about by a metabolic product which is set free by the activity of the retina under the influence of light. Garten ('07, p. 96) supports this theory and thinks that the migration of pigment may also be bound up in some way with this chemical stimulus effect. Now, if this be true for the retina of the frog, there is no reason, simply because the tortoise and lizard retina have only cones, to assume that it is not true for the retina of these animals.

Gaupp ('04, p. 815) is of the opinion that in the migration of the pigment in the protoplasmic processes between the visual elements we have a process very similar to that seen in the melanophores of the skin, where the pigment streams back and forth in the processes of the cells. We have seen in the tortoise eye where the optic nerve has been cut that migration of the pigment, as well as contraction of the cones, still takes place, which is evidence that both the pigment cell and the cone are also directly sensitive to light. Garten's objection that there has been no proof that pigment migration in the retina may be brought about by the direct effect of light because chemical stimuli could possibly play a part has, it seems to me, no weight,

for the simple reason that that is probably just what does take place, not only in the retinal pigment epithelium but in the skin melanophores as well, when stimulated by light. Light causes chemical changes which influence the pigment cells and causes the protoplasm, in which the pigment is carried, to stream.

SUMMARY

1. The retinae of the tortoises, *Chelopus insculptus*, *Chelopus guttatus* and *Chrysemys picta* and of the lizard, *Sceloporus undulatus* contain no rods.

2. In both the tortoise and lizard double, as well as single, cones occur (figs. 6 and 7).

3. Light causes a migration of the pigment and a contraction of the cones in both the tortoise and the lizard retina, the extent of migration in the tortoise averaging 3.6μ and in the lizard 3.1μ . The extent of the contraction of the cones in the tortoise averages 2.3μ . The pigment epithelial cells also flatten in light to the extent of about 2.7μ .

4. Light probably causes the cone nuclei to lengthen and become narrower. Illumination decreases slightly the ability of the outer nuclei to take on stain, but has no effect on the form nor on the ability of the nuclei of the inner granular layer to stain.

5. Illumination has no effect on the form and volume of the ganglion cells, but it reduces the amount of chromatin and Nissl substance so that the cells stain less darkly and more diffusely (figs. 8 and 9).

6. Light causes the pigment to migrate and the cones to contract in the tortoise retina after the optic nerve is cut (fig. 3).

7. Stimulation of the enucleated bulbus with an induced current of moderate strength causes a forward migration as well as a bunching and massing of the pigment. It also causes a slight broadening and contraction of the cones (fig. 10).

8. Passing a constant current of 15 to 20 M. A. through the eye either centrifugally or centripetally for a period of 15 minutes brings about a marked migration of the pigment and in addition a broadening and stretching of the cones. When the

current is passed from the cornea towards the optic nerve (centripetally), a polar effect on the epithelial cell nuclei is observed in that the nuclei are rotated so that the long axis of the nuclei are at right angles to their usual position (fig. 5).

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PLATE 1

EXPLANATION OF FIGURES

All of the figures were drawn with the aid of the camera lucida, and with Leitz oil immersion $\frac{1}{2}$ objective and ocular 4, giving an approximate magnification of 1340 diameters. The figures were then reduced $\frac{1}{3}$ for publication so that as they appear they have a magnification of about 890 diameters.

All of the drawings were made at about 1 mm. distance from the entrance of the optic nerve.

6 Cones of *Chrysemys*. Animal kept in darkness for 24 hours.

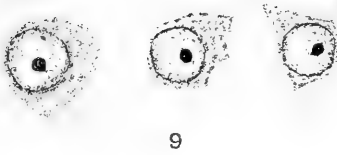
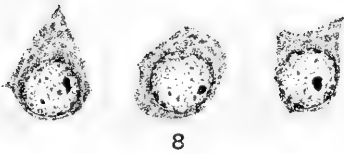
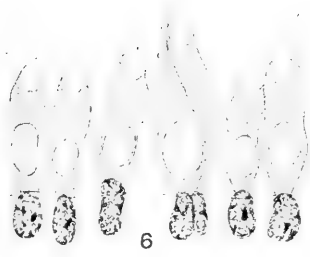
7 A portion of the visual layer of an eye of *Sceloporus undulatus* showing double cones and the two types of single cones.

8 Three ganglion cells from the retina of *Chrysemys picta*. Animal kept in darkness for 24 hours.

9 Three ganglion cells from the retina of *Chrysemys picta*. Individual held in darkness for 24 hours and subsequent exposure to sunlight for 6 hours.

10 Cones from the retina of *Chrysemys picta* showing the effects of stimulation with an induced current for 10 minutes.

11 Two cones from the retina of *Chelopus insculptus* showing the effects of stimulating with a constant current of 18 M. A. for 30 minutes.



EVIDENCE PROVING THE MELANOPHORE TO BE A DISGUISED TYPE OF SMOOTH MUSCLE CELL

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TWO FIGURES

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PRELIMINARY

The melanophores of the lower vertebrates, fish, amphibians and reptiles, are described by most authors as modified connective tissue cells (Fuchs '14). The evidence for such a conception rests chiefly upon a morphological basis. Leydig ('89, '92), showed that no sharp morphological distinction exists between unpigmented, connective tissue cells, inactive, pigmented connective tissue cells and chromatophores (melanophores) containing actively migrating pigment granules.

During the past four years I have been carrying on a series of experiments on the direct physiological responses of the melanophores in fish, chiefly in *Fundulus*. These experiments

have presented an opportunity for occasional morphological and embryological observations also. In attempting to categorize any type of cell it is necessary to consider not only its morphology, but its development and physiology as well. An examination of many data of previous investigators, supplemented by my own observations has led me to the conclusion that the melanophores of lower vertebrates do not belong in the category of connective tissue, but are to be considered functionally modified smooth muscle cells. The object of this paper is to summarize the evidence for my position.

MORPHOLOGICAL EVIDENCE

For our present discussion the following occurrence of involuntary or smooth muscle in vertebrates is of importance, viz., the digestive tract, the vascular system, the eye and the integument. The units of which smooth muscle tissue is composed consist of elongated or spindle-shaped cells which may be branched or forked, containing a single or several oval or elongate nuclei. In certain cases (sphincter pupillae) the cytoplasm is filled with a dense mass of melanin pigment granules. We may therefore say with Franz ('06), (p. 452): "Es ist daher wohl eine zulässige Ausdrucksweise vom Sphincter als von einer Anhäufung spindelförmiger Pigmentzellen zu sprechen."

In the lower vertebrates the melanophores occur chiefly in the derma, more rarely in the epidermis (Pouchet '76, in Rhombus; Müller '60, Eberth '67, Pouchet loc. cit., Biedermann '92 and many others in the frog; Schmidt '12, in *Phelsuma*), on the mesenteries and peritoneum and associated with the blood-vessels. Morphologically we find an entire series extending from relatively simple cells with two or three processes to the familiar, extensively branched forms found in the derma. Zimmerman ('93), for example, noted elongated melanophores between the fin rays in several adult teleosts and I have observed similar simple cells in the tail region of adult *Fundulus* and other teleost species. Franz ('08) has recorded the same condition in the edges of the fins in the larva of *Pleuronectes platessa*. On the

surface of the iris in the eel Steinach ('92) has observed a transition from the stellate melanophores to the spindle shaped cells of the sphincter pupillae. He says (p. 515): "Die verästigten sternförmige Pigmentzellen . . . gehen dicht am Pupillar-rand in einen Ring von besonders dunkeln Pigmentzellen über deren plumpe Körper sich mehr der spindelform nähern."

We may conclude then, that, as regards external and nuclear form and a melanin pigment content, no sharp morphological distinction can be made between melanophores and the smooth muscle cells of the sphincter pupillae.

EMBRYOLOGICAL EVIDENCE

Smooth muscle may be formed either from the mesoderm (mesenchyme), the commonest source, or from the ectoderm, (Nussbaum, M. '01).

Investigations upon the development of the dermal melanophores show a satisfactory agreement. With a single exception¹ they have been found to originate from mesodermal cells (Wenckebach '86, Bolk '10: Ehrmann '96, Eycleshymer '06, Weidenreich '12: Kerbert '77, Todaro '78, Zenneck '94). The chief controversial point in the case of the epidermal melanophores has been as to whether these cells develop from the epidermis in situ, or whether, as unpigmented mesenchyme cells, they wander into the region of the epidermis and become pigmented secondarily. In the first case their origin would be ectodermal and in the second, mesodermal. My own observations are limited to several species of teleosts. In the case of *Fundulus*, epidermal melanophores do not occur, but the origin of the dermal melanophores from wandering mesenchyme cells has been clearly demonstrated by Stockard ('15). Dr. Stockard has very kindly shown me a number of his own preparations and unpublished drawings of the developing melanophores in *Fundulus* embryos. His studies, made in connection with the developing circulatory system, have shown that in this species the yolk melanophores first appear as wandering, actively amoeboid, unpigmented mesen-

¹ Borcea, '09.

chyme cells. From the region of Kupffer's vesicle there develops a fan-shaped area of migrating cells which rapidly spread out on the surface of the yolk. These cells eventually give rise to connective tissue, the yolk melanophores, the endothelial cells and possibly also to the smooth muscle cells in the walls of the larger blood vessels. For some time after the pigment has begun to develop within the wandering cells that are destined to become melanophores, they continue to show active amoeboid movements. In a transparent pelagic egg like that of the scup (*Stenotomus crysops*), these wandering cells at the posterior end of the embryo may be observed with startling clearness.

It is impossible to draw any sharp morphological or embryological distinction between certain pigmented connective tissue cells and chromatophores (Leydig loc. cit.) just as there are morphological transitions between connective tissue and smooth muscle cells (Flemming '97). We can say, then, that typical connective tissue cells, melanophores and smooth muscle cells are all known to develop from mesenchyme and furthermore, that smooth muscle cells (sphincter pupillae) and possibly also melanophores (Borcea loc. cit.) may develop from the ectoderm. The embryological evidence is thus at least not negative.²

PHYSIOLOGICAL EVIDENCE

By far the most striking evidence of the parallelism between smooth muscle and melanophores is furnished by physiological experiments. Franz ('06) first recorded the striking similarity between the physiological responses of the sphincter pupillae in *Acanthias* and the dermal chromatophores of the frog. He noted 1) that both respond to light by contraction, 2) that indifferent gases like N and H elicit no contractions of the chromatophores and that these gases prevent the contraction of the sphincter pupillae upon illumination; 3) that after death, the pigment cells and sphincter are both contracted. He then says (p. 453): "Wir können also in mancher Hinsicht Analogien zwischen den

² It is certainly not without interest in this connection that the chromatophores of cephalopods have been found to develop from single smooth muscle cells by a complicated metamorphosis (Chun '02).

Sphincterfasern und mesodermalen Chromatophoren aufstellen;" and again: "Denn es scheint in mehr als einer Beziehung, dass die Chromatophoren nichts anderes als verknappte Muskelzellen sind."

Many of the older investigations upon the responses of the melanophores to various stimuli were carried out upon living animals. Here the situation was exceedingly complex since so many physiological factors had to be controlled. In a previous paper ('13 b) I have called attention to this objection and have described a method whereby the melanophores of teleosts, especially *Fundulus heteroclitus*, may be removed from the fish and stimulated in a variety of ways without the slightest mechanical injury in manipulation. The technique is exceedingly simple, consisting of the careful removal of the scales with their superficial sheets of dermal melanophores. These scales are readily transferred from one solution to another and, by selecting adjacent scales from the same fish, we obtain very satisfactory physiological units whereby it is possible to test the effects of a series of solutions—the degree of expansion or contraction of the pigment serving as an indicator of relative stimulation. In preparations of this sort in which the circulation and nervous control have obviously been eliminated, I have found that chemical stimuli such as 0.1 N KCl, heat (30° C.), ultra-violet light and induction currents all bring about a more or less rapid contraction of the pigment granules. Furthermore, in indifferent media like Ringer's solution or olive oil, the melanophores remain expanded for long periods. It therefore seems justifiable to consider the contracted phase that of stimulation, since the melanophores respond by contracting to the above familiar series of physiological stimuli. Throughout the following discussion I shall consider the contracted phase of the melanophores as corresponding, physiologically, to the contraction in smooth muscle. The objections to the application of the term 'contraction' in the case of the melanophores, I shall take up at the close of this section.

I. Innervation

The activity of vertebrate smooth muscle is normally controlled through fibers of the sympathetic nervous system. Voluntary motor connections do not ordinarily occur.

The innervation of the melanophores has been satisfactorily demonstrated both histologically (Ballowitz '93) and physiologically (Pouchet '72 and '76, Von Frisch '11, Spaeth loc. cit.) in several species of teleosts. v. Frisch has recently corroborated and amplified the original observations of Pouchet, who first claimed the innervation of the melanophores to be sympathetic. I have repeated the striking experiments of v. Frisch upon *Phoxinus* with *Fundulus*. In this experiment one of the two branches of the sympathetic system is severed immediately behind the body cavity in the region of the haemal arch. Fish so treated lose their power of color adaptation posterior to the point of incision on the operated side, but continue to show normal motor responses. Reciprocally, severing of the spinal cord eliminates motor responses but, provided the operation has been carefully performed, the sympathetic adaptations to different colored bottoms remains normal.

In *Fundulus* I have also found at the base of the medulla a 'contraction center' corresponding with that found by Lode ('90) in the trout and by v. Frisch ('11) in *Phoxinus*. A lightening of the entire body of the fish follows the electrical stimulation of this center.

No satisfactory histological demonstrations of the nerve endings in the melanophores of amphibians and reptiles have been recorded. Bimmerman ('78), Biedermann, loc. cit. and more recently Hooker ('12) have however, all demonstrated, physiologically, the sympathetic innervation in the melanophores of the frog and Carlton ('04) has made similar observations in *Anolis*. There is thus a very satisfactory unanimity of opinion on this question.

II. Effect of Light

Through the investigations of Arnold ('41), Brown-Sequard ('47), Steinach ('92), Magnus ('99), and Franz (*loc. cit.*) we know that the excised iris of certain elasmobranchs (*Acanthias*), teleosts (*Anguilla*) and amphibians (*Rana*) responds to illumination by a contraction of the sphincter pupillae.

Hertel ('07), first succeeded in demonstrating a direct response to stimulation by light in the chromatophores of the cephalopods *Sepiola*, *Octopus* and especially *Loligo*. In this case the reactions of the chromatophores are due to contractions and relaxations of the radially arranged smooth muscles. The active phase is here the expanded one, elicited by the contracting radial muscles i.e., it is the reciprocal of the condition in the melanophores of lower vertebrates. Hertel found that ultra-violet light of 280 $\mu\mu$ produced an almost instantaneous local expansion of all chromatophores. Blue rays of 440 $\mu\mu$ and yellow rays of 558 $\mu\mu$ of equal intensity also gave a distinct, but somewhat slower expansion.

I have shown (*loc. cit.*) that the expanded melanophores of *Fundulus* respond to ultra-violet light (280 $\mu\mu$) by a rapid and reversible contraction. I was unable however, in a series of trials with different regions and intensities of the visible spectrum to obtain contractions of *Fundulus* melanophores. Following practically the same technique as in my experiments with *Fundulus*, Laurens ('15) has recently reported contractions by ultra-violet light in expanded melanophores in pieces of the skin of *Amblystoma* larvae. Hertel (*loc. cit.*) had previously found that the melanophores of *Triton* larvae responded to ultra-violet, blue and yellow rays by contracting. He used the same wave lengths and intensities as in his experiments with cephalopods (*vide supra*). In this case, as in so many of the older experiments, the presence of a complete nerve mechanism and blood supply presents the possibility of secondary complications. In the case of *Fundulus* and *Amblystoma*, however, I believe the results to be free from this objection.

A direct response to light has not thus far been demonstrated in the melanophores of reptiles, though as Fuchs (*loc. cit.*) has emphasized, it will in all probability be found to exist.

We may now summarize the foregoing observations as follows; 1) in certain species of fish and amphibians the sphincter pupillae contracts in response to direct stimulation by light; 2) in certain other species of fish and amphibians the melanophores also respond to direct stimulation by light by contracting.

III. Effect of Electrical Stimulation

Induction currents of sufficient intensity and duration produce contractions in smooth muscle. Such contractions may be seen in strips of frog's stomach prepared according to Meigs ('12), or in preparations of the digestive tube of several species of teleosts.⁵ Beer ('94, '98) observed that the sphincter pupillae of the eel and the frog (*Rana*) contracted when stimulated electrically. The radial muscles of the chromatophores of cephalopods also respond to electrical stimulation by contracting (Brücke '52, Keller '73, Fredericq '78, Klemensiewicz '78, Pouchet '76, Krukenberg '80, Phisalix '92, Steinach '01, Hofman '07, '10, and Fuchs '10). Klemensiewicz (*loc. cit.*) records similar contractions in isolated pieces of the skin of *Loligo*.

Lode (*loc. cit.*) first showed that in excised pieces of the skin of the trout the melanophores contracted upon being stimulated by an induction current. In my own experiments with *Fundulus* the melanophores invariably contracted, reversibly, when proper strength and duration of the current and salt concentration of the mounting medium were selected.

Winkler ('10) found that the melanophores of *Rana esculenta* and *Hyla arborea* contracted when directly stimulated by an induction current. Laurens (*loc. cit.*) has recently verified this observation in large larvae of *Amblystoma opacum*. He finds also that: "When various portions of the body are cut out and directly stimulated either with the central nervous system in-

⁵ Unpublished observations made upon the stomach muscle of *Stenotomus*, *Tautoga*, *Centropristes* and *Fundulus*.

tact or destroyed, a slight contraction of the melanophores is usually induced" (p. 610).

In the case of the reptilian melanophores Bert ('75) and Kruk-enberg ('80) were able to corroborate the original observation of Brücke ('52) who found that direct faradic stimulation of excised bits of dark skin in the chameleon produced a lightening, i.e., a contraction of the melanophores.

Thus the responses of several types of smooth muscle, as well as of the melanophores, in representatives of all three groups of lower vertebrates show a satisfactory agreement in their contraction to faradic stimulation.

IV. Effect of Mechanical Stimulation

By gently pinching or stretching excised pieces of frog or fish³ stomach and oesophagus, powerful contractions may be induced, which are reversible provided the stimulus has not been too violent. Precisely the same reaction follows a similar treatment of portions of the skin of *Loligo*; the chromatophores expand widely. I have verified this observation by Klemensiewicz *loc. cit.* and all of the more recent investigations upon cephalopod chromatophores record a similar phenomenon in *Loligo* and other species.

Fuchs (*loc. cit.*) has called attention to the objection against the expansion observed by many of the older investigators after the surfaces of various teleosts had been more or less violently 'stroked' with a needle. He says (p. 1432): "Aus allen Beobachtungen geht unstreitig hervor, dass einwandfreie Beobachtungen über die direkte mechanische Reizbarkeit der Fischchromatophoren nicht vorliegen." I have shown (*loc. cit.*) that by selecting scales from the lateral portion of *Fundulus* where the melanophores are relatively far apart, it is possible to stimulate a single melanophore repeatedly by exerting a gentle pressure with a fine, fire-polished glass needle. Great care must be exercised not to rupture the delicate cells, for by so doing, the melanin granules are scattered and produce the effect of an 'expansion' recorded by the older observers. In this case it is certain that

mechanical stimulation (gentle pressure) produces a reversible contraction of the melanophores.

As a result of local pressure or pinching with forceps, and along the margins of an incision, the melanophores in the frog contract (v. Wittich '54, '71, Hering l. c., Lister '58 a, '58 b, Fuchs '06, Êtérnod and Robert '08). The darkening of the skin in *Polydectes Reinwardtii*, "nach leichtem Kratzen mit einer Nadel," observed by Siedlecki ('09), is doubtless another case of ruptured melanophores.⁴

The above observations warrant the conclusion that in the radial smooth muscles of the chromatophores of cephalopods, in the smooth muscle of the digestive tract in certain teleosts and amphibia, as well as in the melanophores of these two vertebrate groups, mechanical stimulation (gentle pressure) is followed by a reversible contraction.

V. Effects of Chemical Stimulation

A great many experiments have been carried out upon the effects of various widely differing chemical stimuli upon the melanophores of vertebrates. The method of procedure has been, in most cases, as follows; 1) the substances were brought directly upon the skin of the normal or operated (pithed, etc.) animal, the color-change being considered the criterion of the action of the chemicals; 2) the substances were injected into the circulation, the body cavity, or subcutaneously, the color-change again serving as an indicator of chemical stimulation; 3) the substances were added to the water of the environment, in aquatic forms. In relatively few instances have excised pieces of skin been immersed in the fluid to be tested. As I have repeatedly emphasized, this is the only satisfactory method of determining the direct effect of any stimulus upon the melanophores, for it is only in this way that the circulation and all central nervous

⁴ Observations upon the response to pressure in reptilian melanophores are contradictory and unsatisfactory. Most of the experiments have been made with living animals and the few trials with excised bits of skin were all carried out without regard for the possibility of a darkening resulting from the destruction of the melanophores (Milne-Edwards '34 a, '34 b, Brücke '52, Carlton '04).

control may be simultaneously and effectively eliminated. The objection may be raised that even in excised pieces of the animal it is impossible to destroy the ultimate nerve terminations, hence we are unable to state with certainty that the effect is a direct one and not transmitted through the cut stumps of the sympathetic nerves which remain in such a preparation. I have elsewhere (*loc. cit.*) adduced evidence to show that this objection is certainly invalid in the case of the isolated melanophores of the scales of *Fundulus*. I shall limit the following discussion of the reactions of melanophores to chemical stimuli to cases which are as nearly as possible comparable to the chemical stimulation of isolated smooth muscle.

A. *Inorganic Substances.* Schultz ('97) observed that distilled water acted as a weak contracting stimulus in opened ring preparations of the stomach of the frog. Meigs ('10) has shown that distilled water gives a typical curve of contraction in preparations of longitudinal strips of the frog's stomach.

I have found (*loc. cit.*) that the melanophores of *Fundulus* slowly contract when brought from the living dark fish or from 0.1 N NaCl to distilled water (fig. 1).

Schultz (*l. c.*) noted a relaxation and swelling of the smooth muscle of the frog's stomach in 10 per cent NaCl solution. Meigs (*loc. cit.*) has shown that in solutions of KCl, the stomach muscle of the frog (*R. pipiens*) slowly contracts and loses weight while in NaCl it elongates and absorbs water.⁵ I have observed that the chromatophores in pieces of the mantle of *Loligo* expand immediately upon being immersed in 0.1 N KCl solution. This is obviously due to a contraction of the radial smooth muscles. Furthermore, provided the exposure to KCl has not been too long, when such chromatophores are returned to 0.1 N NaCl or better, Ringer solution, they contract again, the radial muscles are relaxed.

The melanophores of all the species of teleosts with which I have experimented, showed a contraction in 0.1 N KCl solution and an expansion or relaxation in 0.1 N NaCl, dilute sea-water,

⁵ Zoethout ('02) found that KCl produced a contraction in the gastrocnemius of the frog and NaCl a relaxation.

or Ringer's solution.⁶ The effects of other neutral salts of the alkalies were also studied in considerable detail but, since, so far as I am aware, no parallel experiments have been performed upon smooth muscle, a discussion of these results would be irrelevant. It is, however, of interest that both cations and anions of the neutral salts show the same order of physiological effect, the so-called 'lyotropic order,' as in experiments upon striated muscle (Overton '04 and Sewartz '07) and certain colloids (Hofmeister '91, Pauli '99, Höber '14, Chapter 7).

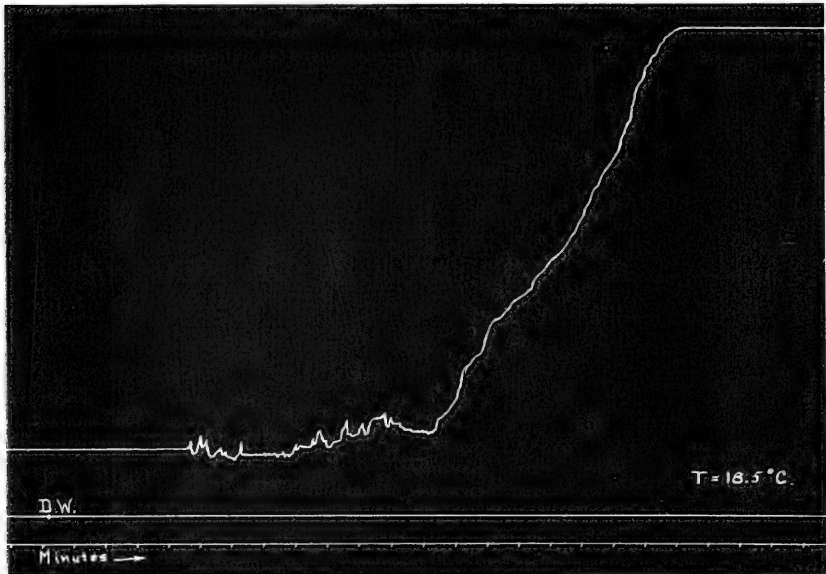


Fig. 1 The slow contraction of a melanophore in distilled water. The upper line represents the movements of the terminal pigment granules in one process of the cell. The lower line indicates time in minutes. At D. W. the cell was immersed in distilled water (from 0.1 N NaCl). The melanophore was completely contracted in twenty minutes. In this case the migration of the pigment granules was followed by means of an ocular micrometer carrying a moveable scale. An empirically selected line of the micrometer scale was kept tangent to the terminal pigment granules by turning the adjusting screw of the ocular. The motion of the screw was transmitted to a set of pulleys and a heart lever and the curve was recorded on a kymograph in the usual way. The actual path of the pigment migration was 0.104 mm, which makes the magnification of the reproduced figure approximately $\times 534$. A detailed description of this apparatus will appear shortly in the *American Journal of Physiology*.

Recently⁶ I have found that the alkaline earths produce a contraction of the melanophores in *Fundulus*. The time for this contraction in isotonic solutions of the neutral chlorides varies in the order



In strontium, calcium and magnesium chloride, the contractions are all reversible, the time required for complete recovery varying in the reciprocal order—



The behavior after treatment with 0.1 N BaCl_2 for ten minutes is peculiar in that, frequently, upon being returned to 0.1 N NaCl , no sign of recovery appears for as long as thirty minutes. After such a longer or shorter period of quiescence, the peripheral melanophores suddenly expand slightly and contract again almost immediately. After a brief interval of inactivity, a second expansion wave appears at the periphery, but this time the melanophores lying nearer the centre of the scale, which had previously remained contracted, show a slight expansion and those at the periphery expand further than the first time. In this way there is set up a rhythmic expansion and contraction wave which, creeping towards the centre of the scale, gradually comes to include all the melanophores. In the course of an hour after being returned to sodium chloride, all the melanophores of a scale are slowly pulsating. This pulsation may continue at ordinary temperatures for as long as four or five hours, the expansion gradually becoming less complete and frequent and eventually ceasing, the melanophores remaining contracted. In cases where the immersion in BaCl_2 has lasted only about five minutes, the pulsations appear upon being returned to NaCl , but

⁶ In scale preparations of *Fundulus*, the small dermal blood vessels always contain a few corpuscles. When a scale is immersed in a KCl solution these corpuscles begin to move within the vessels and this movement continued approximately as long as the melanophores are contracting. On being returned to NaCl , the melanophores expand and the corpuscles return to their original position or pass beyond it. This movement of the corpuscles may be due to a contraction and relaxation of the walls of the larger blood-vessels.

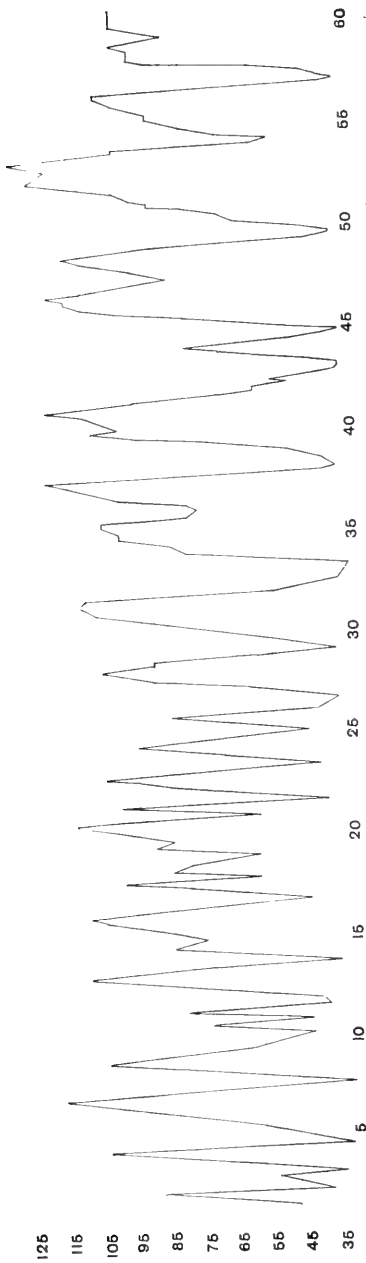


Fig. 2. A typical case of rhythmic pulsation in the melanophore after treating with BaCl_2 . The curve represents changes in the diameter of a single melanophore in the course of an hour. The maximum diameter of this cell was measured by means of an ocular micrometer at various times. These diameters are plotted against the time; 33 divisions of the micrometer represents complete contraction and 130, extreme expansion. The treatment of this cell was as follows:

Experiment 8, 15, 20. June 21, 1915.
7.43 a.m. Scales from an 8.5 cm. female *F. heteroclitus* were immersed in 0.1 N NaCl .

8.14 a.m. 3 scales to 0.1 N BaCl_2 .

8.27 a.m. One scale returned to 0.1 N NaCl ; temperature 19.4°C .

9.14, 15. First observation of rhythmic contractions.

the contractions between expansions are incomplete, the reduction being in this case in the contractions. The disappearance of the pulsations leaves the melanophores expanded instead of contracted. We may therefore say that the melanophores may recover after having been contracted in BaCl_2 , provided the time of immersion in the BaCl_2 has not been more than about five minutes (fig. 2).

Automatic pulsations or rhythmic contractions occur not uncommonly in several types of smooth muscle. Among the lower vertebrates, for example, Schultz (l. c.) has described this phenomenon in ring preparations of the stomach of the frog and Stiles ('01) has studied it in similar preparations of the frog's oesophagus. An examination of their figures shows that the rapidity and frequency of the contractions is not unlike those occurring in the melanophore after having been treated with BaCl_2 and returned to NaCl .

B. *Organic Substances.* Krukenberg ('80) carried out a series of experiments upon the reactions of the chromatophores of *Eledona*, a cephalopod, to organic substances. He found that 5 per cent ethyl alcohol, ether and chloroform (strength not stated in either case) produced a contraction of the radial smooth muscles i.e. an expansion of the chromatophores. When pieces of the skin were washed or returned to sea-water, the chromatophores contracted and regained their irritability. Klemensiewicz ('78) observed a lightening of the skin in *Loligo* and *Eledona* when subjected to vapors of amyl nitrite. The chromatophores in isolated pieces of skin showed the same reaction. Amyl nitrite is a familiar vaso-dilator. The relaxation of the radial muscles in the chromatophores of cephalopods therefore suggests that this may be a typical response for physiologically widely differing varieties of smooth muscle.

I have exposed the melanophores of *Fundulus* to alcohol, ether and chloroform vapors. The scales bearing these melanophores were placed upon glass slides and just covered with Ringer's solution. The slides were then placed over Syracuse glasses containing the organic fluid to be tested and both were covered with small, ground-edged, bell jars and sealed with vaseline.

Under these circumstances the melanophores always contracted.⁷ Control cells under identical sealed jars, remained expanded.

I have also exposed contracted melanophores of *Fundulus* in a contracting mixture of 4 parts of 0.1 N NaCl + 1 part 0.1 N KCl to the vapors of amyl nitrite, using the same technique as in the trials with ether and alcohol. All these melanophores expanded promptly whereas control cells in the same contracting mixture, under a second sealed bell-jar, showed no change.

Weak solutions of atropine or atropine sulphate (0.00025 M in 0.1 N NaCl) produce a prompt expansion of the melanophores in *Fundulus*. The dilating action of this alkaloid upon the pupil is a familiar physiological phenomenon.

Lieben ('06) found that in *R. temporaria*, subcutaneous injections of adrenalin produced a complete contraction of the melanophores in 15 to 20 minutes. The local application of adrenalin upon the swimming web also produced a contraction. These contractions Lieben believed to be independent of the constricting action of the adrenalin upon the blood vessels. When the melanophores of *Fundulus* are immersed in solutions of adrenalin hydrochloride of different concentrations⁸ they invariably contract. Even at a dilution of one part to one million (in 0.1 N NaCl) a contraction appears, though not always in all the melanophores of a single scale.

From the foregoing observations it appears that certain organic substances which typically produce contractions in smooth muscle, cause a contraction in the melanophores of *Fundulus*. Reciprocally, certain other organic substances which produce an expansion or relaxation in isolated smooth muscle, produce an expansion in the melanophores of *Fundulus*.

⁷The contraction depends, however, upon the amount of organic fluid in the Syracuse dish. Under the conditions of these experiments, 3 to 5 drops produced a contraction. Larger amounts inhibited the contraction and produced a narcosis. This became evident when the cells were removed from the bell jars, for a transient contraction appeared, the effect of the dilute stimulus.

⁸A Parke-Davis preparation of adrenalin hydrochloride 1 : 1000 in physiological salt solution was used as the starting point in these experiments. This was diluted with 0.1 N NaCl to the required concentration.

AN ANALYSIS OF THE CONTRACTION IN THE MELANOPHORE

A structural and functional parallelism between smooth muscle and melanophores can scarcely be considered complete without a comparison of the contractions in the two cases. At first sight they have little in common. Indeed the exact mechanical nature of the contraction in the melanophores is still a disputed question. Certain facts are established beyond any reasonable doubt viz: 1) the proximal and distal pigment migrations invariably occur along fixed paths (Kahn u. Lieben '07, Spaeth '13 b); 2) in cases where living cells have been observed under high magnifications it has appeared that the pigment granules are not merely carried along passively by a streaming or flowing of an extremely liquid sort of protoplasm, but that they exhibit active, independent movements within the cell processes (Ballowitz '13 a and b, Degner '12). Exactly what occurs after the pigment granules have reached the centre of the cell, has not been determined as yet with certainty. Either 1) the cell processes remain in situ or 2) the more fluid protoplasm follows the migrating pigment granules and eventually the entire cell becomes rounded up as in a contracted amoeba. In the latter event it is difficult to imagine how the protoplasmic processes find their way back again to the original contour of the expanded cell. Hooker ('12 and '14) has explained away this difficulty by assuming, from histological preparations, the existence of lymph spaces into which the pseudopodia of the amoeboid melanophore creep or flow. Hooker believes these lymph spaces to be fixed and the melanophore to be a typical amoeboid cell. There are serious objections to this view. 1) Hooker's evidence is adduced from fixed preparations and he has been unable to corroborate his observations upon living adult cells; 2) in the many hundreds of living melanophores that I have observed under high magnifications and the most favorable optical conditions (reflected and transmitted light, dark-field illumination, etc.) I have never been able to detect the secondary migration of the fluid protoplasm, though I have repeatedly watched the same cell in a series of expansions and contractions with this as the sole object; 3) the his-

tological preparations of Ballowitz ('93) show nerve terminations on the processes of the melanophores which could not have any physiological significance if the entire cell were withdrawn from these processes; 4) finally I have repeatedly corroborated in *Fundulus*, the observation of Ballowitz ('13) concerning the position of the nucleus following a proximal migration of the pigment granules. Not infrequently in an expanded melanophore, the nucleus may be seen to lie far out from the centre in one of the processes. If such a cell be contracted, all the pigment granules accumulate at the centre, but the nucleus remains in its original position in the process. Under favorable conditions it may actually be seen in the contracted cell, lying in the process, with a considerable area of pigment-free cytoplasm between it and the central mass of melanin granules. In any case, upon reexpansion, the nucleus is found practically to have retained its original position. It is difficult to correlate these facts with Hooker's conception.

At present it is, then, impossible to say that the melanophores are certainly not amoeboid cells in the mature condition, though the weight of the evidence appears to be against such a view.

The question now arises as to what a proximal and distal migration of pigment granules within a stellate cell can have in common with a 'contraction' in smooth muscle. A physical-chemical analysis of the melanophore may serve to clarify the comparison.

Considered as a physical-chemical system the melanophore consists essentially of a colloidal suspension of melanin granules (the disperse phase) in a dispersion medium which is itself an exceedingly fluid sort of protoplasm i.e., an emulsoid sol. 'Contraction' of the melanophore consists of an aggregation of the disperse phase of melanin granules and in 'expansion' there is an increased dispersion of the melanin granules. In other words, when the melanophores are stimulated to contract, we observe the first step in a reversible aggregation or coagulation process, i.e., the aggregation of the melanin granules. Is it possible to consider this phenomenon a reversible coagulation such as occurs commonly in emulsoids? The size of the melanin granules is relatively so large (ca. 0.0004 mm.) that we should rather expect

an irreversible coagulation as we find among suspensions or suspension colloids. The presence of the fluid protoplasm in which the granules are suspended must here be taken into consideration. It is a well known fact (Bechhold '04) that the disperse phase of a suspension colloid or suspension, adsorbs the particles of the disperse phase of an emulsoid when the two are in 'solution' together. This phenomenon has been termed a 'protection' of the coarser phase; the adsorbed emulsoid is the protecting colloid ('Schutzkolloid' of Bechhold). Under such circumstances, the suspension colloid assumes the character of a true emulsoid, i.e., it is protected against the irreversible coagulative effects of electrolytes, heat, etc. Suspension colloids or suspensions, in the presence of emulsoids, thus become far more stable and plastic systems (Neisser und Friedemann '04). The existence of just such a physical-chemical system within the melanophore is a perfectly demonstrable fact. Its reversibility is equally obvious. Thus far, then, we have but analyzed the physical-chemical conditions within the cell based upon the observed facts.

In the case of an increased dispersion in a two-phase system, there is an increased intimacy of relation between disperse phase and solvent; the system approaches a true solution and we speak of an increased solubility of the disperse phase. Reciprocally, in an aggregation or coagulation process, we have a separation of disperse phase and solvent. There is an accumulation of evidence at hand showing "that during the contraction of smooth muscle there is an exchange of fluid between the cells of the tissue and their surroundings" (Meigs '12, p. 543). It is difficult to imagine how this setting free of fluid can occur in a colloidal system such as a smooth muscle cell, except in connection with a reversible coagulation or aggregation process. In other words, evidence for an exchange of fluid during contraction in both striated and smooth muscle must, at the same time, be considered evidence for a reversible aggregation or coagulation process in the cell colloids. The displacement of fluid is as necessary an accompaniment of the act of contraction as is the coalescence of colloidal particles.⁹

⁹ A discussion of the relative merits of the 'swelling' and 'surface-tension' hypotheses of muscle contraction would be irrelevant (Lillie '12).

In the case of the melanophores there is no direct evidence at hand for a loss or exchange of fluid during contraction. There is, however, a visible and reversible colloidal aggregation of melanin granules following a variety of physiological stimuli, all of which elicit contractions in smooth muscle. Similarly, a number of physiological stimuli that produce a relaxation in smooth muscle bring about a dispersion of the pigment granules in the melanophore. Now, from the evidence cited above, we are forced to assume that, during the contraction of smooth muscle there is an invisible aggregation or heaping up of colloidal particles which becomes reversed in relaxation, i.e., when the muscle relaxes, there is an increased dispersion of the invisible colloidal particles. *The aggregation of melanin granules within the melanophore must therefore be considered a visible expression of the colloidal phenomenon that occurs, upon stimulation, in the micro-homogeneous colloidal content of a smooth muscle cell.*

GENERAL APPLICATION OF THE CONCLUSION

Although much of the evidence in the preceding sections has been obtained from experiments with a single species of teleost, *Fundulus*, there is no valid reason for doubting that the mechanism of the responses in the chromatophores of crustaceans and the melanophores of fish, amphibia and reptiles, is identical.¹⁹ In all these cases the color-changes are brought about by an aggregation and dispersion of pigment granules. Chun (l. c.) has shown that in cephalopods the chromatophores develop from a single smooth muscle cell. We may therefore say that it seems highly probable that color changes in crustaceans and cephalopods, as well as in the three groups of lower vertebrates, are brought about by the physiological responses of specialized smooth muscle cells.

The physiological modification in the case of the melanophore may be compared with that of the electric organ in fish where a striated muscle cell becomes so far modified that the normal mo-

¹⁹ The term 'chromatophores' can not be applied to the case of the lower vertebrates until more is known of the physiology of the xanthophores.

tor function disappears completely and the action current, which is normally of relative insignificance, becomes a physiological end in itself and a formidable means of protection. Similarly in the melanophore, the motor function of the smooth muscle cell is lost and there is developed a modified motility, a migration of pigment granules, which may again serve as a means of protection.

SUMMARY

1) Morphological, embryological and physiological evidence is advanced to prove that the melanophores of fish, amphibia and reptiles are not connective tissue cells as has been tacitly assumed heretofore, but functionally modified smooth muscle cells.

2) In the contraction of the melanophore there is an aggregation of melanin granules which is to be considered the visible counterpart of an aggregation of colloidal particles that occurs during the contraction in smooth, and possibly striated muscle.

3) It seems highly probable that color-changes in crustaceans and cephalopods, as well as in the three groups of lower vertebrates, are brought about by the physiological responses of specialized smooth muscle cells.

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THE RELATIVE EFFICIENCY OF VARIOUS PARTS OF THE SPECTRUM FOR THE HELIOTROPIC REAC- TIONS OF ANIMALS AND PLANTS

SECOND COMMUNICATION¹

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SIX FIGURES

I

In 1869 Paul Bert made experiments on the effect of different parts of the spectrum on *Daphnia* to find out whether the vision of these crustaceans is comparable to that of man. He found that the animals "accouraient beaucoup plus rapidement au jaune ou au vert qu' à toute autre couleur," and concluded from this that the green and yellow rays which appear comparatively bright to us appear also brightest to these animals.²

At that time biologists were generally under the influence of the anthropomorphic viewpoint and did not hesitate to interpret the reactions of animals on the basis of human analogies; although it must have occurred to as clear a thinker as Bert that there is no *a priori* reason for assuming that human beings when put into a spectrum must gather in the green or yellow; and experiments in this direction were lacking. This anthropomorphic viewpoint appeared still more plainly in the writings of Graber,³ who experimented with animals which were kept in a box illuminated from above, one-half of which was covered with red, the other with blue-glass. He found that animals which prefer

¹ First Communication, Jour. Exp. Zoöl., 1915, 19, 23. See also Loeb and Wasteneys, Proc. Nat. Acad. Sc., 1915, 1, 44; Science, 1915, 41, 328.

² Paul Bert. Arch. de Physiol., 1869, 2, 547.

³ Graber. Grundlinien zur Erforschung des Helligkeits- und Farbensinnes der Tiere. Prag, 1884.

darkness to light gather under the red; and animals which prefer light to darkness gather under the blue glass. This led him to enunciate the law that animals which are 'fond' of light are also 'fond' of the blue; and animals which are 'fond' of the darkness are also 'fond' of the red. We see again the tacit assumption that animals which collect under blue glass do so because they are 'fond' of this type of light, while animals which collect under the red light do so because they are 'fond' of this type of light.

The field of animal reactions received a different interpretation by Loeb,⁴ who showed that these results can be explained on a purely objective basis without our ascribing to lower organisms sensations the existence of which we can neither prove nor disprove. Loeb showed that the phenomena observed by Bert, Graber, and others can be explained on the assumption that the light automatically orients the animals or determines the direction in which they move, there being two classes of animals, one class being automatically compelled to move to the source of light, the other being compelled to move in the reverse direction; and he pointed out that this phenomenon is the same as the heliotropic reaction in plants, the stem of plants bending to the source of light, the roots bending away from it; or the swarm-spores of algae moving to or from the light. Accordingly he designated the animals going to the light as positively heliotropic, those going away from the light as negatively heliotropic.

As this bending effect in the plant is a purely automatic orientation of the plant, brought about through the influence of the light, so in the animals we are, according to Loeb's theory, dealing only with an orienting effect of the light for the explanation of which merely physicochemical conditions are adequate; without our being compelled to introduce hypothetical sensations as a necessary link in the mechanism. This purely mechanistic conception of the motions of animals to or from the light has recently received a new support by the invention of heliotropic machines by Mr. John Hays Hammond, Jr., in which the two

⁴Loeb, J. *Der Heliotropismus der Tiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen.* Würzburg, 1890. *Sitzungsber. d. Würzburger physikal.-med. Gesellsch.*, Jan. 1888.

retinas are replaced by selenium wire, these machines following a lantern in the dark in the same way as a positively heliotropic animal.

It was easy to interpret the phenomena found by Graber from this heliotropic viewpoint. The botanists had long ago shown that positively heliotropic plants bend readily to the light when behind a blue screen, while they do not do so or only very slowly when behind a red screen; from which they concluded that the rays going through a blue screen had a higher heliotropic efficiency than the rays passing through a red screen.

If the animals were, as Loeb stated, merely positively or negatively heliotropic the light going through blue glass should act like more intense light than that going through red glass, and hence negatively heliotropic animals should gather in the red, positively heliotropic animals in the blue. He could show by a series of experiments that this statement was correct. In this way, purely objective methods and explanations were given for the arbitrary assumption of Graber and the other anthropomorphic biologists that animals moved to or from the light because they were 'fond' of light.

The advantage of this change in viewpoint lies in the fact that it opened this field to the methods of exact experiments and measurements without which no progress is possible; while the attempt to explain reactions by a hypothetical 'fondness' of animals for light or by hypothetical light sensations barred the way to the exact type of investigation.

Recently, however, the old anthropomorphic viewpoint has been resumed by the ophthalmologist Hess,⁵ who has tried to show that all the animals from fish downward suffer from a visual deficiency, namely total color blindness. As a criterion for the presence or absence of color sensations Hess uses (very arbitrarily in our opinion) the heliotropic reactions of animals. Thus in 1909 he confirmed Bert's observation that *Daphnia* collect in the yellow-green part of the spectrum but gave it a different interpretation. By calling attention to the fact that the yellowish-green, which is heliotropically most efficient for *Daph-*

⁵ Hess. Gesichtssinn. Handb. d. vergleich. Physiol., 1913, 4, 555.

nia, appears brightest to the human inflicted with total color blindness, he thinks he has proved that *Daphnia* is also totally color blind.

The number of objections to this kind of reasoning is considerable.⁶

Nobody has yet proved that the heliotropic reactions of animals are determined or even accompanied by any sensations of brightness and it is difficult to see how such a proof can ever be furnished. It is plainly unwarranted to assume that every motion of animals induced by light is accompanied by or is the expression of sensations of brightness or of color. The excised iris of the shark (and of other animals) contracts under the influence of illumination and Magnus has shown that the yellowish-green part of the spectrum is most efficient in this case. It would be arbitrary, to say the least, to state that the excised iris has sensations of brightness and that these sensations make it contract; and yet it is difficult to see why such an assumption should be more arbitrary than a similar assumption in the case of the flagellate *Chlamydomonas* or the heliotropic larvae of *Balanus*. One wonders also whether we are supposed to assume that Hammond's heliotropic machines are guided by sensations of brightness or of color.

The assumption of Hess might be given some consideration if it could be shown that totally color blind human beings are positively heliotropic, i. e., are irresistibly drawn to the source of light; but nobody has ever heard of such a case. The human being is the only one about whose sensations we have definite knowledge and as long as we are unable to prove for the human a connection between positive heliotropism and the sensations of brightness we have no right to take such a connection for granted in the lower animals.

One wonders also what interpretation is to be put on other tropisms, such as galvanotropism or geotropism, if we accept the validity of Hess's viewpoint, since it is only logical to treat all the

⁶ An excellent criticism of Hess's ideas and experiments has been given by W. F. Ewald, *Arch. f. Entwicklgsmech.*, 1915, 37, 581. We are using some of his arguments in this paper.

tropisms from the same general viewpoint. What sensations are aroused in a *Paramecium* which is forced to swim to the cathode under the influence of the galvanic current, or in a *Palaemonetes* which is forced to swim or walk to the anode?

Since Hess starts with an arbitrary assumption, namely that the heliotropism of lower animals is due to their sensations of brightness and that they are totally color blind, it is not unexpected to see him come into conflict with facts in more than one direction. v. Frisch⁷ has shown by experiments which appear to us conclusive that bees (which are also positively heliotropic and which according to Hess are totally color blind) can be trained to go to yellow or blue cardboards distributed among similar cardboards of different shades of gray; while they can not be trained to go to definite shades of gray under similar conditions. Even in *Daphnia* v. Frisch and Kupelwieser,⁸ and Ewald⁹ have been able to demonstrate selective effects of wave lengths different from those found in the totally color blind human.

A second conflict between Hess's view and reality is due to the fact that the most efficient part of the visible spectrum is not the same for all heliotropic organisms. It is known through Blaauw's experiments that the heliotropic curvatures of the seedling of oats are produced most rapidly in the blue part of the carbon arc spectrum. This should force Hess either to the conclusion that the seedlings of oats do not suffer from total color blindness, since the most efficient part of the spectrum for the totally color blind is in the yellowish-green; or to the assumption that only plants are heliotropic, but that animals which show the same reactions to light are not heliotropic. Hess chooses the second alternative by stating that plants are heliotropic, while animals are 'lamprotropic' ($\lambda\alpha\mu\pi\rho\acute{o}s$ = (bright)),¹⁰ i.e., in plants the heliotropic curvature occurs purely automatically, while animals bend or move to the source of light because it is 'bright.' It

⁷ v. Frisch. *Der Farbensinn und Formensinn der Biene*, Zool. Jahrb., 1914, 35, 1, Abt. f. allg. Zool. u. Physiol.

⁸ v. Frisch and Kupelwieser. *Biol. Centralbl.*, 1913, 33, 517.

⁹ Ewald. *Ztschr. f. Psychol. u. Physiol. d. Sinnesorg.*, 1914, 48, Abt. 2, 285.

¹⁰ Hess, loc. cit., pp. 708 and 709.

would be difficult to invent a nicer example of reasoning in a circle; since Hess's assumption that animals have the sensation of brightness is based upon the fact that they move to the light.

Yet we will try to follow Hess even into this circle and select a case already mentioned in a previous note¹¹ and to which we shall return in this paper, namely the case of two green flagellates, *Euglena viridis* and *Chlamydomonas pisiformis*, which are strongly heliotropic, but, being unicellular organisms, of course have no eyes. For *Chlamydomonas* the place of greatest efficiency in the spectrum is in the region of yellowish-green, for *Euglena* it is in the blue. If we follow Hess we must logically conclude from this that *Chlamydomonas* suffers from total color blindness (although it has no eyes), that it is not heliotropic but 'lamprotropic,' and that it is an animal; while its cousin *Euglena* has either a highly developed color sense or is heliotropic and is a plant.

Hess¹² thinks it is inconsistent for Loeb to deny the justification of the assumption that all heliotropic animals are totally color blind and at the same time to state that phenomena of heliotropism are identical in animals and plants. Hess overlooks the fact that the two statements rest on an entirely different basis. The statement that positively heliotropic animals go to the light because they are totally color blind is as we have seen not a fact but an unnecessary and arbitrary assumption which is in conflict with the facts and not even justifiable on the basis of mere analogy, since totally color blind humans are not positively heliotropic. The fact that the region which is brightest to the totally color blind human is at the same time most efficient in certain heliotropic animals admits or demands, as we shall see, an entirely different interpretation.

On the other hand, the statement that heliotropic reactions in animals and plants are identical is merely the expression of the actual observations. Thus Loeb has been able to show that sessile heliotropic animals react to one-sided illumination just like sessile plants, namely by bending towards the source of

¹¹ *Science*, 1915, 41, 328.

¹² *Loc. cit.*, p. 709.

light until the axis of symmetry of their photosensitive organs goes through the source of light (provided only one source of light is given); while movable plant organs, e.g., the swarmspores of algae move to or from the source of light and collect on the side of the light (or on the opposite side) just as do motile heliotropic animals. For the heliotropic reactions of both animals and plants the validity of the law of Bunsen and Roscoe has been proved¹³ and the sense of heliotropic reactions in both groups can be reversed by similar means.¹⁴ It would be artificial to state that because the ones are termed animals and the others plants the identical phenomena in both must be different. Such a view might have been considered at the time of Linné, but today we know that the mechanism of life phenomena in animals and plants is essentially the same. While modern biology, especially since Claude Bernard and Hoppe-Seyler, has tried to establish the essential identity of life phenomena in plants and animals, Hess apparently expects biologists to overlook the progress made in biology and return to the Linnéan viewpoint. In order to maintain his artificial barrier between animals and plants he insists that the wave length which is most efficient in the heliotropic reactions in plants is different from the one most efficient in animals. But even if this were a fact, it would not justify his assumption, since the theory of heliotropism only states that organisms are automatically oriented by the light so that symmetrical elements of their photosensitive surface are struck at the same angle by the light (or that symmetrical elements receive an equal amount of illumination during a properly chosen unit of time). Whether in one case the yellowish-green, in another the blue light is more efficient is secondary.

As a matter of fact, there are heliotropic animals for which the blue rays are as efficient as they are for plants; and there are unicellular organisms, for which the optimum lies in different parts of the spectrum.

¹³ Loeb and Ewald. *Centralbl. f. Physiol.*, 1914, 27, 1165; Ewald, *Ztschr. f. Psychol. u. Physiol. d. Sinnesorg.*, 1914, 48, Abt. 2, 285.

¹⁴ Loeb. *Arch. f. d. ges. Physiol.*, 1906, 115, 564.

From the viewpoint of objective science we accept the fact that in some heliotropic organisms the place of highest efficiency is in that region of the spectrum which for the totally color blind is the brightest, but on this we put a different interpretation, namely the following. The sensations of brightness in the totally color blind human are determined by the rapidity with which visual purple is bleached by the light. The region in the yellowish-green in the carbon arc spectrum appears brightest to the totally color blind human because this region $\lambda = 526 \mu\mu$ has according to Trendelenburg the greatest bleaching effect. Assuming that heliotropic reactions are also due to a photochemical effect, the fact that in certain organisms the region not far from $\lambda = 526 \mu\mu$ is the most efficient in calling forth heliotropism means simply that the photosensitive substance responsible for the heliotropic reaction in these organisms has one peculiarity in common with the visual purple, namely that it is also most sensitive to a region not too remote from $\lambda = 526 \mu\mu$; the two substances may possibly be identical, but this would require a definite proof. The fact that the optimal effect for other organisms lies in the region of blue would indicate that the photosensitive substance in these animals is in all probability different from visual purple. If the effect of light in causing heliotropic reactions were other than chemical we still should be compelled to find a physicochemical and not a psychological explanation for the different heliotropic efficiency of different wave lengths.

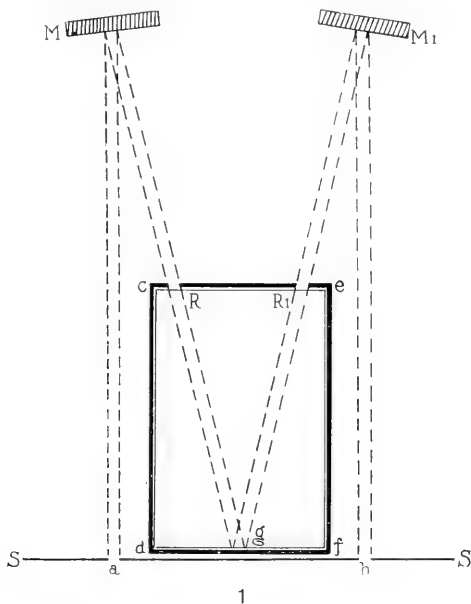
The question to which we intend to confine ourselves in this paper is a very simple one, namely: Is it true that a sharp line of demarcation exists between animals and plants in that sense that for the heliotropic reactions of plants the blue is most effective, while for the heliotropic reactions of all animals a region in the yellowish-green is the most efficient, as Hess claims? In this paper we shall deal with motile organisms, namely first the two unicellular green organisms *Euglena* and *Chlamydomonas* and the larvae of two animal forms, of the annelid *Arenicola* and of the crustacean *Balanus eburneus*.

II. METHODS

When we wish to determine where the most efficient spot of the spectrum for freely moving animals lies, we must realize that in order to get reliable results we must work with organisms which are both very small and very sensitive to light. The organisms must be small so that a large number can be crowded into a narrow region of the spectrum; if this condition is not fulfilled it is extremely difficult if not impossible to make statements concerning the relative efficiency of the different parts of the spectrum which are of sufficient accuracy. Thus attempts to determine the most efficient spot for the heliotropic efficiency of the spectrum for young fish or larger insects can only yield crude approximations. The second condition is that the animals must be very sensitive to light; since Loeb has shown in former papers that only in the case of extreme sensitiveness will the animals go directly to the source of light, while if the sensitiveness is small the animals may go in very irregular paths although the sum total of the motions towards the source of light will prevail. It would be impossible to get a definite result with animals of this kind. Thus experiments on the relative efficiency of different parts of the spectrum with young fish or other animals which are only moderately sensitive are very unreliable.

When we are dealing with positively heliotropic animals distributed in an oblong trough exposed to a carbon arc spectrum the animals will move towards the source of light independently of the nature of the rays by which they are struck; provided intensity and frequency of the waves are above the threshold of heliotropic efficiency. If the animals are evenly distributed in the trough at the beginning of the experiment they will all move towards the source of light and the result should be that at the end of the experiment the animals should all gather equally at the front wall of the trough and their density should be the same on this front wall in the violet-blue and green provided that the rays are sufficiently effective. On this basis it should be difficult to tell whether the blue or the green is more efficient. Since, however, some scattering of light occurs from the surface of the

animals, some blue light will also reach the animals in the green and vice versa. In this way a comparatively denser gathering of animals may occur in that part of the spectrum which is more effective. It is obvious, however, that this method is not very exact since in an aquarium with animals the scattered light from one part of the spectrum can chiefly reach only those individuals which are not too far from this spot.



The second method consisted in the comparison of the relative efficiency of two narrow parts of the spectrum. It was described briefly in a former note.

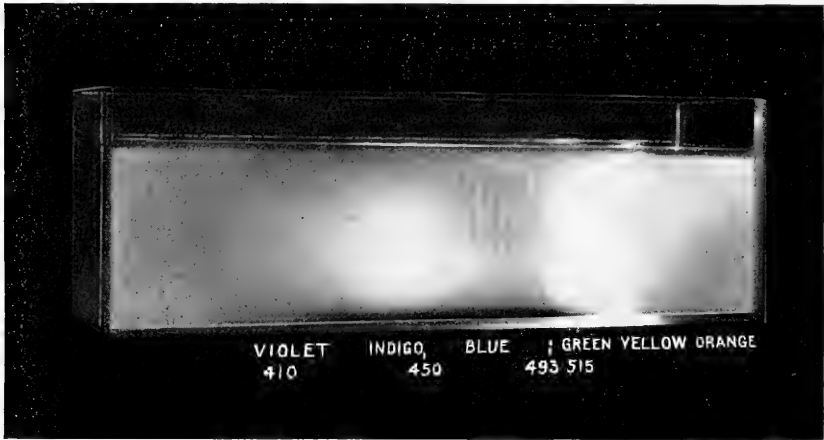
A carbon arc spectrum, about from 18 to 23 cm. wide, was thrown on a black screen SS (see fig. 1) with two slits a and b in the two different parts of the spectrum which were to be compared in regard to their heliotropic efficiency. The two beams of light passing through the slits are reflected by the two mirrors M and M_1 into the square glass trough in such a way as to strike the same region g of the back wall of the trough. The glass trough is surrounded by black paper except at R and R_1 , where the two beams of light enter from the mirrors. Before the ex-

periment begins, all the organisms are collected in the spot g with the aid of an incandescent lamp. As soon as the spectrum is turned on, these organisms are simultaneously exposed to two different beams of light which come from the two mirrors M and M_1 . When one type of light, e.g., that from M , is much more efficient than the other coming from M_1 , practically all the organisms are oriented by the light from M and move toward this mirror, collecting in the region R . When the relative efficiency of the two types of light is almost equal the organisms move in almost equal numbers to R and R_1 . By using as a standard of comparison the same region of the spectrum and successively altering the position of the other slit in the spectrum we were able to ascertain with accuracy the relative efficiency of the different parts of the spectrum for the two forms of organisms. When the two parts of the spectrum which are to be compared are very close to each other it is necessary to deflect the beams with the aid of deflecting prisms, before they reach the two mirrors.

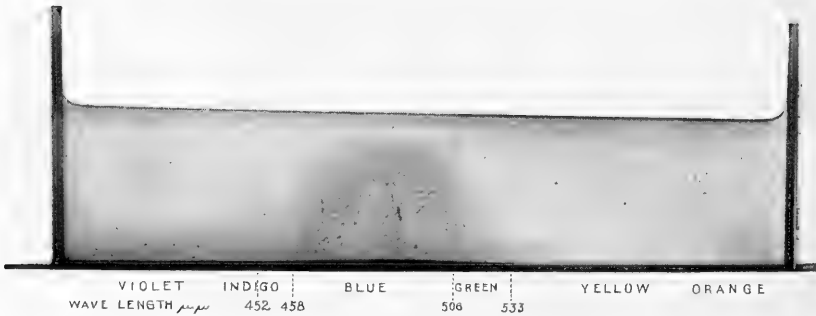
III. THE DISTRIBUTION OF ORGANISMS IN THE CARBON ARC SPECTRUM

The spectrum used was a carbon arc spectrum and its visible part had a width varying from 18 to 23 cm. in different experiments. We used very dense cultures of *Euglena viridis* and filled the trough with this greenish suspension of *Euglena*. After an exposure varying in length between 30 and 180 minutes the results were ascertained, and in some cases the trough was photographed. Figure 2 gives the photograph of the trough after 30 minutes' exposure. A very dense mass of *Euglena* was gathered at the bottom of the trough in front between violet ($410 \mu\mu$) and green ($515 \mu\mu$). In the photograph this mass is visible as a thick, dark, horizontal streak at the bottom. In addition some vertical streaks of organisms are visible in the blue and indigo. The reader will recognize how difficult it is to ascertain the most efficient wave length by this method. We can only say the blue is the most efficient light and the wave lengths $> 515 \mu\mu$ are practically without orienting effect.

Figure 3 gives a photograph of the distribution of the organisms in another experiment after 3 hours in the same spectrum. This longer exposure gives a slightly better result. The dense gathering at the bottom indicated by a horizontal streak is in the blue



2



3

between 458 and 506 $\mu\mu$. It ends at about 533 and becomes very faint at 452 $\mu\mu$. The vertical streaks on the wall, indicating the sticking of the organisms to the front wall, occur again in the blue. These experiments permit us to draw only the con-

clusion that the blue is more effective than the green, yellow, red, indigo, and violet; but they do not permit a more definite statement.

Engelmann states that he found a strong gathering of *Euglena* in the blue between 470 and 490 $\mu\mu$ in a spectrum.¹⁵

When we made similar experiments with *Chlamydomonas pisi-formis*, which is also a chlorophyll-bearing unicellular organism like *Euglena*, we noticed that the gathering went much farther towards the yellow ending at about $\lambda = 560$ or $570 \mu\mu$. The region of maximal gathering seemed to be at about $\lambda = 520 \mu\mu$. A similar result had previously been obtained with *Chlamydomonas* by Loeb and Maxwell.¹⁶

The method is still less definite with larger and rapidly moving animals, and yet it is mainly by such experiments that Hess tried to prove that the most efficient part of the spectrum for heliotropic animals is identical with that which appears brightest to the totally color blind.

IV. EXPERIMENTS WITH THE TWO-BEAMS METHOD

With the aid of two slits (fig. 1) two narrow strips of the spectrum were cut out. Their width was such that in the green part of the spectrum the difference in the wave length of the extreme rays that passed through was about $10 \mu\mu$. With the aid of prisms and mirrors these two parts of the spectrum were made to converge to one spot in the trough where the organisms had previously been collected. It was ascertained which of the two beams of light was more powerful.

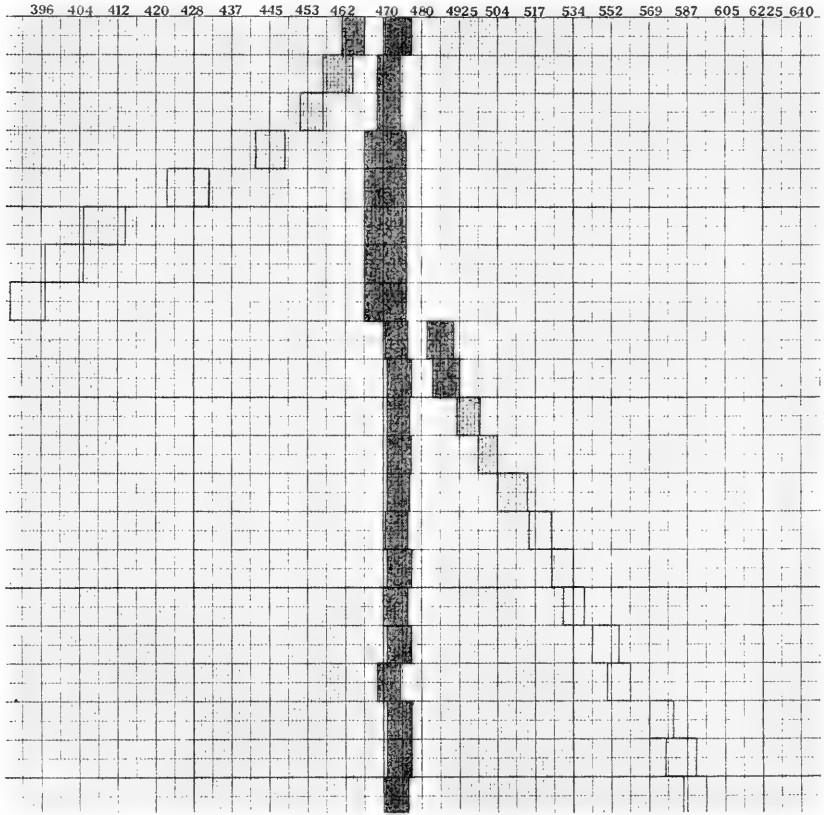
In detail the experiments were as follows. The trough was surrounded with black cardboard in which there was one opening at that spot where it was intended the animals should collect. Then an incandescent lamp was turned on in front of this opening which caused all the organisms to collect at that spot of the trough. When this happened, the spectrum was turned on and the incandescent lamp turned off and the animals were exposed to the two beams of light *a* and *b* selected for com-

¹⁵ Engelmann. Arch. f. d. ges. Physiol., 1882, 29, 387.

¹⁶ Loeb and Maxwell. Univ. Cal. Pub., 1910, Physiol., 3, 195.

parison, after another black cardboard with openings at R and R_1 , to let the two beams of light pass, had been put over the box, and the first cardboard enclosing the animals had been removed. The organisms collected at g were now under the influence of

Euglena viridis
Wave lengths in $\mu\mu$



4

these two beams coming from different directions. As stated before, they moved towards R and R_1 according to the selective efficiency of the two beams of light. The readings were taken after from 15 minutes to 3 hours or more to make sure that the results were permanent. Before a new experiment was made

the organisms were all scattered equally again in the trough. Fresh organisms were used every day.

As stated before, one of the two parts of the spectrum was the same in a group of experiments while the other changed in successive experiments throughout the spectrum. In figure 4 the results of 21 experiments with *Euglena* are plotted. The part of the spectrum which was stationary was situated at about 470 $\mu\mu$ which previous experiments had led us to believe was the most efficient wave length. The different degrees of blackness indicate the denseness of the gathering; the more animals gathered in one spot the darker the oblong representing the experiment. We notice that the oblongs at the region 470 $\mu\mu$ are with two exceptions much darker than all those at other wave lengths. These results indicate that the greatest efficiency is possessed by the rays between 460 and 490 $\mu\mu$ for *Euglena viridis*. The total of all experiments is represented in diagram II, figure 7, where the distribution of this efficiency through the carbon arc spectrum is plotted for this form. The greatest efficiency is indicated by the greatest blackness, the greater blackness indicating the denser gathering.

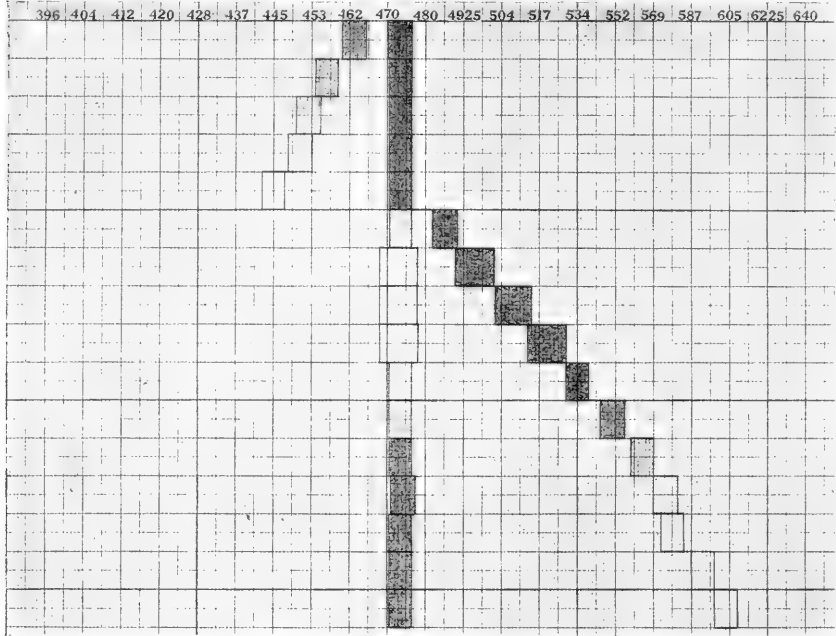
The results with *Chlamydomonas* were entirely different. In figure 5, the region between 470 and 480 $\mu\mu$ was again constant in each determination, while the region compared with this varied in each experiment. It is obvious that in contradistinction to the experiments on *Euglena* the region between 460 and 480 $\mu\mu$ was less efficient than the region from 490 to almost 560 $\mu\mu$.

We, therefore, started another series of determinations in which the region about 534 $\mu\mu$ was constant (fig. 6). We now found that this region was more efficient than any other region in the visible spectrum. The experiments show that the maximal efficiency lies for *Chlamydomonas*, approximately in that part of the spectrum which appears brightest to the totally color blind human.

In the same way long series of experiments were made with the newly hatched larvae of *Arenicola*, an annelid. The experiments in this form suffer from the difficulty that the larvae have a tendency to stick to the glass walls of the trough. We found

that if we keep them in the dark before using them they are more sensitive to light and less liable to stick so soon, and such animals gave clearer results. The most efficient part of the spectrum was situated in the bluish-green in the region of about $\lambda = 495 \mu\mu$. And finally, experiments with this method on the larvae of *Balanus eburneus* yielded the result that the most efficient part of the spectrum lies between $\lambda = 560$ and $\lambda = 578 \mu\mu$.¹⁷

Chlamydomonas pisiformis. Dill.
Wave lengths in $\mu\mu$



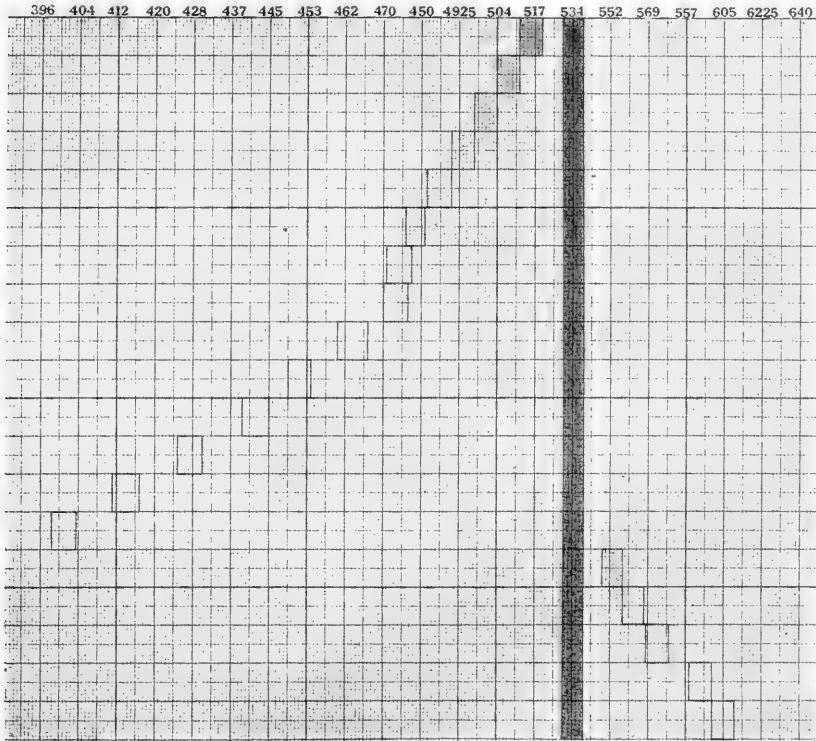
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The relative efficiency of the different parts of the carbon arc spectrum for different organisms is plotted in figure 7. The upper line gives the wave length and below are found the relative efficiency of the various wave lengths as revealed by the two-beams method for *Eudendrium ramosum*, *Euglena viridis*, larvae

¹⁷ These results agree with previous observations by Loeb and Maxwell, Univ. Cal. Pub., 1910, *Physiol.*, 3, 195.

of *Arenicola*, *Chlamydomonas pisiformis*, and larvae of *Balanus*; the greater darkness indicating the greater efficiency. The two most striking facts to us are, first, that there are animals for which the most efficient part of the spectrum is in the blue,

Chlamydomonas pisiformis. Dill.
Wave lengths in $\mu\mu$

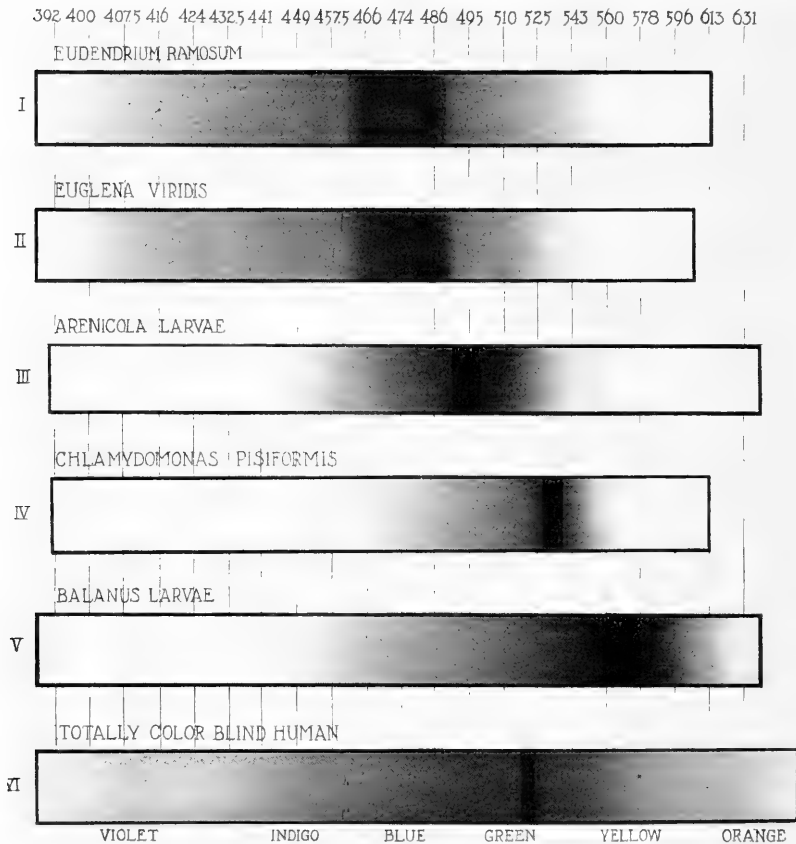


namely *Eudendrium* and the larvae of *Arenicola*; and that among the flagellates, *Chlamydomonas* is most sensitive to yellowish-green, while the closely related *Euglena* is most sensitive to the blue.

The second striking fact is that the place of greatest efficiency does not seem absolutely identical in the organisms of the same

group. In the line VI of figure 7 is represented the relative brightness of the various parts of the spectrum for the totally color blind human after Helmholtz. A comparison with the relative efficiency of the various wave lengths for Balanus larvae and

Wave lengths in $\mu\mu$



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Chlamydomonas (IV and V, fig. 7) shows that the maximal efficiency is not entirely identical in all three cases. We are not prepared to state whether this is entirely due to the inadequacy of the methods.

DISCUSSION AND SUMMARY OF RESULTS

1. As stated in the previous papers, the validity of the Bunsen-Roscoe law for the heliotropic reactions of some (and possibly many or all) organisms suggests that these reactions are due to a chemical action of the light. There seem to exist two types of heliotropic substances (or elements), one with a maximum of sensitiveness in the yellow-green region, and the second with a maximum of sensitiveness in the blue.

2. It would be wrong to state that the one type of photosensitive substances is found exclusively in plants and the other exclusively in animals. As a matter of fact, our experiments have shown that the animals *Eudendrium ramosum* and (the larvae of) *Arenicola* are most sensitive to blue light, which is also most effi-

TABLE I

NAME OF ORGANISM	REGION OF GREATEST HELIO TROPIC EFFICIENCY
	$\mu\mu$
<i>Eudendrium ramosum</i>	460-480
<i>Euglena viridis</i>	460-490
Larvae of <i>Arenicola</i>	about 495
<i>Chlamydomonas pisiformis</i>	about 535
Larvae of <i>Balanus eburneus</i>	560-578

cient for the seedlings of the plant *Avena* (according to Blaauw); while the larvae of *Balanus*, *Daphnia*, and probably many other animals are most sensitive to the yellow-green or yellow part of the spectrum. Of the two green flagellates, *Euglena viridis* and *Chlamydomonas pisiformis*, the former is most sensitive to blue, the latter to greenish-yellow. The two groups of photosensitive substances (or elements) are, therefore, distributed independently of the boundaries between animals and plants. It is quite possible, however, that plants are more generally sensitive to the blue rays of the spectrum, while among animals those may prevail that are more sensitive to yellowish-green or yellow.

3. Table I states the wave lengths in the carbon arc spectrum for which the different organisms investigated by us are most sensitive.

Visual purple is bleached most rapidly by light of the wave length of about 530 $\mu\mu$ (according to Trendelenburg). Neither *Chlamydomonas* nor the larvae of *Balanus* show their maximal sensitiveness exactly at this point; whether the slight deviation is only due to the imperfections of the experimental method or due to the fact that the photosensitive substance is not identical with visual purple can not be decided on the basis of the material which is at present available.

We find likewise that those organisms which react best to the blue part of the spectrum do not all have their greatest sensitiveness in the same spot of the blue. Thus the seedlings of oats are most sensitive to a region of $\lambda = 466 \mu\mu$, the animal *Eudendrium* and the flagellate *Euglena* for a region near $\lambda = 460$ to $490 \mu\mu$, while the larvae of *Arenicola* are most sensitive to $\lambda = 495 \mu\mu$.

THE REACTIONS OF THE MELANOPHORES OF
AMBLYSTOMA LARVAE—THE SUPPOSED
INFLUENCE OF THE PINEAL ORGAN

HENRY LAURENS

Osborn Zoological Laboratory, Yale University

SIX FIGURES

INTRODUCTION

Although the literature dealing with the chromatophores is very voluminous, nevertheless there are still many points concerning which our knowledge is far from complete. One of these, and a fundamental one, because it lies at the foundation of our comprehension of the physiology of the pigment cells, is the relation between them and the nervous system. We know much about the reactions to various stimuli of the chromatophores of many animals but when we come to compare them it is found that they are so diverse that it is almost impossible to lay down any general rule which will cover all cases. In some animals light causes a contraction of the pigment cells, in others it has no noticeable effect, and in still others it produces expansion. Attempts have been made, of course, to explain these different results, and in the excellent review by Fuchs ('14) this is often done, with, it must be admitted, not always marked success. The particular case in which we are interested concerns the reactions to light and to darkness of the melanophores of *Amblystoma* larvae.

Babak ('10) obtained very interesting results regarding the melanophores of these larvae. He found that there was a difference between the reactions of the pigment cells of normal and blinded *Axolotl* (*A. mexicanum* Cope, *A. tigrinum* Laurenti) larvae to light and darkness. In diffuse light, according to Babak, the melanophores of normal seeing larvae contract, those of

blinded larvae expand. In darkness the melanophores of normal larvae expand, while those of blinded larvae contract. This opposite reaction of the melanophores of normal and blinded larvae he found, however, not to occur until the larvae had attained a certain stage of development, about 17.0 mm. long. Babak believes, (Laurens '15, p. 592) that before this period the retina has not acquired the pigment motor function which it later has, so that the melanophores simply respond to direct stimulation, which is therefore the same in both normal and blinded individuals. After this period, by means of the control which the eyes have gained through the central nervous system, the sense of the reaction of the melanophores is reversed, the effect of indirect stimulation through the eyes being opposite to that of direct. Babak's explanation of why there should be this difference between the reactions of normal and blinded larvae, or in other words, why the effect of indirect stimulation of the melanophores should be opposite to that of direct is briefly as follows (Laurens, '15, p. 623): the chromatophores of normal Axolotl larvae in both phases of their movement—expanding and contracting—are governed by the central nervous system, and this double innervation is conditioned upon the retinae which have opposite influences upon the nervous system according as to whether they are illuminated or darkened. The darkened retinae exert a positive influence on the chromatophores through the nervous system, just as the illuminated retinae do, but in the reverse direction. The destruction of the retinae has an entirely different result from that obtained by darkening them. In other words, the retinae in complete darkness are active and exert a positive influence which is directly opposite to that caused by illumination.

Babak, however, does not believe that these two opposite effects of the retinae upon the chromatophores are either of them inhibitory, but that they are two kinds of tonic influences. The impulse bringing about the expansion of the chromatophores originates in the darkened retinae, and is so strong that it overcomes the tendency of the darkened chromatophores to contract and brings about their expansion. On the other hand, the

impulse for the contraction of the chromatophores originates in the illuminated retinae and is in turn so strong that it overcomes the tendency of the illuminated chromatophores to expand and brings about their contraction.

The results which were obtained from a study of the reactions of the melanophores of larvae of *A. punctatum* and of *A. opacum* (Laurens '15) were such that this explanation of Babak's could not be applied to them. They threw no doubt, however, on the assumption that both phases of the movement of the melanophores are normally under the control of the nervous system by means of the eyes, although one of the influences of the retinae must be admitted to be inhibitory, and opposite in effect to an impulse which causes the pigment cell to contract.

The results of my work showed that the melanophores of normal and eyeless larvae react primarily in identically the same way, expanding in light and contracting in darkness, the only difference being that the reactions come about more quickly in the normal than in the eyeless larvae (p. 585 and table 2). Secondly, however, the melanophores of the normal larvae are found to be in the opposite conditions in both light and darkness to what they were in before, for after having been kept for from three to five days in light the melanophores are contracted, and after having been for five days or more in darkness, the melanophores are expanded. The melanophores of the eyeless larvae do not show these secondary reactions.

It was assumed (pp. 624-625), to explain these secondary reactions of the melanophores of normal seeing larvae, that, although the primary effects of indirect stimulation of the melanophores through the eyes were the same as those of direct stimulation, in the case of light the constant illumination or stimulation of the retinae had the result of causing impulses to be started, the end effects of which were opposite to those of direct stimulation and in this way the secondary contraction of the melanophores was brought about. These impulses were supposed to have their immediate cause in certain photo-chemical changes taking place in the retinae. In the case of darkness the same thing was supposed to take place, in that, due to the

long continued absence of light, chemical changes in the retinae started impulses which reaching the melanophores caused them to secondarily expand. Briefly, the seat of the causes of the secondary changes were assumed to be in the retinae, for which there was abundant experimental proof, just as Babak assumed that the reactions of the melanophores of the normal larvae were due to the influence of the eyes, which were opposite in effect to direct stimulation of the melanophores themselves.

Now Fuchs ('14, p. 1545) considers that Babak's explanation of the differences between the reactions of the melanophores of normal and blinded Axolotl larvae is unsatisfactory and seeks to explain it by advancing a theory based on the results of von Frisch's work on the minnow *Phoxinus* (von Frisch '11, p. 374). As his chief objection to Babak's explanation, he points out that the latter's contention that the expansion of the pigment cell is as much an active process as its contraction, and that therefore the condition of rest is one of medium pigment contraction, is untenable. For all that is known concerning the distribution of pigment in the chromatophore makes it necessary to regard the expanded condition as that of rest, while a condition of medium contraction must be considered as the result of a tonic condition of excitation, no matter where the tonus arises.

Fuchs therefore offers the following explanation of Babak's results, seeking in the first place to show why the melanophores of young Axolotl larvae expand in the light. Substances, he says, which are perhaps products of inner secretions, but which at any rate are the results of the ordinary processes of life, arise and cause the melanophores to contract. Now if the young larvae are removed from all possibility of external stimulation, then under the influence of these metabolic products the melanophores contract. This is the reason why the melanophores are contracted in darkness. But if a light stimulus, at this time, be allowed to act on the parietal organ, then through the stimulation of this organ an impulse is started which inhibits the endogenously produced contraction and the melanophores expand. Gradually, as the larvae grow older, the eyes develop and gain an influence (i.e., a pigment motor function) over the pigment cells.

The stimulation of the retinae (illumination) produces a contraction of the melanophores, and as the eyes continue to develop and finally gain the upper hand in the sense life of the animal, so the impulses started by illumination of the retinae become stronger and finally overcome entirely the inhibitory influence of the parietal organ, so that in light the melanophores are contracted. Now, if the influence of the eyes is removed, by blinding the larvae, then the parietal organ is again in complete control, and the melanophores therefore expand in the light.

The remainder of this theory of Fuchs is as interesting as what has gone before. He goes on to say, that whether all larvae have a functional parietal organ, and whether the functioning power decreases as the development of the animal proceeds—which he considers probable—can be learned only experimentally. If this is found to be true then, he believes, it will be easy to understand why in adult animals the eyes have no particular influence over the color changes. For when the eyes have overcome the opposite influence of the parietal organ then after the extinction of its influence the function of the eyes must naturally in this respect also be less, because their antagonist, the stimulation of which causes the pigment cells to expand, is lacking. In other words, Fuchs would claim that the pigment motor function of the eyes, by means of which contraction of the pigment cells is brought about, is developed to offset an opposite effect of the parietal organ, and after this has been overcome it disappears. Moreover in his theory, nothing is said concerning the direct stimulation of the pigment cells by light, and no indication is given as to just how the chromatophores are stimulated after the influence of the eyes as well as that of the parietal organ has been extinguished.

EXPERIMENTAL

The work, the results of which will be given in the succeeding pages, was undertaken to put to experimental test this theory of Fuchs. More particularly to see whether the parietal organ in young normal seeing larvae can be shown to exert any inhibiting influence upon the melanophores, which later disappears

after the pigment motor function of the eyes has developed, but which in eyeless larvae is still present, and remains so until the larvae reach a certain stage of development when, according to the letter of Fuchs' theory, it would be expected to decrease and finally disappear.

It should be mentioned, of course, that this theory of Fuchs was put forward to explain the results obtained by Babak concerning the reactions of the melanophores of the Axolotl larvae. As has been pointed out, these are different in certain points from those obtained by me with the larvae of *A. punctatum* and *opacum*. But the main fact with which Fuchs' explanation is concerned is the same, viz. that in larvae deprived of their eyes the melanophores expand when the larvae are illuminated. If his explanation, that this is so because of the stimulation of the parietal organ, by virtue of the fact that impulses going out from it inhibit an endogenously produced contraction of the melanophores, holds for larvae of the Axolotl, it must hold also for larvae of *A. punctatum* and *opacum*. The same applies to his belief that when the eyes are present their stimulation starts impulses which are opposite in effect to those sent out by the parietal organ and stronger, so that these are rendered of no avail.

To test this hypothesis of Fuchs several methods of experimentation were employed and these will be taken up in order. But before proceeding to describe and discuss their results it seems desirable that some idea be had concerning the development of the so-called parietal organ, and its position at this time and later. To do this it is hardly necessary to more than call attention to the accompanying figures. In figure 1, which is a view of the extreme anterior portion of a sagittal section of a 5.3 mm. larva of *A. punctatum* there is seen the beginnings of the development of the epiphysis and paraphysis. In figure 2, of a 6.6 mm. larva, development has proceeded only a little further. In figure 3, which is of an 8.0 mm. larva, the epiphysis and paraphysis are both seen as evaginations, the former of the diencephalic wall, the latter of the telencephalic, the two cavities of the brain being partially divided by the velum transversum. In

figures 4 and 5 (of larvae 14.5 and 34.0 mm. long respectively), the later development of these parts of the roof of the brain are shown. The condition seen in figure 5 is typical of older larvae and of adult *Amblystoma*, although it must be said that the open-

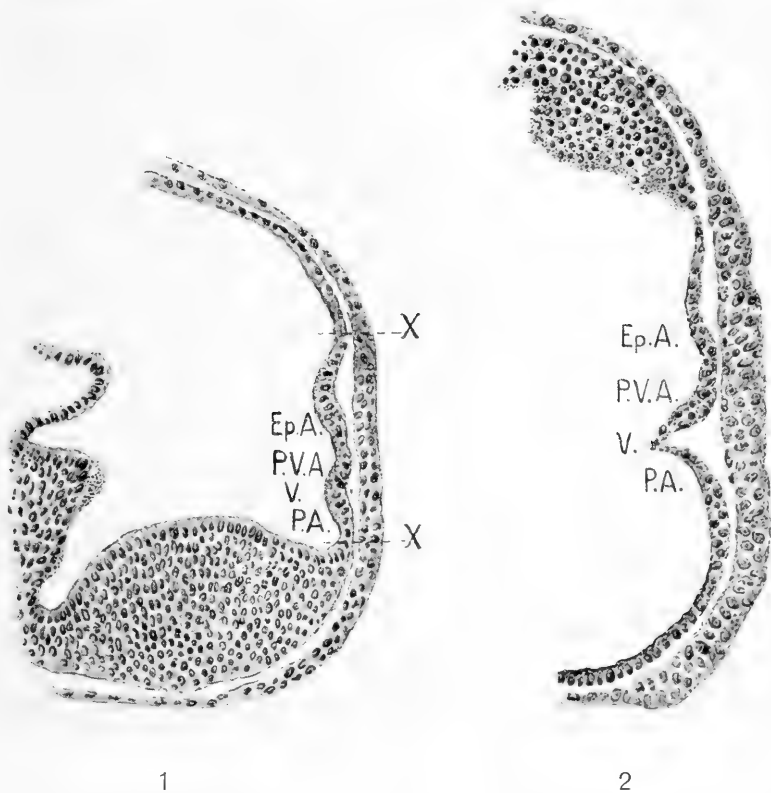


Fig. 1 The anterior end of a sagittal section of a 5.3 mm. larva of *A. punctatum*. *Ep.A.*, epiphysal arch; *P.A.*, paraphysal arch; *P.V.A.*, post-velar arch; *V.*, velum.

Fig. 2 The anterior end of a sagittal section of a 6.6 mm. larva.

ing into the stalk of the epiphysis cannot always be made out with great distinctness, so that there is a question as to whether it may not later be solid instead of hollow as here represented. In no case, however, was the epiphysis found not attached to the brain. Its cavity is more or less incompletely divided by septa.

The paraphysis, which is much larger and more conspicuous than the epiphysis, has a wall of a single layer of cells and a large irregular cavity with branching tubules with which the blood vessels of the choroid plexus are in intimate relation.

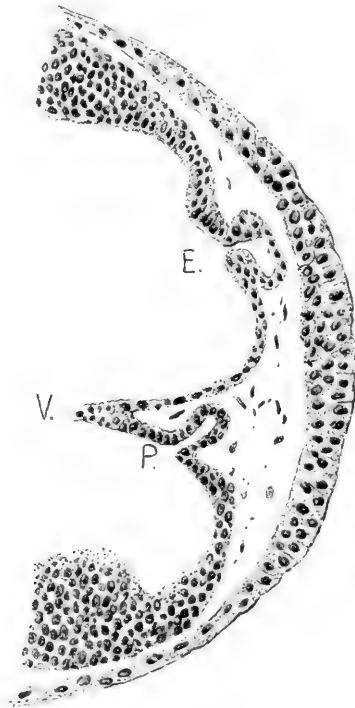


Fig. 3 The anterior end of a sagittal section of an 8.0 mm. larva. *E.*, epiphysis; *P.*, paraphysis; *V.*, velum.

These figures also serve the purpose of showing that the epiphysis, as in other Urodeles, is relatively poorly developed in *Amblystoma* and moreover that there is no pineal or parietal organ or eye. The paraphysis on the other hand reaches a high degree of development and with the surrounding blood vessels can be seen through the skin and brain case of the larvae, particularly

when the melanophores are contracted or when they are scarce. Figure 6 is given to show this, where, it will be admitted, it has very much the appearance of a brow-spot or "Scheitelfleck" and might be taken for such.

From reading over the literature one is soon forced to the conclusion that it seems doubtful whether a parietal organ in any Urodele could have an influence on the reactions of the melano-



Fig. 4 The anterior end of a sagittal section of the head of a 14.5 mm. larva.

phores, and this particularly for the reason that a parietal organ as such does not exist. There seems to be in all a small epiphysis (Studnička '05, and Warren '05). Nevertheless, although there is no parietal organ and only a small epiphysis, there was still the chance, considering the results obtained by von Frisch ('11), that in this region of the brain there might be a 'center' the stimulation of which would have an inhibitory influence on the melanophores.

The first of the methods of experimentation and the one which seemed at first thought to be the most promising, but which failed for reasons which will be given later, was to remove that portion of the undifferentiated nervous system from which the epiphysis arises. This was accordingly carried out on larvae measuring between 5.5 and 6.1 mm. The operation is a simple one requiring but slight practice. The embryo to be operated on was placed in 0.02 per cent NaCl in a watch glass, the bottom

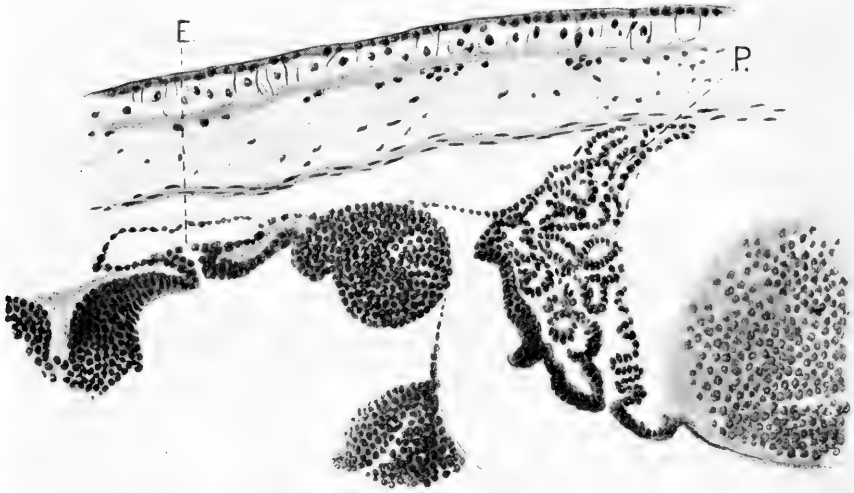


Fig. 5 A sagittal section of a portion of the head of a 34.0 mm. larva to show the position and relative development of the epiphysis and paraphysis.

of which was covered with paraffin. In this a little pocket was dug near the middle of the dish and into this the embryo was slipped so that the anterior end was directly upwards. Under the binocular microscope, with a small pair of iridectomy scissors, the epidermis and then the desired portion of the undifferentiated nervous system was removed. The extent of the portion removed is indicated approximately in figure 1 between the dotted lines x—x. This operation was performed on 10 embryos between 5.5 and 6.1 mm. long. On 10 others in addi-

tion the optic vesicles were also removed by the method described in former papers (Laurens '14, p. 196 and '15, p. 579).

A complete series of experiments were carried out on these 20 larvae together with control normal seeing and eyeless larvae,

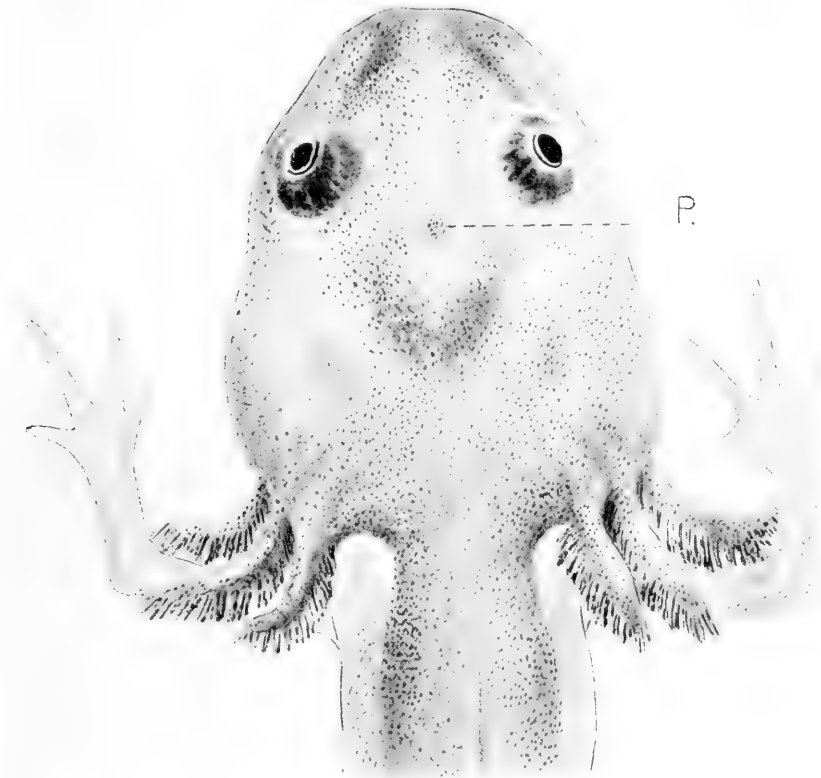


Fig. 6 The anterior end of a 42.0 mm. larva to show the appearance of the paraphysis (with the surrounding blood vessels) as seen from the outside.

for the purpose of seeing whether there was any difference in their reactions to darkness, light and backgrounds and in the time when these reactions first made their appearance (Laurens '15, pp. 583-595). The results were in complete agreement with my earlier ones. But unfortunately upon sectioning the

larvae and examining the brains it was found that in nearly every case either complete or partial regeneration of the parts concerned had taken place. In some cases only a small epiphysis was present, while in others no difference between this region in the operated larvae and in the normal larvae could be detected. In only 2 larvae sectioned was there no sign of an epiphysis. Therefore another method of getting rid of the epiphysis, etc. had to be resorted to.

This was to remove it from older larvae after differentiation of the nervous system had taken place. This operation was successfully carried out on 28 normal larvae ranging in length from 12.5 to 40 mm., and in 8 eyeless larvae ranging in length from 12.0 to 30.0 mm., from which the optic vesicles had been removed when they were about 5.5 mm. long. In addition to removing the epiphysis the roof of the diencephalon was also cut out in 3 normal and 3 eyeless larvae when they were about 12.5 mm. long.

After the experiments to test the various reactions of these larvae to darkness, light and background had been finished several of them were killed, sectioned and the brain examined for indications of regeneration of the parts involved. Some of the others were allowed to live until they had metamorphosed, others not quite as long. Eventually they were all of them sectioned and studied. In only one was there any sign of an epiphysis.

The carrying out of the operation necessary to remove the epiphysis is not a particularly difficult one. The larvae were anaesthetised by placing them in 0.02 per cent chloretone. With a pair of iridectomy scissors a flap of skin was cut and left attached at one end so that it could be folded back, after which the roof of the brain case was removed in small pieces until the desired portions of the brain were sufficiently exposed to be removed. Out of a total of 43 operations 28 normal and 8 eyeless larvae survived and grew.

Again the experiments carried out on larvae operated on in this way showed no differences between those which have already been reported in such detail for normal and eyeless larvae. In addition to simply placing the larvae in darkness and in light on various backgrounds there was carried out a series of experi-

ments in which a narrow beam of light, reflected from a Nernst glower by a mirror and concentrated with a lens, was thrown on the region of the brain from which the epiphysis arises. The results of these experiments give additional evidence concerning the ability of the melanophores to respond to direct stimulation by light.

When *Amblystoma* larvae are placed in darkness and observed by means of a faint red light, they remain motionless for the greater part of the time if there are no other larvae present or other animals which may serve as possible food. It is therefore a simple matter to throw such a beam of light upon any portion of the dorsal or lateral surface of the body and keep it there. Naturally, at times the larvae move by alternately crawling and swimming with the snout close to the bottom of the vessel in the characteristic nosing fashion, nevertheless, the animals can usually be followed with the narrow beam of light until they again come to rest.

It was found that in no case when the beam of light was thrown upon the region of the epiphysis of normal seeing or eyeless larvae did an expansion of the melanophores over the whole body take place. On the other hand the result of such local illumination at any place on the body results in an expansion of the melanophores stimulated. These experiments are rather tedious because of the time consumed in carrying them out. In table 2 (Laurens, '15, p. 585) it will be seen that it takes from $1\frac{1}{2}$ to 2 hours for the melanophores of normal larvae with eyes to expand when the larvae are placed in the light, and 2 to 3 hours for the same thing to happen in eyeless larvae. In these experiments with local illumination no expansion was observed taking place in less than 2 hours and in most cases an illumination lasting 3 hours or more was necessary.

These experiments on local illumination were carried out on both normal and eyeless larvae with and without the epiphysis and the roof of the diencephalon. In some of them not only was the beam of light thrown on the region of the head under which the epiphysis is, but the brain was exposed and directly illuminated. In addition it was also carried out on larvae in which

the central nervous system had been completely destroyed by boring it out. In all, the results were the same, expansion of the melanophores illuminated by the beam of light, and no effect on the pigment cells of the remainder of the body.

These results show conclusively that the expansion of the melanophores of the larvae of *A. punctatum* caused by light are due primarily to the direct stimulation of the pigment cells themselves and not to the inhibitory action of the nervous system. The nervous system certainly helps to bring about the expansion, just as it helps to bring about the contraction of the melanophores when the larvae are placed in darkness, for, when the reaction times of normal and eyeless larvae are compared it is seen that those of the former are shorter (Laurens, p. 585, table 2). The experiments on larvae in which the central nervous system was cut through, and partially or totally destroyed (pp. 614-616) could be considered sufficient proof against the assumption of the action of an inhibiting center in *Amblystoma*, such as Fuchs suggests. But there seemed sufficient reason for carrying out experiments to test just this point. These have afforded us additional evidence concerning several things mentioned in my former paper and have added information not there contained. One interesting fact which was brought out and which seems worthy of remark is that although the roof of the diencephalon is cut out with the epiphysis, nevertheless the secondary reactions of the melanophores, and the reactions to background, which are certainly dependent on the presence of the eyes, still come about. As we know, the median portion of the diencephalon has no nervous elements in it, these being in the lateral walls. In a few experiments which were carried out on larvae after cutting the lateral walls, the secondary reactions of the pigment cells, as well as the reactions to black and white backgrounds did not take place.

DISCUSSION AND CONCLUSIONS

Fuchs' idea that the parietal organ, or, as we may now say, the epiphysis, and the surrounding region of the roof of the brain has an inhibitory influence on the melanophores does not hold

for the larvae of *Amblystoma punctatum*. It has already been pointed out that Babak's explanation of the reactions of the melanophores of the Axolotl larvae also cannot be applied to those of the melanophores of *A. punctatum*.

When one considers the relative anatomical insignificance of the epiphysis in the Urodeles (Studnička, '05 and Warren, '05) it is hardly surprising that this organ should be found to have no influence upon the melanophores. In the teleosts and particularly in the reptiles, the epiphysis reaches a high degree of development with a distinct parietal organ, so that in these animals it is possible that it may have an important function in connection with the reactions of the melanophores (Fuchs, pp. 1442 and 1651, and von Frisch, '11, p. 374). Nevertheless, it cannot be assumed from the results of von Frisch's experiments,—which are the only ones that have been carried out previously to specifically test this point—that even in the minnow *Phoxinus*, the parietal organ is responsible for the inhibiting effect produced by stimulation with light. For he found even after the parietal organ had been extirpated (controlled by microscopic sections) that stimulation with light of this region still produced an expansion of the melanophores, while shading it caused the melanophores to contract. Von Frisch further found that when the portion of the roof of the brain which extends from the point of junction of the epiphysis with the brain to the posterior commissure is also removed, that illumination may still sometimes result in an expansion of the melanophores, although sometimes there is no change at all. From these results von Frisch is finally forced to conclude that in the region of the diencephalon there must be light perceiving cells which function as an inhibiting center, and from which nerve fibers run to the deeper portions of the brain which are in this way connected with the pigment motor apparatus so as to bring about this reaction of the melanophores. Perhaps, he thinks, these cells are particularly numerous in the parietal organ, perhaps they are identical with the sense cells and perhaps the connecting nerve fibers constitute the 'Tractus pinealis.' But they are not limited to the parietal organ, else, when it was

removed, the reactions of the melanophores would cease to take place.

As an interesting fact von Frisch found this region of the roof of the diencephalon to be also particularly easily stimulated by other means than light, e.g., electric currents (pp. 335, 336, and 376) in this particular fish *Phoxinus*.

Von Frisch (p. 377) is led to make the suggestion, later taken up by Fuchs, that perhaps in other animals the parietal organ may be found to have an influence on the pigment cells and that thereby an explanation may be found for some of the perplexing diverse results, e.g., in tadpoles, where darkness causes the melanophores to contract and light causes them to expand, while the opposite reactions take place in the adult frog, and in Axolotl larvae, citing Babak's results on normal and blinded individuals. Also for the reactions of the melanophores of *Salamandra maculosa* larvae and of *Triton cristatus* adults which, however, show an expanded condition of the melanophores in light and in both normal and blinded individuals.

However, he has himself (p. 378) carried out experiments with *Salamandra* larvae and adult Tritons with different results than he hoped for. For in these animals illumination of the head region did not give as clear results as in *Phoxinus*. Furthermore he received the impression that local illumination of any portion of the body results in an expansion of the melanophores of the whole body. In other words the illumination of the portion of the body under which the epiphysis is has no different effect from illumination of any portion of the body.

Experiments such as von Frisch suggests should be carried out on tadpoles and frogs, in some of which the parietal organ has been destroyed, in others of which the influence of the eyes has been removed, etc. In these animals the pineal organ attains a comparatively high degree of development (*Studnička*, p. 110 ff.) Furthermore these results should be compared with those obtained with such an animal as *Diemyctylus*, where the reactions of the larval melanophores are also opposite to those of the adult, but where there is no pineal organ and where the epiphysis is not highly developed. The results of such experi-

ments would certainly afford a great deal of evidence concerning the influence of the parietal organ on the melanophores. Such experiments should also be carried out on reptiles, where the parietal organ reaches a high degree of development, and in some animal where the color changes of the skin are well marked.

The nervous system must be admitted to exert a very important effect upon the chromatophores of any animal, and this effect is for the most part conditioned upon the eyes, which have been shown to play such an important part in the color changes of many animals (see, for example, the reactions which have been described for the melanophores of *Amblystoma*, or the effects observed by von Frisch, '11 and '12, of blinding fish). Take away the eyes and the major part of the controlling influence of the nervous system over the pigment cells of the skin is lost. This of course does not apply in full to those cases where the eyes have, or are believed to have, lost control over the chromatophores and where other things than light, such as temperature, tactile stimuli, etc., are believed to be of greater importance.

But even in such a case, as Biedermann has shown, the reactions of the melanophores to light are controlled by the nervous system in that this exerts a tonic influence upon the pigment cell. Biedermann demonstrated that the pigment cells of the frog as soon as they are released from the influence of the nervous system have the tendency to expand. When he cut through the connection between the thalamus and the mid-brain in *Rana esculenta* and *R. fusca*, as well as in *Hyla*, he found that the melanophores expanded, and that when the frogs (particularly *Hyla*) operated on in this way are kept for weeks in darkness as well as in diffuse light no change takes place in the melanophores. Briefly, that light has under these conditions little, if any, effect on the melanophores, unless direct sunlight is used.

It is my belief that the general function of the chromatophores is to expand when illuminated and to contract again when in darkness. But when the melanophores are under the influence

of the nervous system, which they normally are, it may be, owing to the nature of things, environmental conditions (background) or psychic factors, etc., that the sense of the reaction to a light stimulus is reversed and contraction is brought about.

What causes the melanophores to contract in darkness is another matter, it happens whatever its cause may be. From the conflicting evidence that we have we are forced to speculate regarding its cause. It may be due to chemical substances, such as Fuchs suggests, which arise as a result of the ordinary processes of life (inner secretions, or what not). It may be due to chemical changes in the skin, brought about by darkness, (or the absence of light). It may be due, on the other hand, in some cases at least, to what may be called for want of a better term, a simple relaxation, for the reason that when pieces of the skin are removed from some animals (the frog, Biedermann; *Anolis*, Carlton) the melanophores contract. (Laurens, '15, p. 599). But that this contraction may also be due to chemical changes is perfectly possible.

The expanded condition of the melanophores in darkness which comes about in animals in which, due to the control of the nervous system, the pigment cells contract in light, is probably not a specific reaction to darkness but one which is due to the lack of the nervous impulse which light sets up. The pigment cells have become subordinated to the influence of the nervous system, and when the condition of excitation ceases (in darkness) the melanophores expand. It is interesting, in this connection, that Hargitt could observe, in tree frogs, no definite effect of darkness (and of low temperature) on the pigment cells.

The contracted condition of the pigment cell is claimed by many to be its active state. Arguments are put forward to support this view from the fact that muscle contracting stimuli also cause the chromatophores to contract. But light (and under certain conditions high temperature) is the adequate stimulus for the chromatophores of animals and light causes the melanophores in many animals to expand, both when under the control of the nervous system and when released from this control, perhaps in all when the influence of the nervous sys-

tem is removed. The fact that Hertel ('07) obtained contraction of the chromatophores when he locally stimulated the skin of Triton larvae with ultra violet rays is another matter, since these rays probably always cause the pigment cells to contract (Laurens, '15, p. 599). That he also obtained contraction when he locally stimulated with yellow and red light, may be due to one or more of several things. First, that the intensity of the light was high; second, since the light was focussed, that the pigment cells were subjected to a high temperature; and third, that the contraction of the pigment cells stimulated was a reflex action. This last possibility has less in its favor than the others, since there was no indication of spreading. Ballo-witz's ('14) results of contraction are probably due to the effects of the high intensity of the light that he used, about 1000 candles, hardly to a heat effect (see p. 200). Moreover the melanophores that he experimented with were taken not from the skin but from the "Hirnhaut." Finally the results of Hooker ('12) are also, it seems to me, capable of explanation.¹ Hooker found, when the melanophores of the frog (*R. fusca*) were completely deprived of their nervous connections, either in the body or when bits of skin were placed in hanging drop cultures, that for a day the reactions of the melanophores were the same as when they were under normal conditions. But after this period of time had elapsed the reactions of the pigment cells to light and darkness were reversed. Hooker offers no explanation of this curious fact. An explanation for the primary reactions, those lasting for the first day, may be found in the fact that sunlight was used as the source of light. It is therefore highly probable that the contraction of the melanophores was a heat effect and not due to the light at all. When the melanophores are placed in dark-

¹ Opportunity is taken to call attention to the fact that Hooker is misquoted on p. 598 of my former paper ('15) though rightly so on p. 628. Also that Ballo-witz is incorrectly quoted on p. 598. The statement concerning his results should read that when the melanophores are removed from the body they contract, but when placed in salt solution they partially expand. When they are now illuminated they contract. The opportunity is also taken to point out a mistake in table 4, p. 589 where under 'black background' a II. reaction is indicated as follows: "contraction (3-4 hrs.*)" This should be struck out.

ness the pigment expands again, not because of a specific stimulating effect of darkness, but simply because the heat stimulus ceases to act. Why, after a day, these isolated melanophores show exactly the reverse reactions to a heat stimulus that they did before is not entirely clear to me. That it may be due to an increase in the acidity of the cells, both of the pigment and surrounding tissue cells, in other words to a change in the H ion content, is not absolutely impossible.

Steinach's results, judging from the results of Biedermann's work which show the dependence of the chromatophores upon the central nervous system, were probably also obtained by illuminating the animals with sunlight. The effects therefore are to be referred to the action of heat and not to light.

The chief function of the chromatophores is probably to enable the animals possessing them to adapt themselves to the intensity and color of their background. To make this possible the pigment cells must be under the control of the eyes which regulate through the nervous system, the movements of the pigment suspended in their cytoplasm. It is very possible that in some animals the eyes may have no (or very little) influence over the chromatophores as Biedermann has pointed out to be the case in the frog where according to him, light plays a small part in the color changes. In some animals conditions are of course different from those in others. Von Frisch ('11 and '12) has shown this to be the case in fishes. The minnow *Phoxinus* does not possess in any marked degree the power of adaptation to background, but the melanophores respond more to the relative intensity of the light. In *Salmo* the response of the melanophores to different backgrounds is very marked. In *Crenilabrus pavo* this is also the case. But the control of the nervous system is in each of a different nature. In *Phoxinus* when the eyes are removed the melanophores expand in the light and contract in the dark, which is opposite to the conditions seen in the normal fish. In *Salmo*, when blinded, the melanophores are under all conditions expanded, and in *Crenilabrus* the melano-

phores expand in the light and contract in the dark just as they do in the normal fish, only more extensively.

The reactions of *Amblystoma* larvae to different backgrounds show that this is an important function of the melanophores. *Amblystoma* larvae are nearly always found in pools in the woods in which the bottom is covered with leaves which are either dark brown or black. Occasionally they may be found in ditches in a field, but here the bottom is of black mud. Over such a background, as my experiments have shown, the larvae are very dark, almost black, due to the complete expansion of the melanophores. But over a white background the larvae are pale, exhibiting, to be sure, not such a marked adaptation, but at any rate the melanophores are contracted. Also over an indifferent background, when the illumination is constant and bright, the melanophores contract ($\frac{1}{8}$ to $\frac{1}{4}$ expansion), so that the larvae are more nearly of the color of the bottom. The fact that the time required for the changes in the melanophores to come about is long and that therefore the usefulness to the individual in the way of protection, of the ability of the melanophores to change according to the background, is of doubtful value, is an argument that can be answered by the statement that probably such conditions in change of background are not very likely to occur in nature. But the fact that if they do occur the melanophores can react to the change shows that the ability to change is in the nature of an adaptive one. I have carried out experiments in a large aquarium, directly in front of a window in my room, to further test this matter. In one series of experiments the bottom of one half of the aquarium was of black mud or of black leaves, the other half of whitish sand, the larvae being free to swim from one to the other. Under these conditions the larvae are of course not at all adapted to the background for they do not remain in any one portion of the aquarium long enough for the melanophores to react adaptively. But if the aquarium is divided by a glass partition placed at the dividing line between the two different colored bottoms then the adaptive changes of the melanophores which have been described, do come about and the coloration of the larvae is strikingly

similar to the bottom, particularly in the case of the black condition, so that they are very difficult to see. If the partition is now again removed so that the larvae can swim through the whole aquarium freely then the difference in coloration between the larvae that have been over the one or the other background is very apparent.

The theory that the melanophores in an aquatic animal can have any function in regulating the temperature has been shown by Bauer ('14) to be highly improbable. Also it does not seem possible to suppose that the contraction or expansion of the pigment cells in the skin has anything to do with the reception of a light stimulus by sensory nerve endings. It is well known that phototactic reactions can be induced in animals, which have been deprived of their eyes, by illuminating the skin. The amphibians show this faculty very well (Parker '03, Laurens '11). But as Parker ('09) has shown only a very few fish show this ability on the part of the integumentary nerves stimulated by light to result in phototactic responses. These reactions are supposed to be started by the action of light on receptors in the skin, either directly as light energy (heat) or indirectly as chemical energy set free by the photochemical changes started by the action of the light on the skin, which stimulate the sensory nerve endings. The fact that in most fishes no such reactions take place, although the pigment motor function is well developed, argues against the pigment cells having any such universal function in aiding or retarding the rapidity of perception. Experiments were carried out by me ('14) to test just this point as to whether the condition of the pigment cell—contracted or expanded—had anything to do with the sensitiveness of eyeless *Amblystoma* larvae to light with negative results, and the conclusion was reached that the condition of the pigment in the skin melanophores had nothing to do with the sensitiveness of the larvae to light, although it was dependent upon the fact as to whether they had been kept in light or in darkness before their phototactic reactions were tested.

To conclude then this brief and fragmentary discussion it seems to me that the reactions of the pigment cells in the skin

to light are in most cases adaptive. The primary reaction of the melanophore is to expand when illuminated which it always does when directly stimulated with light of ordinary intensity and sometimes when the stimulus is indirect and through the eyes. Fuchs (p. 1651) closes his review of the color changes of the reptiles with the statement that in those animals which do not show expansion of the pigment in the light, either the parietal organ has lost its function in the course of phylogeny or ontogeny, or the eyes have gained a regulatory influence over the light reactions which results in changing the original light reaction (expansion) into the directly opposite reaction (contraction). I should change this statement and applying it to the chromatophores of all animals say, that in those animals in which the pigment cells do not expand in light of ordinary intensity and under normal conditions of temperature but contract, this is due to the controlling regulatory influence of the eyes, by means of which the reactions of the melanophores are made adaptive. In other words, when the pigment cells contract in the light, this reaction is one that is bound up with the nervous system in that an impulse started in the retinae is sent out which is opposite to that produced by direct stimulation. Such is the case in frogs, in *Diemyctylus*, and in *Phoxinus*, *Salmo*, etc. Usually, however, the melanophores expand in the light, due in most part, to their direct stimulation, although the eyes still show an influence in that in many cases the expansion is not maximal as it is when the eyes are removed, and that when the eyes are present the reactions come about more quickly.

SUMMARY

1. The epiphysis of *Amblystoma punctatum* larvae has no influence on the reactions of the melanophores to light and darkness. There is no parietal organ.
2. The view is expressed that the reaction to light of ordinary intensity of the pigment cells in the skin of animals is to expand. When this does not take place it is due to the controlling regulatory influence of the eyes.

3. The reactions of chromatophores are believed to be adaptive in that they either respond to the relative intensity of the light or to the color and intensity of the background.

4. The control over the melanophores which the eyes possess is of course most important, for by this means the reactions of the melanophores are able to be adaptive.

5. Indirect stimulation of the melanophores through the eyes is not by any means always opposite in effect to direct stimulation, but only so when the conditions are such that it is necessary that the chromatophores contract in the light in order that the reaction be adaptive.

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THE CONTROL OF SEX BY FOOD IN FIVE SPECIES OF ROTIFERS

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SIX FIGURES

It has been shown in the American and English rotifer, *Hydatina senta*, that food conditions are the controlling factors in regulating the parthenogenetic production of the two sexes. When the parthenogenetic females were fed upon a diet of the colorless flagellate, *Polytoma*, they produced female-producing daughters exclusively even through a period of twenty-two months and extending through many scores of generations. However, when the parthenogenetic females were suddenly transferred from the *Polytoma* diet to a diet of the green flagellate, *Chlamydomonas pulvisculus*, they produced in many instances as high as 80 per cent, or higher, of male-producing daughters within a few hours. In a few selected experiments the percentage of male-producing daughters reached 100 per cent when the diet of colorless *Polytoma* was suddenly changed to a diet of the green *Chlamydomonas*.

. If the production of the sexes can be regulated in this rotifer by the diet it is of considerable interest to know whether the diet can regulate the production of the sexes in other species of rotifers. Furthermore, it is quite important to determine whether it was the stimulus produced by the change of food from the *Polytoma* to the *Chlamydomonas* diet that caused the male-producing daughters to be suddenly produced or whether it was a sufficient quantity of more easily assimilated food that changed the mechanism of the daughters from female to male-producers, or perhaps some other factor.

The colorless *Polytoma* was reared in stable tea (horse manure) solution in a subdued light while the green *Chlamydomonas* was reared in bouillon solution in direct sunlight. It is very probable that their values as foods would be considerably different, in as much as *Polytoma* lacks chlorophyl and consequently cannot carry on photosynthesis while *Chlamydomonas* possesses chlorophyl and manufactures and stores starch in its cell. Thus the colorless flagellate would contain neither starch nor sugars while the green flagellate might have more or less of each.

In order to get new data on these two problems the former experiments upon *Hydatina senta* were carefully reviewed and new experiments carried out upon the following rotifers: from the order of Ploima the species, *Branchionus pala*, *Diaschiza sterea*, *Diglena catellina*, and from the order of Scirtopoda the species *Pedalion mirum*. These species were identified by Harry K. Haring, Custodian of the Rotatoria in the United States National Museum, to whom I am greatly indebted for the favor.

Hydatina senta

The results obtained from numerous experiments carried out upon this species from New Jersey and from England have already been published in detail and need not be repeated again. However, as no diagrams have been published of the American form, diagrams have been drawn to the same scale of a female and a male individual, a parthenogenetic female-producing egg, a parthenogenetic male-producing egg, and of a fertilized egg. These are shown in figure 1. A general review of the results of the former experiments have been put into a plotting in diagram 1, showing that a continuous diet of *Polytoma* caused only female-producing females to be produced for nearly two years but when the food was suddenly changed to the green *Chlamydomonas* a high percentage of male-producing females appeared within a few days. An important feature of these experiments, which does not show in the diagram, is the place where the stimulus is effective in changing the females from female-pro-

ducers to male-producers. The mother of the female-producing or male-producing daughters is the individual influenced by the diet. If the mother is fed the colorless diet all of her daughters

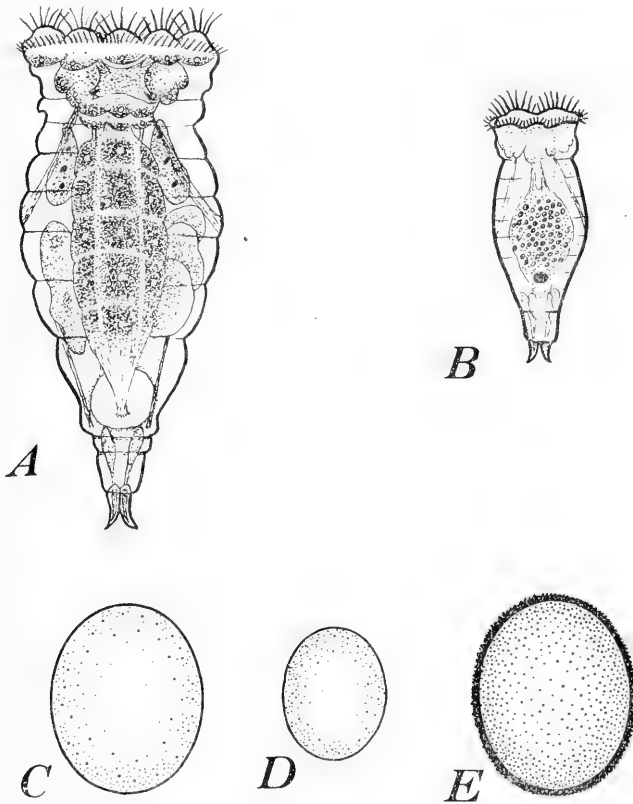


Fig. 1 *Hydatina senta*. *A*, female; *B*, male; *C*, parthenogenetic egg which develops into a female; *D*, smaller parthenogenetic egg which develops into a male; *E*, fertilized egg, which is often called resting or winter egg, and always develops into a female. This egg if unfertilized would have formed a small parthenogenetic egg which would have developed into a male.

will produce females but if the diet of the mother is changed from the colorless flagellates to the green flagellates she will begin soon to produce daughters which will produce males.

Brachionus pala

In August of 1908 a small pond was found at Cold Spring Harbor, Long Island, New York that contained great numbers of this species of rotifer. The pond was located in a small sunny pig pasture in which there were kept a few pigs. These animals lay in the pond a large part of the time during the hot days and consequently the water became somewhat foul and furnished an ideal culture medium for the rotifers and the va-

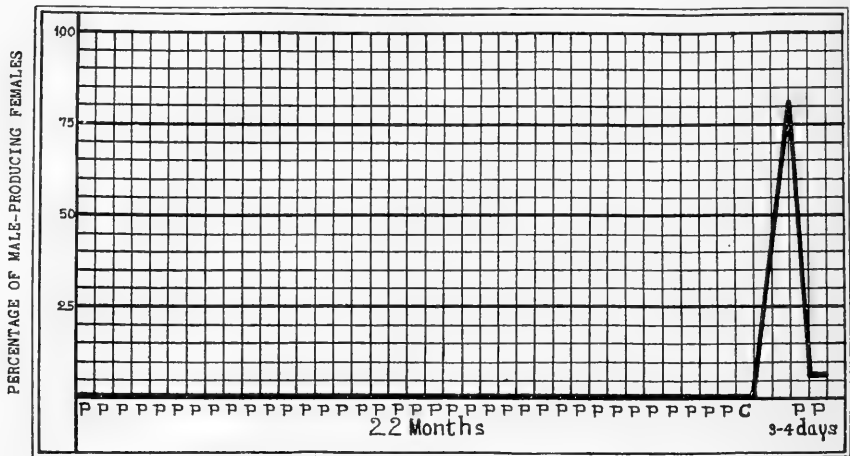


Diagram 1 *Hydatina senta*. Showing that a continuous diet of *Polytoma* through a long period of time yielded only female-producing females but when the diet was suddenly changed to *Chlamydomonas* male-producing females appeared at once. P indicates a *Polytoma* diet, C indicates a *Chlamydomonas* diet.

rious micro-organisms on which they fed. No experiments were carried out at that time but some mud from the bottom of the pond was taken, dried, put into a paper bag, and stored in an ordinary laboratory room.

In February of 1915 some of this dried mud was put into a solution of 10 cc. of bouillon and 140 cc. of tap water and placed near a window. Within a few days various protozoa were numerous in the jar and a few females of the rotifer, *Brachionus pala*, were found. These females undoubtedly had hatched

from the winter eggs which are the fertilized eggs. Such eggs of *Hydatina senta* have been found to live six years in old culture water and three years in a dried state. When dried they have withstood for several days the extreme low temperature of liquid air which is about -191°C . and also they have withstood as high a temperature as $+110^{\circ}\text{C}$. for a few hours. Moreover, it has been found that species of rotifers that produce fertilized eggs cannot themselves be dried and later be revived by placing in water as is true of those rotifers which do not produce fertilized eggs.

These females of *Brachionus pala* continued to live and to reproduce rather slowly in the culture jar of bouillon and water. From this stock jar females were taken and many preliminary experiments were carried out in attempts to find optimum food conditions. Finally it was found that when bouillon cultures, inoculated with the miscellaneous green flagellates that developed from the same mud from which the rotifers developed, were placed in direct sunlight they developed very fine food cultures for the rotifers. In order to prevent the temperature of the culture jars from rising too high in the direct sunlight and thereby killing all the flagellates as well as the rotifers that might be in them, the jars were placed in a large pan through which tap water flowed. In this way the temperature could be regulated at will and was not allowed to rise above 35°C . to 37°C . The green flagellates seem to be the most active at this temperature and as the rotifers could eat them only in an active state all the cultures were usually kept in this pan. At night and on cloudy days the temperature would be the same as the room temperature of about 20°C .

The stock solution of bouillon was made by boiling one Armour's beef bouillon cube in 400 cc. of tap water.

The bouillon culture of green flagellates was allowed usually to stand from several days to several weeks in direct sunlight with occasional additions of fresh bouillon. During this time several inoculations with the rotifers were made and usually at the end of a few days there would be a good culture of both the green flagellates and the rotifers. After some time if no

fresh bouillon was added the whole culture of the flagellates and rotifers became balanced, that is the flagellates reproduced at such a rate as to keep the rotifers moderately supplied with food. When they were thus supplied moderately with food only females were found in the culture jar but when fresh bouillon was added to the general culture it furnished additional food for the flagellates causing them to multiply very rapidly and to produce enormous numbers of themselves in the jar. With this great increase of food the rotifers also began to eat ravenously and soon produced a great number of females which to a large proportion produced male offspring. In this manner of increasing the food by adding fresh bouillon males could be produced in enormous numbers within a short time. These males copulated with many of the young females and within a few days the majority of the adult females were carrying fertilized eggs. Soon the cultures were depleted of food by the great increase of rotifers and then nearly all the rotifers died of starvation but left thousands of fertilized resting eggs.

It has been shown by Lauterborn that if the male egg is fertilized in *Asplanchna* it produces the winter or resting egg. The same fact has been shown by Whitney and Shull to be true of *Hydatina senta* and recently it has been shown by Moro to be true also of *Brachionus pala*.

These experiments were done in mass cultures many of which contained many thousands of individuals and countings were made of the female-producing and male-producing females at various intervals. These two kinds of females were easily distinguished from each other because after they had extruded the eggs they carried around the eggs attached to their bodies. The male-producing females carried small eggs and the female-producing females carried large eggs. The details of the experiments are in table 1. Drawings of the females carrying the different eggs and also of a male are in figure 2. In diagram 2 a plotting is seen of experiment 1, and in diagram 3 a plotting is seen of a part of experiment 3.

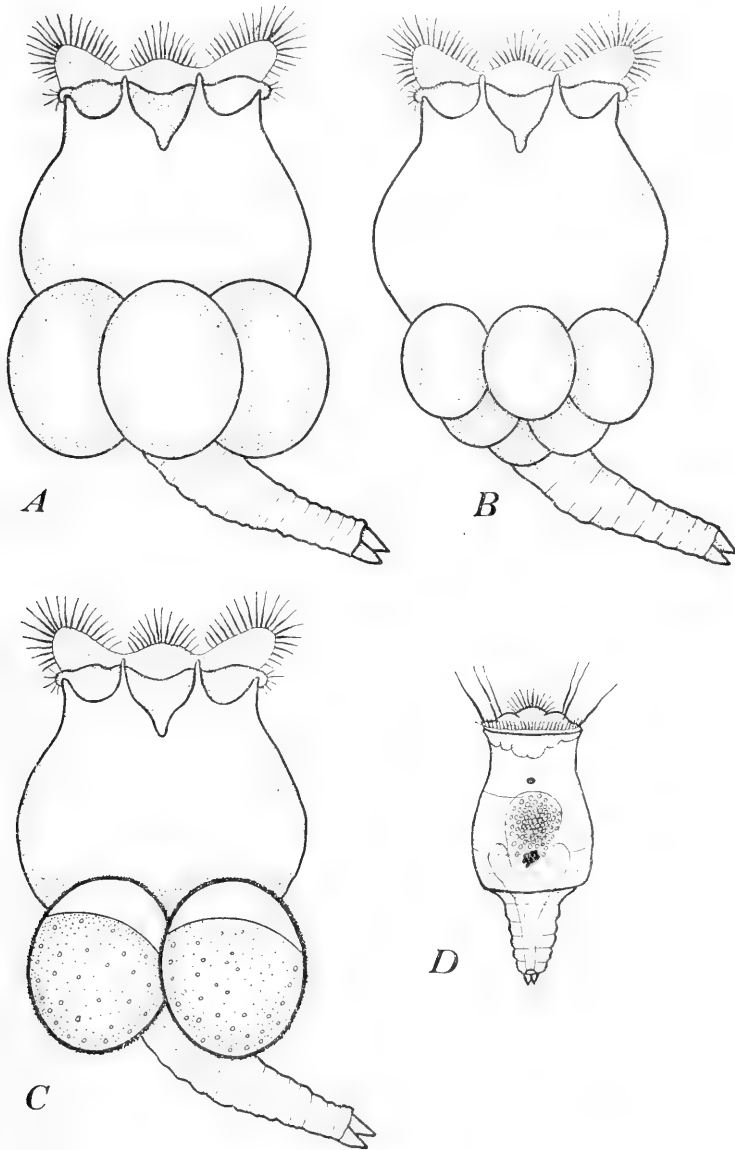


Fig. 2 *Brachionus pala*. A, female with attached parthenogenetic female eggs; B, female with attached parthenogenetic male eggs; C, female with attached fertilized eggs; D, male.

TABLE 1

Experiments with Brachionus pala and a mixed diet of miscellaneous green flagellates showing that when the flagellates were caused to be very abundant by the addition of fresh culture media the percentage of the male-producing female rotifers was very high and the percentage of the female-producing female rotifers was very low but when no fresh culture media was added and the flagellates occurred only in moderate numbers the percentage of male-producing females was very low and the percentage of the female-producing females was very high.

		1915	CULTURE WATER	♀ ♀	♂ ♀	% ♂ ♀
Experiment 1	April	6	{ 15 cc. bouillon 135 cc. tap water mixed green protozoa			
	April	14	15 cc. bouillon added.....			
	April	17	20	0	0
	April	18	15 cc. bouillon added.....	60	0	0
	April	19	18	2	10
	April	20	15 cc. bouillon added.....	28	12	30
	April	21	70	30	30
	April	23	95	5	5
	April	25	95	5	5
	April	28	200	1	0.5
	May	1	15 cc. bouillon added.....	200	1	0.5
	May	3	15 cc. bouillon added.....			
	May	7	65	35	35
	May	11	97	3	3
	May	19	15 cc. bouillon added.....	100	0	0
	May	21	15 cc. bouillon added.....			
	May	22	70	30	30
	May	24	100	100	50
June	1	100	0	0	
			Total.....	1218	224	
Experiment 2	March	8	{ 15 cc. bouillon 135 cc. tap water mixed green protozoa			
	April	19	15 cc. bouillon added.....	200	0	0
	April	24	15 cc. bouillon added			
	April	26	200	200	50
	April	30	200	2	1
	May	3	15 cc. bouillon added.....			
	May	5	80	20	20
	May	6	15 cc. bouillon added.....			
	May	7	70	30	30
May	11	99	1	1	
			Total.....	849	253	

TABLE I—Continued

		1915	CULTURE WATER	♀ ♀	♂ ♀	% ♂ ♀
Experiment 3	April	19	{ 15 cc. bouillon 135 cc. tap water mixed green protozoa 15 cc. bouillon added..... 15 cc. bouillon added..... 15 cc. bouillon added..... 15 cc. bouillon added..... 15 cc. bouillon added..... Total			
	April	22				
	April	24				
	April	26				
	May	25		95	5	5
	May	28				
	June	1				
	June	2		5	95	95
	June	9		50	0	0
				150	100	
Experiment 4	April	19	{ 20 cc. bouillon 130 cc. tap water mixed green protozoa 20 cc. bouillon added..... 20 cc. bouillon added..... 20 cc. bouillon added..... 20 cc. bouillon added..... 20 cc. bouillon added..... Total.....			
	April	22				
	April	24				
	April	26				
	June	1		100	0	0
	June	3				
	June	6		50	50	50
					150	50
Experiment 5	April	19	{ 20 cc. bouillon 130 cc. tap water mixed green protozoa 20 cc. bouillon added..... 20 cc. bouillon added..... 20 cc. bouillon added..... 20 cc. bouillon added..... 20 cc. bouillon added..... Total.....			
	April	22				
	April	24				
	April	26				
	April	28				
	June	1				
	August	20		200	0	0
	August	21				
August	27	100	100	50		
				300	100	

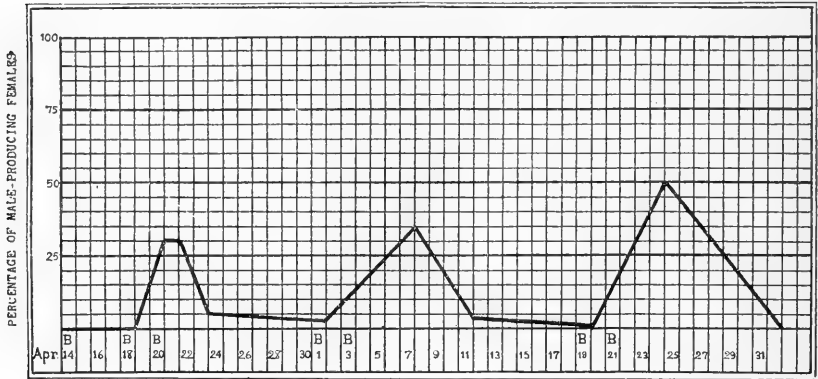


Diagram 2 *Brachionus pala*. Experiment 1 of table 1. Showing the production of a high percentage of male-producing females when the food conditions were changed by the additions of bouillon. *B* indicates the addition of bouillon.

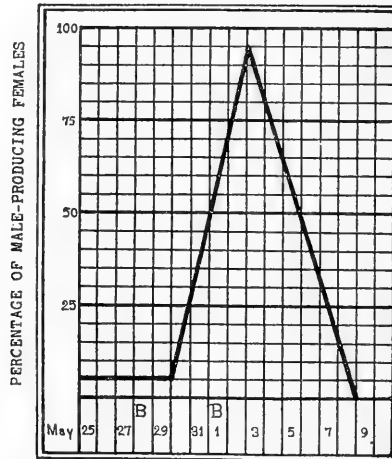


Diagram 3 *Brachionus pala*. Experiment 3 of table 1. Showing the production of a high percentage of male-producing females when the food conditions were changed by the additions of bouillon. *B* indicates the addition of bouillon.

Diaschiza sterea

In March of 1915 four fertilized eggs of *Diaschiza sterea* were isolated from a battery jar containing miscellaneous varieties of animals growing in a stable tea (horse manure) solution. This jar was made in September of 1914 and had been left standing since that time without a renewal of stable tea. These fertilized eggs were put into fresh tap water for a few hours and then later a little stable tea was added to the water. Within a short time the eggs hatched and produced females. These females were fed upon the colorless flagellate, *Polytoma*, and reproducing readily produced a culture of many females but no males.

The *Polytoma* was reared in a solution of stable tea as follows: 800 grams of fresh horse manure mixed with 1200 cc. of tap water, was cooked in an autoclave at 15 lbs. steam pressure for one hour and then 1000 cc. of the liquid was pressed out of the cooked manure. This solution, hereafter called stable tea, was used as a stock supply. One part of this stable tea and three parts of sterilized water were mixed and inoculated with *Polytoma*. Every day one-half of this culture was poured off and replaced by a fresh solution of one part stable tea to three parts water. In this manner a vigorous growing pure culture of *Polytoma* was daily maintained.

The *Diaschiza sterea* is a small rotifer, no larger than a small paramoecium, and on account of their small size it was found impossible to make individual pedigreed cultures of them. Consequently all the experiments were made in mass cultures either in small watch crystals or in depression slides. In a weak solution of the *Polytoma* culture (1:2) the female rotifers grew very readily but produced entirely female offspring. However, when these females were transferred to a weak bouillon solution (2 cc. or 5 cc. of the stock bouillon solution to 148 cc. sterilized water) to which pure cultures of *Chlorogonium*¹ had been added males appeared within a few days varying in pro-

¹This green flagellate formally has been known as *Chlorogonium euchlorum* but recently Wille has reclassified it as *Chlamydomonas euchlorum*. Engler u. Prantl, Pflanzenfam. Nachtrage I Teil, 2d Abt., 1911, p. 18.

portion to the females from 10 per cent to 60 per cent. Diagrams of a male and a female and the different eggs are in figure 3. The details of the experiments are in table 2 and a plotting of experiments 10 to 12, and 16 and 17, are in diagram 4.

The *Diaschiza sterea* are bottom feeders eating only microorganisms that settle and remain on the bottom of the culture dishes. As both *Polytoma* and *Chlorogonium* did this to a considerable extent they furnished very good food material. *Chlamydomonas* was tried as a food but the rotifers soon died, apparently from starvation.

At this time another series of experiments was made to determine whether the males were caused to appear by the stimulus of the new food or by the stimulus of the bouillon in which the new food and the rotifers were placed. In order to determine this point some culture water from the *Polytoma* cultures in which the rotifers were thriving was filtered and the female rotifers put back into it. Then the *Chlorogonium* which had been cultivated in a bouillon solution (1 part of the stock bouillon solution to 1 part water) were thoroughly washed by placing them in test tubes filled with sterilized water and centrifuging on a large centrifuging machine. This process would, of course, collect the *Chlorogonium* in a small mass at the end of the tube. This washing process was repeated three or four times until the *Euglena* were undoubtedly thoroughly freed from any bouillon on their external parts. These washed *Chlorogonium* were then fed to the rotifers that had been put back into the filtered *Polytoma* culture water. Males soon appeared and in some experiments as many as 40 per cent were found. These experiments demonstrate that the stimulus which caused males to be produced was in the *Chlorogonium* itself and not in the bouillon. The details of the experiments are in table 3. All the experiments with this species of rotifer were done at room temperature on a table in subdued light.

Diglena catellina

In July of 1915 a few females of the rotifer, *Diglena catellina*, were found in some general mixed culture jars which were probably inoculated by a collection of various organisms collected

in a towing from a small pond in Middletown. These rotifers are also very small and only mass culture experiments were made.

TABLE 2

Experiments with Diaschiza sterea and two diets of pure cultures of Polytoma and Chlorogonium showing that only females were produced in the colorless diet of Polytoma but when the female rotifers were transferred to a diet of Chlorogonium males appeared soon in the cultures.

EXPERIMENTS	TIME	CONTROL REARED AND CONTINUED IN STABLE TEA CULTURE CONTAINING POLYTOMA				FEMALES REARED IN STABLE TEA CULTURE CONTAINING POLY- TOMA TRANSFERRED TO BOUL- LON CULTURE CONTAINING CHLOROGONIUM			
	Hours	Fe- males isolated	Estimated number of offspring			Fe- males isolated	Estimated number of offspring		
			♀	♂	% ♂		♀	♂	% ♂
1.....	60	40	400	0	0	20	140	60	30
2.....	60	40	400	0	0	20	140	60	30
3.....	60	40	400	0	0	20	160	40	20
4.....	60	40	400	0	0	20	160	40	20
5.....	60	40	400	0	0	20	180	20	10
6.....	90	40	500	0	0	20	180	120	40
7.....	90	40	500	0	0	20	210	90	30
8.....	90	40	500	0	0	20	225	75	25
9.....	90	40	500	0	0	20	210	90	30
10.....	90	40	500	0	0	20	150	150	50
11.....	90	40	400	0	0	20	90	60	40
12.....	90	40	400	0	0	20	120	80	40
13.....	90	40	400	0	0	20	90	60	40
14.....	90	40	400	0	0	20	130	70	35
15.....	90	40	400	0	0	20	140	60	30
16.....	90	40	400	0	0	20	80	120	60
17.....	90	40	400	0	0	20	80	120	60
18.....	90	40	400	0	0	20	120	30	20
19.....	90	40	396	4	1	20	105	45	30
20.....	90	40	396	4	1	20	96	54	36
21.....	90	40	396	4	1	20	135	15	10
22.....	90	40	396	4	1	20	90	60	40
23.....	90	40	396	4	1	20	111	39	26
24.....	90	40	396	4	1	20	81	69	46
25.....	90	40	396	4	1	20	105	45	30
26.....	90	40	396	4	1	20	111	39	26
27.....	90	40	396	4	1	20	126	24	16
Total.....		160	1696	4		540	3565	1735	32

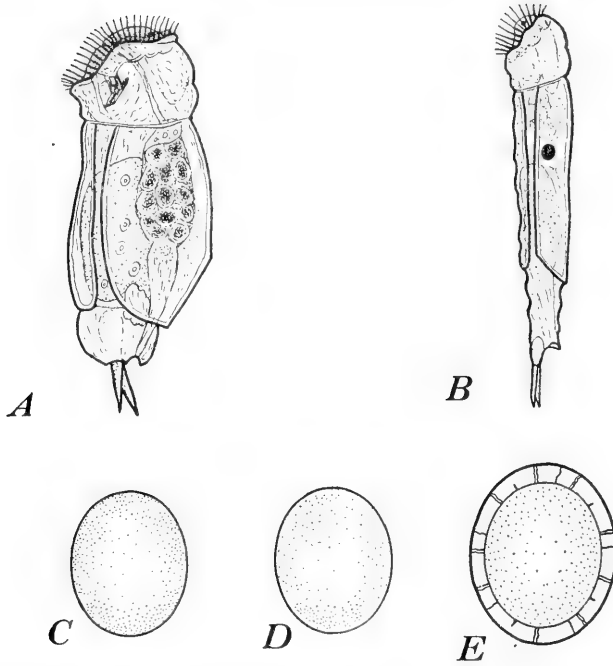


Fig. 3 *Diaschiza sterea*. *A*, female; *B*, male; *C*, parthenogenetic female egg; *D*, parthenogenetic male egg; *E*, fertilized egg.

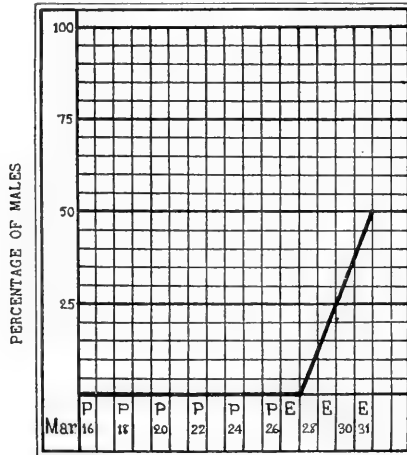


Diagram 4 *Diaschiza sterea*. Experiments 10, 11, 12, 16, and 17 of table 2. Showing the percentage of males produced when the diets were changed. *P* indicates the feeding of a *Polytoma* diet, *G* indicates the feeding of a *Chlorogonium* diet.

The female rotifers were placed in a weak solution of *Polytoma* culture water which contained many *Polytoma*. Here

TABLE 3

Experiments with Diaschiza sterea showing that it was the food and not the culture media that caused the males to be produced

EXPERIMENTS	REARED AND CONTINUED IN STABLE TEA CULTURE CONTAINING POLYTOMA		ESTIMATED NUMBER OF OFFSPRING			FEMALES REARED IN STABLE TEA CULTURE CONTAINING POLYTOMA TRANSFERRED TO FILTERED STABLE TEA CULTURE WATER TO WHICH WAS ADDED EQUILLON FED CHLOROGONIUM THAT HAD BEEN WASHED		ESTIMATED NUMBER OF OFFSPRING		
	TIME	Females isolated	Estimated number of offspring			TIME	Females isolated	Estimated number of offspring		
			♀	♂	% ♂			♀	♂	% ♂
1.....	4	10	125	1	1	4	12	80	20	20
2.....	4	10	125	1	1	4	12	100	25	20
3.....	4	10	125	1	1	4	12	100	25	20
4.....	4	10	125	1	1	4	12	106	18	15
5.....	4	10	125	1	1	4	12	87	37	30
6.....	4	10	125	1	1	4	12	81	43	35
7.....	4	10	125	1	1	4	12	112	12	10
8.....	4	10	125	1	1	4	12	112	12	10
9.....	4	10	125	1	1	4	12	93	31	25
10.....	14	20	2000	0	0	4	20	210	90	30
11.....	14	20	2000	0	0	4	20	162	87	35
12.....	14	20	2000	0	0	4	20	175	75	30
13.....	14	20	2000	0	0	4	20	120	60	30
14.....	14	20	2000	0	0	4	20	130	70	35
15.....	14	20	2000	0	0	4	20	175	75	30
16.....	14	20	2000	0	0	4	20	120	80	40
17.....	14	20	2000	0	0	4	20	140	60	30
18.....	14	20	2000	0	0	4	20	130	70	35
19.....	14	20	2000	0	0	4	20	120	80	40
20.....	14	20	2000	0	0	4	20	140	60	30
21.....	14	20	2000	0	0	4	20	160	40	20
22.....	14	20	2000	0	0	4	20	140	60	30
23.....	14	20	2000	0	0	4	20	150	50	25
24.....	14	20	2000	0	0	4	20	160	40	20
25.....	14	20	2000	0	0	4	20	150	50	25
26.....	14	20	2000	0	0	4	20	160	40	20
27.....	14	20	2000	0	0	4	20	180	20	10
28.....	14	20	2000	0	0	4	20	170	30	15
29.....	14	20	2000	0	0	4	20	150	50	25

they lived and produced many daughters which produced in their turn daughters. This was continued for several weeks and many hundred females were produced but not a single male

TABLE 4

Experiments with Diglena catellina showing that when the rotifers were fed the colorless Polytoma diet no males were produced but when the females were transferred to a diet of miscellaneous green flagellates males soon appeared.

EXPERIMENTS	TIME	CONTROL REARED AND CONTINUED IN STABLE TEA CULTURE CONTAINING POLY- TOMA				FEMALES REARED IN STABLE TEA CULTURE CONTAINING POLYTOMA TRANSFERRED TO A MIXED STABLE TEA AND BOUILLON CULTURE CON- TAINING MISCELLANEOUS GREEN FLAGELLATES			
	Hours	Females isolated	Estimated number of offspring			Females isolated	Estimated number of offspring		
			♀	♂	% ♂		♀	♂	% ♂
1.....	72	20-30	200	0	0	20-30	160	40	20
2.....	72	20-30	200	0	0	20-30	170	30	15
3.....	72	20-30	200	0	0	20-30	180	20	10
4.....	72	20-30	200	0	0	20-30	160	40	20
5.....	72	20-30	200	0	0	20-30	170	30	15
6.....	72	20-30	200	0	0	20-30	180	20	10
7.....	72	20-30	200	0	0	20-30	170	30	15
8.....	72	20-30	200	0	0	20-30	160	40	20
9.....	48	15-20	150	0	0	15-20	105	45	30
10.....	48	15-20	150	0	0	15-20	105	45	30
11.....	48	15-20	150	0	0	15-20	120	30	20
12.....	48	15-20	150	0	0	15-20	135	15	10
13.....	48	15-20	150	0	0	15-20	120	30	20
14.....	48	15-20	150	0	0	15-20	120	30	20
15.....	48	15-20	150	0	0	15-20	120	30	20
16.....	90	50	500	0	0	15-20	160	40	20
17.....	90	50	500	0	0	15-20	170	30	15
18.....	90	50	500	0	0	15-20	180	20	10
19.....	90	50	500	0	0	15-20	160	40	20
20.....	90	50	500	0	0	15-20	140	60	30
21.....	90	50	500	0	0	15-20	170	30	15

individual appeared during this time. However, when some of these females were taken from the Polytoma culture and placed in a weak culture water of bouillon and stable tea (10

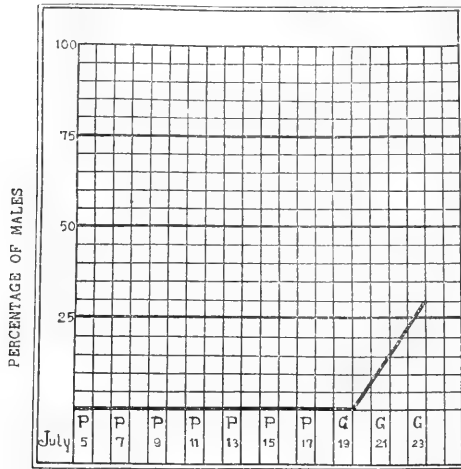


Diagram 5. *Diglena catellina*. Experiment 20 of table 4. Showing the percentage of males produced when the diets were changed. *P* indicates the feeding of a *Polytoma* diet, *G* indicates the feeding of a mixed green flagellate diet.

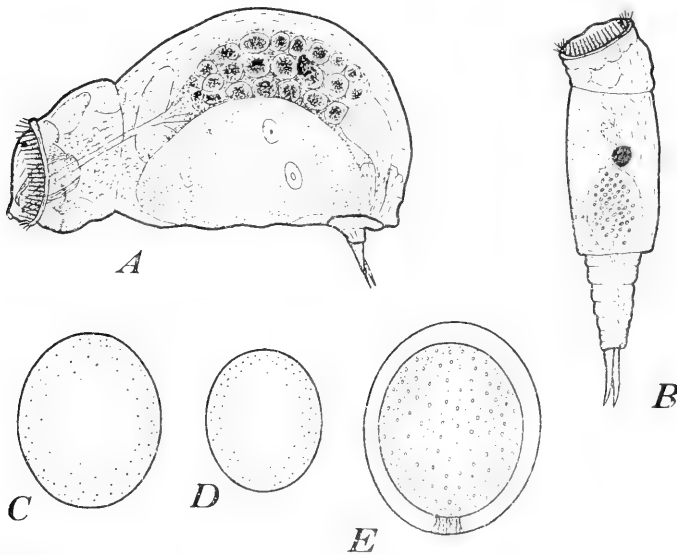


Fig. 4 *Diglena catellina*. *A*, female; *B*, male; *C*, parthenogenetic female egg; *D*, parthenogenetic male egg; *E*, fertilized egg.

cc. bouillon, 2 cc. stable tea, and 138 cc. tap water) in which there were growing a mixed culture of various green flagellates many male individuals appeared within a few days.

The average percentage of males produced in this species is lower than it is in the preceding species. This may have been due to the inferior quality of these flagellates as a food for this particular species of rotifer, or as these rotifers are also bottom feeders perhaps they could not obtain a superabundance of food as many of the flagellates were more or less free-swimming.

These experiments were all done at room temperature on a table in subdued light. Diagrams of a female and a male rotifer and the different eggs are in figure 4, a plotting of experiment 20 is in diagram 5, and the details and the results of the experiments are in table 4.

Pedalion mirum

In February of 1915 some of the same dried mud that was collected at Cold Spring Harbor in 1908 and produced *Brachionus pala* in February of 1915 was put into weak stable tea water (3 cc. stable tea added to 147 cc. water) and after a few days several females of the jumping rotifer, *Pedalion mirum* were found. After considerable experimenting a suitable method was found by which large numbers of these rotifers could be readily reared.

In some experiments weak solutions of stable tea alone were used and in others bouillon was added to the stable tea solution. All the cultures were inoculated with a miscellaneous collection of green flagellates and then kept in direct sunlight as much as possible. The temperature was not allowed to rise above 37°C. This was rendered possible by placing the culture jars in a large pan through which water flowed from the tap. At night, however, the temperature was that of the room, 20°C.

After the cultures had been progressing for a few days the flagellates, mostly *Chlamydomonas*, and the rotifers became more or less balanced and only female rotifers occurred. How-

ever, when new stable tea or bouillon was added to the balanced jars a rapid increase in the number of flagellates took place. This furnished a superabundance of food for the rotifers and they became loaded with eggs. This superabundance of food could be maintained for several days and in the meantime a great number of the rotifers were seen to be carrying small male eggs. Some females had as many as eighteen of these eggs attached to the outside of their bodies. A little

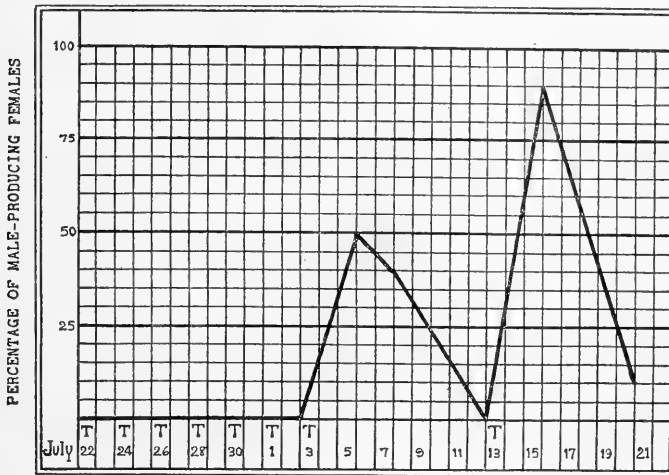


Diagram 6 *Pedalion mirum*. Experiment 1 of table 5. Showing the production of a high percentage of male-producing females when the food conditions were changed by the additions of stable tea. *T* indicates the additions of stable tea.

later there would be found a very few females carrying female eggs, a few carrying male eggs, and a large number carrying one fertilized egg on the inside of the body. In some jars as many as 90 per cent of the females bore male or fertilized eggs.

After a time nearly all the flagellates were eaten and nearly all the rotifers would die but usually a few survived and as the flagellates would begin to increase again in a few days there would be produced again a balanced culture. In such cul-

tures only female rotifers were usually found. If new stable tea or bouillon was added another epidemic of males was produced. Diagrams of the different females carrying eggs and also a diagram of a male are in figure 5. A plotting of experiment 1 is in diagram 6 and the details of the experiments are in table 5.

TABLE 5

Experiments with Pedalion mirum and a mixed diet of miscellaneous green flagellates showing that when the flagellates were caused to be very abundant by the addition of fresh culture media the percentage of the male-producing female rotifers was very high and the percentage of the female-producing female rotifers was very low, but when no fresh culture media was added, thus causing the flagellates to occur in moderate numbers, the percentage of male-producing females was very low and the percentage of the female-producing females was very high.

		1915	CULTURE WATER	♀ ♀	♂ ♀	% ♂ ♀	
Experiment 1	July	22	{ 2 cc. stable tea 150 cc. tap water Miscellaneous green flagellates				
	July	24		2 cc. stable tea added....			
	July	26		2 cc. stable tea added....			
	July	28	2 cc. stable tea added....				
	July	30	2 cc. stable tea added....				
	August	2	200	0	0	
	August	5	100	100	50	
	August	7	120	80	40	
	August	8	160	40	20	
	August	10	270	30	10	
	August	12	398	2	0.5	
	August	13	2 cc. stable tea added....				
	August	14	70	30	30	
	August	15	10	90	90	
	August	18	60	40	40	
	August	20	90	10	10	
Total.....				1558	342		

TABLE 5—Continued

		1915	CULTURE WATER	♀ ♀	♂ ♀	% ♂ ♀
Experiment 2	July	24	2 cc. stable tea 150 cc. tap water Miscellaneous green flag- ellates			
	July	24		2 cc. stable tea added . . .		
	July	26	2 cc. stable tea added . . .			
	July	28	2 cc. stable tea added . . .			
	July	30	2 cc. stable tea added . . .			
	August	2	5 cc. bouillon added	200	0	0
	August	5	5 cc. bouillon added	200	0	0
	August	7	200	0	0
	August	8	160	40	20
	August	10	291	9	3
	August	11	2 cc. stable tea added . . .			
	August	13	2 cc. stable tea added . . .			
	August	14	90	10	10
	August	17	60	40	40
	August	18	50	50	50
August	20	90	10	10	
			Total	1341	159	
Experiment 3	July	28	75 cc. old stable tea cul- ture 75 cc. tap water 5 cc. bouillon Miscellaneous green flag- ellates			
	August	5		2 cc. stable tea added . . .		
	August	7	2 cc. stable tea added . . .	70	30	30
	August	8	100	0	0
	August	10	100	0	0
	August	11	2 cc. stable tea added . . .			
	August	13	2 cc. stable tea added . . .			
	August	14	50	50	50
	August	16	60	40	40
	August	18	400	1	0.25
			Total	780	121	

TABLE 5—Continued

		1915	CULTURE WATER	♀ ♀	♂ ♀	% ♂ ♀
Experiment 4	July	27	{ 75 cc. old stable tea culture 75 cc. tap water 5 cc. bouillon Miscellaneous green flagellates			
	August	2	5 cc. bouillon added.....			
	August	3	5 cc. bouillon added..... 2 cc. stable tea added....			
	August	5	2 cc. stable tea added....			
	August	7	2 cc. stable tea added....	40	160	80
	August	10	396	4	1
	August	11	2 cc. stable tea added....			
	August	13	2 cc. stable tea added....			
	August	14	140	60	30
	August	15	2 cc. stable tea added....			
	August	16	100	100	50
	August	18	200	0	0
	Total.....			876	320	
Experiment 5	July	28	{ 40 cc. old stable tea culture 110 cc. tap water 5 cc. bouillon Miscellaneous green flagellates			
	August	5	2 cc. stable tea added....			
	August	7	2 cc. stable tea added....	96	4	4
	August	8	285	15	5
	August	11	2 cc. stable tea added....			
	August	13	2 cc. stable tea added....			
	August	14	160	40	20
	August	15	2 cc. stable tea added....			
	August	16	150	50	25
	August	18	90	10	10
	August	20	200	0	0
Total.....			1081	119		

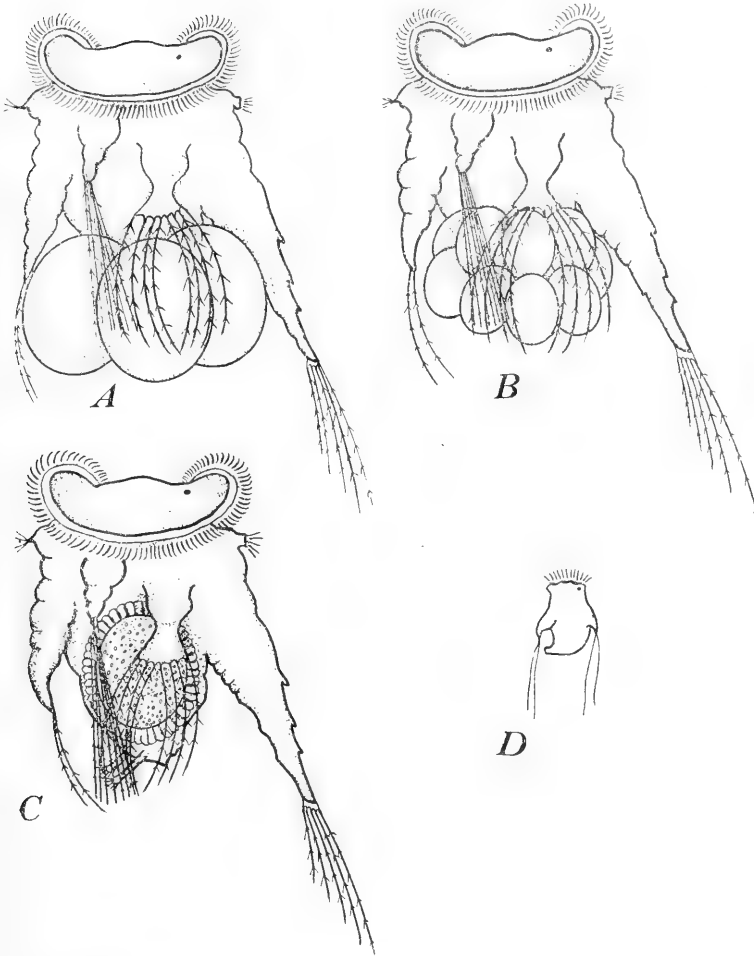


Fig. 5 *Pedalion mirum*. A, female with attached parthenogenetic female eggs; B, female with attached parthenogenetic male eggs; C, female with the fertilized egg inside her body; D, male.

THE INFLUENCE OF A SINGLE DIET

In all of the preceding experiments there were used either two diets, one of colorless flagellates alternated with one of green flagellates or a mixed diet containing miscellaneous species of protozoa, principally green flagellates. During the experiments with the mixed diets when at times they consisted of nearly all of one species of flagellate, *Chlamydomonas*, it was suspected that one favorable diet could be so manipulated as to yield either female-producing or male-producing rotifers. In order to test this hypothesis pure cultures of the green *Chlamydomonas* were made in mixtures of bouillon and stable tea solutions. These cultures were inoculated with the rotifer, *Brachionus pala*, and several experiments were started and carried through to completion.

The flagellate, *Chlamydomonas*, passes through several stages in its life cycle, some of which are to the advantage of the rotifers and others of which are distinctly to the disadvantage of the rotifers as far as being of food value. In favorable culture solutions the individuals of *Chlamydomonas* are very active during the sunny part of the day but do not reproduce very much. However, at night, having reached their full size, many of them become motionless and begin to divide inside of their outer envelope. Here each divides three times resulting in eight small individuals. Sometime in the morning hours the parent envelope breaks and the eight young come out and soon become separated from each other. These young individuals are active during the day while increasing in size and at night having reached their full size they become quiescent, begin to divide, and before morning break into eight small individuals. There is considerable variation in this life cycle. Some may be in the eight-cell stage and break out in the early evening while others may be in such stages in the late morning but the large majority go through the cycle as indicated.

When the *Chlamydomonas* are small in size they are the most readily eaten by the rotifers. As they reach full size, although they may be very active, they are not eaten at all by the rotifers.

In fact in some old cultures where there were great numbers of these full sized flagellates in an active state but not dividing the rotifers died from starvation. The rotifers are transparent and one can see the food in their stomachs after they have eaten.

By adding fresh stable tea or bouillon or both to the cultures enormous numbers of the small sized *Chlamydomonas* were produced and in a short time the jars were swarming with female rotifers each of which was carrying many small male eggs. Then if no fresh stable tea or bouillon was added nearly all the flagellates were soon eaten, and consequently nearly all the rotifers died. After a short time the few flagellates that had escaped would increase their numbers considerably and the few rotifers that had escaped starvation could thus get a moderate amount of food. Soon the flagellates and the rotifers would form a balanced culture and continue thus for many days. In this balanced condition nearly all female-producing females were produced. However, if fresh stable tea or bouillon were added again to the culture an enormous increase in the numbers of the small sized *Chlamydomonas* took place accompanied by a rapid increase of the female rotifers. Soon a very high percentage of these females would be carrying male eggs. Thus it is shown that a moderate amount of the diet *Chlamydomonas*, will cause only female-producing females to be produced but that an abundant diet or a superabundant diet of the same *Chlamydomonas* will produce in some experiments as high as 90 per cent of male-producing females.

The details of these experiments with a pure culture of *Chlamydomonas* are seen in table 6 and a semi-diagrammatic plotting of the average results of the nine experiments are seen in diagram 7. Diagrams of the different stages in the life cycle of *Chlamydomonas* is seen in figure 6.

TABLE 6

Experiments with Brachionus pala and a pure diet of Chlamydomonas showing that when the Chlamydomonas were caused to be very abundant by the addition of fresh culture media the percentage of the male-producing female rotifers was very high and the percentage of the female-producing female rotifers was very low but when no fresh culture media was added, and thereby causing the Chlamydomonas to occur in moderate numbers the percentage of male-producing females was very low and the percentage of the female-producing females was very high.

	1915	CULTURE WATER	♀ ♀	♂ ♀	% ♂ ♀
Experiment 1	September 22	{ 10 cc. bouillon 140 cc. tap water Chlamydomonas			
	September 23	10 cc. bouillon added.....			
	September 25	10 cc. bouillon added.....			
	September 27	10 cc. bouillon added.....			
	September 28	3 cc. stable tea added....			
	October 2	10 cc. bouillon added.....			
	October 11	20	80	80
	October 13	20	180	90
	October 20	10 cc. bouillon added.....	95	5	5
	October 28	10 cc. bouillon added.....			
	October 31	80	120	60
	November 1	50	150	75
	November 5	200	0	0
	Total.....	465	535		
Experiment 2	October 2	{ 3 cc. stable tea 10 cc. bouillon 137 cc. tap water Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added.....			
	October 21	40	160	80
	October 28	10 cc. bouillon added.....	95	5	5
	November 1	10 cc. bouillon added.....	200	0	0
	November 8	20	80	80
	November 16	98	2	2
	Total.....	453	247		

TABLE 6—Continued

	1915	CULTURE WATER	♀ ♀	♂ ♀	% ♂ ♀	
Experiment 3	October 2	{ 3 cc. stable tea 10 cc. bouillon 137 cc. tap water Chlamydomonas				
	October 4		10 cc. bouillon added.....			
	October 6		10 cc. bouillon added.....			
	October 11		10 cc. bouillon added.....			
	October 21		40	160	80
	October 22		40	360	90
	October 28		10 cc. bouillon added.....	97	3	3
	November 1		10 cc. bouillon added.....			
	November 8		20	80	80
	November 16		100	0	0
			Total		297	603
Experiment 4	October 2	{ 3 cc. stable tea 10 cc. bouillon added 137 cc. tap water Chlamydomonas				
	October 4		10 cc. bouillon added.....			
	October 6		10 cc. bouillon added.....			
	October 11		10 cc. bouillon added.....			
	October 22		10 cc. bouillon added.....			
	October 23		20	180	90
	November 8		10 cc. bouillon added.....	95	5	5
	November 10		3 cc. stable tea added....			
	November 14		10 cc. bouillon added.....			
	November 20		25	75	75
			Total		190	260
Experiment 5	October 2	{ 3 cc. stable tea 10 cc. bouillon 137 cc. tap water Chlamydomonas				
	October 4		10 cc. bouillon added.....			
	October 6		10 cc. bouillon added.....			
	October 11		10 cc. bouillon added.....			
	October 22		10 cc. bouillon added.....			
	October 23		40	160	80
	October 24		40	160	80
	November 8		10 cc. bouillon added.....	95	5	5
	November 10		3 cc. stable tea added....			
	November 12		10 cc. bouillon added.....			
	November 18		20	80	80
	Total		245	406		

TABLE 6—Continued

	1915	CULTURE WATER	♀ ♀	♂ ♀	% ♂ ♀
Experiment 6	October 2	{ 10 cc. bouillon 3 cc. stable tea 137 cc. tap water Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added.....			
	October 22	10 cc. bouillon added.....			
	October 23	40	160	80
	October 24	20	180	90
	November 8	{ 10 cc. bouillon added..... 3 cc. stable tea added...	97	3	3
	November 10	10 cc. bouillon added.....			
	November 12	20	80	80
	November 20	100	0	0
		Total.....		277	423
Experiment 7	October 2	{ 10 cc. bouillon 3 cc. stable tea 137 cc. tap water Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added			
	October 22	10 cc. bouillon added.....			
	October 26	30	170	85
	November 8	{ 10 cc. bouillon added..... 3 cc. stable tea added...	98	2	2
	November 10	10 cc. bouillon added.....			
	November 12	20	80	80
	November 14	15	85	85
	November 20	98	2	2
		Total.....		261	339
Experiment 8	October 2	{ 10 cc. bouillon 3 cc. stable tea 137 cc. tap water Chlamydomonas			
	October 4	10 cc. bouillon added.....			
	October 6	10 cc. bouillon added.....			
	October 11	10 cc. bouillon added.....			
	October 22	10 cc. bouillon added.....			
	October 26	20	180	90
	November 8	{ 10 cc. bouillon added..... 3 cc. stable tea added...	98	2	2
	November 10	10 cc. bouillon added.....			
	November 12	50	50	50
	November 16	10	90	90
	November 20	100	0	0
		Total.....		278	322

TABLE 6—Continued

		1915	CULTURE WATER	♀ ♀	♂ ♀	% ♂ ♀
Experiment 9	October	2	{ 10 cc. bouillon 3 cc. stable tea 137 cc. tap water Chlamydomonas			
	October	4	10 cc. bouillon added.....			
	October	6	10 cc. bouillon added.....			
	October	11	10 cc. bouillon added.....			
	October	22	10 cc. bouillon added.....			
	November	1	40	160	80
	November	8	{ 10 cc. bouillon added..... 3 cc. stable tea added...	95	5	5
	November	10	10 cc. bouillon added.....			
	November	12	40	60	60
	November	13	15	85	85
	November	20	99	1	1
	Total.....			289	311	

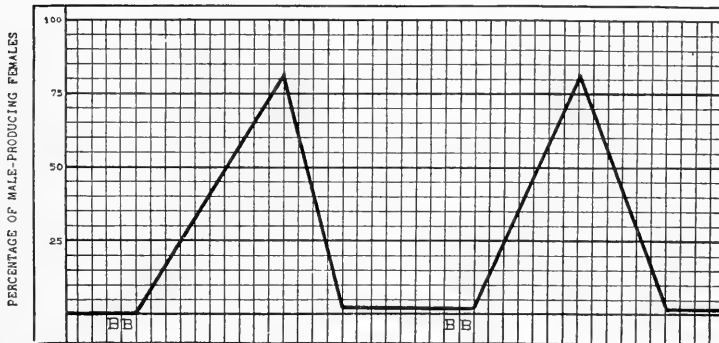


Diagram 7 *Brachionus pala*. Experiments 1 to 9 of table 6. Showing the production of a high percentage of male-producing females when the food of pure cultures of *Chlamydomonas* was made superabundant by the additions of bouillon. B indicates the addition of bouillon.

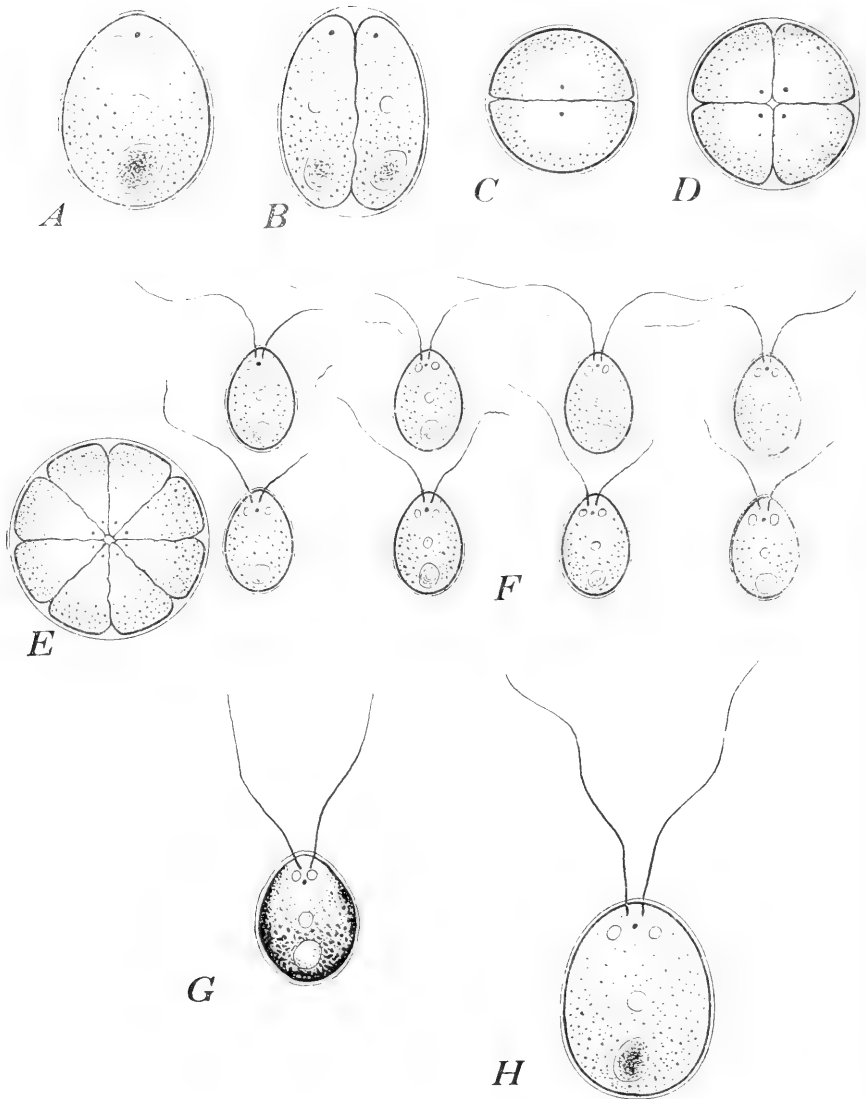


Fig. 6 *Chlamydomonas pulvisculus*. A, mature individual in quiescent stage; B, two cell stage, side view; C, two cell stage, end view; D, four cell stage, end view; E, eight cell stage, end view; F, the eight active cells after they have broken out of the parental envelope. This is the stage in which the rotifers can eat the *Chlamydomonas* the most readily; G, a later stage; H, mature active stage which the rotifers seem unable to eat.

GENERAL DISCUSSION

Probably the production of the male-producing females in all the experiments with the five different species of rotifers can be explained by the quality of the food in the diet. In the experiments with *Hydatina senta*, *Diaschiza sterea*, and *Diglena catellina* the colorless flagellate, *Polytoma*, very likely furnished a moderate amount of nutrition so that the rotifers lived and reproduced at a moderate rate. When the rotifers were transferred to the green diet they probably ate about the same amount of food but might have digested and assimilated it more easily and faster, and consequently the formation of eggs was more rapid. However, in some way, as yet unknown, the green diet changed the mechanism of the eggs in such a manner that instead of developing into female-producing females they developed into male-producing females. Moro has shown in her chemical experiments with *Brachionus pala* that a mother forms and extrudes the eggs that develop into male-producing daughters very much faster than a mother that is forming and extruding eggs which develop into female-producing daughters. The ratio was 4 to 1.

In my experiments with *Brachionus pala* and a pure *Chlamydomonas* diet it has been found that a mother produces very few eggs that develop into male-producing daughters when she is kept on a scanty diet but when the diet of the same flagellates in the same culture water is made abundant or superabundant the mother produces many eggs which develop into male-producing daughters. Thus showing that there is something in an abundant diet that causes a mother to produce male-producing daughters.

Moro concludes that sudden changes of temperature, either the raising or the lowering of it, causes mothers to produce male-producing daughters. In *Hydatina senta* Punnett, Whitney, and Shull have found no evidence to support this contention. In the recent experiments with *Diaschiza sterea* and *Diglena catellina* the temperature was that of the room at all times. These rotifers in the *Polytoma* diet produced only fe-

males but when they were put upon the green diet at the same temperature they produced many males. In the experiments with *Pedalion mirum* and *Branchionus pala* which were carried on partly in direct sunlight the temperature varied from 20°C. to 37°C. on nearly every day because both sets of experiments were carried on, luckily, in periods of many successive days of sunshine. When the green flagellates were scanty or in the mature stages, regardless of the daily changes in temperature from room temperature to 37°C. during the mornings and from 37°C. to room temperature during the evenings, very few male-producing females were produced. However, when fresh *Chlamydomonas* culture media was added to the jars in which the rotifers were living an enormous increase in the numbers of *Chlamydomonas* occurred and soon, in some jars, as high as 95 per cent of male producing females were found. In former observations upon *Hydatina senta* it has been noted that at the higher temperatures of about 25°C. to 28°C. its green food was very active and could be readily obtained by the rotifers and as many as 80 per cent produced were male-producers but at the lower temperatures of 9°C. to 12°C. the greater part of its green food became quiescent in which stage it was impossible for the rotifers to eat it and all the females produced were female-producers. The general conclusion can be drawn that the changes in temperature unless accompanied by changes in the amount of the diet are not potent factors in regulating the production of female and male-producing female rotifers.

SUMMARY

1. In pedigreed cultures of *Hydatina senta* a diet of the colorless flagellate, *Polytoma*, which is probably a poor diet, causes only female-producing daughters to be produced, whereas a diet of the green flagellate, *Chlamydomonas pulvisculus*, which is probably an optimum food, causes nearly all male-producing daughters to be produced.
2. In mass cultures of *Branchionus pala* a scanty diet of miscellaneous green flagellates caused nearly all female-producing fe-

males to be produced whereas a superabundance of this same green diet, produced in the jars by the addition of fresh culture media, caused in some experiments as high as 95 per cent of male-producing females to appear.

3. In mass cultures of *Diaschiza sterea* living in stable tea water at room temperature the diet of *Polytoma* caused only females to be produced but when the rotifers were transferred to bouillon water containing an abundant diet of *Chlorogonium* many males were produced.

4. In mass cultures of *Diaschiza sterea* living in stable tea water at room temperature the diet of stable tea reared *Polytoma* caused only females to be produced but a diet of a bouillon reared *Chlorogonium* which had been washed and was added to the filtered stable tea water caused many males to be produced, thus showing that it was the diet and not the change of culture water that caused males to be produced.

5. In mass cultures of *Diglena catellina* at room temperature the diet of *Polytoma* caused only females to be produced but a diet of miscellaneous green flagellates caused many males to be produced.

6. In mass cultures of *Pedalion mirum* a scanty diet of miscellaneous green flagellates caused nearly all female-producing females to be produced whereas a superabundant diet of the same green flagellates, produced in the jars by the addition of fresh culture media, caused in some experiments as high as 90 per cent of male-producing females to be produced.

7. In mass cultures of *Brachionus pala* a scanty diet of pure cultures of *Chlamydomonas pulvisculus* caused nearly all female-producing females to be produced whereas a superabundance of this same *Chlamydomonas* diet, produced in the jars by adding fresh culture media, caused in many experiments as high as 90 per cent of male-producing females to be produced.

8. The colorless diet of *Polytoma* causes female-producing females to be produced and the green diets of *Chlamydomonas*, *Chlorogonium*, or miscellaneous green flagellates causes male-producing females to be produced.

9. A scanty or poor diet of pure cultures of the green flagellate, *Chlamydomonas pulvisculus*, causes nearly all female-producing females to be produced whereas a copious or superabundant diet of the same pure cultures of the flagellates causes nearly all male-producing females to be produced.

10. A poor or scanty diet causes only female-producing females to be produced but a plentiful diet of the right kind causes nearly all male-producing females to be produced.

December 6, 1915

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THE RHYTHMIC PULSATION OF THE CLOACA OF HOLOTHURIANS¹

W. J. CROZIER

THIRTY-ONE FIGURES

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¹ Contributions from the Bermuda Biological Station for Research, No. 43.

I. INTRODUCTION

Holothurians possessing respiratory trees are provided with a cloacal chamber which acts as a pump supplying these organs with sea water. The most obvious manifestation of this activity is the rhythmic opening and closing of the anus. This pulsation has never been studied in detail,² though it presents morphological and physiological features of considerable interest.

I have previously (Crozier, '15) brought forward reasons for believing that the cloacal region of a typical aspidochirote holothurian, *H. surinamensis*, contains within itself the essential mechanism of its pulsation, though there is, superimposed upon this independently pulsating complex, a control of its activity by the animal as a whole. It was found that rhythmic cloacal movements were maintained for about three days in the case of small pieces cut from the aboral end of *H. surinamensis*, but that in the excised pieces pulsation was continuous and failed to exhibit certain interruptions which are characteristic of these movements in the intact animal. It was noted that Edwards ('09, p. 215) recorded the observation of anal movements in the larvae of *H. floridana* before the appearance of the respiratory trees. Therefore it seemed that the anal pulsating mechanism might be regarded as constituting another unit in the series of independent effectors, such as pedicellariae and spines, which go to make up the echinoderm neuro-muscular equipment.

The ability of the isolated posterior ends of pedate holothurians to maintain their pulsation for relatively long periods I have further verified by observations upon the following species occurring at Bermuda: *Cucumaria punctata* Ludw., *Holothuria cap-tiva* Ludw., *H. rathbuni* Lamp., and *Stichopus moebii* Semper.

The problem of the present investigation was to determine the physiological characteristics of cloacal pulsation in holothurians, particularly in the case of the aboral ends removed from the control of the animal as a whole by amputation; and to discover to what extent the laws of rhythmic movement, especially as

² The early observations on the pumping activity of the holothurian cloaca are collected by Ludwig ('89-92, p. 387).

derived from the study of the medusae, are applicable to these movements in an echinoderm, in which the neuro-muscular conditions are in some respects similar. The account here given is essentially of a preliminary kind. This work has, I believe, resulted in the discovery of a type of material peculiarly favorable to quantitative studies, especially upon the nature of ionic actions.

The animal principally employed for this study was *Stichopus moebii* Semper,³ both because of its availability in unlimited quantity, and since its large size greatly facilitated operative experiments. Unless indication is made to the contrary, it is to be understood that in the subsequent pages this species is always referred to.

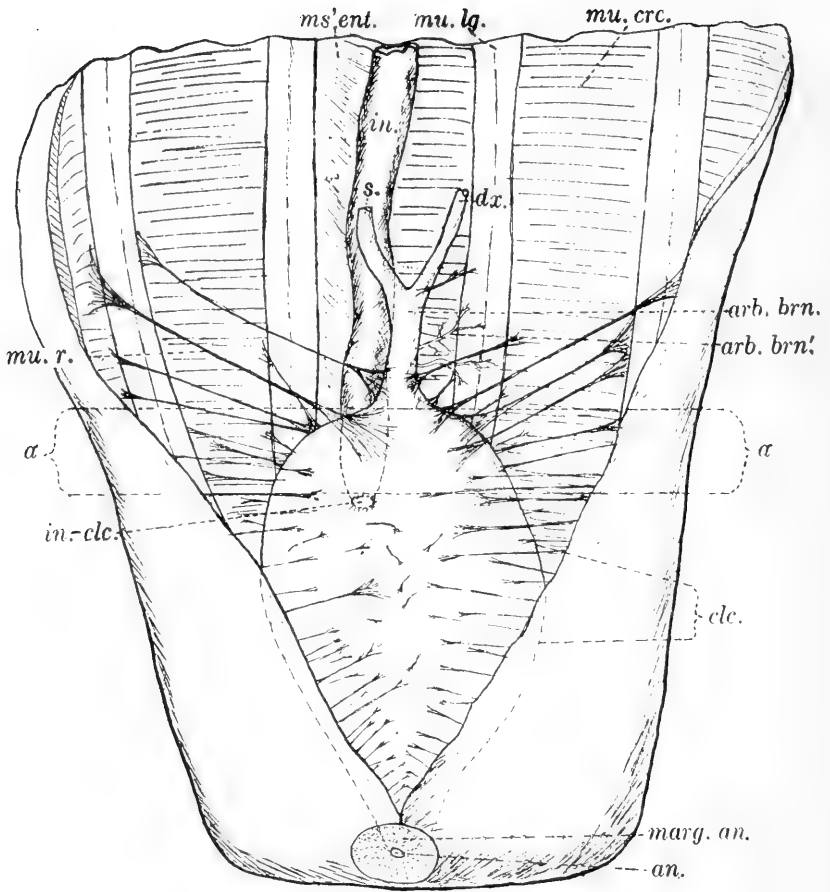
To Prof. E. L. Mark I am again indebted for the privileges of the Bermuda Biological Station and the abundant fauna to which it gives access. The work was carried out during the summer of 1914. I wish to record, in addition, my obligation to Mr. L. B. Arey for much incidental assistance. Prof. G. H. Parker has read this manuscript, and has materially aided me both by inspiration and by advice, for which my best thanks are due.

II. PULSATION IN THE INTACT ANIMAL

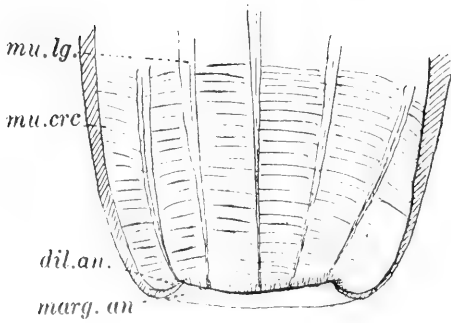
a) Morphology of the pulsating parts. The parts concerned in pulsation will first be described, and, following this, their normal operations, as a basis upon which to interpret the experiments.

The general relations of the contractile parts in the aboral end of *Stichopus* will be evident from figures 1 and 2. As is necessary in any pulsating system which does not include an elastic supporting structure against which the muscles can work, there are essentially two sets of opposed muscles: 1) the radiating muscles, producing cloacal expansion, and 2) the circular

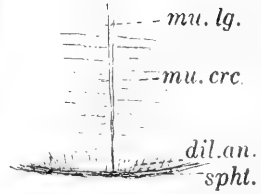
³ I agree with Clark ('01) that the black, spotted, and uniform reddish-brown forms of this *Stichopus* are specifically identical. They are physiologically indistinguishable, except in the matter of pigment formation, and in this all intergrades were found among the approximately 2000 individuals examined.



1



A



B

2

muscles of the cloaca, sphincter, and body wall, producing contraction of the cloacal region.⁴

The conditions are, however, much more complex than this simple statement indicates, and it is necessary to consider the distribution of the muscles more in detail. The third limb of the intestine opens into the cloacal chamber (*in.-clc.*, fig. 1) on its ventral side. This opening is guarded by a sphincter, which is kept closed except during defecation. At its anterior end the cloaca gives rise to the stalk of the respiratory trees, also guarded

Fig. 1 The posterior end of *Stichopus moebii* opened along the mid-dorsal inter-radius; the body wall is somewhat distorted, so that only four of the five longitudinal muscles are seen. From a living preparation ($\times 2\frac{1}{2}$).

<i>an.</i> , anus.	<i>ms.'ent.</i> , mesentery.
<i>arb.brn.</i> , trunk of respiratory tree.	<i>mu.crc.</i> , circular muscles of the body wall.
<i>arb.brn'</i> , small derivatives of the respiratory trees, originating rather far down the stalk. When full of water these small branches pass backward into the space about the posterior end of the cloaca.	<i>mu.lg.</i> , longitudinal muscles of the body wall.
<i>arb.brn.dx.</i> , right respiratory tree stem.	<i>mu.r.</i> , radiating muscles of the cloaca. Some of them are shown cut. Only a few of the many strands present are drawn. Some of the strands are of connective tissue, and are not contractile.
<i>arb.brn.s.</i> , left respiratory tree stem.	α --- α , level of amputation in experiments with the isolated cloaca, showing the range of this level.
<i>clc.</i> , outline of cloaca.	
<i>in.</i> , intestine.	
<i>in.-clc.</i> , union of intestine with the cloaca.	
<i>margin.an.</i> , anal brim.	

Fig. 2 *A.* The body wall of *Stichopus moebii* divided along the dorsal inter-radius, and the cloaca removed ($\times \frac{5}{6}$). *B.* A portion of the anal brim flattened out ($\times 1\frac{2}{3}$).

<i>dil.an.</i> , dilators of the anus.	<i>mu.lg.</i> , longitudinal muscles.
<i>margin.an.</i> , anal brim.	<i>spht.</i> , circular fibers of the sphincter.
<i>mu.crc.</i> , circular muscles.	

⁴ Jordan ('13, '14) has dealt with the contractile properties of the so called 'connective tissue' of the skin of holothurians, and states his belief that this tissue by its degree of slow tonic contraction acts in conjunction with the locomotor muscles. The pulsation movements which we are considering are, as compared with the progression movements, very rapidly executed, so that (aside from their rôle in maintaining a tense support for the radiating muscles) these contractile elements of the integument are not discussed in detail. The relation of salts to their degree of tonus will be studied in a subsequent paper.

by a sphincter (Bordas, '99). Posteriorly the cloaca and body wall are continuous in the region of the anal sphincter. The longitudinal muscles of the body wall are reduced to very fine threads just before they reach the anal sphincter, but there are, in addition to the circular fibers of the sphincter, certain contractile elements set perpendicularly to them. This last mentioned set of muscles assists in the opening of the sphincter; its members are homologous to the radiating muscles of the cloaca.

Concerning the innervation of these structures it is difficult to get precise information, since the nervous elements are refractory to ordinary neurological technique. I was unable to secure methylene-blue preparations in the case of *Stichopus*. The cloaca is referred to (e.g., Haanen, '14, p. 223) as of ectodermal origin, in agreement with which is the fact that the inner cloacal surface shows, though to a lesser degree, the pigmentation of the outside of the animal. It is, therefore, possible that the cloaca (including the radiating muscles [?] and sphincter) is innervated from the radial nerve strands (Ludwig, '89-92, p. 70), in common with the body-wall muscles. The gut of holothurians is supplied with special nerve strands from the nerve ring, and the cloaca may receive a nervous supply from this source. The holothurians have no aboral nerve ring.

With reference to the finer histological condition of the nervous apparatus, Bethe ('03, p. 22) remarks that "über die histologischen Verhältnisse des Nervensystems ist bei den Echinodermen so gut wie Nichts bekannt," but it seems certain that a subepithelial plexus is present in the form of a true nerve-net (Jordan, '14, p. 380). This would appear to be the condition in many autonomous organs of invertebrates, and especially in structures which exhibit rhythmic contraction (Alexandrowicz, '13).

b) The sequence of movements. Peristaltic movements appear in the intestine, respiratory trees (as noted by Henri, '03), cloaca, anal sphincter and body wall of *Stichopus*. All but the first of these organs are concerned with the transport of water. In ordinary inspiration the movements appeared in the following order: 1) the anal sphincter opened, 2) a wave of opening moved anteriorly along the cloaca, 3) the stalk of the respiratory trees

opened when the wave had run to this level, with the result that the whole cloaca was now wide open; 4) the anus then closed, and 5) the body in the region of the cloaca, which had previously increased in diameter, now began to contract in such a way as to indicate that a wave of constriction was passing forward on the cloaca. This does not mean that the anal sphincter is the seat of origin of the pulsation wave, but is merely a description

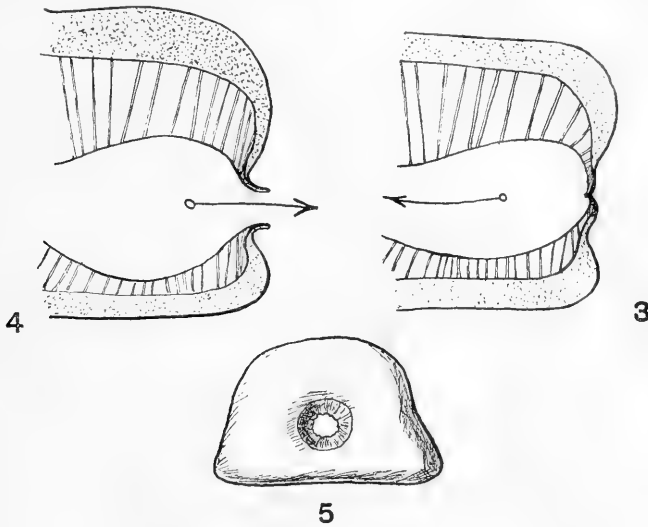


Fig. 3 Diagrammatic longitudinal section through the cloacal end, showing the relation of the sphincter during the pumping of water into the respiratory trees. Arrow indicates the course of the water.

Fig. 4 Showing the form of the anal brim during spouting. The arrow gives the direction of the current.

Fig. 5 View of the posterior end of *Stichopus* ($\times \frac{5}{12}$). The action of the anal dilators is shown by the irregular form of the edge of the brim.

of the pulsation as seen from the outside of the animal. When the anus was closed, and especially immediately after its sphincter had contracted and the constriction of the cloaca and body wall were increasing the pressure on the fluid within the cloacal chamber, the edge of the sphincter appeared to be acting after the manner of heart-valves, as indicated in figure 3. This was verified by the inspection, from the cut end, of amputated cloacae. Yet, when the cycle of movements began again with the opening

of the sphincter, its edge was pulled back sharply, a process which was also evident at the termination of spouting (fig. 4) and subsequently; this is the function of the minute muscles above described, which are placed on the coelomic face of the anal brim perpendicularly to the circular constrictors of the sphincter. The opening of the sphincter therefore involves not only the relaxation of the circular muscles but also the contraction of a set of antagonistic dilators, whereas in closing the action of these sets of muscles is reversed. During the opening of the anus the edge of the sphincter showed very clearly the action of the dilator muscle strands (fig. 5). The closure of the brim was usually not quick enough to catch all the water within the cloaca at the end of an expansion period, because the general contraction movement slightly preceded the constriction of the sphincter, so that some of the contained water escaped before the brim was completely closed. The time relations of the phases of a single pumping movement are indicated by the measurements summarized in table 1.

TABLE 1
Time relations of the phases of anal pulsation, in seconds

PHASE	LENGTH OF STICHOPUS	
	20 cms.	30 cms.
(1) Sphincter open.....	2.5-3.5	3.2-4.0
(2) Sphincter closed.....	2.4-2.8	2.6-2.9
(3) Expiration.....	9-10	13-15

In all the measurements of pulsation rate given in this paper, a complete 'pulsation' begins with the opening of the anal sphincter and ends with the start of the next succeeding opening movement. In practice this interval could be timed with extreme accuracy, since, immediately the anal sphincter began to open, the whitish interior of the cloaca became visible in the center of the dark brown or black field presented by the general surface of the animal. The slowness of the movements also greatly aided precision in their measurement. Five to ten pulsations were timed with a stop-watch, and the result reduced to

“time for ten pulsations;” where less than 10 successive pulsations could be timed, the observation was repeated two or three times; closely agreeing times were always obtained in successive determinations.

When the cycle of movements comprising a single pumping operation had been carried out some 6 to 10 times in succession, an interruption occurred, in the form of a wide opening of the anus persisting for some seconds (table 2), during which the water previously pumped in was forced out of the respiratory trees. The ratio of the number of inspirations to an expiration varied directly with the size of the animal.⁵ This expiration, or ‘spouting,’ was accompanied by retraction of the tentacles, constriction

TABLE 2

Stichopus moebii; occurrence of spouting in relation to the number of cloacal pulsations between each expulsion of water; / indicates an expulsion of water

LENGTH	TIME FOR TEN PULSATIONS	NUMBER OF PULSATIONS BETWEEN SPOUTING	AVERAGE NO.
<i>cms.</i>	<i>seconds</i>		
19	54	5/6/6/6/7/5/8/5	6.0
23	66	6/7/6/8/6/10/6	7.0
25	60	9/8/10/9/7/8/10	8.5
30	75	9/10/10/11/8/7/9/10	9.2

of the buccal sphincter, and a general body contraction. The interesting point in this connection, however, is that when the central edge of the anal sphincter was watched closely it was seen that the sphincter continued to pulsate at a normal rate, though with greatly reduced amplitude, in spite of the fact that it was being held wide open by the body-wall muscles and the strongly contracted radiating strands. The first inspiration succeeding a spouting act was usually of greater vigor than the following ones.

The pumping movements also ceased, the oral sphincter contracting firmly, when the posterior end of the animal was stimulated tactually, especially when the papillae were touched. Stimuli of various kinds applied to the animal's surface resulted in reactions which included the temporary constriction of the

⁵ A similar observation was made by Hérouard ('89, p. 689).

anal sphincter.⁶ Closure of the anus was also a preliminary operation when *Stichopus* was beginning to engage in locomotion.

The control of anal rhythm by the animal as a whole includes, then, (1) the cessation of pulsation by causing the anus to constrict (either in response to stimuli, or in locomotion), and (2) the great restriction of the amplitude of pulsation (in 'spouting') due to the contraction of the radiating muscles and the muscles of the body. Of these modes of control only one, contraction in response to stimulation from the outside, persists in the isolated cloaca.

As regards the function of cloacal pumping in holothurians, it is clear from the work of Bordas ('99) and Winterstein ('09) that the water drawn in and out of the respiratory trees serves to supply the coelomic fluid with oxygen, to remove excretory products, and at the same time to control the amount of fluid contained within the animal, upon which its locomotor movements depend.⁷ In connection with the respiratory function of pulsation it is of interest to note that if by repeated mechanical stimulation, pulsation is prevented from occurring for some minutes, the movements which occur when pulsation is resumed are not increased in rapidity, but are of greater amplitude than those normally seen in undisturbed specimens. This was also noted by Pearse ('08, p. 272) in the case of *Thyone*, and he drew the conclusion that the animal was in this way making up for its preceding oxygen deficiency. This deduction by no means follows, however, because when repeatedly stimulated holothurians decrease greatly in volume (cf. Crozier, '15) by expelling water from the anus, and when left undisturbed after such stimulation they tend to return to their original size. In doing this the first pulsations of the new series are more vigorous than usual, as is also found after each normal water expiration. The increase in amplitude observed after forced cessation of pulsation is therefore only remotely connected with respiration; this

⁶ These reactions were similar in every respect to those of the previously described *H. surinamensis* (Crozier, '15 [?]).

⁷ The movements of holothurians have recently been analyzed by Jordan ('14) from the standpoint of his conception of a 'Hohlorganartig' animal.

view is supported by the low oxygen requirements of echinoderms, to be considered subsequently, and the known presence of other modes of respiration than that associated with the respiratory trees. The phenomenon of increased amplitude of pulsation subsequent to forced inactivity in a pulsating structure is also well known in the vertebrate heart, and may be seen, for example, in such curves as those plotted by Vernon ('10) to show the recovery of the heart-beat after perfusion with protoplasmic poisons.

The occurrence of anal rhythmic pulsations in holothurians is not an isolated phenomenon, for such movements (with a suggested similar function) occur among Enteropneusta (Willey, '99, p. 244), both in the adult⁸ and in Tornaria (Willey, '99, p. 306), and in decapod crustacea (Miller, '10), etc. In the lobster and crayfish the muscular arrangements for producing anal rhythm are in a general way similar to those in holothurians, since in the former case circular muscles and radiating muscles running to the body wall also occur; furthermore, the coördinating mechanism is a local one (Miller, '10, '12).

c) Correlation with size. It has long been a matter of general knowledge that the activity of animals varies with their size, smaller animals being more active than larger ones. This rule holds conspicuously for the execution of rhythmic movements by animals of the same species. The rapidity of breathing movements, etc., are not, however, simply proportional to the reciprocal of length or any other body measurement, but the empirical curve expressing the relation between size, or weight, and activity is almost invariably of a rather complex hyperbolic type. Polimanti ('13) has recently reviewed the literature of this subject, and has supplied an additional example in the respiratory movements of Octopus. The equation derived from his data is of the form:

$$y = a + bx + cx^2 + \dots$$

where y = weight, and x = respiration rate.

⁸ I have observed that the amputated posterior end (4-5 mm. long) of the Bermudan Ptychodera sp. will pulsate in sea water. A sphincter ani is present.

The rate of rhythmic pulsation of the anal sphincter for animals of different sizes was observed in *Stichopus*, *Holothuria surinamensis*, *H. captiva*, and *Cucumaria punctata*. The results are given in figures 6 to 9. The data in the case of *Stichopus* (fig. 9) are more irregularly distributed than with the other

Relation between size and pulsation-rate

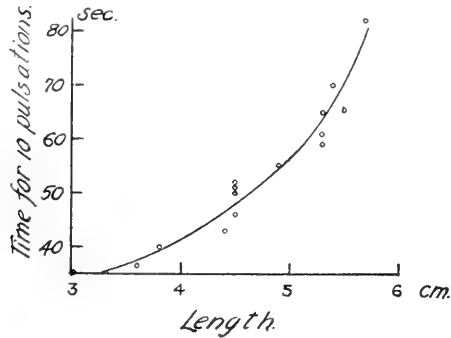


Fig. 6 *Cucumaria punctata*. Temp. = 24.5°

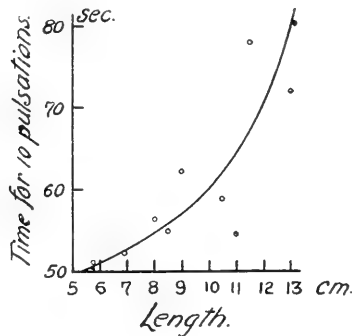


Fig. 7 *Holothuria surinamensis*. Temp. 25.0°

forms. This is due to the fact that in the instances measured the individuals of the other species were of more homogeneous history, in that their small size made it possible to keep numbers of them in a single aquarium, so that their pulsation rates were

all measured at one time. Because of their bulk the specimens of *Stichopus* had to be studied separately, often at intervals of some days, so that various factors, including temperature differences, probably entered, tending to lack of uniformity in the results.

Relation between size and pulsation-rate

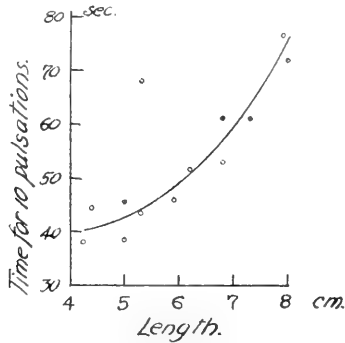


Fig. 8 *Holothuria captiva*. Temp. = 26.0°

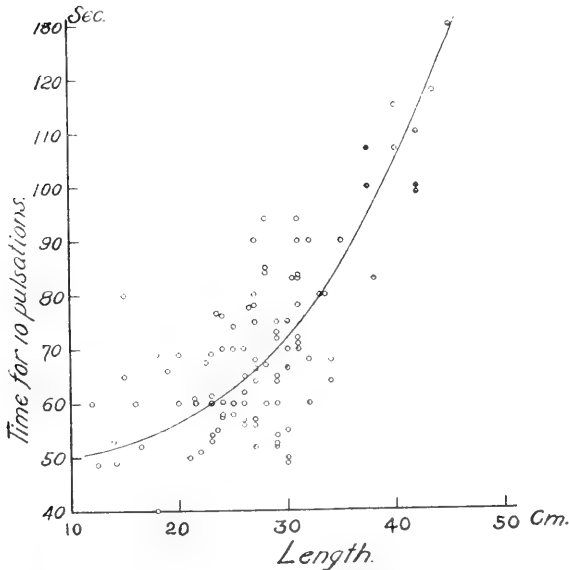


Fig. 9 *Stichopus mobii*. Temp. = 24°-26°

The smooth curves in figures 6 to 9 are in a general way similar to that of Polimanti ('13) for the rate of respiratory movements in Octopus. The weight of *Stichopus* is a simple function of length, such that

$$\text{Weight in grams} = (0.034 \pm) \times (\text{length in cms.})^3$$

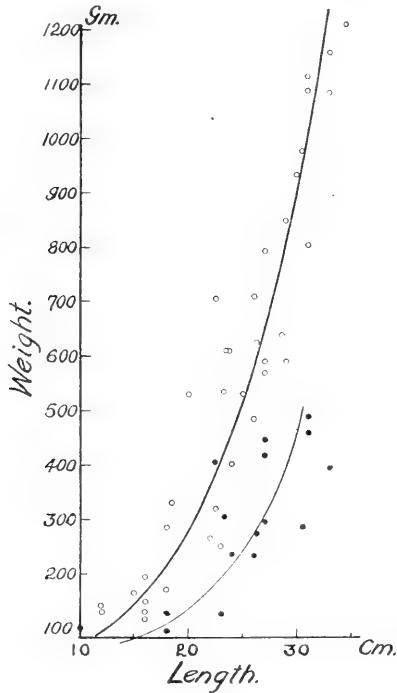


Fig. 10 Relation between weight and length in *Stichopus*: —○—○—, weight including that of the body fluids; —●—●—, weight of the integument alone.

(fig. 10); this type of relation between weight and length is also true for the other species. If pulsation-rate were plotted against weight the resemblance to Polimanti's curve would be increased. As shown in figure 10, the weight of the integument alone is too variable to give smoother results; the variation is produced mainly by the fact that the skin seems to absorb water to a varying extent, depending upon its rigidity. The curves relating size

to pulsation-frequency are of the same type as that found by plotting Mayer's ('06, p. 8) data on diameter vs. pulsation-rate in *Cassiopea*.

In attempting to account for the relation of pulsation-rate to size, it is possible to adopt the view that the rapidity with which an animal executes a given act is a measure of the amount of energy available for the performance of that type of work, and that when operating under similar conditions the relative energy content of different individuals in the same species may in this way be compared. According to this interpretation, larger animals must contain less motor energy, proportionately, than do smaller ones of the same kind. The observations of Tashiro and Adams ('14), that the cardiac ganglia of large *Limuli* have a lower output of CO_2 per gram of nerve-substance than do the (smaller) corresponding ganglia of smaller *Limuli*, and those of Child ('13, p. 140), who found by the KCN method that young (small) individuals of *Planaria* had a higher rate of metabolism than larger ones, lend support to this general idea.

III. COÖRDINATION OF THE PULSATING COMPLEX

The pulsation of the cloaca exhibits a high degree of coördination in the action of a number of individual effectors. The questions arise, From what center, if any, does the stimulus to pulsation proceed, and by what means are the various muscles brought to act in appropriate sequence?

The following observations and experiments bear upon the answers to these questions. The points to be considered are (1) the cycle of pumping movements in the cloaca, and (2) the cessation of these movements, with the exception of those of the anal sphincter, during the expulsion of an expiratory stream. It may be mentioned here that during defecation the sphincter movements are not interrupted, and as a result the faecal masses present beaded constrictions at regular intervals along their length, the constrictions (fig. 11) being formed by the pressure of the sphincter as it attempts to close. This applies to *Stichopus* and *Holothuria*; but in *Cucumaria* there are no

definite castings, because the ejected material is discharged in fragments during the course of an expiratory act.

a) *Effects of autoevisceration.* When *Stichopus* undergoes autoevisceration the cloaca ruptures in the region of the termination of the intestine, and the gut is passed out, pulling with it more or less of the respiratory trees; the whole then being autotomised,

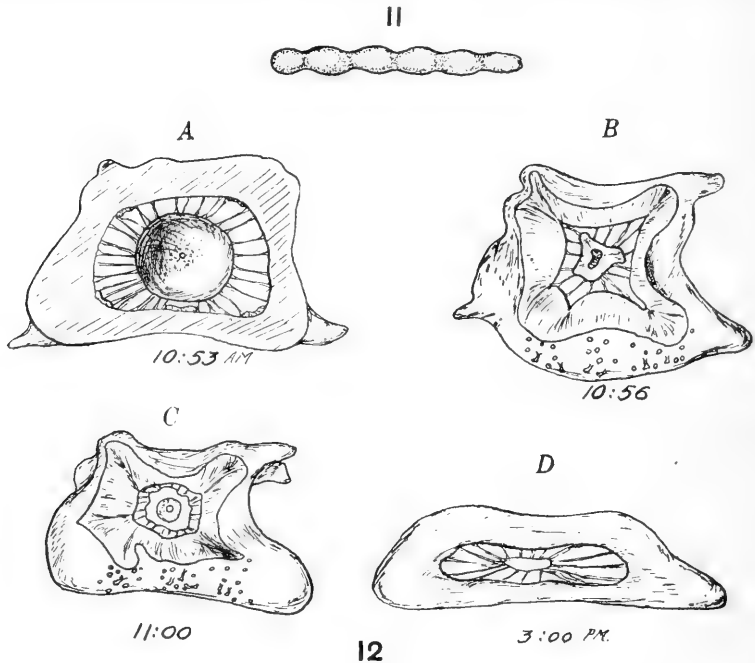


Fig. 11 A casting of *Stichopus* ($\times \frac{5}{12}$), showing the constrictions, somewhat exaggerated, due to the continuance of anal rhythm during defecation.

Fig. 12 Stages in wound closure, drawn from one preparation, at the periods indicated.

the animal which remains consists of the dermo-muscular tube and the cloaca. Such an animal continues to exhibit cloacal pulsation, the water, however, being now pumped into the body-cavity directly. The rate of pulsation in eviscerated animals was normal, up to at least twelve hours after autotomy, and the characteristic interruption of the inspiratory movements after every sixth to tenth one, by the expulsion of water, continued as

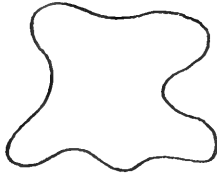
before evisceration. The general tonal depression of the animal involved some decrease in pulsation amplitude.

The stimulus to spouting, then, does not necessarily originate from a state of tension in the full respiratory trees.

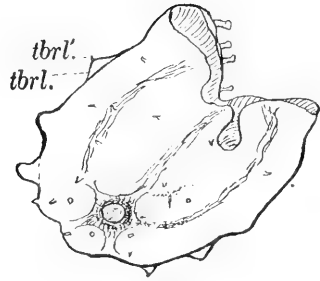
b) *Effects of amputation.* The cloacal end of *Stichopus* when amputated at the level α — α (fig. 1) contracted firmly. But if it was then put into sea water, or into one of a variety of salt solutions subsequently to be described, it very shortly opened up and continued to pulsate rhythmically, though with a gradually decreasing frequency and amplitude, for as much as thirty hours. The duration of pulsation depended upon the size of the excised piece, the volume and composition of the surrounding solution, and the existing temperature.

About one minute after excision the cut edges of the body wall began to bend inward (fig. 12 B). The cut edge of the cloaca itself was flared outward, while its lumen was closed by the contraction of a powerful circular muscle about midway between respiratory trees and anus. The radiating muscle-strands near the plane of the cut were relaxed, but they contracted when pinched. The flared anterior end of the cloaca was then pulled toward the anus, while the cut edge of the body wall was bending inward. These processes, tending to close the remnant of the coelom, had a highly protective appearance. Some three to four hours after the inbending of the edge of the cut, the body wall in this region became flabby and relaxed, and a progressive degeneration, which involved swelling and mucoid disintegration, began at the cut edge. After about five to six hours the inter-radii became sunken inward, so that a cross section of the pulsating piece had the appearance shown in figure 13; in addition, the previously inturned edges of the body wall were now relaxed. At this time, that is after about six hours' isolation in sea water, the excised cloacae looked like the one sketched in figure 14.

The history of individual preparations, made in the way described in the preceding paragraph, was followed under various conditions until they ceased to live. I shall refer here merely to the performance and fate of cloacal pieces contained in one liter of sea water. The time required for the execution of 10 rhythmic



13



14

Fig. 13 Cross-section of a cloacal piece, showing the sunken condition of the inter-radii.

Fig. 14 Sketch of a pulsating piece, from above ($\times \frac{5}{12}$).

tbl., one of the characteristic wart-like tubercles.

tbl.', the delicate tactile papillae which they bear (not noted by previous writers).

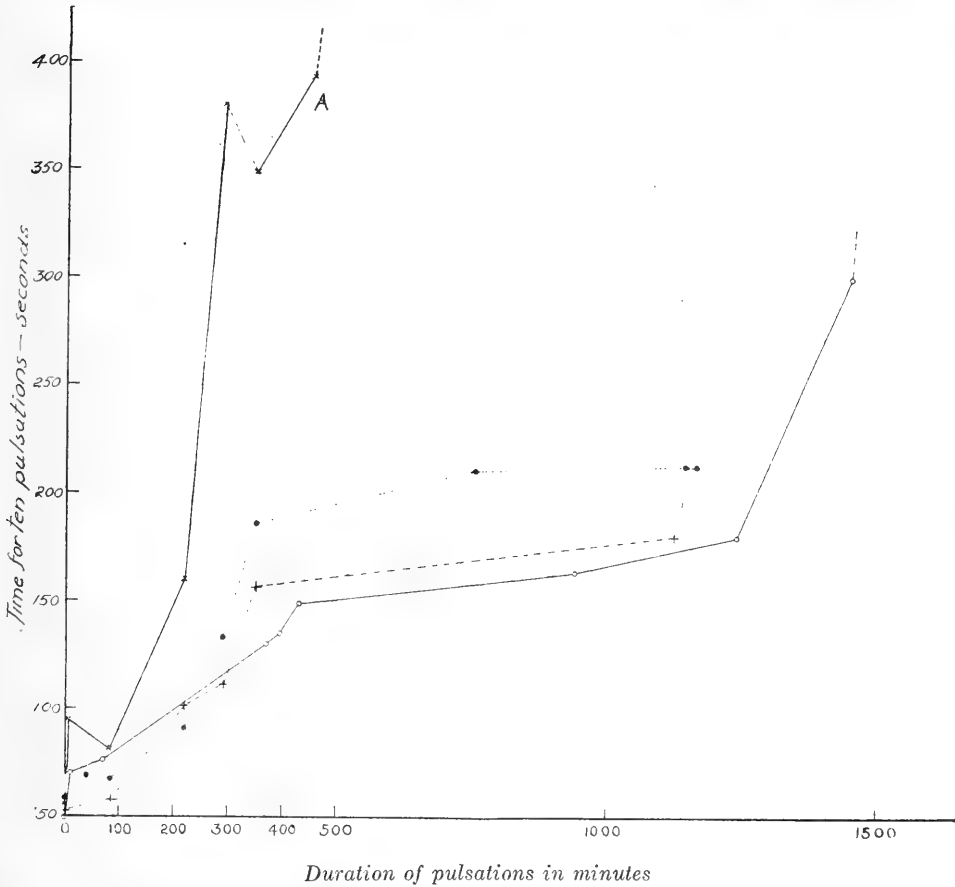
movements of the anal sphincter was measured at intervals of time subsequent to the isolation of the pieces. Exhaustion curves obtained in this way are illustrated by figure 15. A typical example is given in detail:

Experiment 33.3. June 21. *Stichopus moebii*; 24 cms. long; normal pulsation, 58 seconds for 10 movements. Temperature varied between 26° and 24°C.

MINUTES ELAPSED	SECONDS REQUIRED FOR TEN MOVEMENTS	NOTES
0		Cloacal end amputated; put in 1000 cc. sea water
2		Sphincter opening; beginning to pulsate
5		Pulsation becoming more regular
40	81.0	Amplitude fuller
85	68.5	Normal
220	91.5	
290	133.0	
350	176.0	Closure no longer complete
755	210.0	
1145	212.5	
1165	212.0	Barely pulsating. Soon stopped

There were in general two modes of exhaustion; one, a rapid type (marked 'A' in fig. 15), the other showing a sort of 'fatigue level.' The first type of exhaustion was exhibited by pieces of

shorter length than that of the other 'normal' pieces, i.e., cloacae cut off below the level α — α (fig. 1). It is possibly of significance that the earlier part of these exhaustion curves bears a superficial similarity to those of autocatalytic reactions, which might be taken to indicate that there is here an autocatalyst of exhaustion, represented by fatigue products. The sharp upward



bend of the curves near the time of cessation of pulsation indicates that pulsation continues until some critical condition is arrived at within the pulsating mechanism, beyond which spontaneous rhythm is not possible. The exhausted condition was

not reversible experimentally, though sphincters which had ceased to beat rhythmically in sea water would pulsate once in response to a touch or to the local application from a pipette of a small volume of a stimulating solution, and they could momentarily be revived by immersion in sea water free of Ca; possibly the mechanical stimulation involved in the last experiment was responsible for part of the pulsation.

A very significant fact about the pulsation exhibited by the isolated cloacal ends, is that the rhythm was not interrupted at intervals corresponding to the expulsion of the cloacal stream in the intact *Stichopus*, but was indeed perfectly continuous, unless complete contraction was induced by some especially applied stimulus. This condition is intelligible in view of the fact above noted that in the normal animal the edge of the sphincter was observed to pulsate with faint amplitude during spouting. It follows that the stimulus to spouting has its origin outside the cloaca.

The early parts of the exhaustion curves show a peculiarity which must be noted. Immediately after amputation and immersion in sea water, the contracted musculature of the cloaca began to relax, and by two minutes, at most, had begun to open. Very commonly it then remained wide open for some seconds, and when pulsation was resumed the rate of sphincter movement was very slow and closure incomplete. The rate of movement was soon improved, however, coincident with the institution of contractions and relaxations of the cloaca, which were of maximal amplitude. It would seem that the coördination of the members of the pulsating complex is disturbed by cutting this complex away from the rest of the animal, and that some little time must elapse before harmonious interaction can again be established. The full amplitude of the pulsation was usually revived very suddenly.

Rhythmic pulsation in the isolated posterior ends was similar in all essential respects to that in the intact animal. The time relations of the several phases of movement are given in table 3, which may be compared with table 2, showing the normal condition in the intact *Stichopus*. In the isolated pieces it could

be observed that the cycle of movements constituting a complete pulsation began at the cut end of the cloaca.

TABLE 3

Time relations, in seconds, of the phases of pulsation for the isolated cloaca in sea water

EXAMPLE	1	2
Open phase.....	5.6-7.3	7.0-8.0
Closed phase.....	3.0-5.7	5.2-5.6

The direction of the current of water produced by the isolated cloaca was studied with the aid of carmine suspended in sea water, and also by small 'flags' of mucous or bits of thread attached to the inner edge of the sphincter. The current was directed anteriorly, as in the intact animal. The maximum fluid pressure developed in the amputated pieces was ascertained by inserting a glass tube of appropriate size into the anus, which then contracted tightly about the tube. The pressures were never more than 1 cm. of sea water. In the intact animal the cloacal pressure is much greater, since the body muscles then play a greater part in the pumping.

The pulsating sphincter exhibited a refractory period, such that if the sphincter were touched at the very beginning of an opening movement, it continued to open to its normal extent; but if touched at the edge when more than one-half open, the brim closed down promptly, though some local retraction was evident at the point stimulated (fig. 16).

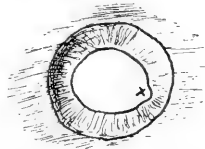
It was pointed out in a previous section that the rate of cloacal pulsation was correlated with the size of the holothurian. It was important to learn if this correlation persisted in the excised posterior ends. The rate of pulsation in cloacal pieces of equivalent size (i.e., amputated at the level α — α in fig. 1) derived from 10 *Stichopi* of increasing lengths is tabulated in table 4, from which it will be seen that, though some trace of this general effect may be maintained, it is by no means clear cut; the rate of pulsation in the isolated posterior ends was very rapidly reduced to an approximately uniform level soon after excision.

TABLE 4

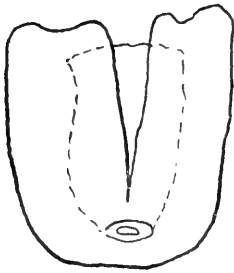
Relation of pulsation rate in the excised cloacae to the size of the animals from which they were obtained. Temperature = 26.0°

NO.	LENGTH <i>cms.</i>	TIME FOR TEN PULSATIONS	
		Before amputation <i>seconds</i>	After amputation <i>seconds</i>
1	23.5	55.3	88.6
2	24.0	58.0	70.1
3	26.0	64.3	88.0
4	27.0	80.0	90.2
5	28.0	84.1	100.2
6	28.3	85.0	89.5
7	29.0	65.4	85.0
8	30.0	70.0	84.3
9	31.0	78.5	86.4
10	31.0	83.0	80.8

16



17



18

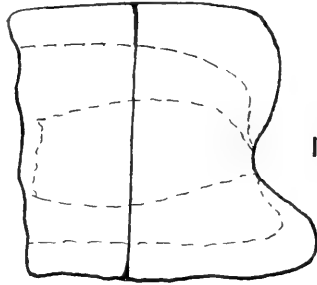


Fig. 16 Reaction of the opening sphincter to tactile stimulation at the point x ($\times 1\frac{2}{3}$).

Fig. 17 For explanation, see text.

Fig. 18 For explanation, see text.

The rate of rhythmic movement is, however, very much conditioned by the length of the cloaca included in the cut off piece. In table 5 is given a summary of the history of three posterior ends of different lengths cut from animals of the same size, which illustrates this point.

TABLE 5

Dependence of pulsation rate and duration upon the length of the excised piece. A = complete cloaca; B = about three-fourths of cloaca; C = one-half of cloaca; each from a Stichopus 26 cm. long

TIME ELAPSED SINCE AMPUTATION	TIME FOR TEN PULSATIONS		
	A	B	C
	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
<i>minutes</i> 10	71	93	115
70	81	90	123 ¹
250	99	125	∞

¹ Irregular movements.

This suggests that the stimulus to pulsation originates in the anterior part of the wall of the cloaca with its associating radiating muscles, in the sense that the structure which of itself pulsates in sea water faster than any other part of the cloaca is located there, and that its activity carries with it that of the other contractile parts. A somewhat parallel case is found in the vertebrate intestine, where the transmission of pulsation depends upon the myenteric plexus (Cannon and Burket, '13); Alvarez ('14) found that the rate of pulsation of strips from the small intestine varied inversely with the distance from the pylorus. The localization of the origin of pulsation in the cloaca of *Stichopus* will be further considered later in this paper.

Implied in the above description is the view that the fastest beating member of a pulsating complex determines the rate of movement of the whole. This idea was put forward by Loeb ('00^a p. 29) in his explanation of the reversal of pulsation in the tunicate heart; the neatest demonstration of the operation of this principle is probably found in an experiment of Mayer ('11, p. 7), who grafted together a large and a small *Cassiopea* and found that the faster beating medusa determined the pulsation rate of the whole mass.

The possibility presents itself that the stimulus to pulsation normally arises outside the cloacal region, and that the cut-off pieces continue to pulsate from a sort of 'habit' or 'organic memory.' It, indeed, is possible to impress rhythms upon holothurian structures, as I have discovered in certain experiments upon the physiology of the 'shading reflex.' Shadows were cast upon the anterior end of a *Holothuria surinamensis* at 0.25 minute intervals, to which the animal reacted (cf. Crozier, '14, '15) by more or less complete retraction of the tentacles and contraction of the buccal sphincter; after the 115th successive stimulation and reaction, the rhythmic shading was discontinued, but the animal *continued to retract the tentacles, etc., at very nearly 0.25 minute intervals for the next succeeding 3 minutes* (i.e., 11 times), after which the 'reactions' became of irregular occurrence. This observation was repeatedly confirmed.⁹ But the fact that the rhythm of the isolated anal parts could be caused to stop, by appropriate sensory stimulation, and then to resume again in perfectly reversible fashion, argues against this interpretation, as does indeed the whole behavior of the pieces in different salt solutions.

c) *Mutilation experiments.* Stichopus having the oral end, including the nerve ring, amputated, continued to exhibit rhythmic anal movements like those of the whole animal. As long as the new anterior end remained closed by the close approximation of the inturned edges of the cut, so that some internal fluid

⁹ Quantitative investigation of this phenomenon is contemplated, and should provide important data relative to the physico-chemical nature of 'protoplasmic memory.' Somewhat comparable rhythms of short period, impressed by experiment, are not unknown among plants. It is especially to be noted that the condition here described in *Holothuria* is one of 'positive memory,' in contrast to the 'negative memory' [the terms are my own] studied by Piéron ('09, and subsequent papers), who investigated the law according to which the sensitivity of snails to rhythmic shading was abolished. Piéron was, I believe, dealing with a condition primarily of sensory exhaustion. Both 'kinds of memory' are capable of mechanistic analysis, but that exhibited by *Holothuria* is more advantageous for experimental work. I venture to predict that the study of the impression of short-period rhythms upon animals, rather than the investigation of 'tidal memory' (of *convoluta*, etc., cf. Kafka, '14, Chap. 8), will afford the clue to the dynamics of primitive associative memory.

pressure could be produced, the typical spouting movements also occurred. Animals with the anterior end excised, however, rapidly lost tone and, in the case of *Stichopus*, died within a day or so. With *Holothuria surinamensis*, as appears also to be true of *Thyone* (Scott, '14, p. 289), anal rhythm becomes slow and weak soon after the amputation of the anterior end. This is associated with a general loss of tone and a totally quiescent condition, which is only removed upon the regeneration of a new anterior end (Crozier '15 [?]). The stimulus to 'spouting' is therefore probably derived from a condition of general body tension, resulting from the pumping of water into the interior of the body when the muscular integument presents a volume of definite size. The stimulus is not, necessarily at least, conditioned by a state of tension in the respiratory trees alone, since the eviscerated animals behaved in this respect as did the complete ones.

The muscles concerned in pulsation of the amputated cloacal end have previously been enumerated. Experiments were carried out to determine the significance of each of these. The results may be briefly stated thus:

Cutting out the cloaca, so that only the dermo-muscular tube and sphincter remained, resulted in complete cessation of movement. Scraping away the radiating muscles and connective-tissue strands had the same effect. Cutting the radiating muscles on only one side gave preparations which pulsated at normal rates; but in these preparations the side of the cloaca and brim on which the muscles were still intact closed and opened before the other side, the rest of the cloaca lagging behind in such a way as to give the impression of being "dragged along" with the intact half. The anal brim when cut out by itself remained open in sea water and did not pulsate, though it reacted, by a single closure, to delicate mechanical stimulations and to small volumes of various stimulating solutions.

If preparations of this sort, i.e., isolated sphincters—pieces deprived of the cloaca—or posterior ends in which all the radiating muscles had been cut, were placed in solutions of unusual Na/Ca content, they did exhibit rhythmic movement for some

minutes. These effects were secured in solutions of the following composition:

1) 95 cc. sea water + 5 cc. $\frac{N}{10}$ sodium citrate. Cloacae with the radiating muscles cut pulsed for about fifteen minutes, but irregularly; parts of the anal sphincter closed before others, so that the anus presented a ragged outline.

2) Van't Hoff solution without Ca. Effect the same as in (1), but less marked. In both solutions isolated sphincters pulsed for several minutes.

3) $\frac{M}{16}$ NaCl. Cloacal ends with the radiating muscles cut pulsed slowly, but the sphincter did not open at all.

4) 100 cc. $\frac{M}{16}$ NaCl + 2 cc. $\frac{N}{10}$ NH₄OH. Slow pulsations, with incomplete closure of the anus.

In none of these tests did the pulsation last for more than a few minutes. These experiments are not, of course, decisive as regards an answer to the question of the relation of Ca to pulsation. Continuous stimulation resulting from the action of an exciting solution upon ectodermal sense organs, or upon muscles, might, in connection with the refractory period, produce the same result.

The integumentary nerve-net does not play a *necessary* part in the transmission of the wave of pulsation, since cloacal pieces having the integument cut in various ways (figs. 17 and 18) pulsed with complete coordination after some preliminary re-adjustment following the disturbances of the operation. To give an example:

Experiment 84.2. July 22, 3.44 p.m. a cloacal piece, pulsating in sea water at the rate of 105 seconds for 10 pulsations, was cut as shown in figure 18, so that two separate rings of the integument were each connected with corresponding parts of the cloaca by the radiating muscles.

3.52 Posterior part pulsating feebly.

3.57 Both parts beating. Pulsations of normal amplitude; rate, 96.9 seconds for 10 movements.

4.10 103.9 seconds for 10 movements.

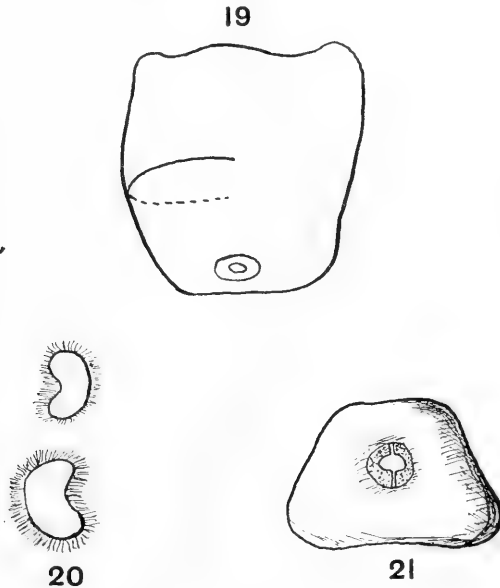
4.30 115 seconds for 10 movements.

8.45 Pulsating in irregular fashion.

The cloaca itself is therefore able to control the coordination of the pulsating system.

That the integumentary part of the apparatus does normally enter into transmission is indicated by tests made under the following conditions: 1) with the skin completely cut through on one side (fig. 19), and 2) with cloacal sphincter cut into lateral halves (fig. 20).

In the first case the sphincter and cloaca on the cut side lagged behind the opposite side, both in systole and in diastole.



Figs. 19, 20, 21 For explanation see text

In the second instance the brim no longer pulsated in coördinated manner and ceased to move after a few minutes. If one of the halves was gently pinched, it reacted alone, by the constriction of its circular muscles; whereas if stimulated more vigorously, or if a nearby point on the skin was stimulated, both halves of the brim contracted, the one nearer to the irritated place contracting sooner.

The effects of stimulation are thus conducted in a radiating fashion, such as would result if an ectodermal nerve-net were operating, and I conclude that such a net is present.

The rôle of the cloaca in pulsation, and especially of its anterior end, could be demonstrated very clearly. Isolated cloacal ends immersed in sea water containing $\frac{M}{200}$ caffeine presented this condition: During the early stages of the history of a piece in such a solution the anal sphincter pulsed at a normal rate of 10 movements in 70 to 80 seconds, but the cloaca was tightly contracted excepting immediately in the region of the anus (fig. 21). If now in such a preparation the contracted anterior end of the cloaca was exercised, all rhythm very promptly ceased. This points to the conclusion that the stimulus to pulsation originates at the anterior end of the cloaca with its associated radial muscles and is conducted posteriorly along the cloaca even when the cloaca itself is not pulsating. The fact that in certain salt solutions (e.g., NaCl M 5/8) the cloaca continued to beat after the anus had ceased, serves to strengthen this idea. The excised cloaca alone in sea water pulsed, though in a somewhat irregular manner. In recovery from the immediate effects of amputation, the cloaca and anal sphincter, and especially the former, began to pulsate before the body-wall portion of the complex.

It is taken for granted in this discussion that the coördinating mechanism is essentially nervous in character, and this view is supported by the experimental results. It has been suggested, however, that there is a chemical basis of coördinated pulsation in the case of the vertebrate intestine; Weiland ('12) claims to have extracted from the mammalian digestive tube a substance which, acting on Auerbach's plexus, leads to coördinated rhythmic movement. It must be admitted that the analysis of peristalsis upon the basis of the tonus idea (cf. Cannon, '11) does not account for the inception of the stimulus to contraction, and that therefore some further link is needed in the chain of pulsation processes.

d) Summary. The course of events in a pulsation cycle may be pictured as beginning with the opening of the anterior end of the cloaca, due to the relaxation of its circular muscles and the contraction of the associated radiating fibers. The stimulus

derived from their contraction is transmitted posteriorly¹⁰ by the integumentary nerve-net, while the wave of opening travels posteriorly along the cloaca, until finally the anal sphincter opens. Upon the contraction of the sphincter a wave of constriction travels anteriorly on the cloaca, forcing out the contained water.

IV. RELATION OF PULSATION TO TEMPERATURE

The large size of *Stichopus* precluded any attempt to employ the entire animal in a series of temperature experiments, both because there were no large thermostats available and because the thickness of its integument is prejudicial to the rapid establishment of uniform temperature conditions throughout the animal. For these tests therefore I employed *Holothuria surinamensis* and *H. captiva*; they were subjected to temperature changes in beakers of thin glass contained in a heating or cooling bath. Temperatures were measured by an enclosed-scale thermometer reading to 0.01°, placed close to the cloacal end of the animal. The instrument was calibrated. The results of one experiment, typical of all others, are given in figure 22.

This curve is entirely characteristic of the temperature curves found in connection with many other biological phenomena, in that it is of an exponential character, with a temperature coefficient (12.5°–22.5°) of about 2.4 ±.

In attempting to obtain temperature coefficients of pulsation rate in the case of the amputated cloacal ends of *Stichopus*, there entered a very considerable time factor. The method of procedure consisted in (1) obtaining records of rhythm in pieces which had recovered from operative shock and were subjected to fairly slow temperature changes (table 6), and (2) in subjecting similar pieces to sudden changes of temperature and estimating rhythm-rate after 5 to 10 minutes had passed and thermal equilibrium had presumably been established (table 7). The

¹⁰ Experiments on *Stichopus* confirm the results obtained with *H. surinamensis* (Crozier, '15 [?]), to the effect that the responses to sensory stimuli in the posterior region of the animal tend to be conducted posteriorly toward the anus, which often reacts before there is visible any local response to the stimulating agent.

time factor enters in two ways, for in addition to the slow exhaustion which occurs even at the normal temperature and is itself probably affected by temperature, extreme thermal conditions also exercised a characteristic effect of their own which was manifested in a kind of hysteresis. By a condition of hysteresis I mean that subjecting a holothurian or its isolated cloacal end to

TABLE 6

Experiment 33.2. June 21. Stichopus moebii, 31 cms. long; 83 seconds for ten pulsations

TIME	MINUTES	TEMPERATURE	SEC./10 P.	NOTES
		<i>D. g. C.</i>		
8.40				Cut off end. Open phase prolonged. Parts dissociated. No movement.
8.46	6	24.5	80.8	
9.04	14	25.0	122.0	
9.06	16	26.5	118.0	
9.07	17	27.6	105.0	
9.08	18	28.7	98.0	
9.10	20	29.4	100.0	
9.11	21	29.7	135.0	
9.12	22	29.8	143.0	
9.15	25	30.0	150.0	
9.19	29	32.0	164.0	
9.25	34	34.0	∞	

TABLE 7

Experiment 39.4. June 24. Stichopus moebii 29 cms. long; 74 seconds for 10 pulsations, at 24.3°. Fifteen minutes after excision, plunged successively into water at different temperatures

TEMPERATURE	TIME FOR TEN PULSATIONS
<i>Deg. C.</i>	<i>seconds</i>
25.0	94
14.0	198
16.0	187
17.0	186
19.5	140
21.2	127
19.6	144
20.0	155
15.0	192

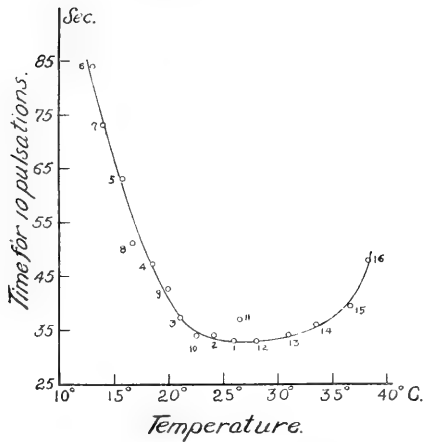


Fig. 22 *Holothuria captiva*. Relation of pulsation rate, in the intact animal, to temperature. The numbers on the curve indicate the succession in which the observations were taken.

a temperature of, say, 10°, produced an anaesthetised-like condition which persisted for a considerable time after its removal to some higher temperature and thermal equilibrium had in all probability been reached. By way of illustration:

Experiment 39.2. June 24. *Stichopus moebii*, 27 cms. long; 78 seconds for 10 pulsations at 25.0°; slowly cooled.

TIME	MINUTES ELAPSED	TEMPERATURE	TIME FOR TEN PULSATIONS	NOTES
<i>P.M.</i>		<i>Deg. C.</i>	<i>seconds</i>	
3.25	0	25.0	64	Six minutes after amputation
3.33	8	25.0	88	
3.37	12	22.0	109	
3.45	20	12.0	215	
4.00	35	10.0	∞	Closed; no pulsation
4.20	55	17.0	∞	Beginning to open slightly
4.30	65	18.2	∞	Opening slowly
4.45	80	18.5	216	Not opening completely
4.50	85	18.9	188	Fuller amplitude

Experiment 34.1 June 21. *Stichopus moebii*, 29 cms. long; 76 seconds for 10 pulsations at 25.0°; cloaca amputated; during the following 18 minutes, slowly heated up to 30°; held at 30° for 10 minutes, with the following results.

ELAPSED TIME	TIME REQUIRED FOR TEN PULSATIONS	NOTES
<i>minutes</i>	<i>seconds</i>	
2	135	
5	146	
8	198	
10	∞	Wide open; no pulsation

Then cooled down slowly (for 19 minutes) to 20.0°; pulsations (slowly revived) required 150 seconds for 10 movements (the usual rate 60 minutes after amputation being 60 to 75 seconds for 10 pulsations). All movements ceased in about 10 minutes more.

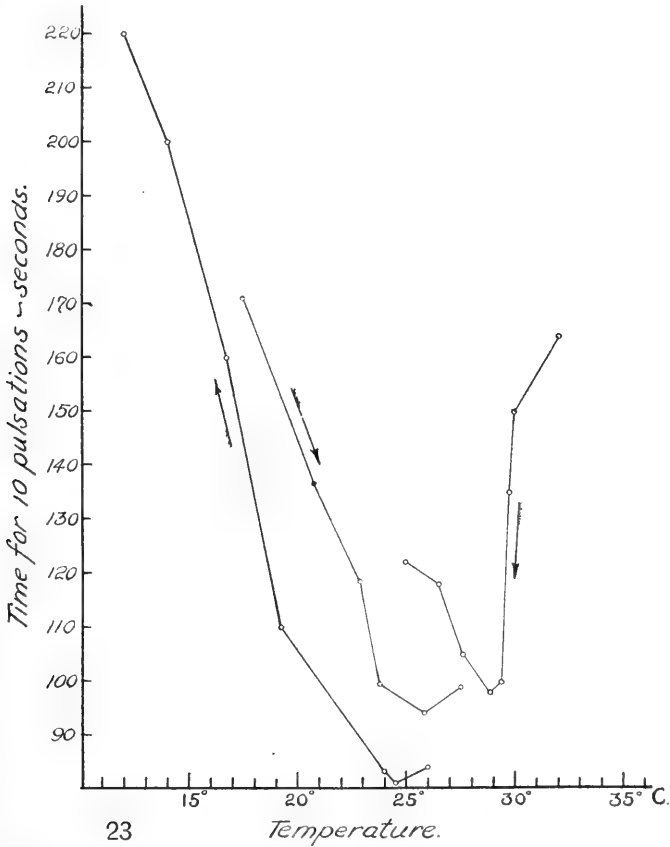
These experiments were checked by many others of similar type which gave essentially the same result.

The main object of these tests being to ascertain the magnitude of complications possibly introduced into the experiments by temperature effects, there is given in figure 23 some of the curves derived from different tests under several of the possible conditions of temperature change, and in figure 24 are collected the data from all the temperature experiments with isolated cloacae.

The discussion of these results with regard to the significance of the temperature coefficient would be unprofitable,¹¹ since (in view of the source of complication above noted) decisive evidence could be obtained only by the study of isothermal exhaustion curves. But it may be pointed out that the hysteresis effect to which attention has been called indicates that some physical alteration of the substance of the pulsating tissues has occurred at the temperatures which produce this hysteresis effect. It is difficult to believe that this influence is discontinuous, and, as Adrian ('14) has most recently pointed out, the van't Hoff equation holds only in homogeneous systems in which no alteration of the components is induced by temperature changes. In other words, there is a time-factor to be considered when working with temperatures removed from the normal. It is the neglect of this

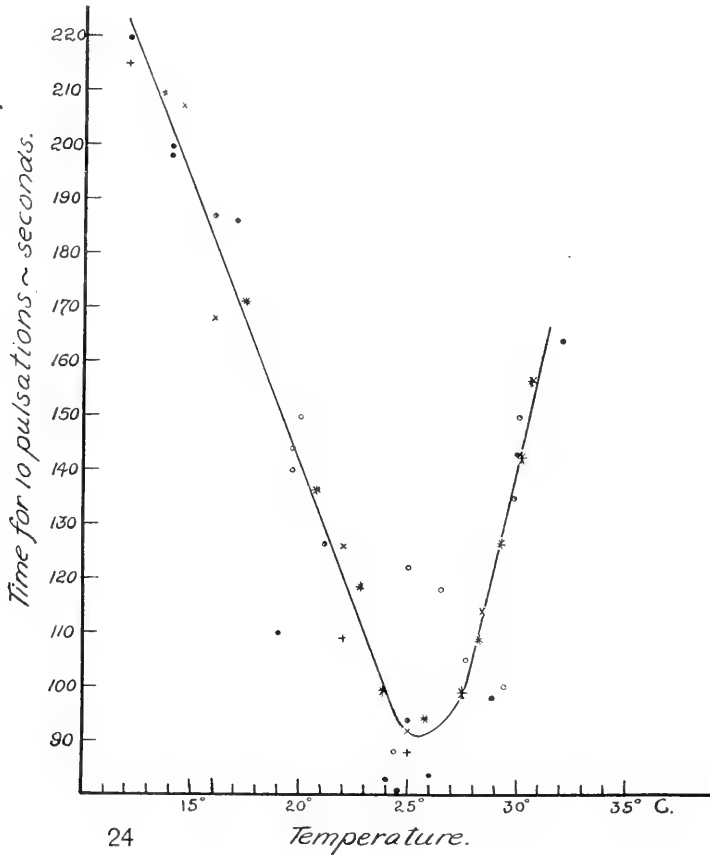
¹¹ Pütter ('14) has recently given an exhaustive treatment of the temperature-variability curve from the standpoint of the linked reactions occurring in living systems.

factor which renders valueless for exact purposes much of the experimental evidence concerning the influence of temperature upon marine animals (e.g., as in the paper of Mayer, '14^a); it is simply incorrect to say that an animal dies at such and such a temperature, for it dies at T° after being heated thereto at a certain rate and kept at T° for a certain length of time.



The curves (figs. 23 and 24) show that there is a rather sharp maximum of pulsation rate in the neighborhood of 26° . No pulsation was apparent below 12.5° , nor above 32.0° . These limiting temperatures hold for the rates of heat change, etc., used in these experiments; there is no more a definite upper

limit to the physiological temperature scale than there is a lower one (Cameron and Brownlee, '13). On either side of the minimum in "time for 10 pulsations" the curves are rather steep and of exponential character, though this is obscured in the collected



data (fig. 24). The range of laboratory temperatures, obtained from temperature measurements in connection with each experiment, was from 24° to 27°, hence very little, if any, complication due to a temperature effect entered into the subsequent experiments.

It is interesting that the temperature of maximum pulsation rate should be so near the temperature, at this time of year, of the sea water in which the animals were living; this was 24° to 27° in the day time.

V. ACTION OF DEPRESSING AGENTS

a) *Anaesthetics*. The effects of typical anaesthetics upon rhythm in the isolated cloaca of *Stichopus* are illustrated by the following:

Chloroform

Experiment 46.1. July 29. *Stichopus moebii*, 21.5 cms. long; before excision of cloaca, 61 seconds for 10 pulsations.

TIME ELAPSED AFTER AMPUTATION	NOTES	TIME FOR TEN PULSATIONS
<i>minutes</i>		<i>seconds</i>
3	Began to pulsate in sea water	
11		56
15	Transferred ² to sea water half-saturated with CHCl ₃	
20		113
21		120
22	Pulsation stopped	∞
24	Washed, and put in normal sea water	∞
40	Recovering amplitude	105
57	Amplitude fully recovered	130
90	Normal	145

² Previous tests had shown that transferring the pieces from one solution to another had no effect on pulsation rate, even though during handling the sphincter was closed.

Ether, ethyl alcohol and ethyl carbamate gave similar results, i.e., the depression of rhythm was, within limits, reversible. Magnesium sulphate to the extent of 0.25 M added to sea water stopped pulsation in about 10 to 12 minutes. Urethane and chloretone were compared quantitatively, with the result that when freshly isolated cloacal pieces were immersed in $\frac{M}{200}$ chloretone in sea water they ceased to pulsate in less than 3 minutes, whereas in $\frac{M}{200}$ urethane pulsation lasted for as much as 80 minutes.

In all cases of anaesthetisation the quiescent condition showed the anal sphincter in diastole and the cloaca itself more or less closed, that is, with the radiating muscles relaxed.

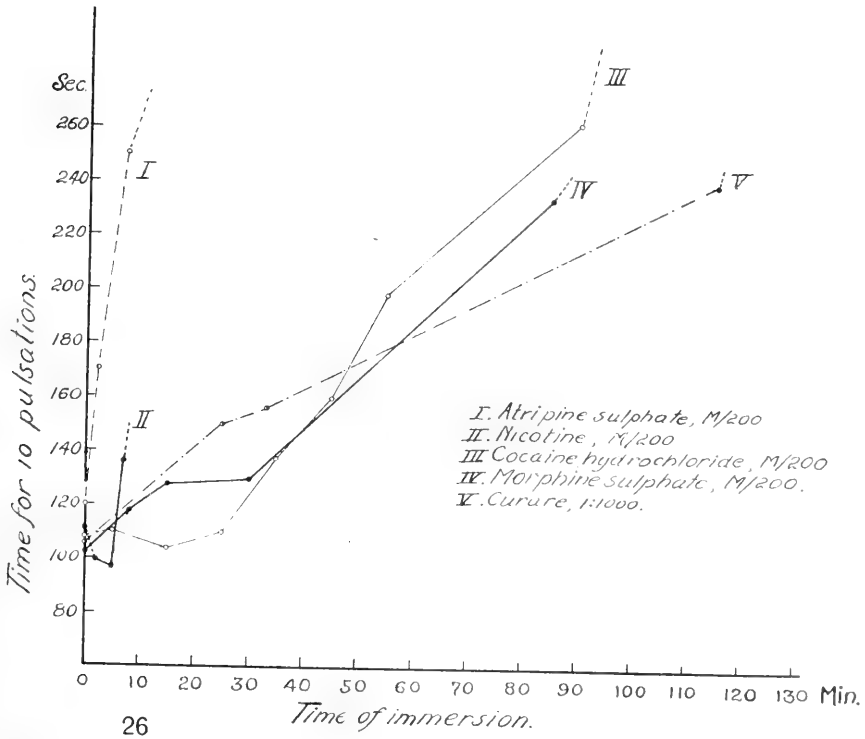
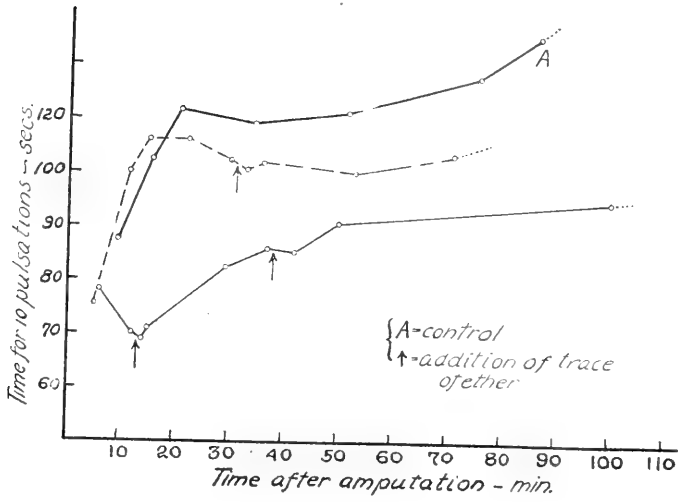
Traces of anaesthetics commonly induce a temporary acceleration in the rate of rhythmic movements. This is also the case with the cloaca of *Stichopus* (fig. 25). The effect on pulsation *rate* was less evident than the concomitant increase in amplitude and vigor of contraction. The effect lasted only a few minutes.

TABLE 8
Volume of solution in each case = 250 cc.

SUBSTANCE	CONCENTRATION	DURATION, IN MINUTES, OF		NOTES: CONDITION OF ANAL SPHINCTER AFTER STOPPAGE
		Pulsation	Irritability	
Curare.....	1 : 1000	130	170	Circular body muscles contracted, longitudinal ones relaxed; = open
Caffeine.....	M/200	25	160	Cloaca contracted; sphincter open
Nicotine.....	M/200	8	12	Contracted
Cocaine hydrochloride	M/200	90	100	All muscles relaxed
Atropine sulphate.....	M/200	28	60	Wide open; radiating muscles strongly contracted
Morphine sulphate....	M/200	140	165	Relaxed
Sea water control.....	540±	1100±	Sphincter ± open, all muscles relaxed

b) Alkaloids. The influence of depressing alkaloids was also studied; in each instance the history of five individual isolated cloacal ends being followed in detail and the general result checked by less detailed observations upon other pieces. The results with regard to pulsation and irritability to mechanical stimulation are summarised in table 8. Representative individual curves of exhaustion in these solutions are plotted in figure 26.

The powerful effects of nicotine and atropine are comparable to their influence on many other types of smooth muscle.



VI. RELATION OF PULSATION TO OXYGEN AND METABOLIC PRODUCTS

a) *Oxygen*. It has previously been shown that holothurians would live for relatively long periods in sealed jars of boiled sea water (Crozier '15). This was in conformity with the observations of Moore and his collaborators ('12, '14), showing the small amounts of dissolved oxygen used up in the activity of some marine animals, including echinoderms. The isolated cloacal ends of *Stichopus* continued to pulsate for over 700 minutes in jars of sea water which had been boiled, sealed and subsequently cooled to room temperature out of contact with the air. In preparing this water the evaporation was very slight, but possibly some faint increase in alkalinity was induced. The course of a typical experiment is illustrated in figure 27 (B), plotted from the record of experiment 78.3, with which may be compared the control (A), begun at the same time.

Experiment 78.3. July 10-11. *Stichopus moebii*, 20 cms. long; 60 seconds for 10 pulsations at 23.5°; volume of boiled sea water, 550 cc.

MINUTES ELAPSED	TIME FOR TEN PULSATIONS	NOTES
	<i>seconds</i>	
0		Cloacal end amputated and put in sealed jar of boiled sea water
10	78	
20	78	
35	78	
50	91	
65	88	
95	92	
350	109	Amplitude decreasing
740	170	Pulsations feeble and irregular
752		Taken out of jar and transferred to fresh sea water. Not pulsating
764	150	Closure nearly complete but more or less irregular
778	180	Irregular movements; no wide-open phase
800		Twitching irregularly; barely alive

The dependence of pulsation upon oxidations is probably indicated, however, by the high toxicity of KCN, as, for example, in

Experiment 71.2. July 25. *Stichopus moebii* 27 cms. long; cloacal end amputated and immersed in 200 cc. sea water + 5 drops $\frac{M}{10}$ KCN.

TIME OF IMMERSION	TIME FOR TEN PULSATIONS	NOTES
minutes	seconds	
5	80	Not closing completely
10	147	Closed phase very brief
17	84	Full amplitude
23	111	Closure not complete
38	∞	Irregular movements
53		Dead

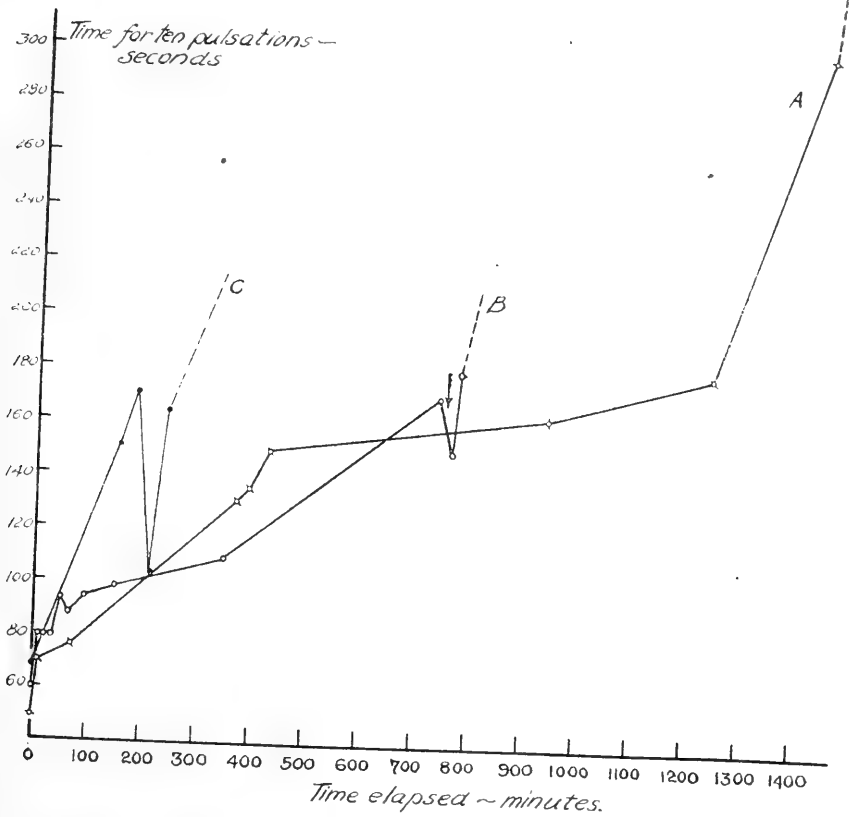


Fig. 27 A = control, in sea water. B = in sealed jar of boiled sea water. C = sea water (300 cc.) + 5 per cent urea (5 cc.).

Note: At the point marked ↓, 'B' had ceased to pulsate, and was transferred to normal sea water, in which it temporarily revived.

This effect was uniformly obtained in other experiments of similar nature.

b) Carbon dioxide. Carbon dioxide was a powerful agent in suppressing pulsations. Tests were made by adding to sea water small volumes of rain water charged with CO₂. The carbonated water was slightly acid aside from its H₂CO₃.

Experiment 79.2. July 11. Three freshly amputated cloacal ends were immersed each in 200 cc. of sea water to which 2 cc. of 'carbonated water' had been added. They did not pulsate in this mixture. After ten minutes immersion the pieces were transferred to normal sea water. Pulsations revived in 5 to 7 minutes.

Concordant results were obtained by adding CO₂-water to solutions containing pulsating pieces, the pulsations being rapidly reduced in amplitude and usually stopped within a few minutes when from 1 to 2 per cent of the 'carbonated water' had been added.

c) Urea. Urea likewise had a depressing effect upon pulsation. Fosse ('13) identified urea in echinoderms and their excretory products.

Experiment 38.2. June 24. *Stichopus moebii*, 27 cms. long; 68 seconds for 10 pulsations at 25.1°; cloacal end amputated and placed in 300 cc. sea water + 5 cc. $\frac{M}{10}$ urea.

IMMERSION	FOR TEN PULSATIONS	NOTES
<i>minutes</i>	<i>seconds</i>	
153	151	Very long open phase
184	170	Irregular amplitude
214	103	Somewhat improved
242	164	Open phase long
360	∞	Sphincter half open; reacts only slowly to tactile irritation

This particular experiment is plotted at *C* in figure 27; it was checked by four other experiments, which yielded the same result.

d) Light. With *Holothuria surinamensis* and *H. captiva* it was previously found (Crozier, '14, '15) that light exerted a distinctly toxic influence on the animals. It was therefore expected that sunlight would affect in some way the pulsation rate

of the isolated cloacal ends. *Stichopus* is much less sensitive to photic irritation than is *Holothuria*, and no influence of light upon either rate or amplitude of pulsation could be detected. In *Holothuria* it has been shown with some degree of probability that the green fluorescent integumentary pigment acts as a photosensitizer (Crozier, '14); hence a photic effect upon cloacal pulsation would be much more probable in this case.

Isolated cloacal extremities of *Stichopus* which had ceased to pulsate regularly in sea water, though otherwise in apparently good condition, were placed in moderately bright sunlight. This did not stimulate to pulsations. When such pieces were shaded, the sphincter reacted by closing fairly tightly; the reaction time was about 1.2 seconds at 25.0°. Pieces which were pulsating slowly (130 seconds \pm for 10 movements) gave the same reaction, the sphincter closing promptly when the shadow was cast during the open phase, but failing to react during a period of closure. Light did not accelerate the rate of movement in normally pulsating cloacal ends.

The isolated cloacal extremity of *H. surinamensis*, however, gave the following result:

Experiment 53.2. Seven specimens of *Holothuria surinamensis* had the posterior ends (1.5 cms. \pm) removed. After remaining about 30 minutes in diffuse daylight, their pulsation rates were observed (column *a*). Four of the pieces were then placed in bright sunlight (*b'*), the other three remaining in the diffuse daylight (*b*). After 15 minutes the pulsation rates were again determined (*b, b'*):

Time for ten pulsations, seconds

IN DIFFUSE LIGHT.		AFTER FIFTEEN MINUTES IN BRIGHT SUNLIGHT	
<i>a</i>	<i>b</i>	<i>b'</i>	Notes
80		137	Very irregular Brim only beating
90		54	
84		∞	
130		∞	
70	72		
81	80		
76	84		

The cloacae exposed to light did not recover on return to the shade. Light exerted its typical effect upon the excised cloacal ends, and at a rate which is comparable to that at which it causes the disappearance of sensitivity to shading in the intact animal (Crozier '14, '15).

e) *Summary.* Lack of oxygen would appear to be much less powerful as a depressant of rhythmic activity than the presence of urea,¹² CO₂, and other typical metabolic products (the effects of acids will be discussed subsequently in a separate section), though the suppression of oxidations by KCN is rapidly effective in stopping movement. In those cases in which light produces toxic modifications in the animal (*H. surinamensis*, *H. captiva*), this agent also causes rapid cessation of rhythm.

VII. INFLUENCE OF OSMOTIC PRESSURE

a) *Dilutions of sea water.* According to Henri et Lalou ('03), the membranes of *Stichopus regalis* which are exposed to contact with water are almost perfectly semi-permeable, the salt concentration in the ambulacral¹³ and perivisceral fluids being normally slightly less than that in the sea water. They found that *S. regalis* readily accommodated itself to moderate dilutions of the sea water in which it was placed.

In the experiments about to be described it was found that diluted and concentrated sea water exerted characteristic effects upon cloacal pulsation. It should be remembered that in these experiments with the isolated cloacal ends the medium had access to the internal surfaces of the body wall and cloaca, and further that the cut surfaces of the integument and cloaca at the exposed end were freely open to its action.

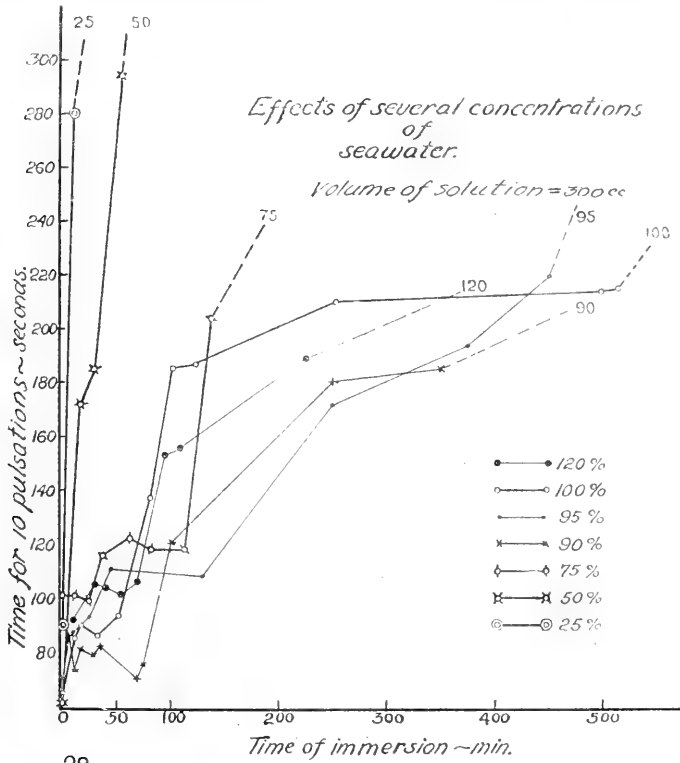
The pulsation-rate of amputated cloacal ends was observed in sea water at the following volume percentage concentrations: 0, 25, 50, 75, 90, 95, 100, 110, 120.¹⁴ The history of typical indi-

¹² According to Henri et Lalou ('03) the membranes of *Stichopus regalis* are impermeable to urea, but in my experiments a cut surface was exposed to the action of the fluid. Furthermore, the remnant of the body cavity was freely open.

¹³ From the Polian vesicle.

¹⁴ The dilutions from 100 per cent were made by the addition of rainwater; the 110 per cent and 120 per cent solutions were made by evaporation of sea water to the required volume. Normal Bermuda sea water contains about 36.8 p.p.m. salt (Mark, '13).

vidual cases is plotted in figure 28. The tests indicated that above 50 per cent concentration, mixtures of sea water with rain water tended to preserve about the normal pulsation-rate, but that in these mixtures the duration of pulsation was less than that of the control (100 per cent); below 50 per cent sea water



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the pulsation-rate and duration fell off rapidly. Above 100 per cent concentration the duration of pulsation was also curtailed. The general nature of these effects did not differ whether (a) the cloacal pieces were immersed in the solution immediately after amputation, or (b) after pulsation in the amputated end had been allowed to start in sea water; procedure 'a' was followed in the cases recorded.¹⁵ In these experiments the pieces

¹⁵ With this method of immersion, the pieces were in the condition shown at A in figure 12, and the solution therefore had free access to the interior parts of the cloacal end.

to be immersed in a given volume of diluted or evaporated sea water were first washed (30 to 60 seconds) in a stream of sea water of the corresponding concentration. The volume of solution containing a single isolated cloacal end was in each case 300 cc.

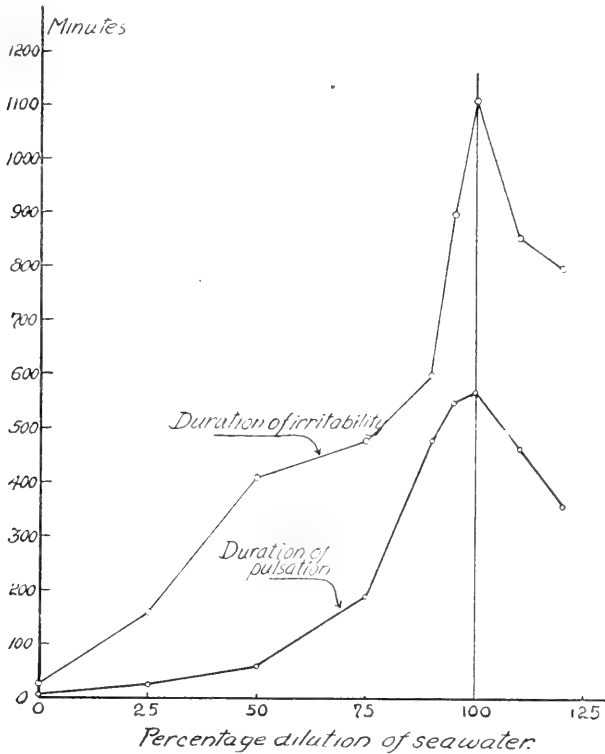


Fig. 29 Based on experiments with 45 specimens of *Stichopus*

Five individual pieces were carefully studied at each concentration of sea water and their behavior checked by less detailed observations upon a number of other examples. The average times for the continuation of pulsation and of irritability to mechanical stimulation under the conditions just specified (at 25° to 27°) are given graphically in figure 29. As regards the condition of the cloacal sphincter after the cessation of pulsation, this difference was noted between the action of rain water

and the various dilutions of sea water, namely that in the former case the sphincters remained wide open, whereas in the latter instances the sphincters (as in 'normal' exhaustion in 100 per cent sea water) were more or less contracted, though with the rain-water effect it was not a matter of the contraction of the anal dilators, but rather of the more complete relaxation of the circular constrictors.

With rain water it was found that the brownish skin pigment rapidly made its appearance in the water surrounding a beating cloacal end even before pulsation ceased. This effect became apparent after immersion of about 5 minutes. It was, under certain circumstances, found with other concentrations of sea water, but (as will be shown subsequently) it could be inhibited by the presence of non-electrolytes. This reaction may indicate either that the permeability of the superficial cells had been very highly modified, or that the cells surrendering their coloring matter were dead; inasmuch as the pigment loss did not occur more at the region of the cut end of the pieces than at any other place, and could be inhibited by the addition of sea water, and further inasmuch as the region of the cut end did not give off any visible amount of pigment into normal sea water, it might reasonably be held that the latter alternative is not necessarily the correct one. It is possible that at concentrations much removed from that of normal sea water the cells of *Stichopus* became rather highly permeable for salts while still alive. This possibility was suggested by the very evident amounts of chlorine found in rain water in which pulsating pieces had lain. If this permeability for salts could be proved over the lower range of concentrations here dealt with, the form of the lower parts of the curves in figure 29 might readily be accounted for; between 0 per cent and 95 per cent these graphs might result if the critical internal conditions (salt concentrations?), beyond which no spontaneous pulsation is possible and irritability ceases, were approached (1) by the intake of water and at the same time (2) by the exit of salts.

It is stated by Mayer ('14^b, p. 40) that the rate of nerve conduction in operated *Cassiopea* is accelerated by slight dilution

of the sea water (down to 80 per cent of the original concentration), whereas the rate of pulsation in intact medusae (p. 28) is not increased in this way; but in his tables no records are given of the pulsations of entire medusae in sea water of the critical concentrations (95 per cent, 90 per cent). It might appear from the records in my figure 28 that there is some tendency on the part of diluted sea water to preserve in the excised cloacal ends of *Stichopus* a higher rate of pulsation during the later stages of their history, than is the case with the 'control' pieces in 100 per cent sea water. But, as a matter of fact, this is not so, since there was enough variation in these records and in the controls to prohibit a conclusion of this sort. The matter was studied more carefully with 90 per cent and 95 per cent sea water; no increase in pulsation frequency was discoverable upon diluting to these concentrations sea water in which amputated cloacal ends were pulsating.

b) Non-electrolyte solutions. It was found by Loeb ('00^a) that in solutions of non-conductors the isolated center of *Gonionemus* did not exhibit rhythmic contractions. This has in general been the experience of others employing a variety of pulsating mechanisms, namely, that in a medium free from salts but with its osmotic pressure made up to normal by dissolved non-electrolytes, rhythmic contractions are either not initiated or do not continue for any length of time. According to Mayer ('14^b) this physiological inefficiency, of sugars and the like, is also apparent in nerve-conduction in *Cassiopea*.

The results of experiments in which the isolated cloacal end of *Stichopus* was placed in solutions of non-electrolytes may be given very briefly. Pieces immersed in solutions of sucrose, lactose, maltose, or glycerin theoretically isosmotic with sea water (i.e., 0.9 to 1.0 M) did not pulsate for more than about 15 minutes, the actual time for the duration of pulsation varying from 2 to 25 minutes. Irritability to mechanical stimulation disappeared after about half an hour. Such pieces did not recover on return to sea water. In these tests the amputated cloacal end was allowed to begin its pulsation in sea water and to continue there for about 10 minutes; the pieces were then

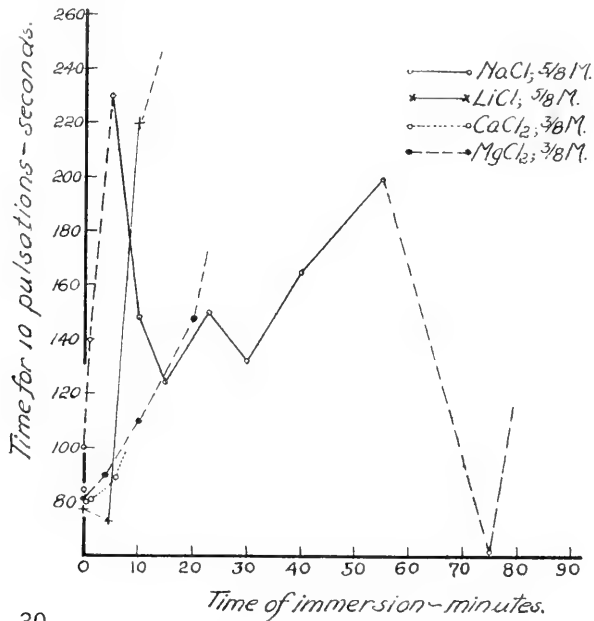
washed (inside and out) with a stream of fresh water¹⁶ before being put in the non-electrolyte solution. No evidence was had of an increase in muscular tone in sugar solutions, though pulsation usually ceased with the sphincter in the contracted condition. I had previously found (Crozier '15) that glycerin and maltose would stimulate the skin of *Holothuria surinamensis* in a sensory way; this effect probably had nothing to do with the action of sugar solutions upon pulsation, since the glycerin and maltose solutions did not behave differently from those of sucrose, etc., which had been found not to stimulate. Pulsations, where they did occur, were always of lower frequency in sugar solutions than in the controls in sea water.

VIII. ION EFFECTS

a) *Single salts.* The relation of the salts of sea water to cloacal pulsation and general irritability was studied by observing the action of single electrolytes at equivalent concentrations (i.e., isotonic with sea water), and the effects of various combinations of these substances in the proportions at which they occur in sea water. The method of procedure consisted in placing in the desired salt solution freshly amputated cloacal ends of which the pulsation rate had been determined previous to removal from the animal. Before immersion the pieces were rapidly washed by a stream of rain water from a wash bottle. Check experiments showed that washing the outside and inside of the cloacal end in this way did not have any effect upon the rate or duration of subsequent pulsation of pieces reimmersed in sea water. Since the excised pieces were immersed while in the condition shown in A, figure 12, that is, before the inturning of the cut edges had closed the cavity containing the radiating muscle strands, the solution had abundant opportunity to gain access to the elements concerned in pulsation. Care was taken that the cavity became filled with the solution surrounding the immersed piece and that no air remained in it. Since the pumping movements maintained a current of fluid through the cloaca, the solution

¹⁶ Check experiments showed that the washing had no effect on pulsation, at least on its duration.

was by this means (as well as by the movements of the body wall) efficiently stirred as long as the piece continued to pulsate; local diffusion changes were thus avoided. One disadvantage of this whole method lies in the fact that nothing could be done to prevent the action of the solutions upon the cut surfaces along the plane of amputation. The magnitude of this effect could not be estimated.



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Individual records illustrating the action of the single salt solutions in which cloacal pulsation occurred are plotted in figure 30. Table 9 contains a summary of the results of these experiments. With NH_4Cl and KCl , although the anal sphincters were tightly closed, some irregular pulsation of the body wall usually was evident for about the length of time indicated in table 9.

The order of the disappearance of tactile irritability parallels that of the stoppage of pulsation in these salt solutions. The completeness of the correspondence argues for a close similarity in the mechanisms of stimulation by internally and externally generated agencies.

TABLE 9
Effects of single salts

SALT	M. CONCENTRATION	DURATION OF	
		Pulsation	Irritability
		<i>minutes</i>	<i>minutes</i>
NaCl.....	5/8	75	105
LiCl.....	5/8	12	15
NH ₄ Cl.....	5/8	(10) 0	12
KCl.....	5/8	(3) 0	10
CaCl ₂	3/8	5	7
MgCl ₂	3/8	20	30
MgSO ₄	9/10	17	24

Averages from five series of tests. The figures in parenthesis opposite NH₄Cl and KCl mean that, while the sphincter of cloacal pieces immersed in these solutions did not pulsate, the body part did so, irregularly, for the number of minutes noted.

TABLE 10

Summarizing the results of tests made upon five isolated cloacal ends of Stichopus moebii with each of the salts indicated

SALT	NaCl		LiCl		NH ₄ Cl		KCl	
	5/8	5/16	5/8	5/16	5/8	5/16	5/8	5/16
Concentr., molecular.....	5/8	5/16	5/8	5/16	5/8 (10)	5/16	5/8 (3)	5/16
Duration of pulsation.....	75	50	12	35	0	0	0	0
Duration of irritability...	105	80	15	38	12	41	10	15

Time—Minutes.

For the chlorides of the alkali metals and radical (table 10), the order



was obtained with reference to their ability to preserve pulsation and irritability. The significance of this series, in terms of the aggregation state of protoplasmic materials, has been treated in a comprehensive manner by Höber ('14, pp. 471 et seq.). A beautiful demonstration of the action of the cations of neutral salts in this series has recently been given by Spaeth ('13) for the chromatophores of *Fundulus*. In solutions of NaCl and LiCl the pieces came to rest with the anal sphincter in an expanded condition but with the radiating cloacal muscles con-

tracted. This was unlike the condition in solutions of NH_4Cl and KCl , where both cloaca and anal sphincter were tightly contracted. The absence of any pulsation in $5/8 \text{ M NH}_4\text{Cl}$ and in $5/8 \text{ M KCl}$ is partly a secondary result, since solutions of these salts are powerful sensory stimulants for the skin of holothurians (Crozier, '15), which produced contractions from which the cloacal ends did not recover before the toxic action of the solutions led to death.

That the single electrolytes LiCl , NH_4Cl , and KCl are *toxic*, is also indicated by the results of tests in which several concentrations of these substances were compared (table 10). These experiments showed that with LiCl the duration of rhythmic movement and of irritability was greater at $5/16 \text{ M}$ than at $5/8 \text{ M}$ concentration, and that with NH_4Cl and KCl the duration of irritability was also greater at the lower concentration, even though in the case of the more dilute solutions an osmotic factor (see previous section) was also working to produce death.

NaCl is thus the only single constituent of sea water which will maintain pulsation and irritability for any considerable length of time, and in this respect it is only partially imitated by LiCl —a fact further proven by the physiological incompleteness of a van't Hoff solution made up with LiCl in place of NaCl , in which the pulsation of a cloacal end endured for about 45 minutes.

The comparative effects of MgCl_2 and MgSO_4 were of particular interest. Spaeth ('13, p. 553) found that MgSO_4 exerted a more powerful action upon the melanophores of *Fundulus* than did an iso-ionic MgCl_2 solution. Table 11 contains the data from experiments in which MgCl_2 and MgSO_4 were compared at several concentrations by means of their action on cloacal pulsation in *Stichopus*. The result of these tests was that MgSO_4 appeared to have a distinctly higher anaesthetic power than MgCl_2 .

b) *Mixed salts.* As the basis for the experiments made with mixed salts there was employed a van't Hoff solution of the composition: $5/8 \text{ M}$ [$100 \text{ NaCl} + 7.8 \text{ MgCl}_2 + 3.8 \text{ MgSO}_4 + 2.2 \text{ KCl} + 2.5 \text{ CaCl}_2$] (cf. Mayer, '11, '14 b). The solutions were made up in rain water. The averages obtained for the time of

TABLE 11

SALT	MgCl ₂		MgSO ₄	
Concentrated, molecular.....	5/16	3/8	5/16	9/10
Duration of pulsation, minutes.....	14	20	14	17
Duration of irritability, minutes.....	37	30	26	24

duration (a) of pulsation and (b) of irritability to mechanical stimulation, in the several salt mixtures studied, are given in table 11. By way of comparison, data taken from Bethe's account of the effects of sea water constituents upon pulsation in medusae (Bethe, '08) are given in the last column of table 12. The cloacal ends of holothurians are in some ways a better indicator for use in studies of this nature than are medusae, since the former pulsate continuously and not, as the jelly fishes do, in more or less interrupted groups of movements.

TABLE 12

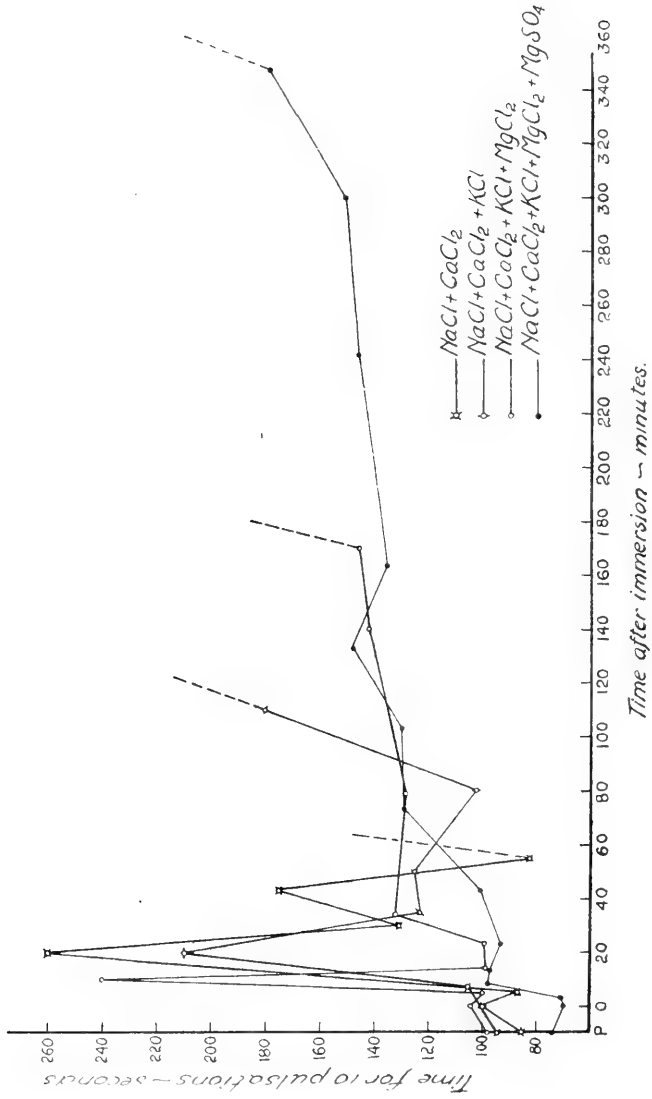
Summarizing the results of tests with salt mixtures. For details, see text

SOLUTION		DURATION OF		R.
		Pulsation	Irritability	
		<i>minutes</i>	<i>minutes</i>	
1	NaCl.....	75	100	27
2	NaCl + CaCl ₂	60	280	95
3	NaCl + KCl.....	50	90	47
4	NaCl + KCl + CaCl ₂	120	260	150*
5	NaCl + MgCl ₂	42	60	1
6	NaCl + KCl + CaCl ₂ + MgCl ₂	104	300+	
7	van't Hoff solution.....	360	480	1200±
8	"7" made alkaline.....	600		
9	Sea water.....	540		(10 days)

In column 'R' are given the corresponding data for the pulsation of the medusa *Rhizostoma* (Bethe, '08, p. 573).

* This solution contained CaCO₃.

A notable peculiarity of the pulsation curves of pieces immersed in NaCl solution (compare previous section, and figure 30) is the irregularity of the pulsation rate. During the early history of pieces in NaCl, the pulsation rate was also very low. By reference to figure 31 it will be seen that, in the experiments



plotted, only in the presence of MgSO_4 is the sodium effect completely abolished. This was quite uniformly observed. MgSO_4 , in combination with NaCl , KCl , and CaCl_2 , led to pulsations of a normal character, whereas MgCl_2 did not do so, not completely at least, even in mixtures where the quantity of Mg normally derived from $\text{MgSO}_4 + \text{MgCl}_2$ was "made up" by a calculated amount of MgCl_2 . The deficiency of solutions lacking magnesium lay in the fact that, as Loeb ('06) observed with the medusa *Polyorchis*, in these mixtures the sphincters tended to contract permanently, in more or less tetanic fashion. MgCl_2 , and especially MgSO_4 , and more particularly both together, acting with the other salts of sea water, led to normal relaxations after each systole, and thus tended to preserve a normal rate and duration of pulsation. Solutions containing NaCl and MgCl_2 or MgSO_4 , or both, did not maintain rhythmic movements as long as controls in NaCl ; CaCl_2 and KCl were necessary for the complete balance of the solution, as regards pulsation.

This applies to artificial salt mixtures. They were practically neutral in reaction. An attempt was made to approach the problem from the other side, by precipitating the SO_4 out of sea water by BaCl_2 . Solutions prepared in this way preserved pulsation for not quite so long (about 4 hours) as did the neutral van't Hoff mixture. The effect noted in the absence of MgSO_4 in artificial salt combinations may therefore have been due, in part, to the particular C_{H} of those mixtures (cf. Loeb, '10).

It is unnecessary to go into the analysis of many of the results obtained with salt mixtures, since they are so similar to those which have been found for other pulsating structures (Robertson, '10). Certain findings with reference to the significance of Ca in pulsation may however be mentioned. From table 12 it will be seen that the antagonism between Na and Ca was a very imperfect one as regards the duration of pulsation, though good as determined by the preservation of irritability. Between Na and K there was no antagonism. $\text{Na} + \text{K} + \text{Ca}$ formed a nearly completely balanced solution, aside from the K and Ca tetanising effects, which interfered with normal diastolic relaxation. The preservation of irritability was not very much less

efficient in $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ than in the same + MgCl_2 . The depressant action of salts which diminish the active number of Ca ions has frequently been assumed to be due to their effect on the Ca^{++} concentration, but the results of Salant and Hecht ('15) indicate that this conclusion is not well founded. Experiments of the following type showed that these well known relations obtained also with the cloaca of *Stichopus*:

Experiment 45.2. June 29. Five preparations from *Stichopus moebii* were allowed to begin pulsating in sea water. They were then transferred to a 0.1 per cent solution of oxalic acid in sea water. Pulsations of the sphincter ceased in 3 minutes, though the cloaca and body wall contracted rhythmically for about 4 minutes longer. The sphincter remained open; it contracted once in response to each light touch, for 5 minutes more.

Experiment 75.2. July 20. Three cloacal preparations immersed in a solution of the following proportional composition: 100 cc. sea water + 3 cc. $\frac{N}{10}$ tartaric acid. Pulsation lasted 15 to 20 minutes. Ceased with the cloaca and sphincter in the *open* phase.

Experiment 74.2. July 20. Five cloacal preparations immersed in a solution of the following composition: 100 cc. sea water + 5 cc. $M\ 5/8$ sodium citrate. Pulsations continued for a little over two hours, ceasing in the *open* phase. The movements, while they lasted, were of almost abnormal amplitude and vigor. Cessation found the sphincter in the *open* phase.¹⁷

Experiment 74.1. August 3. Three cloacal preparations immersed in a solution of the composition 100 cc. sea water + 2 cc. $M\ 5/8$ CaCl_2 . Pulsations continued for about 5 hours, at the end of which time the sphincters were all tightly closed, though they responded to a touch by opening and closing once.

Further increase in the amount of CaCl_2 in sea water stopped pulsation (with the characteristic calcium tetanus) in a shorter time.

The efficiency of salt mixtures in preserving pulsation was notably improved by making them alkaline, especially with NH_4OH . NH_4Cl was found to be very toxic, but solutions of the composition: 100 cc. sea water + 2 cc. $\frac{N}{10}$ NH_4OH preserved pulsation for about 20 hours, when the volume of solution for each piece was 500 cc. NaOH or KOH substituted for the

¹⁷ In this solution the tube feet moved incessantly, in sharp contrast to their usual quiescent condition in sea water.

NH_4OH did this for about 15 hours. The amplitude of pulsation in these mixtures was greater than in controls run in sea water. It is suggested that alkalis favored the continuance of rhythm by assisting in ionic exchanges at the surface of the contractile elements; probably this was accomplished in part in a secondary way, namely, by the neutralization of acid metabolic products.

c) *Hydrogen-ion concentration.* The hydrogen-ion concentration of sea water is given by various authors as lying between 0.5×10^{-8} and 1.5×10^{-8} (Höber, '14, p. 195). The sea water used in experiments with *Stichopus* was faintly alkaline to neutral red and neutral to tropäolin "000." Its C_{H^+} was thus about 10^{-8} . Increasing the C_{H^+} by the addition of acid (HCl)¹⁸ led to the rapid cessation of pulsation movements.

Experiment 95.2. July 24. Sea water to which $\frac{N}{10}$ HCl had been added until just neutral to Congo red (i.e., $C_{\text{H}^+} = \text{ca. } 10^{-4.5}$) was tested with five fresh cloacal preparations. Pulsations of an irregular character were manifested for about 15 minutes in two of these tests. The anal sphincter did not pulsate, but remained about one-third closed.

In transferring the cloacal preparations to a desired solution, they were lifted out of sea water; they thereupon ceased beating (contracting as the result of mechanical stimulation)¹⁹ and if returned to normal sea water about two minutes usually elapsed before pulsation began again. It was sought to utilize this fact and control the hydrogen-ion effect more closely (as was done by Dale and Thacker, '14, in analyzing the automaticity of different regions of the frog heart) by discovering the concentration which would just permit pulsation to begin and continue for about a minute. For the sphincter this concentration was attained by mixing equal volumes of sea water and sea water made just barely acid to Congo red: the mixture had therefore a C_{H^+} of $\text{ca. } 10^{-6}$. This limit is very close to that found by Bethe ('09, p. 261) for the pulsations of medusae.

¹⁸ The effect of certain organic acids was also studied, but not with sufficient completeness to warrant discussion in this place.

¹⁹ No pulsations were ever observed out of sea water.

Since it was not practicable to employ a perfusion method, experiments dealing with the action of the C_{H_2} in solutions which permitted the continuance of pulsation for some time were not attempted, as it was found that such solutions were modified in the direction of neutrality by contact with the tissue for about half an hour.

It may be of interest to note that, although the function of cloacal pumping is in part respiratory, no increase in pulsation rate was induced by an increase in C_{H_2} ; cloacal rhythm in *Stichopus* thus resembles the (partly) respiratory movements of the arms of barnacles (Roaf, '12).

IX. SUMMARY

The rhythmic pulsation of the cloacal chamber and anal sphincter of *Stichopus moebii* is dependent upon the continuous generation of stimuli within the cloaca, and particularly at its anterior end. The mechanism whereby the radiating muscles of the cloaca, the circular muscles of the cloaca and anal sphincter, the anal dilators, and the muscles of the body wall are brought to act in orderly sequence in the pumping of water into the respiratory trees is likewise locally contained. The aboral ends of *Stichopus moebii*, *Holothuria surinamensis*, *H. captiva*, and *Cucumaria punctata* continue to pulsate for many hours after they have been amputated at the level of origin of the respiratory trees. In such amputated parts a complete pulsation movement begins with the opening of the anterior end of the cloaca; a wave of opening runs aborally along the cloaca; the anal sphincter then opens and afterward closes; a progressive constriction of the cloacal chamber begins at the closed anal sphincter and runs forward; at the termination of the pulsation the whole cloaca is closed.²⁰

Normally the pulsation of the cloaca is interrupted by two means: (1) by complete constriction of the anal sphincter, in response to sensory stimulation and during locomotion, and (2) by holding the cloaca and anal sphincter open, during 'spouting.'

²⁰ Doubtless the nervous arrangements for the production of this kind of sequence in movements is similar to that involved in the use of the lantern in the locomotion of *Echinus* (Gemmill, '12).

In the latter case the edge of the sphincter continues to pulsate. Pulsation continues during defecation. The isolated cloacal ends do not 'spout;' they contract in response to mechanical, chemical, and photic (shading) stimuli. The stimulus to spouting in the intact holothurian is probably derived from a condition of tension in the body wall.

The radiating muscles and connective-tissue strands of the cloaca are necessary for the performance of pulsation. They appear to act in connection with an integumentary nerve net.

The results of experiments upon the cloacal termination of *Stichopus* are in essential agreement with the data derived from many previous studies of pulsating structures, such as those of medusae, ctenophores, the arthropod heart, and the vertebrate heart and intestine. The rhythm has a temperature coefficient of the order of magnitude of that for chemical processes. The rate of pulsation is related to the size of the animal (in *Stichopus*, *Holothuria surinamensis*, *H. captiva*, and *Cucumaria punctata*) in such a way as to suggest that the larger animals, which pulsate more slowly, possess relatively less energy than do smaller ones of the same species. Pulsation of the amputated aboral end is readily depressed by urea, carbon dioxide, acids, and KCN; it is resistant to lack of dissolved oxygen. Either dilution or concentration of sea water curtails the duration of pulsation. Rhythmic movements do not continue for more than a few minutes in non-electrolyte solutions. The relation of pulsation to the salts of sea water is essentially like that in other well known pulsating systems; NaCl + CaCl₂ + KCl (in the proportions found in sea water) enables pulsations and irritability to continue longer than with NaCl or with NaCl + CaCl₂; but magnesium, and particularly MgSO₄ (at least in neutral salt mixtures), must be present to insure normal diastole. Calcium is intimately concerned in contraction. In the series



the preservation of pulsation and irritability is successively less. The pulsating mechanism is extremely susceptible to increase in the hydrogen-ion concentration. The addition of NH₄OH, or other alkalies, to normal sea water assists in the preservation of pulsation and irritability.

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REACTIONS TO LIGHT IN VANESSA ANTIOPA, WITH SPECIAL REFERENCE TO CIRCUS MOVEMENTS

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TWENTY-ONE FIGURES

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INTRODUCTION

One of the most thorough pieces of work, which have been done on the reactions to light in butterflies, is that reported by Parker ('03) on the mourning-cloak butterfly, *Vanessa antiopa*.

This investigator found that these butterflies are highly positive in their reactions to light, but that when they come to rest in bright sunlight they ordinarily orient with the head directed away from the source of light. He found, however, that when one eye is painted black they do not orient, but continuously creep or fly in curves with the functional eye toward the center. Such reactions are usually called circus movements. This behavior, the author asserts (p. 463), is in accord with the view "that the orientation of an organism in light is dependent upon the equal stimulation of symmetrical points on its body."

A number of other investigators have, also, recorded experiments with other organisms in which circus movements have been observed. Reactions of this nature have been reported in experiments of three sorts: those in which one eye has been prevented from functioning, either by being blackened, or by being injured; those in which one antenna has been removed; and those in which certain parts of the brain or of the inner ear have been destroyed.

In these experiments it has been found that photo-positive animals, usually turn continuously toward the functional eye, while photo-negative animals usually turn in the opposite direction. This is especially true in those cases in which one eye has been covered. Holmes ('01 and '05) and his students, McGraw ('13) and Brundin ('13), maintain that they have observed this behavior in the following organisms: *Hyalella dentata*, *Talorchestia longicornis*, *Orchestia agilis*, two species of bees, the robber fly, *Asilus*, *Tabinus*, a Syrphid, *Ranatra*, *Notonecta*, several beetles, *Stenopelmatus*, three species of flies, a number of species of butterflies, and the amphipod, *Orchestia pugettensis*. In all these cases, positive animals turned toward the functional eye, while negative animals turned toward the covered eye. This, however, was not found to be true in all of the species investigated. Holmes and McGraw ('11, p. 370) state that several species of butterflies, among them *Vanessa antiopa*, frequently went in circles toward the covered eye, while Brundin ('13 p. 346) maintains that in positive specimens of the amphipod, *Orchestia traskiana*, "circus movements will occur

as often toward the blackened eye as toward the normal eye." Similar results have also been obtained with animals in which one eye was injured. Rádl ('01, p. 458) extirpated one eye of the water scavenger beetle, *Hydrophilus*, and found that it deflected toward the side of the injured eye. Hadley ('08, pp. 180-199) seared with a hot needle the surface of one eye of larval lobsters in all stages of development, and maintains (p. 198): "The immediate results following this destruction of photo-reception in one eye are: (1) The production of rapid rotations, often at the rate of 150 per minute on the longitudinal axis of the body, which are invariably in a determined direction. (2) A type of progression in which the larva continually performs 'circus movements' or turns toward the side of the injured eye." Since these animals vary in the sign of their reaction to light at different stages of development, it is interesting to note that Hadley maintains that the circus movements made by animals of all ages were all in the direction of the blinded eye. Mast ('10, p. 132) found that "*Planaria* with one eye removed, either by gouging it out or by cutting off one side of the anterior end obliquely, turn continuously from the wounded side for some time, evidently owing to the stimulation of the wound, since, after this is healed, they tend to turn in the opposite direction."

The destruction of the function of one eye is however not always followed by circus movements. Rádl ('03, pp. 58-64) states that *Calliphora vomitoria* is apparently not affected in its behavior by having one eye covered, while *Musca domestica*, although performing circus movements at times, can also "run rather long distances in one direction." Carpenter ('08, pp. 483-491) blackened one eye of *Drosophila ampelophila*, and reported that now and then one performed circus movements, but he says (p. 486), "This conduct was exceptional, and was never persisted in except in the case of a single insect which had long been active and showed signs of fatigue." They usually, however, deflected somewhat toward the functional eye as they proceeded toward the light. To quote further (p. 486), "They crept in a fairly direct path toward the light, although a tendency to deviate toward the side of the normal eye regularly

occurred. The insects generally moved in a peculiar, jerky manner. The tendency to diverge from the direct path toward the side of the uncovered eye was overcome by a series of short, quick turns in the opposite direction, which kept them headed toward the light." Mast ('11, p. 222) found that the toad, *Bufo americanus*, with the lens removed from one eye, hops or walks toward a source of light, usually deflecting slightly toward the injured eye. Some individuals, however orient nearly, if not quite, as accurately after the operation as before. Thus, it is evident that there are numerous exceptions to the idea that the destruction of one eye is followed by circus movements.

Moreover, it has been found that some animals which make circus movements modify their behavior after having had a certain amount of experience, and move directly toward the light. Holmes ('05), in a detailed description of the behavior of one specimen of *Ranatra* with the right eye blackened, says that in the first ten trials before an electric light, it made many circus movements, and showed a "marked tendency to turn to the left." In the next four trials it turned directly toward the source of light and in the succeeding ten trials it reached the light by a nearly straight path. After an interval of fifty minutes, eleven more trials were made, "and it had not forgotten in the meantime how to reach the light by the most direct means," for it went to the light in every case in a nearly straight course. The author also states that other specimens of *Ranatra* and *Notonecta* showed this same modification. Brundin ('13, pp. 334-352) observed similar reactions in the amphipods, *Orchestia traskiana* and *Orchestia pugettensis*, except that being negative the animals turned toward the blackened eye. Mast tested on two successive days a toad with one eye destroyed. He says ('11, p. 222): "The following day this toad was again exposed: it now went toward the source of light even more nearly directly than on the preceding day." Thus, it is clear that the reactions of at least some of these mutilated organisms may become modified as the result of repeated trials.

This is apparently not true of some animals. Rádl cut out one eye of *Hydrophilus*, and states that, though it lived for

several weeks in an aquarium, it never moved in a straight line, but always in a course curved toward the side of the injured eye. He says ('01, p. 458): "Es hat darnach noch mehrere Wochen in meinem Aquarium gelebt, bewegte sich aber niemals gerade sondern immer nur in einem Bogen concav nach der Seite des extirpirten Auges." This investigator ('03, p. 62) also observed a fly, *Dexia carinifrons*, on the second day after its eye was blackened and found its behavior was similar to that exhibited immediately after the eye was covered, that is, it moved continually toward the functional eye.

The second group of experiments, as previously stated, refer to insects with one antenna removed. V. L. Kellogg ('07, pp. 152-154) removed the left antenna from a male silk worm moth, and found that when such an animal was placed three or four inches from a female it "moved energetically around in repeated circles to the right, or, rather, in a flat spiral, thus getting (usually) gradually nearer and nearer to the female." Males with the right antenna removed turned continually to the left. In the same year, Barrows ('07, pp. 515-537) removed the terminal segment from one antenna of some fruit flies, *Drosophila ampelophila*, and then, after twenty-four hours without food exposed them to the odor of fermenting banana. He maintains that they moved in circles toward the uninjured antenna in all but a few cases in which they deflected in the opposite direction.

The third group of experiments mentioned comprises those in which parts of the brain and inner ear have been injured or removed. In these cases it is also maintained that the animals make circus movements.

It can thus be seen that great diversity exists among the results obtained by the various investigators in their experiments on animals with the sense organs on one side destroyed. Among these, those which refer to the eyes are of greatest immediate interest to us. In these experiments it was found that while photo-positive animals usually turn toward the functional eye and photo-negative animals toward the non-functional eye, some turn in the opposite direction and others orient fairly accurately,

while still others make circus movements for a period and then orient fairly accurately.

This marked lack of harmony between the results obtained may in some measure, at least, be due to the fact that the number of sources of light was not the same in all of the experiments: Parker does not state the conditions under which the specimen of *Vanessa antiopa* used by him made circus movements. Rádl presumably performed his experiments before a window, i.e., under conditions in which the animals received some light from many different directions. The same probably held also for the work of Holmes on amphipods and several insects. In some experiments, however, as in those performed with *Ranatra* and *Notonecta*, he worked in a 'darkened room,' and used for a source of light a sixteen candle-power incandescent lamp. Brundin and Carpenter also used a similar source of light. It is significant indeed that in every case where a single source of light on the same horizontal plane with the organism was used, at least some trials are described in which no circus movements were made, the animals moving in a fairly straight course toward the light. This was true of *Ranatra*, *Notonecta*, *Drosophila*, *Bufo americanus*, *Orchestia traskiana*, and *Orchestia pugettensis*. On the contrary, in none of the experiments but one, where the light conditions were not sharply defined, have the investigators recorded any other behavior than movements in circles. This single exception is that described by Rádl, in which *Calliphora vomitoria* and *Musca domestica* with one eye blackened ran for some distance directly toward a window.

The experiments described in the present paper show that in the case of *Vanessa antiopa*, at least, a knowledge of the number of sources of stimulation is of great importance in a discussion of circus movements; for the same animals, which, in a horizontal beam, moved toward the source of light in a fairly straight course, performed circus movements continuously when placed before a window, or when the single source of light was placed above the animal so that the light was non-directive.¹ The

¹ The term 'non-directive light,' as used in this paper, denotes diffuse illumination.

reactions under the former conditions seem to indicate that both eyes are necessary for orientation; those under the latter, that only one eye is necessary. Consequently, if the butterflies had been studied only in front of a window, the conclusions would necessarily have been erroneous.

Circus movements have been held by many to have a very important bearing on the question as to the nature of the process of orientation.

Holmes discusses this question rather fully. He takes the position that the performance of circus movements indicates a *direct or indirect* connection between the impulses set up by light in the two retinas and the tension of the muscles of the legs or appendages on the two sides of the body, and that this is a "sort of mechanical reflex process." To him the pleasure-pain theory explains those cases in which orientation occurs in these asymmetrical animals. He says ('11, p. 54): "In most crustacea, as in most insects, orientation is effected through the unequal action of the appendages on the two sides of the body. In a form which is positively phototactic, light entering one eye sets up impulses, which, passing through the brain and nerve cord, cause, directly or indirectly, movements predominantly of flexion of the legs of the same side and of extension of the appendages of the opposite side of the body. If this is a sort of mechanical process, we should expect that, in a positively phototactic form, if one eye were destroyed or blackened over, the animal would move continuously toward the normal side." Mindful of the fact that the *Ranatra*s and *Notonectas* in time straightened their courses, and followed the light nearly as precisely as if they had the use of both eyes, he also concludes that "Phototaxis may fall, to a certain extent, under the pleasure-pain type of behavior. . . . Light, in some animals, is followed much as an object of interest is pursued by a higher animal" ('11, p. 55). To these conclusions Brundin ('13) assents.

According to Carpenter, the local action theory of tropisms would explain circus movements, were it not that some animals with one eye blackened can orient as accurately as if both eyes were functional. The pleasure-pain theory, he holds, explains

this behavior. He says ('08, p. 486): "It is clear that the tropism theory, with its assumption of local action of stimulus on the side exposed to its effect, does not furnish a complete explanation of these reactions. . . . A 'pleasure-pain' reaction appears to inhibit and dominate a 'tropic' reaction."

To Rádl, circus movements are an evidence of inequality in the tension of the muscles on opposite sides of the body, produced by the blackening of one eye. He says ('03, p. 63): "Bei einem Tier, dem ein Auge geschwärzt wurde, erschlaffen etwas die Muskeln an der Körperseite, wo das Auge nicht sieht; da sich nun die Muskeln der anderen Seite kräftiger bewegen, so erfolgt eine Bewegung in einer nach der Seite dieser stärker arbeitenden Muskeln gekrümmten Bahn."

Parker ('03, p. 463), as has been previously stated, maintains that the circus movements he observed in *Vanessa antiopa* are in accordance with the view "that the orientation of an organism in light is dependent upon the equal stimulation of symmetrical points on its body." He says further: "Should the eyes be the parts stimulated, any interference with one of these ought to result in a disturbance of the direction of the butterfly's locomotion. Thus, if the cornea of one eye were blackened, the insect in locomotion, being positively phototropic, ought to move as though that eye were in shade, namely in a circle, with the unaffected eye toward the center."

To Barrows, who worked on the reactions of *Drosophila* to odors, circus movements can only be explained by the 'tropism theory.' He says ('07, p. 535): "It seems impossible to explain the movements under these conditions in any other way than on the basis of the tropism theory. This theory has been stated in several ways. As applied to chemical stimulation, Verworn ('99, p. 429) declares: 'The word chemotaxis is applied to that property of organisms that are endowed with the capacity of active movement by which, when under the influence of chemical stimuli acting unilaterally, they move toward or away from the source of the stimulus.'"

V. L. Kellogg ('07) and Bohn ('11) agree with Loeb, whose views are given in the next paragraph, and Bohn even cites circus movements as one of his criteria for tropisms.

Loeb ('06, p. 140) attempts to refute any notion of a pleasure-pain type of behavior in lower organisms, and accepts the phenomenon of circus movements as a fact in support of his theory in explanation of the orientation of animals. This is discussed fully in the Mechanistic Conception of Life. ('12, p. 35-62.) He holds that the orientation of animals is controlled unequivocally by external agents, and that in orientation to light, there are two essential factors, the continuous action of light and the symmetrical structure of the organisms. According to his view, which may be called the 'continuous action theory,' the tension of the muscles of the appendages on the two sides of the body is controlled through direct reflex arcs by the photochemical changes produced by light in the two retinas. He says ('12, p. 39): "When two retinae (or other points of symmetry) are illuminated with unequal intensity, chemical processes, also of unequal intensity, take place in the two optic nerves (or in the sensory nerves of the two illuminated points). This inequality of chemical processes passes from the sensory to the motor nerves and eventually to the muscles connected with them. We conclude from this that with equal illumination of both retinae the symmetrical groups of muscles on both halves of the body will receive equal chemical stimuli and thus reach equal states of contraction, while when the rate of reaction is unequal, the symmetrical muscles on one side of the body come into stronger action than those on the other side. The result of such an inequality of the action of symmetrical muscles of the two sides of the body is a change in the direction of movement on the part of the animal."

It is clear that in this theory it is assumed that light is effective in orientation through its continuous action, that after orientation has occurred, light *continues* to stimulate the photosensitive areas, and through direct reflex arcs, continues to affect the muscles of the appendages on the two sides of the body. These assumptions, as stated above, are, according to Loeb, supported by the behavior of animals with the sense organs functional only on one side. He quotes Parker as follows ('06, p. 140): "Loeb has pointed out that the orientation of an organism in light is dependent upon the equal stimulation of symmetrical points on

its body. Should the eyes be the parts stimulated, any interference with one of these ought to result in a disturbance of the direction of the butterfly's locomotion. Thus, if the cornea of one eye were blackened, the insect in locomotion, being positively phototropic, ought to move as though that eye were in shade; namely, in a circle, with the unaffected eye toward the center."

Mast holds that the precision with which some organisms with but one functional eye perform circus movements appears to add support to the 'continuous action theory,' but he also says ('11, p. 222), as a result of his work on the toad, "These results show that, in this form and in all other forms which orient after one eye is destroyed, difference of effective intensity on opposite sides does not regulate orientation."

A glance at these various views shows that the movement of animals in circles when one eye is blackened, or when one antenna is removed, has been held by most of the investigators to support the view that the orientation of animals is in accord with the 'continuous action theory' described above. This theory is opposed by one that may be called the 'change of intensity theory,' the adherents of which hold that in some organisms, at least, light does not produce orientation through its continuous action, but by stimuli dependent upon the time rate of change of intensity. According to this theory, an organism going toward a source of light, may turn to one side; but when this occurs, then, immediately the photosensitive surfaces are exposed to a change of intensity, and this causes a reaction which results in reorientation, after which the orienting stimulus ceases.

The chief points at issue between the two theories concern the following questions: (1) Does light function in orientation through its continuous action, or through a change of intensity? (2) Does an animal, when oriented, continue to be affected by the same stimulus that is effective in producing orientation? and (3) Is bilateral symmetry essential in the process?

In view of the bearing that circus movements have on the theories as to the mechanism of normal orientation in animals, and in view of the conflicting results recorded by previous workers it seemed desirable to make a more thorough and a more extended

study of this phenomenon than has been done previously. Moreover, such a study should throw light on the question as to whether or not the path of nerve impulses resulting in a given reaction can be altered, as well as on the very important problem of modifiability in behavior in general.

The mourning cloak butterfly, *Vanessa antiopa*, was chosen to begin with because the results secured with this animal by Parker are widely known and frequently quoted. This work is to be followed by a more general study of the phenomenon in question.

Before entering upon a discussion of these experiments I wish to express my very sincere appreciation of the kindness of Professor S. O. Mast in suggesting this problem to me and in so unselfishly aiding me throughout the course of the work.

METHODS

The butterflies used were all reared in the laboratory from larvae secured from both the June and the August broods in Massachusetts, New York, and Pennsylvania. No difficulty was experienced in keeping them in excellent condition for long periods. They were kept in the laboratory in a large glass case, and fed on honey and a weak solution of maple syrup in water. At frequent intervals the insects were picked up and dropped on filter paper soaked in the latter sweet mixture. If the proboscis was not extended at once, it was uncoiled with a pin, and when once the tip touched the liquid, the animal continued to feed until its abdomen was swollen to an extent which seemed dangerous. Since these butterflies pass the winter in the imago state, it is not surprising that six specimens lived from August until the latter part of February. These were the survivors of a lot of about thirty which were received at the same time. Had proper care been taken, it is likely that nearly all would have lived through the winter in the laboratory. The wings of the butterflies were usually clipped to prevent their escape. This was in no wise injurious, for animals with clipped wings lived and thrived as well as those whose wings were intact, and they behaved in the same manner.

As already stated, three methods have heretofore been used to prevent the functioning of one eye; extirpation, searing with a hot needle, and covering with asphalt varnish. The latter method was used exclusively in the present work, because it was believed that fewer disturbing factors would be introduced thereby.

In the early part of the work, one eye was covered with one or two coats of the asphalt varnish. After having made some experiments with animals treated thus, it was found, to my surprise, that insects with both eyes covered in this way still oriented fairly precisely, and went toward the source of light. Thus it is evident that the varnish as used did not exclude all of the light. The eyes were then painted repeatedly until the coats were so thick as to be distinctly evident when the observer was several feet from the butterflies. Under these conditions the animals were indifferent to light. Warned by this experience, the blinded insects used in all future experiments were so treated that it seemed certain that the eye was in every case effectively covered. Moreover frequent examinations were made to make sure that the varnish had not cracked or fallen off; and new coats were from time to time applied to make assurance doubly sure.

In work of this sort it is important that the varnish be of such nature that it does not injure the eye in any way. The effect of the covering was consequently repeatedly tested by removing it from the eyes after it had been on for some time. Insects thus tested behaved as did those whose eyes had not been blackened, showing no effect from the varnish.

The supply stock of butterflies was ordinarily kept in a large cage which was four feet high, four feet long and two feet wide. This was fastened against a south window in such a way that the window formed one side of the enclosure. The opposite side was also of glass and faced a small laboratory room. The other two sides, the top, and the bottom, were of wood. Careful observations were made on the insects in this enclosure, from time to time, throughout the whole period over which the experiments extended. But a much more thorough investigation of

the behavior in light was made in a dark room under accurately controlled environmental conditions. In these experiments the animals were exposed in a horizontal beam produced by means of a 110 volt Nernst glower. The glower was mounted in front of a small opening in a light-proof box that was painted dead black inside, so as to form a non-reflecting background. It was placed 10 cm. from and at the same level with the top of a table on which the animals were tested. By means of screens the light from the glower was so cut down as to produce a sharply defined beam of the size desired. The edges of this beam could be clearly seen on the black top of the table. This beam was the only light in the room, and this was in large part absorbed by means of dull black paper hung over the exposed walls. There was consequently very little light in the room aside from that in the beam. Under these conditions therefore, the animals were exposed in a beam of light from a single, small and concentrated source.

The limits of this beam were very apparent in the dark room in which the experiments were made. The nature of the source of light and the sharply defined character of the beam are important, for experiments described later demonstrate that the behavior of animals with one eye blackened depends to a marked extent upon whether there are one or more sources of light present.

Not only was the behavior of the animals described by the observer, but the butterflies, themselves, were forced to make permanent records of their own behavior. This was done by allowing them to walk on sheets of paper which had been covered with soot from an oil lamp. These sheets measured 20 x 25 cm., but in some experiments, a number of them were placed side by side until the area was as large as desired. The tracings made by the insects were made permanent by means of a coat of shellac. The butterflies were frequently allowed to walk over the sheets of paper covered with soot, and then the same experiment was repeated without the use of the blackened paper. The same results were secured in both cases. This shows that the behavior was not affected by the soot. This method of hav-

ing the animals make permanent records of their own behavior is most valuable, for the records can be kept indefinitely and studied, thus giving opportunity to recognize many significant features which otherwise might have been overlooked at the time the experiments were performed. It would be of value, no doubt, to the keenest observer.

BEHAVIOR OF NORMAL SPECIMENS

In the study of normal animals in the cage referred to above, Parker's observations were confirmed. It was found that the insects were highly positive in their reactions to light. During the day, in the absence of direct sunlight, they were usually in active movement, flying against the window. Occasionally an animal would fly around the cage, but this was exceptional. When at rest the butterflies were usually grouped on the window side of the cage, where they assumed various positions on the bottom of the window sash, some facing the light, others in a horizontal position at right angles with the rays, some hanging on the sash in a vertical position with their heads up, and others hanging with their heads down.

When the sun was so situated that the butterflies were exposed directly to its rays, and they were undisturbed, they usually ceased their active movements and oriented very definitely. They turned so as to face directly away from the sun and spread their wings to their fullest extent, exhibiting behavior similar to that described by Parker. This position was retained indefinitely unless the insects were disturbed.

In a beam of light in the dark room the responses were quite different. In making observations under these conditions the animals were placed in the beam at various distances from the glower so that they faced the source of light. As soon as they were released they usually darted directly toward the glower and continued until they reached the edge of the table. The insects were always found to be highly positive in all intensities in which they were tested. They never exhibited the slightest indication of negative reactions. They never came to rest with the head directed from the light and the wings spread, as they usually did in direct sunlight.

BEHAVIOR OF SPECIMENS WITH BUT ONE FUNCTIONAL EYE

A. BEHAVIOR IN NORMAL CONDITIONS OF ILLUMINATION

In normal conditions of illumination the behavior of butterflies with but one functional eye was very different from that described above. Such specimens were tested on the floor of the cage referred to previously, on a table before a window, and in a beam of light in the dark room. Before a window and on the floor of the cage it was found that whenever they moved they turned continuously toward the functional eye, exhibiting behavior similar to that described by Parker. The periods of activity, which in some cases lasted for several minutes, alternated with periods of rest in which the animals remained practically motionless, as if recovering from fatigue. But the point that is of especial interest is that they continued to make circus movements from day to day, and that they did not learn to orient. Two insects with one eye blackened were kept for twenty-three days, and although they were observed many times each day no modification in their behavior was detected. In this respect, however, the reactions in a beam of light differed greatly.

B. BEHAVIOR IN A BEAM OF LIGHT

1. Description of reactions—deflection, circus movements, and orientation

Under the conditions of illumination described in the preceding paragraph, the animal receives light from all sides, and all the large areas of the functional eye are approximately equally illuminated in every position assumed by the insect. When exposed to the light in a beam the animal receives light from only one direction, and consequently every movement that is not directed toward the glower produces a change in the illumination of different large areas of the uncovered eye. This may account for the difference in behavior observed under the two conditions of illumination.

The behavior in a beam of light of *Vanessa* with one eye blackened was studied in 46 different individuals and many of

these were tested on several successive days. In nearly all cases the animals were forced to record their reactions on carbon paper, as previously described. In all tests the butterflies were placed in the beam so that they faced the light directly. The results obtained varied considerably in different individuals and also in the same individual under different conditions. In some respects, however, there was but little variation.

Nearly all of the butterflies tested turned toward the functional eye immediately after they were exposed, regardless of the luminous intensity or the axial position with reference to the

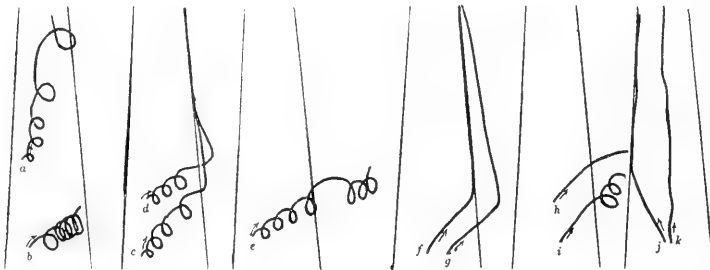


Fig. 1 Reproduction of various trails made by different specimens of *Vanessa* with the left eye blackened when exposed in a beam from a Nernst glower. The diverging straight lines represent the limits of the horizontal beam. The arrows indicate the direction of motion. Their trails show that there is great variation in the reactions of different individuals under the same conditions.

direction of the rays of light. Some of them continued to turn in this direction making repeated circus movements² (fig. 1, *a* and *b*) until they became fatigued and stopped, or until they reached the edge of the beam, where many turned sharply toward the glower and traveled along the edge of the beam toward the source of light, as is shown in figure 1, *c*. A few, however, did not turn toward the light when they reached the edge of the beam, but passed into the shaded region, continuing to make circus movements (fig. 1, *e*). Others did not make circus movements, but turned until the longitudinal axis made a certain

² In the present paper the term 'circus movements' with no further explanation means continuous movement toward the functional eye.

angle with the rays of light, and then continued until they reached the edge of the beam. Here they usually turned sharply toward the glower and moved along the edge of the beam toward the source of light (fig. 1, *f*) but occasionally they continued to turn here and made circus movements (fig. 1, *i*), and sometimes they did not respond at all when they reached the edge of the beam, but continued until they had passed into the shaded region from 2 to 5 cm. when they usually turned and proceeded directly toward the glower, remaining in this region (fig. 1, *g*). On a few occasions, however, they did not turn when they reached the edge of the beam, but proceeded on in the shaded region indefinitely (fig. 1, *h*). A few animals did not turn toward the functional eye, but oriented fairly accurately and walked toward the glower in a nearly straight course (fig. 1, *k*). Several specimens in some trials turned toward the blackened eye, crossed the beam, and on reaching the edge turned and walked along it toward the source of light (fig. 1, *j*).

Many insects, as the trials proceeded, showed an increase in accuracy of orientation. This was evident in three respects: (1) in the number of circus movements made, (2) in the angle of deflection, and (3) in the promptness with which they oriented at the edge of the beam.

The above general description may perhaps be made clearer if the reactions of one organism are described in detail. This animal designated as butterfly 10/25-a (left eye blackened) was tested on three successive days.

On the first day this butterfly was given twenty trials (fig. 2). In every one it turned toward the unblackened eye immediately upon being placed in the beam. In the first trial it crossed the beam at an angle of approximately 95 degrees with the rays of light, and passed into the shaded region. After it had gone 6 cm. in this region it turned to the left (the blinded eye) and walked toward the glower in a slightly zig-zag course, remaining, however, in the comparative darkness to the right of the beam. In the second trial, after crossing the beam at an angle of nearly 80 degrees, it again went to a point 6 cm. beyond the edge of the beam, but then it turned sharply to the right (toward the func-

tional eye) and performed a circus movement. This was followed by a fairly straight course for 7.5 cm. At this point the organism turned again to the right as if to make a circus movement but did not complete it, turning instead to the left toward the source of light. In the third trial the insect made a circus movement as soon as it was placed in the beam and then crossed the beam at an angle of about 95 degrees with the rays of light, and went 3 cm. into the shaded region where it turned toward the blackened eye and moved in a course nearly parallel with the edge of the beam. In the fourth the behavior was like that in the preceding trial except that after the organism passed the

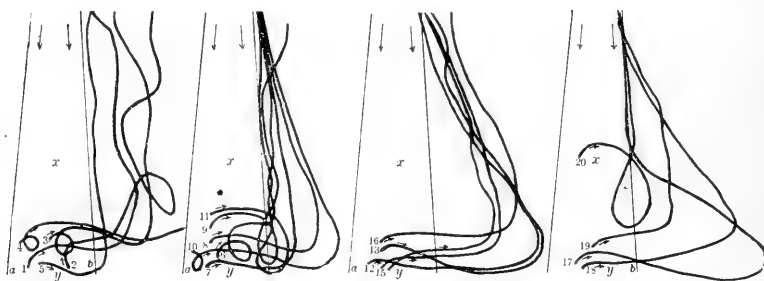


Fig. 2 Reproduction of 20 successive trails made by butterfly 10/25-a (left eye blackened) on the first day of the tests. *a* and *b*, limits of horizontal beam of light; 1-20, trails made in successive trials; small arrows, direction of movement of animal; large arrows, direction of rays of light; illumination at *x*, 624 mc.³ at *y*, 250 mc.

edge of the beam it did not turn toward the glower, but continued on in a fairly direct course until it reached the edge of the table. In the fifth trial the butterfly continued across the beam at about the same angle as in the previous trials until it had gone 2.5 cm. beyond the edge. At this point it turned toward the blackened eye and moved fairly directly toward the glower. In the sixth the organism again made a circus movement immediately upon being placed in the beam. It then crossed the beam at right angles with the rays, and on reaching the right

³ Throughout this paper the abbreviation 'mc.' will be used to indicate meter-candles.

edge, immediately turned toward the blackened eye and moved along the edge of the beam toward the glower. In the seventh, eighth, and in the twelfth to the nineteenth trials the behavior of the butterfly was essentially the same as in the fifth, but it usually went further in the shaded region before turning toward the glower, this distance varying from 2.5 to 14 cm. After orientation, however, it continued to move in all cases fairly directly toward the glower. In the tenth and third trials, the behavior was essentially similar. In the ninth, eleventh, and twentieth the reactions were also very much alike, the organism in each trial curving gradually toward the functional eye, in this way passing beyond the edge of the beam into the shaded region outside, and then coming back to the edge again. On reaching the edge of the beam the second time the butterfly turned much more sharply toward the functional eye, thus completing a circus movement and at the same time arriving at the edge of the beam a third time. When this occurred, the insect turned toward the glower and moved along the edge of the beam toward the source of light.

These reactions in the trials on the first day of the tests show: (1) that *Vanessa* with but one functional eye tends to turn toward this eye when placed in a beam of light; (2) that it can orient; (3) that orientation does not usually occur in the beam, but does occur either at the edge of the beam or several centimeters beyond it; (4) that after circus movements have been performed in a given trial the animal often orients and moves directly toward the source of light; and (5) that a change in illumination seems to favor the performance of circus movements, since, out of 8 circus movements, 4 were made almost immediately after the insect was placed in the beam and before it had reached the edge of the beam, 3 were made at the edge of the beam, and only 1 was made elsewhere.

On the second day in all of the first eight trials, except the fifth, the butterfly assumed an angle of about 90 degrees with the rays, and then traveled across the beam and into the shaded region for a distance of from 1.5 to 9 cm. where orientation occurred (fig. 3). In the fifth it continued on to the right in

a moderately straight course until it fell off the table. The behavior in the next three trials was very much alike, the organism performing a circus movement upon first being placed in the beam, and then, after having gone a few centimeters beyond the edge, it turned and went toward the glower. The eleventh trial is interesting in that, although the organism was started very much nearer to the glower, and consequently in much stronger

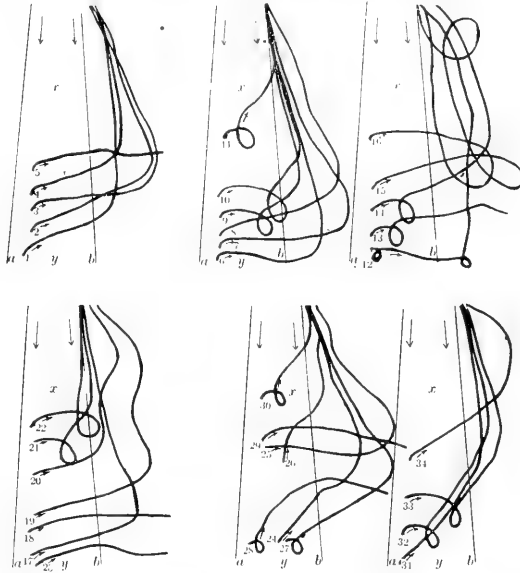


Fig. 3. Reproduction of 34 successive trails made by butterfly 10/25-a (left eye blackened) on the second day of the tests. *a* and *b*, limits of horizontal beam of light; 1-34, trails made in successive trials; small arrows, direction of movement of animal; large arrows, direction of rays of light; illumination at *x*, 624 mc.; at *y*, 250 mc.

light, it, after having performed a circus movement, deflected at an angle of only 40 degrees with the rays of light, while in several of the previous trials in which it had started further away from the source of light it deflected at a much greater angle. In the twelfth trial the butterfly made a circus movement when first started and then after having gone 1.5 cm. beyond the edge of the beam it again performed a circus move-

ment. This was followed by a zig-zag course nearly parallel with the edge of the beam. This circus movement is worthy of notice for it was made in the shaded region outside the beam, when the animal was in very weak light. It should also be noted that the diameter of the curve made is very nearly the same as the diameter of the curve made in the beam in comparatively strong light, when the insect was first started in this trial. This peculiarity will be correlated later with the results of other experiments. In the thirteenth trial after performing a circus movement in the beam the organism continued to the right in a fairly straight course to the edge of the table. In the fourteenth a circus movement in the beam was made, and then the animal went 7 cm. beyond the edge and oriented, moving toward the glower. In the fifteenth it crossed the rays of light and made a circus movement to the right of the beam. It then went toward the glower in a fairly straight line, but before reaching the source of light it made another circus movement. In the sixteenth a circus movement was made to the right of the beam. This was followed by a zig-zag course toward the glower. The behavior in the succeeding eighteen trials was essentially similar to that described above. It should be noted, however, that in the twenty-fourth trial the butterfly after moving to the right until the edge of the beam was reached turned more sharply toward the functional eye at this point. It did not, however, perform a circus movement, but gradually turned to the left. This sharp turn toward the functional eye on reaching the edge of the beam seems to support the conclusion arrived at from the trials on the previous day, namely, that change in illumination tends to favor the performance of circus movements.

These trials on the second day thus confirm strongly the conclusions drawn from the reactions on the first day, and they show moreover that after a certain amount of experience the angle of deflection tends to decrease, for on the first day the average angle between the path of the butterflies and the rays of light was 100 degrees while on the second day it was only 89.5 degrees.

The reactions on the third day (fig. 4) differed very markedly from those described for the first two days in several respects.

In all but the fourth and fifth trials the organism turned toward the functional eye, crossed the beam at a definite angle which was smaller than on the preceding days, and, on reaching the edge of the beam, turned at once to the left and walked along this edge toward the glower. In the fourth trial it responded very much like a normal specimen, walking down the center of the beam in a fairly straight line. In the fifth it deflected toward the blackened eye. No circus movements were made in any of the trials on this day.

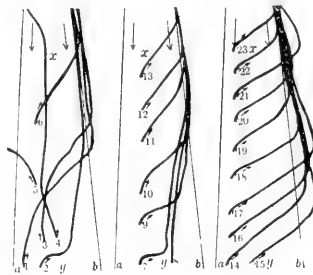


Fig. 4 Reproduction of 23 successive trails made by butterfly 10/25-a (left eye blackened) on the third day of the tests. *a* and *b*, limits of horizontal beam of light; 1-23, trails made in successive trials; small arrows, direction of movement of animal; large arrows, direction of rays of light; illumination at *x*, 906 mc.; at *y*, 266 mc. Compare figures 2, 3 and 4 and note that the insect on the third day made no circus movements, while on the two preceding days, it made numerous ones. Note also that the angle at which it deflected with the rays of light decreased.

- By comparing all of the reactions observed during the three days it will be seen that modification occurred in three different respects, as follows: (1) On the first two days there were numerous circus movements; on the third day there were none whatever; (2) On the first two days the butterfly usually passed into the shaded region a considerable distance before it turned and went toward the glower; on the third day it turned toward the glower promptly on reaching the edge of the beam; (3) The angle of deflection was greatest on the first day and least on the third, the average angle at the edge of the beam for the three days being respectively 100, 89.5, and 41.5 degrees. The reac-

tions of three other insects showing similar behavior are presented in figures 5, 6 and 7.

The results presented in these figures as well as in the preceding ones seem to show that butterflies with but one functional eye improve in the accuracy of orientation with experience. This conclusion and others are strongly supported by the results

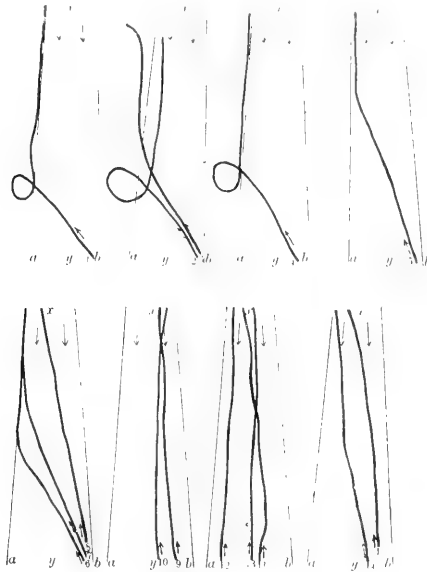


Fig. 5 Reproduction of 15 successive trails made by butterfly 7/29-c (right eye blackened). *a* and *b*, limits of horizontal beam of light; 1-15, paths made in successive trials; small arrows, direction of movement of animal; large arrows, direction of rays of light; illumination at *x*, 4892 mc.; at *y*, 544 mc. Note that this insect made three cirrus movements in the first four trials, while in the next eleven trials it made none.

obtained in all of the tests made. These are briefly summarized in table 1.

This table will be clearer if a brief explanation of some of the data is given. In the columns headed 'Direction turned' is stated the direction toward which the butterflies turned *immediately* after they were placed in the beam. The average angle of deflection was ascertained in the following way. The angle

between the rays of light and the trail of the insect at the edge of the beam in each of the trials was measured. This angle is termed the 'angle of deflection.' The average then was computed for a number of the first trials on each day, this number being equal to the number of trials on that day on which fewest trials were given. The columns marked 'Place where orientation

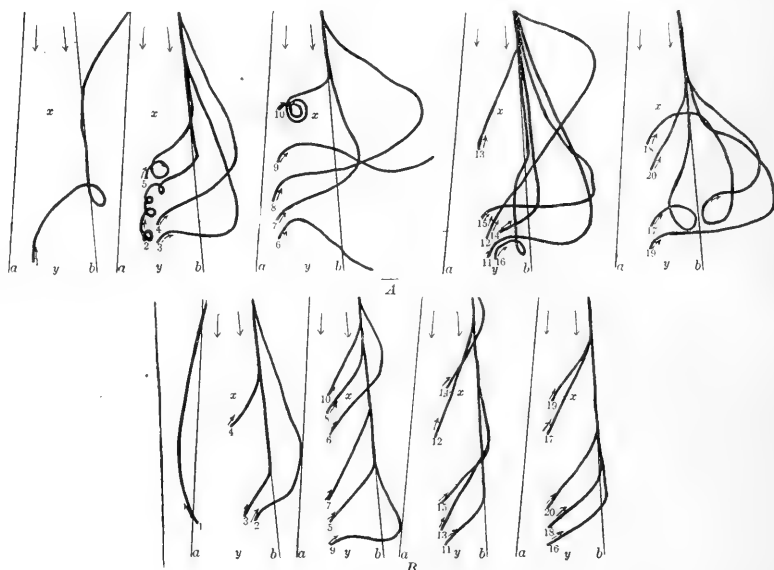


Fig. 6 Reproduction of 40 trails made by butterfly 10/1-b (left eye blackened). *A*, 1-20, trails made in successive trials on the first day of the tests; *B*, 1-20, trails made on the second day of the tests; *a* and *b*, limits of horizontal beam of light; small arrows, direction of movement of animal; large arrows, direction of rays of light; illumination at *x*, 925 mc.; at *y*, 266 mc. Note that this insect modified its reactions in that it made numerous circus movements in the trials on the first day, but made none in the trials on the second day.

occurred' also demand some explanation. By 'Orientation' is meant the assumption of an axial position with the head pointed directly toward the glower followed by movement in this direction. If the animal turned and moved directly toward the source of light before it reached the edge of the beam it is said to have oriented 'in the beam.' If it, however, went more than one centimeter beyond the edge before it turned toward the glower it is



said to have oriented in the 'shaded region.' When orientation occurred either precisely at the edge of the beam or within one centimeter beyond the edge it is considered to have occurred 'near the edge of the beam.' In those trials in which the insect either continued to perform circus movements or passed on outside the beam into the shaded region beyond, in a more or less straight course with no turn toward the source of light, 'no orientation' is said to have occurred.

An examination of this table shows that out of a total of 3077 trials the butterflies turned toward the functional eye in 2699 trials, and away from it in 207 trials, while in 171 trials they

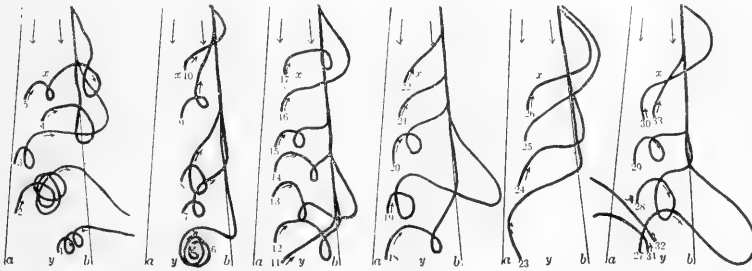


Fig. 7. Reproduction of 33 successive trails made by butterfly 10/14-b (left eye blackened) on the first day of the tests. *a* and *b*, limits of horizontal beam of light; 1-33, paths made in successive trials; small arrows, direction of movement of animal; large arrows, direction of rays of light; illumination at *x*, 1510 mc.; at *y*, 250 mc. Note that this insect modified its behavior in that it performed circus movements in 13 out of the first 16 trials, but made circus movements in only 6 of the next 16 trials.

moved toward the glower without first turning toward one side or the other. This indicates clearly that there is in *Vanessa* with one eye blinded a strong tendency to turn toward the functional eye.

The table shows also that in 2399 of the 3077 trials individuals with but one functional eye oriented and moved fairly directly toward the light, and that in 287 trials orientation occurred in the beam of light, indicating strongly that both eyes are not necessary in this process.

It shows, moreover, that in 16 of the 27 individuals tested on more than one day orientation occurred in more trials of the last day than in those of the first, and that in 18 of the 27 individuals orientation at the edge of the beam occurred more promptly during the trials on the last day than it did during those on the first. This is well illustrated by the reactions of butterfly 10/25-a, described in table 1 and in figures 2, 3, and 4.

It shows, furthermore, that in 20 of the 27 individuals circus movements decreased in number, and that in 20 the average angle of deflection was less in the trials of the last day than it was in those of the first. Although not shown in table 1, 10 individuals performed fewer circus movements in the last trials of the first day of the tests than in the first trials on this day.

This seems to indicate clearly that with practice there is in Vanessa with but one functional eye improvement in the accuracy of orientation in three respects, as previously stated: (a) increase in promptness of orientation, (b) decrease in the number of circus movements performed, and (c) decrease in the angle of deflection.

If this is true, then it is evident that orientation is not dependent upon the stimulation of both retinas by equal amounts of light energy. This conclusion is strongly supported by the fact that in 171 out of 3077 trials the organism with but one functional eye did not turn either to the right or the left, but moved fairly directly toward the source of light. It is moreover supported by the results obtained in observations on: the relation between the degree of curvature in circus movements and the luminous intensity, relation between the angle of deflection and the luminous intensity, and reorientation after changing the direction of the beam of light. These are discussed in the following paragraphs.

2. Relation between the degree of curvature in circus movements and the luminous intensity

According to the 'continuous action theory' smaller curves should be made in the strong light in the beam than are made in the weak light outside the beam, for the adherents of this theory, as stated above, hold that the tension of the muscles of the legs

on the two sides of the body varies with the relative amount of light energy received by the two retinas. No such relation, however, was at all evident in the observations on *Vanessa*. Curves of the same size were made in both positions, and very frequently those in the region of low illumination outside the beam were smaller than those in the region of comparatively high illumination in the beam. This is well seen in the ninth trial made by animal 10/14-b on the third day (fig. 8). In this case the butterfly, while in the beam, began to perform a circus movement of a diameter of 6.5 cm. By the time it was half

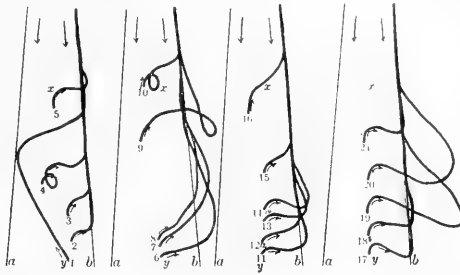


Fig. 8 Reproduction of 21 successive trails made by butterfly 10/14-b (left eye blackened) on the third day of the tests. *a* and *b*, limits of horizontal beam of light; 1-21, paths made in successive trials; small arrows, direction of movement of animal; large arrows, direction of rays of light; illumination at *x*, 1510 mc.; at *y*, 250 mc. Note that in trial 9 this insect turned very much more sharply toward the functional eye while in the shaded region to the right of the beam than it did while in the comparatively strong light in the beam.

completed the animal was 3 cm. beyond the edge, and in the weak light to the right of it. On reaching this point, the insect turned sharply to the right, and made another circus movement of a diameter of only 1.5 cm. i.e., in weak light the organism turned more sharply toward the functional eye than in strong. Similar reactions were observed in many other cases.

3. Relation between the angle of deflection and the luminous intensity

a. Effect of beginning the trials in different intensities. It has been shown that in those trials before the Nernst glower in which circus movements are not performed continually, *Vanessa antiopa*

usually turns until it assumes a certain angle with the rays of light, and that it then proceeds diagonally across the beam. If orientation is dependent upon the relative amount of light energy received by the two eyes, as demanded by the 'continuous action theory,' the degree of deflection ought to be greater in high illumination than in low, for if only one eye is functional, the greater the intensity, the greater the difference in the amount of energy received by the two eyes. This was tested by measuring the angles of deflection in different intensities of light in each one of the trials made by all of the insects. The results of some of these tests are recorded in figure 9 and in table 2.

TABLE 2

Angles of deflection made in different intensities of light by four butterflies with one eye blackened

NUMERICAL ORDER OF TRIALS	BUTTERFLY 9/22-a		BUTTERFLY 10/1-3-b		BUTTERFLY 10/8-a		BUTTERFLY 10/1-4-b	
	Intensity of light in meter candles at the point where the trials were begun	Angle of deflection	Intensity of light in meter candles at the point where the trials were begun	Angle of deflection	Intensity of light in meter candles at the point where the trials were begun	Angle of deflection	Intensity of light in meter candles at the point where the trials were begun	Angle of deflection
1	380	50	257	80	234	85	234	50
2	380	85	383	80	445	110	275	30
3	624	50	758	50	445	90	624	55
4	2153	60	758	70	624	85	234	30
5	380	95	791	40	275	80	234	30
6	448	65	936	50	791	70	634	50
7	624	50	234	40	383	70	634	50
8	1223	50	337	40	1044	70	936	95
9	2153	65	624	35	218	50	257	90
10	380	75	936	50	234	85	337	90
11	448	80	257	50	257	85	416	75
12	624	60	314	60	634	70	234	100
13	839	65	416	60	291	70	337	80
14	1497	55	624	50	1044	70	624	85
15	2883	80	936	45	416	60	936	110
16	380	65	257	65	234	60		
17	547	80	337	45	624	50		
18	624	75	624	35	259	60		
19	711	80	257	110	1044	50		
20	1497	95	257	50	218	60		

By referring to this figure, which represents the course of a given individual in different intensities of light it will be seen that the angle of deflection is essentially the same in all, in spite of the fact that the illumination varied from 76 to 3397 mc. This is a typical case. It seems to show that the degree of deflection is within wide limits independent of the intensity of the light.

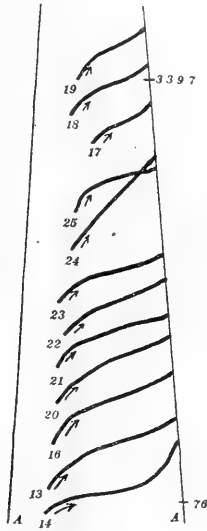


Fig. 9 Diagram showing the angles of deflection made by butterfly 10/8-b (left eye blackened) when exposed in light of different intensities. A A, limits of horizontal beam of light; 14-25, successive trials; 76 and 3397, intensity of light in meter candles at the corresponding points. Note that the insect deflects at about the same angle with the rays of light, no matter whether the trials are begun in an intensity of 76 mc. or in an intensity of 3397 mc.

The results presented in table 2 support this contention. They demonstrate that, while the degree of deflection varies greatly in different individuals and in the same individual under different conditions, there is no apparent correlation between it and the intensity of the light. Since the degree of deflection is a measure of the difference in tension of the muscles of the legs on the two sides of the body these results also show that there is no apparent correlation between this difference in tension and the intensity

of the light. This conclusion receives still further support from the results obtained in non-directive light which are reserved for discussion later. Before entering upon further discussion based on table 2 we will describe experiments as to the effect upon behavior of changes of intensity.

b. Effect of sudden changes of intensity on the angle of deflection. The previous experiment in which the position of the glower was unchanged, but in which the intensity of the light varied in different trials was supplemented by others. In some of these the light was increased after the butterflies had oriented. In others it was suddenly decreased. The insects with only one eye functional were placed in a beam of light, and, as soon as

TABLE 3
Effect upon the angle of deflection of suddenly increasing the illumination from 104 mc. to 1400 mc.

DESIGNATION OF BUTTERFLIES	NUMBER OF TRIALS IN WHICH ANGLES WERE INCREASED	NUMBER OF TRIALS IN WHICH ANGLES WERE DECREASED	NUMBER OF TRIALS IN WHICH NO EFFECT WAS EVIDENT
10/20-21-a.....	5		
10/6-9-b.....	3	2	8
10/8-c.....	8	1	3
10/8-9-a.....		1	1
10/1-7-c.....	5		3
Total.....	21	4	15

they had assumed a definite direction of locomotion, the glower was suddenly moved closer to the organisms, or was suddenly moved further away. In this way the intensity of the light was suddenly changed from 104 mc. to 1400 mc. and vice versa. Some butterflies turned suddenly toward the functional eye, others turned toward the blinded eye, and still others did not respond. The reactions of the animals to sudden increase of intensity are given in table 3; those to a sudden decrease of intensity in table 4.

Table 3 shows that in 21 out of 41 trials, the insects immediately turned toward the functional eye when the light was suddenly increased; that in 4 they turned toward the blinded side; and that in 15 there was no response.

Table 4 shows that four butterflies were tested, making 23 trials in all, and that in 12 of these there was no response, while in 8 the butterflies turned toward the blackened eye, and in 3 toward the functional eye.

The results recorded in these two tables show clearly that a sudden increase of intensity tends to cause the butterflies to turn sharply toward the functional eye and that a sudden decrease tends to cause them to turn in the opposite direction.

The reactions, as described above, upon a sudden change in the intensity of the light are very puzzling until the behavior of the individual animals is carefully examined. In one of the butterflies, 10/8-c, whose reactions are given in table 3 and in

TABLE 4

Effect upon the angle of deflection of suddenly decreasing the illumination from 1400 mc. to 104 mc.

DESIGNATION OF BUTTERFLY	NUMBER OF TRIALS IN WHICH ANGLES WERE INCREASED	NUMBER OF TRIALS IN WHICH ANGLES WERE DECREASED	NUMBER OF TRIALS IN WHICH THERE WAS NO APPARENT EFFECT
10/1-7-c.....	1	4	4
10/8-9-a.....	1		2
10/8-c.....	1		4
10/20-21-a.....		4	2
Total.....	3	8	12

figure 10, the angle of deflection did not change in three trials, but in seven out of the other nine trials made, it increased, at once, when the intensity was suddenly increased. *Immediately afterwards, however, as shown in figure 10, the organism turned toward the glower, and deflected at a smaller angle with the rays of light than it had before the intensity had been increased.* In one trial, though, at the sudden increase of intensity, it decreased the angle, and deflected only slightly in the beam. In another trial it increased the angle and went out of the beam.

The behavior of this last animal gives, I think, the clue to the explanation of the fact that when the intensity of the light is suddenly changed the angle of deflection decreases in some animals, while in others it increases. The fact that this butterfly

(fig. 10) increased the angle of deflection at the sudden change of intensity, and then immediately decreased the angle of deflection, although the insect was closer to the glower, is very significant in slowing that it is not the higher intensity which caused the increase in the angle of deflection, but that it is the *sudden change* from a lower to a higher intensity. Consequently, the sudden increase in the angle of deflection that occurs in some cases, seems

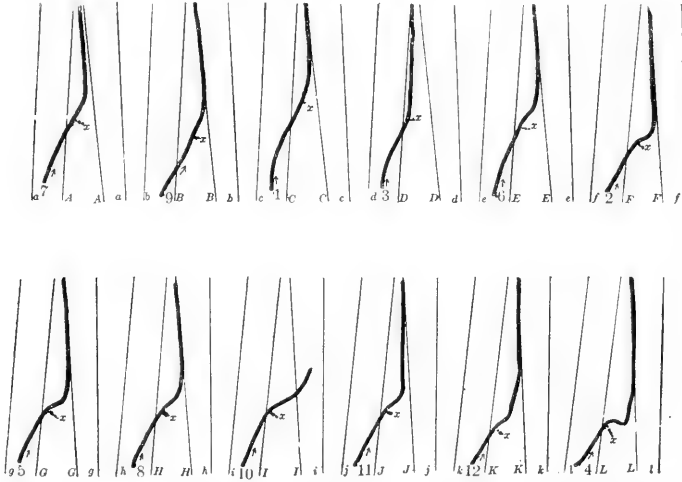


Fig. 10 Semi-diagrammatic reproduction of the records made by butterfly 10/8-c (left eye blackened) when the illumination was suddenly increased from 104 mc. to 1400 mc. The individual trials are numbered. The limits of the beams of high intensity are designated by the capital letters; the beams of low intensity by the small letters. X, position of the animal at the time the illumination was changed; arrows, direction of movement of animal. Note that the insect usually turned sharply toward the functional eye immediately after the illumination was increased, and later again in the opposite direction.

to be a shock reaction and not the result of unequal amounts of light energy received by the two retinas, as is demanded by the 'continuous action theory' of orientation. Furthermore, according to this theory the angle of deflection should be greater in high than in low intensity. This was, however, not found to be true, as is shown in table 2 and figure 9. By referring to this table it will be seen that the angle of deflection in 2000 mc. and in 200 mc. was essentially the same, whereas, according to

the 'continuous action theory' it should be ten times greater in the former intensity than in the latter. Moreover, this theory is not supported by the results obtained when the illumination is *gradually* increased after the animals have become oriented in a beam of light. The condition mentioned is fulfilled in the experiments already described in which the insects with one functional eye were tested in a beam from a stationary glower. In figures 4 and 9 it can be seen that after the butterflies had become oriented and were moving toward the source of light at a definite angle with the rays they were gradually approaching the glower and consequently the illumination was at the same time being gradually increased. On the basis of the 'continuous action theory,' one would expect the butterflies while in the beam to curve gradually toward the functional eye, increasing the angle of curvature as they approached the glower. On the contrary, they moved while in the beam in fairly straight courses, and the angle of deflection remained practically unchanged as the organisms drew nearer to the source of light.

This work shows conclusively that the angle of deflection does not vary with the intensity of the light, thus indicating most strongly that orientation in *Vanessa* is not necessarily dependent upon the relative amounts of light energy received by the retinas. It also seems to show that the assumption that the tension of the muscles controlled by the two retinas varies with the amount of light energy received, does not hold. This conclusion is further supported by the fact that *Vanessa* with only one eye functional can reorient toward either side and so follow a source of light as its position is changed, as is shown in the following section.

4. Reorientation after changing the direction of the beam of light

To ascertain whether or not butterflies with but one functional eye can reorient they were placed into a horizontal beam of light and after they had assumed a definite axial position and were moving toward the source of light at a definite angle to the direction of the rays, as previously described, the source was moved to a second position at the same distance from the animal, but

such that the rays of light were now at right angles to their former direction. When this was done, it was found that the butterflies usually reoriented. That is, they usually turned until they again assumed the same axial position with reference to the direction of the rays of light that they had taken before the glower was moved. This occurred no matter if the glower was moved to the right or to the left. Thus, it is evident that in the process of reorientation the animals may turn either toward the blinded or the functional eye (figs. 11 and 12).

Thirty-one butterflies were used in this experiment, and it was found that, of these, twenty-two reoriented both to the right and to the left. Nine, however, although they reoriented toward the functional eye, did not turn toward the blackened eye. Three of these were kept and tested for several successive days. The behavior shown in these tests is recorded in table 5.

These results show clearly that *Vanessa* with only one eye functional can reorient, and in this process can turn either toward the blinded eye or toward the functional eye. They also show that the behavior may become modified since those insects that do not reorient by turning toward the blinded eye when first tested are able to do so after a certain amount of experience (fig. 12, 1-3). Moreover they seem to support the conclusion derived from results described previously that the performance of circus movements seems to be favored by a sudden change in light conditions, since in four of the trials described in table 6 the butterflies either performed circus movements, or apparently began to perform them, when the position of the glower was suddenly changed.

The ability of the butterflies with only one eye functioning to reorient, and so follow the source of light as its position is changed is important in a consideration of the factors in the process of orientation. It has a direct bearing on whether or not orientation in light is dependent upon stimulation of symmetrically located photosensitive areas, as demanded by Loeb's theory of orientation. In reorientation, such as has been described, only one retina is affected by light. The chemical changes taking place in the two photosensitive areas are not at all identical.

TABLE 5

Behavior of three butterflies tested for reorientation on several successive days

DESIGNATION OF BUTTERFLIES	NUMERICAL ORDER OF DAYS ON WHICH TESTS WERE GIVEN	NUMERICAL ORDER OF TRIALS	BEHAVIOR WHEN GLOWER WAS MOVED TO THE SIDE OF THE COVERED EYE	BEHAVIOR WHEN GLOWER WAS MOVED TO THE SIDE OF THE FUNCTIONAL EYE
7/4.....	1	1	Circus movement toward functional eye, followed by movement toward glower	Reoriented
	2	1	Circus movement toward functional eye, followed by movement toward glower	Reoriented (See fig. 12, 4)
		2	Circus movement toward functional eye, followed by movement toward glower	
		3	Reoriented	
	3	1	Failed to reorient	Reoriented
		2	Failed to reorient	Reoriented
		3	Reoriented	Reoriented
		4	Reoriented	Reoriented
	4	1	Reoriented	Reoriented
		2	Reoriented	Reoriented
10/21-b....	1	1	Failed to reorient	Reoriented
		2	Failed to reorient	Reoriented
		3	Failed to reorient	Reoriented
		4	Failed to reorient	Reoriented
		5	Failed to reorient	Reoriented
		6	Reoriented	Reoriented
	2	1	Reoriented	Reoriented
10/24-a....	1	1	Reoriented	Reoriented; immediately performed circus movement toward functional eye; then went toward glower
	2	1	Reoriented	Reoriented
	3	1	Circus movement toward functional eye, followed by movement toward glower	Reoriented
	4	1	Sharp turn toward functional eye as if to make circus, but turned and moved toward glower	Behavior similar to that shown when glower was moved to side of covered eye

See figure 12, 1-3

The organism is non-symmetrical, only one eye being functional. And yet the butterfly moves toward a source of light, deflecting, it is true, toward the functional eye, and when the position of the light is changed, the animal also changes the direction of its motion to correspond with the change in position of the source of light. This behavior bears little resemblance to that which would be exhibited were the organism such that it could only

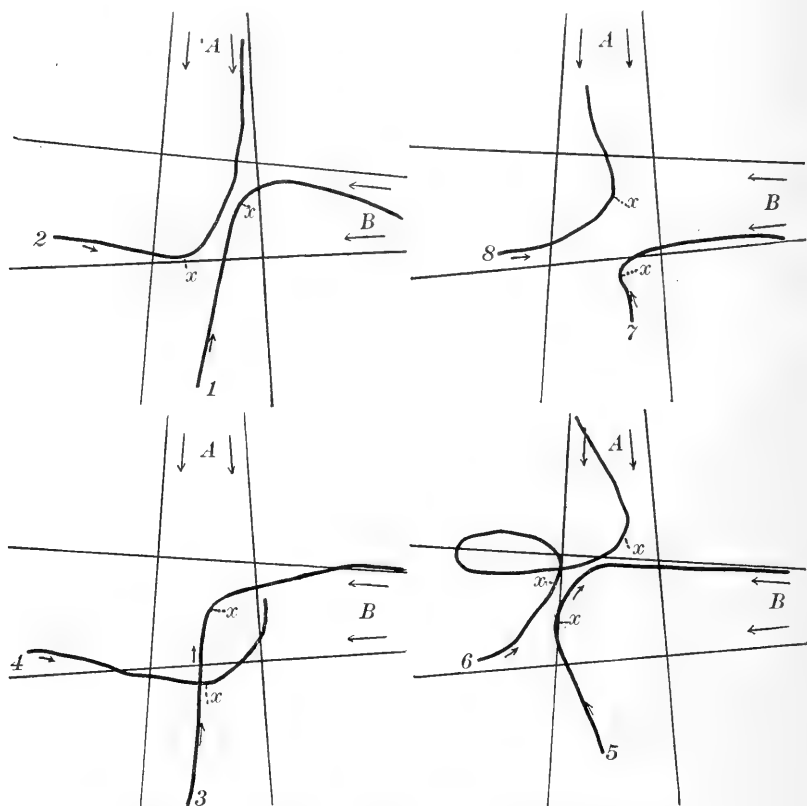


Fig. 11 Reproduction of trails made by four butterflies showing reorientation. Large arrows, A and B, direction of rays of light in the two positions of the glower; small arrows, direction of movement of the butterflies; x, position of animals when the direction of the rays was changed; 1 and 2, trails made by butterfly 7/29-d; (left eye blackened); 3 and 4, trails made by butterfly 7/16-c (left eye blackened); 5 and 6, trails made by butterfly 7/29-b (left eye blackened); 7 and 8, trails made by butterfly 9/22-b (right eye blackened).

orient if both eyes were stimulated equally. Were this hypothesis true, the animal with only one eye functional could not orient,

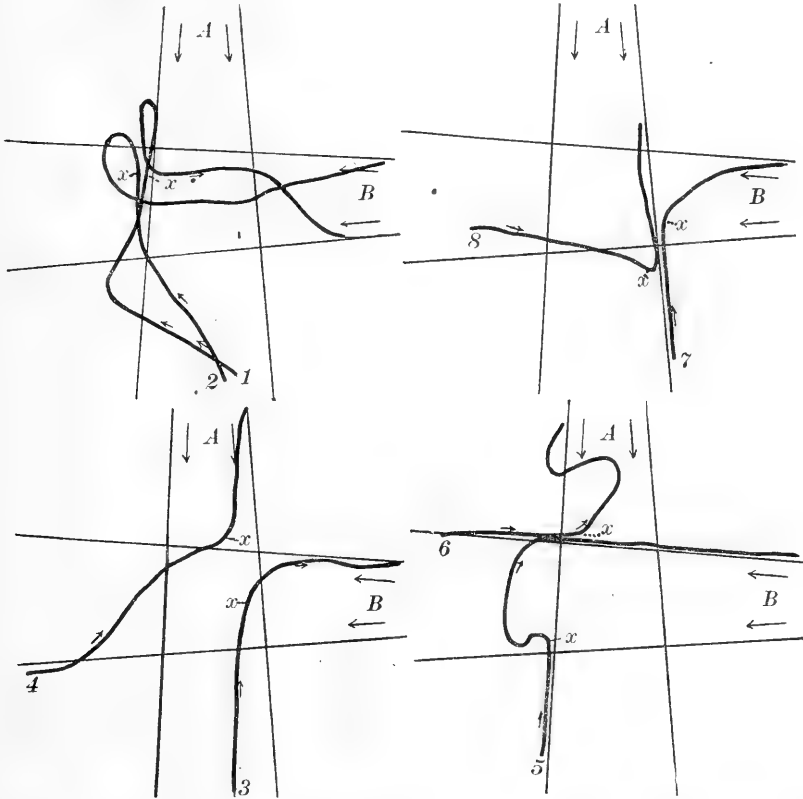


Fig. 12 Reproduction of trails made by three butterflies showing reorientation. Large arrows, *A* and *B*, direction of rays of light in the two positions of the glower; small arrows, direction of movement of the butterflies; *x*, position of the animals when the direction of the rays was changed; 1-4, trails made by butterfly 7/4 (right eye blackened); 5-6, trails made by butterfly 10/21-b (left eye blackened); 7-8, trails made by butterfly 10/24-a (right eye blackened). Note that butterfly 7/4 (right eye blackened) failed to reorient promptly toward the side of the blackened eye in the first two trials, but that in the third it did reorient promptly in this direction, showing marked modification in behavior. This figure and the preceding one are presented not merely to show the accuracy with which *Vanessa*, with only one eye functional, reorients upon change in position of the source of light, but also to show some of the peculiarities exhibited by different individuals.

but would be compelled to perform circus movements continually, regardless of the position of the source of light.

This experiment, however, throws no light on the nature of the stimulus effective in orientation. Reorientation could conceivably occur in organisms with but one functional eye, whether light exerts its orienting stimulus through its continuous action, as demanded by the 'continuous action theory,' or through a change of intensity, as demanded by the 'change of intensity theory.'

C. EFFECT OF THE COVERING OF THE EYE OWING TO CONTACT

We have clearly demonstrated that Vanessa with but one functional eye tends to turn toward this eye when it is placed in a beam of light, and that this tendency decreases with experience. Are these responses due to the elimination of the action of light on the blinded eye or to the effect of the contact of the covering on this eye? This question was answered very conclusively by placing in darkness specimens with one eye covered. In this experiment a wooden box (45 x 55 x 59 cm.) lined with black cloth was used. In the bottom of this box were placed sheets of paper covered with soot. The butterfly to be tested was placed in the enclosure upon the sheets of paper, and an opaque cover was drawn over the top of the apparatus. All the experiments were performed in the dark room, many of them late at night. The precautions taken were of such a nature that the observer feels confident that no light could possibly have reached the organisms.

Under these conditions it was found that the animals made circus movements, but in the reverse direction from that in which they moved in the presence of light. With few exceptions, the butterflies turned continuously *toward the blinded eye*, and in most of the exceptional cases they showed a decided tendency to curve somewhat in this direction. Only in a few cases did they move in a straight line. In some of these the course was nearly straight for eight or ten centimeters or more.

This general statement of the behavior is illustrated by the following detailed description of the reactions of two insects.

1. Butterfly 7/16-c (right eye blackened) was tested in darkness ten hours after the eye had been covered. It moved continually toward the *blinded eye* (fig. 13).

2. Butterfly 7/11 (right eye blackened) was tested immediately after the eye had been covered and again on each of the following two days. In the first trial it turned continuously toward the blackened eye. In the second and third it went in straighter courses, but showed a decided tendency to curve to

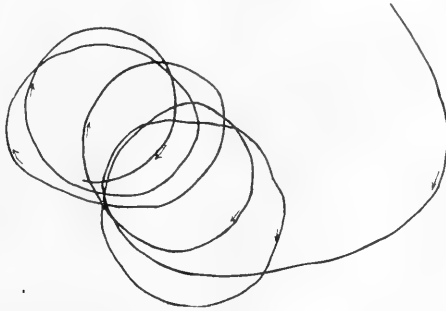


Fig. 13 Reproduction of tracings made in total darkness by butterfly 7/16-c (right eye blackened) showing continuous turning toward the blackened eye. Arrows indicate direction of movement. This record was made ten hours after the eye was covered.

the right (fig. 14, *A*). On the second day three trials also were given, and in each of these the organism turned continuously toward the blackened eye (fig. 14, *B*). On the third day in the first five of the ten trials given this insect again turned continuously toward the blinded eye, and in the other five it deflected in the same direction, but not so strongly as in the first five trials (fig. 15). It can thus be seen that the behavior exhibited on the first day was, in general, retained on the succeeding days of the tests.

Similar results were obtained in observations on a number of other specimens. These may be summarized as follows: Eight butterflies were tested for ten minutes each day for ten consecu-

tive days; three for four days; three for three days; one for two days; and thirteen on only one day. All of these animals usually turned continuously toward the blackened eye. The behavior was strikingly uniform. This continuous movement toward the

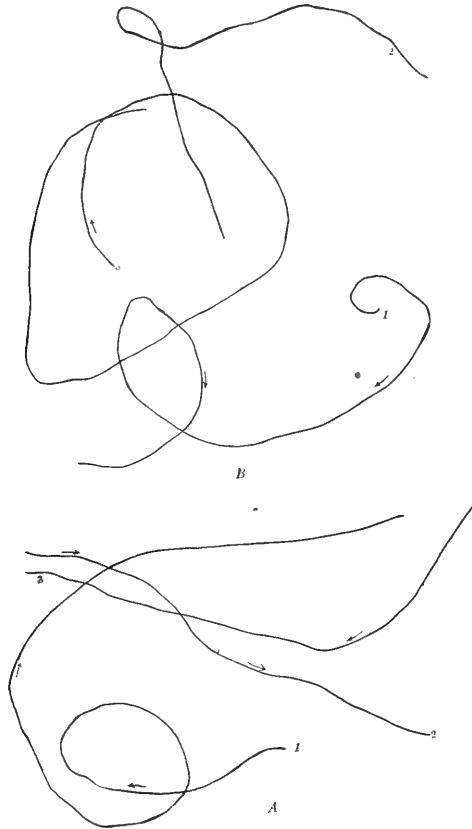


Fig. 14 Reproduction of trails made by butterfly 7/11 in total darkness (right eye blackened). *A*, 1-3, trails made in three successive trials immediately after the eye had been covered. *B*, 1-3, trails made in three successive trials twenty-four hours later.

blackened eye under conditions in which no light can affect the uncovered eye shows conclusively that the covering of the eye exerts a stimulus of some sort upon the organism. It also shows that the deflection toward the functional eye in light is not due

to the contact stimulus exerted by the covering of the blackened eye, for in light the insects turn toward the functional eye. It seems to demonstrate, moreover, that there is but little, if any, permanent modification in the behavior on successive days, for essentially similar reactions were observed in all of the insects during the whole period over which the tests extended.

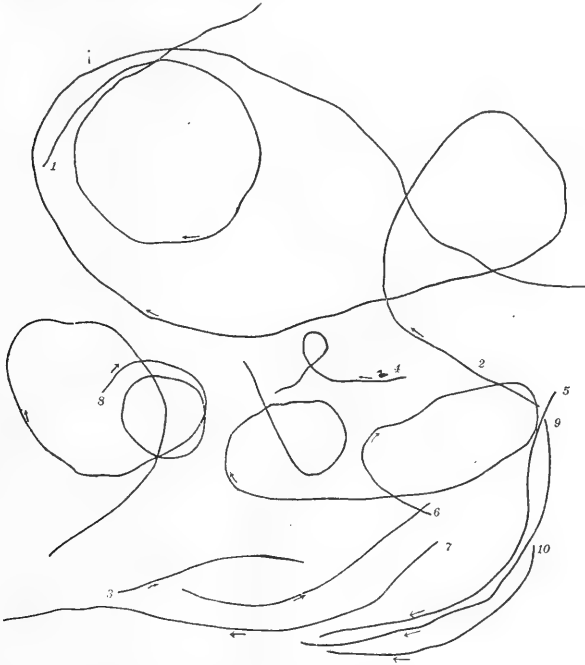


Fig. 15. Reproduction of trails in total darkness made by butterfly 7/11 (right eye blackened) in ten successive trials forty-eight hours after the eye had been covered. Compare the preceding figure with this one, and note that in the tests on three successive days this butterfly usually turned continuously toward the blackened eye, showing little, if any, modification in behavior.

The results obtained in these experiments throw light on the puzzling results which Holmes and McGraw obtained in their experiments with butterflies. They used a jar, the bottom and sides of which were lined with white paper. This was covered by a cone of the same material, and at the apex of this cone, an electric light was placed. When butterflies with one eye

blackened were placed in the center of the enclosure, specimens of some species went uniformly in circles toward the uncovered eye, while those of other species, among them *Vanessa antiopa*, moved continuously toward the blinded eye, although all were positive. Since insects which are positive in their reactions to light usually go in circles toward the uncovered eye, when one eye is blackened, while those which are negative go in circles toward the blackened eye, the above results were apparently inexplicable. Since, however, *Vanessa antiopa*, which is highly positive, goes in darkness in circles toward the blinded eye, it is clear that the results secured by Holmes and McGraw are to be explained by the fact that at times the stimulus exerted by the covering of the eye was strong enough to overcome the stimulus exerted by light on the uncovered eye. When the butterflies went toward the functioning eye, they were responding to light, while when they went in circles toward the blinded eye they were responding to the stimulus exerted by the covering of the blackened eye.

The above statement probably applies equally well to the peculiar results which Brundin obtained. These are described in the following manner ('13).

"In positive specimens of *Orchestia traskiana*, circus movements will occur as often toward the blackened eye as toward the normal eye. All specimens used for this experiment were strongly positive. There is no way to account for this variability, except that the animal might be made temporarily negative by having one of the eyes covered over. The fact, however, that as soon as the blackening is removed from the eye of one of these 'apparently negative' specimens, its reactions to the light is decidedly positive, seems to throw considerable doubt upon this hypothesis."

It seems very probable that the circus movements toward the blackened eye performed by this amphipod were due to a stimulus produced by the covering of the blinded eye and were not due to light.

It is possible, also, that the above described behavior in darkness of *Vanessa* may be suggestive in connection with the peculiar

results secured by Hadley with larval lobsters. He found that these animals, at all ages, moved in circles *toward the blinded eye*, although they vary in the sign of their reaction to light at different ages. He says ('08, p. 197): "In the lobster larvae all the progressive reactions which took place immediately following the blinding of one eye were positive. In certain cases it appeared that either the operation itself, or the effects of blinding changed the index of reaction from negative to positive. In all these instances, whether the previous reaction had been negative or positive, the resulting behavior was the same; a series of revolutions, or circus movements, or a progression in which the direction of turning indicated that the influence of light on the unblackened eye was to cause greater activity of the swimming appendages on that side of the body, while blinding invariably had the opposite effect. In other words, the reaction of the blinded positively reacting lobster larvae corresponds with those of Holmes's negatively reacting amphipod, *Hyalella dentata* (Smith) but not with his positively reacting amphipods."

Hadley is the only investigator who records continuous movements only toward the non-functional eye in individuals both in the positive and in the negative state. It is therefore probable that the circus movements described by him were due not to the withdrawal of the light stimulus from one eye, but to the stimulation produced by searing the eye with a hot needle, which was the method used by him in blinding his animals. These experiments ought to be repeated and the organisms tested on succeeding days. Besides, this method of blinding the lobster should be supplemented by entirely cutting off the eye stalk and testing for several days in succession the young lobsters so operated on, thus eliminating the possible effect of injury on the response.

D. BEHAVIOR IN NON-DIRECTIVE LIGHT

In the preceding pages we have discussed the behavior of Vanessa in a horizontal beam of light, and in the absence of light. We shall now describe its reactions in a field uniformly illuminated from above. When the butterflies are placed in a beam of light, all of the light that reaches them emanates practically

from one point, so that the illumination of the functional eye varies with every change in position of the animals. The question naturally arises as to whether or not many of the reactions observed under these conditions are due to this change in the illumination. This was settled conclusively by placing the butterflies with one eye blackened in light so arranged that the illumination of all of the large portions of the uncovered eye was essentially the same in all of the positions assumed by the insects, i.e., in which the light was non-directive.

To accomplish this the box described in the preceding section was used. Over the top of the box there was drawn an opaque cloth cover, in the center of which a circular hole, 3 cm. in diameter, was cut. A 16 c.p. electric lamp was placed directly over this hole in contact with the cloth. The butterfly was then

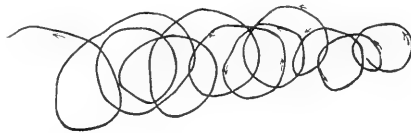


Fig. 16 Reproduction of trail in non-directive light, 6 mc., made by butterfly 7/16-c (right eye blackened) immediately after the eye was covered. Note the continuous turning toward the functional eye.

placed upon the papers in the bottom of the enclosure. Some light was reflected from the sides and bottom of the black box, but the amount of this reflected light was comparatively very small, and, moreover, it was approximately equal on all sides of the animal. The luminous intensity at the bottom of the box was 6 mc.

Thirty-one butterflies were tested in this apparatus in non-directive light soon after one eye had been blackened, and nine were also given trials on several successive days. The behavior exhibited by these animals is recorded in figures 16 and 17 and in table 6.

A study of this table and the figures show that if *Vanessa antiopa* with one eye blackened is placed in non-directive light it tends to turn continuously toward the functional eye, and that

TABLE 6

Behavior of butterflies with one eye blackened, in non-directive illumination of 6 mc. when tested on several successive days

DESIGNATION OF ANIMALS	NUMERICAL ORDER OF DAYS ON WHICH TESTS WERE GIVEN	BEHAVIOR
7/21-6.....	1	Circus movements; circles 6 cm. in diameter; in several trials it went in more or less straight courses for as much as 18 cm. and then curved toward the functional eye
	2	Not tested
	3	Not tested
	4	Continuous circus movements; circles 5 cm. in diameter
	5	Circus movements; circles 15 cm. in diameter; in several trials went in more or less straight courses for as much as 18 cm. and then curved toward the functional eye
	6	Circus movements; circles 5 cm. in diameter
7/23-4.....	1	Circus movements; circles 20 cm. in diameter
	2	Circus movements; circles 5 cm. in diameter; in several trials went in more or less straight courses for as much as 10 cm.
	3	Circus movements; circles 6 cm. in diameter; in several trials went in more or less straight courses for as much as 15 cm.
	4	Circus movements; circles 5 cm. in diameter.
7/23-4-e.....	1	Circus movements; circles 5 cm. in diameter, in several trials went in more or less straight courses for as much as 10 cm.
	2	Same behavior as on first day
	3	Circles 8 cm. in diameter in direction of <i>blackened eye</i>
	4	Behavior similar to that shown on first day
7/11-4.....	1	Circus movements; circles 4 cm. in diameter
	2	Circus movements; circles 3 cm. in diameter.
7/11-3.....	1	Circus movements; circles 10 cm. and 5 cm. in diameter
	2	No circus movements made; animal moved in more or less straight courses for as much as 15 and 16 cm. and then stopped, showing, however, a decided tendency to turn toward the functional eye
	3	Circus movements; circles 10 cm. in diameter; in several trials went more or less straight courses for as much as 15 cm.
7/24-3.....	1	Circus movements; circles 5 cm. in diameter
	2	Circus movements; circles 10 cm. and 3 cm. in diameter; with sharp turns toward blackened eye also
	3	Circus movements; circles 3 cm. in diameter
7/15-2.....	1	Repeated circus movements
	2	Four circus movements; circles 2 cm. in diameter; it then went in two zig-zag courses, which were each 20 cm. long
10/20-a.....	1	Circus movements; circles 2 cm. in diameter
	2	Circus movements; circles 10 cm. and 3 cm. in diameter; nine circus movements in all; it also made three other trials, in which it went in a zig-zag course for as much as 45 cm.
7/24-4.....	1	Circus movements; circles 3 cm. in diameter; in several trials it went in more or less straight courses for as much as 10 cm.
	2	Not tested
	3	Not tested
	4	Circus movements; circles 5 cm. in diameter; walked 18 cm. in a more or less straight course

this tendency is in general retained for several successive days, although some modification in reactions is evident. This statement, that there is some change in behavior, is supported by the fact that of the nine animals tested on more than one day five continued to perform circus movements on the first day of the tests, but on succeeding days in some trials went in courses which were more or less straight for several centimeters.

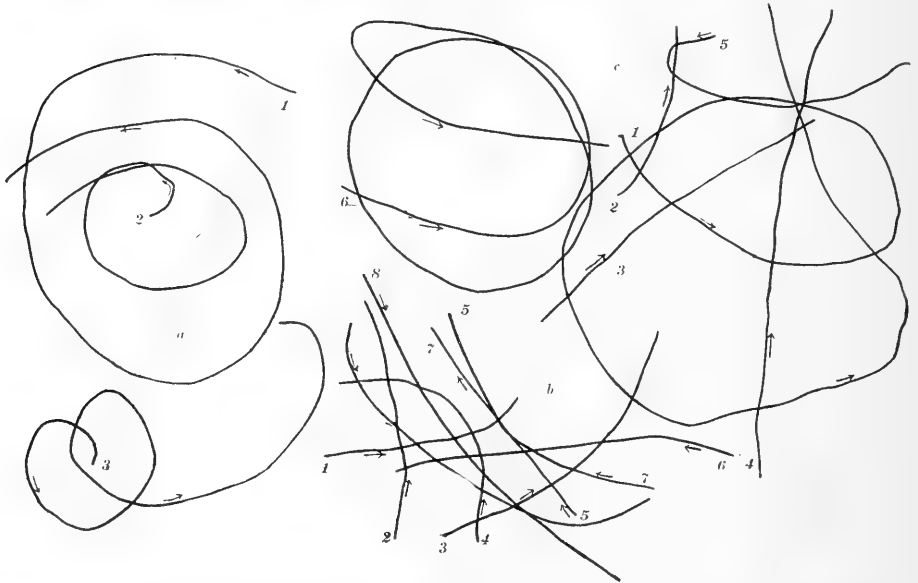


Fig. 17 Reproduction of trails made in trials on three successive days in non-directive light of 6 mc. by butterfly 7/11-3 (right eye blackened). *a*, trails made in three trials on the first day, *b*, trails made in eight trials on the second day, *c*, trails made in six trials on the third day. Note that the insect moved in rather straight courses on the second day, while on the first and third days it usually turned continuously toward the functional eye.

From time to time throughout the entire period, the animals used in the experiment above were tested in a horizontal beam. In the beginning some of them made circus movements, and some did not, but at the close of the experiment none of them made circus movements. This is of great interest, for in non-directive light these insects continued to make circus movements throughout the entire period. Under both conditions, only one

eye was functional. But when placed in non-directive light, essentially the same surface of the functional eye was continuously illuminated; while in a horizontal beam, the surface of the eye illuminated changed whenever the animal turned in either direction. The absence of circus movements in the beam of light must, therefore, have been due in some way to the change in the illumination of different regions of the eye, produced by the lateral movements.

This conclusion is further supported by the fact that all of the animals with one eye blackened used in the experiments described in the present paper, if placed before a window performed circus movements continuously, whenever they moved. In some cases the organisms were kept under these conditions for as long as two weeks, and frequent observations revealed no modification in their behavior. Yet when these same insects were placed in a horizontal beam from a glower they made no circus movements after a certain amount of experience. When a butterfly with one eye blackened, is placed before a window, the light conditions resemble, in many respects, those present in non-directive light. A change in position on the part of the organism, does not involve the illumination of an entirely different area of the functional eye. In every position all large areas of the eye are approximately equally illuminated, and no two positions involve the illumination of entirely different areas of the eye, for the sources of reflected light are countless, and extend on all sides of the animal.

No definite conclusion can, however, be drawn as to the nature of the stimulus which regulates the movement of the butterflies in non-directive light. Superficially it would appear to be due to the continuous action of light. Yet, it must be remembered, that with every change in the axial position of the butterfly, although there may be essentially no change in the illumination of the surface of the eye, there must be accompanying changes of intensity upon certain of the *ommatidia*. There is just one possible type of reaction in which the same *ommatidia* would be illuminated equally in all of the positions assumed by the insect, and that is movement of the organism in a circle with its center

directly below the center of the lamp. But since this did not occur, it is evident that there must be changes of intensity of light upon certain ommatidia coincident with every change in position of the head.

The fact that some butterflies with one eye blackened, when placed in non-directive light, at times moved in a more or less straight course, especially when tested twenty-four hours or more after the eye was covered, also demands attention. In these straightened courses, the movement is either controlled entirely or in part by internal factors, or it is the result of a balanced effort of two stimuli; one, light, tending to cause the organism to turn in one direction, and another, the varnish, tending to cause it to turn in the opposite direction.

E. RELATION BETWEEN THE DEGREE OF CURVATURE IN CIRCUS MOVEMENTS AND THE INTENSITY OF NON-DIRECTIVE LIGHT

If the assumptions made by the adherents of the 'continuous action theory' are valid, i.e., if orientation in organisms is dependent upon the relative amount of light energy received by the two retinas, and if the tension of the muscles controlled by the retinas varies with the amount of light energy received, then the circles made by animals with but one functional eye in non-directive light of high intensity should have a smaller radius than those made in light of low intensity, for the amount of light energy received by the functional eye would be greater in the high intensity than that received in light of low intensity, and, consequently, the inequality between the amounts of energy received by the two retinas would be greater under the former condition than under the latter.

This was tested in two experiments. In one, non-directive light of two fairly high intensities was used; in the other, the insects were first exposed to light of very low intensity (0.5 to 0.07 mc.) and then the intensity was gradually decreased. These experiments were performed in the light-tight box previously described. In the first experiment the lower intensity was produced by placing a 16 c.p. lamp over the opening of the box, and by interposing resistance in the circuit until the intensity of this

light was reduced to 2 mc. The higher intensity was produced by replacing the 16 c.p. lamp with two Nernst filaments side by side. The luminous intensity produced in this way was 460 mc. The insects were tested first in the light of low intensity, and then in that of high intensity. They were kept in darkness for twenty minutes before being exposed to either of these two lights. In the second experiment a white sheet of paper measuring 20 x 17 cm. fastened over a piece of board of the same size, was placed above the center of the box at an angle of forty-five degrees with the plane of the bottom. A horizontal beam of light from a Nernst glower was thrown on this sheet of paper, and reflected into the box below. By altering the elevation of the reflecting surface, and also by varying the distance between the glower and this surface, the intensity of the light in the box could be changed. In this way light of very low intensity was produced.

The results obtained in these experiments may be illustrated by a description of the behavior of two animals.

Butterfly, 10/13-a (left eye blackened), after having been in darkness twenty minutes, was exposed to non-directive light of an intensity of 2 mc. It made four circus movements, turning continuously toward the functional eye, and then stopped. It was picked up and dropped a second time, on the smoked papers in the bottom of the box. In this, the second trial, it again performed circus movements in the same direction. Similar reactions were observed in all the rest of the ten trials made (fig. 18). After a stay of twenty minutes in darkness, the source of light of high intensity was used, and the insect was given ten trials. In all of these it either made circus movements in the same direction as in the lower intensity, with a much smaller degree of curvature, or walked in fairly straight courses, as is shown in figure 19.

Instead of moving in smaller 'circles' in light of high intensity than in that of low intensity, as the 'continuous action theory' demands, the organism showed the reverse behavior, for the smaller 'circles' were all made in the lower intensity instead of in the higher.

Butterfly 10/3-a (right eye blackened) was placed in non-directive light of very low intensity, and this intensity was then still further decreased. It was found that circus movements toward the functional eye continued to be performed until the intensity reached 0.24 mc. When it was, however, still further decreased, the courses became more and more direct, until at 0.09 mc. the animal moved in a fairly straight line. When the intensity was lowered still further the butterfly deflected toward the *blackened eye*, and when it had reached 0.0265 mc. the insect moved in 'circles,' turning toward this eye.

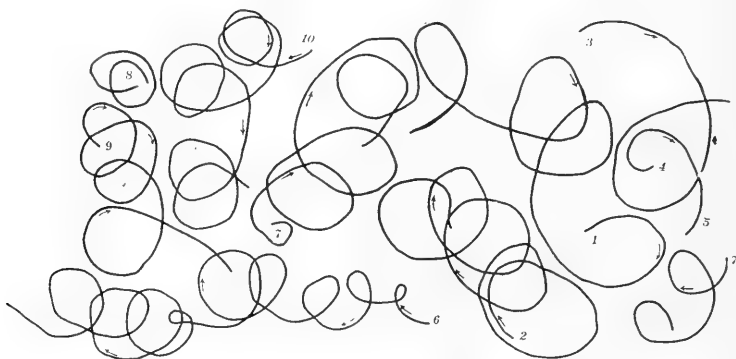


Fig. 18 Reproduction of trails made in non-directive light of low intensity, 2 mc., by butterfly 10/13-a (left eye blackened). Note that the animal moves in small 'circles' continuously toward the functional eye.

The reactions described above are typical of those exhibited by the twenty-one butterflies exposed to non-directive light of different intensities, as is shown in tables 7 and 8.

From table 7 it can be seen that out of sixteen animals tested in non-directive illumination of two intensities, one 230 times greater than the other, in not a single case did an animal move in smaller 'circles' in the higher than in the lower intensity. On the contrary, four butterflies actually made much smaller 'circles' in the weak light than they did in the strong light, and three animals made slightly smaller 'circles' in the weaker light. Moreover, one insect which made circus movements in the weaker light did not make any at all in the stronger, but rather went in

TABLE 7

Showing the relative degree of curvature between the circus movements made in non-directive light of low intensity and those made in that of high intensity

DESIGNATION OF BUTTERFLIES	NON-DIRECTIVE LIGHT OF AN INTENSITY OF 2 MC.	NON-DIRECTIVE LIGHT OF AN INTENSITY OF 460 MC.
9/22-c	Circus movements	Fairly straight courses with a decided curve toward uncovered eye
9/22-b	Circus movements	Circus movements with larger angles of curvature
9/21-a	Circus movements	Circus movements with slightly larger angles of curvature
9/22-d	Similar circus movements	
10/1-a	Circus movements	Circus movements with much larger angles of curvature
10/1-b	Fairly straight courses although circus movements are made	Fairly straight courses with numerous turns toward the blackened eye
10/1-c	Fairly straight courses in fifteen trials	Fairly straight courses in fifteen trials
	Under both conditions a decided tendency to turn toward the functional eye though numerous turns are made toward the blackened eye	
10/1-c-6	Circus movements in both cases—records indistinguishable	
10/13-a	Circus movements	Circus movements with much larger angles of curvature
10/14-18-a	Fairly straight courses under both conditions	
10/16-a	Trials indistinguishable	
10/20-a	Circus movements	Circus movements with much larger angles of curvature
10/21-b	Circus movements	Circus movements with slightly larger angles of curvature
10/24-a	Circus movements	Circus movements with slightly larger angles of curvature
10/28-a	Circus movements in both cases—records indistinguishable	
10/31-a	Circus movements	Straightened courses

fairly straight courses with a decided curve toward the uncovered eye. In the case of seven individuals the trials made in the two conditions of illumination were indistinguishable.

In table 8 are given the results obtained when non-directive light of a very low intensity was used.

TABLE 8

Amount of decrease in intensity of non-directive light necessary to force the butterflies to cease making circus movements toward the functional eye and to make circus movements in the opposite direction

DESIGNATION OF BUTTERFLIES	LOWEST INTENSITY IN WHICH CIRCUS MOVEMENTS WERE MADE IN THE DIRECTION OF THE UNCOVERED EYE	LIMITS OF INTENSITY WITHIN WHICH THE COURSES WERE NEARLY STRAIGHT		* INTENSITY IN WHICH THE INSECTS FIRST BEGAN TO MOVE IN CIRCLES TOWARD THE BLINDED EYE
1	0.24 mc.		0.09 mc.	0.0265 mc.
2	0.15 mc.	0.1 mc.	0.043 mc.	0.265 mc.
3	0.52 mc.	0.4 mc.	0.273 mc.	0.18 mc.
4	0.114 mc.	0.47 mc.	0.036 mc.	0.025 mc.
5	0.077 mc.	0.046 mc.	0.014 mc.	0.011 mc.

This table shows, in general, that the animals placed in non-directive light of low intensity, half a meter-candle or less, make circus movements toward the functional eye; that if the intensity of the light is then decreased, the insects deflect less and less until their courses become relatively straight; and that if the intensity is then still further decreased, they move in 'circles' toward the blackened eye.

It must be concluded from these reactions that it is probably true that in light below certain limits, the effect of the light and that of the covering of the eye, may become equal. But, if this is true, it does not necessarily mean that the nature of the stimulus produced by the contact of the covering with the eye and that caused by light in the opposite eye is the same. It is possible that while the covering probably stimulates through its continuous action, light may stimulate by virtue of change of intensity. This may well be so, and yet the effect of the two

stimuli may be neutralized by their simultaneous action on the organism.

These experiments in non-directive light of different intensities show conclusively that there is no relation between the degree of curvature of cirrus movements and the intensity of the light above certain very low limits. They thus support most strongly the conclusion reached before, that orientation in *Vanessa* is not dependent upon the relative amount of light energy received by the two retinas.

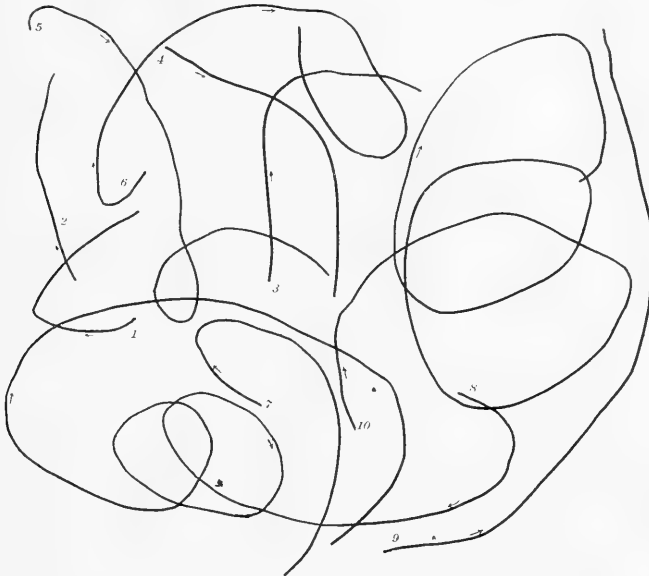


Fig. 19 Reproduction of trails made in non-directive light of high intensity, 460 mc., by butterfly 10/13-a (left eye blackened). Compare the preceding figure with this one and note that in light of high intensity the animal does not move in courses with a smaller degree of curvature than it does in light of low intensity, thus exhibiting reactions not in accordance with Loeb's theory of orientation.

In addition these results contradict an apparently possible explanation of the reactions in the horizontal beam of light. Superficially it would seem that these reactions may be due simply to a balanced effect of two stimuli acting unilaterally, one, light, acting continuously and tending to cause movement toward the functional eye, and the other, the covering of the

blackened eye, tending to cause movement toward the non-functional eye. However, the lack of correspondence between the size of the circles made and the intensity of non-directive light shows that the organism does not respond to differences in intensity as would be demanded by this theory. This conclusion is further supported by the fact that a gradual change in the intensity of light does not affect the angle of deflection. Consequently orientation can not be due to a balanced effect of the action of the covering on one eye and the action of light on the other.

F. EFFECT OF ILLUMINATING ONLY ONE EYE

1. *Effect of illuminating the entire surface of one eye*

The experiments described previously in which one eye was thrown out of function by being blackened were supplemented by others in which one eye was prevented from functioning simply by not being illuminated. This was accomplished by methods somewhat similar to those used by Holmes and McGraw ('13). These investigators held insects between the fingers over a "thin horizontal disc rotating on a pivot, like the turntable of the microscopist," with the head pointing either toward or away from the center. "An electric light was so placed that the rays of light fell upon one side of the body. These insects attempted to turn toward the light, and by the action of their legs, caused the disc to rotate in the opposite direction. When the animals became quiet they could, generally, be caused to resume their activity by pulling them slightly backward."

Holmes and McGraw draw very decided conclusions from the results of these experiments. They say (p. 373): "There can be little doubt that light exercised a continuous stimulating influence upon their [the butterflies] activity. It is not possible, we believe, to construe phototaxis entirely in terms of differential sensibility. Responses to the shock of transition, whether in the direction of an increase or a decrease of stimulus, may play a part in the orientation of many forms, but the continuous stimulating influence of light appears to be, in several cases, at least, the factor of major importance."

Feeling that the methods, as described above, were scarcely adequate to justify the conclusions drawn, a somewhat similar piece of apparatus was constructed with the important modifications that the light conditions were sharply defined, and that the animals were suspended above the disc by means of a mechanical holder, instead of being held with the hand. The object of this experiment was to ascertain the nature of the stimulus effective in orientation, that is, whether light produces orientation through its continuous action or through a change of intensity. A way of determining this would be to exclude one of these possible methods of stimulation and to test the effect of the other alone. Consequently, if the latter method is to be excluded, the organism should be so held that it would be subjected to no changes of intensity. This condition is met only if the precautions described above, or similar ones, are taken.

That this condition might be fulfilled and that the direction of movement of the animal might be detected, a circular piece of thin black card board, 10 cm. in diameter, was suspended in a horizontal position by means of a hat pin which was held in a support that offered as little resistance to the easy movement of the pin as possible. To the bottom of the disc a thin cork was glued so that the pin pierced the cork as well as the disc. The butterflies were suspended above the disc by means of a holder which clamped the wings firmly together. The holder was then adjusted so that the insects faced the center of the disc, and were at such a distance above the disc that they could just touch it with their feet, and yet not get a firm grip upon it. A Nernst glower was so situated that the rays from it struck the right eye of the animal at right angles to the long axis of the body. By means of screens the beam of light was made so small that the area of a cross section of it was but slightly larger than the surface of the illuminated eye. In line with the glower and the head of the animal a mirror was so placed that it reflected the shadow of the head of the animal down upon the table which supported the whole apparatus, as is shown in figure 20. A screen was placed around the image so that as little light as possible might be reflected into the room. Under these conditions the

head, only, was illuminated. Any movement of the legs of the insect could, however, be instantly detected, for, as the legs moved the head was 'bobbed' simultaneously up and down. Thus, by observing the reflected shadow of the head, the periods of activity of the animal could be ascertained.

The results obtained were essentially the same in all of the seven experiments performed. They may be illustrated by the following detailed description of part of one of them. After the

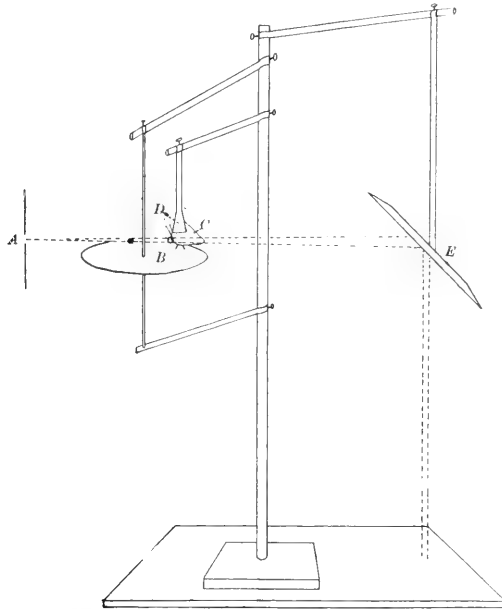


Fig. 20 Diagram to show apparatus used in experiments in which the butterfly is suspended above a rotating disk. *A*, Nernst glower; *B*, disc; *C*, butterfly; *D*, holder clamped over wings of butterfly; *E*, mirror.

butterfly was placed in the mechanical holder, with the right eye illuminated by the beam of light, it remained quiet for 390 seconds; then became active and continued to move for 15 seconds; after which periods of rest and activity alternated as follows: quiet 240—active 10—quiet 60—active 30—quiet 60—active 5—quiet 60—active 30—quiet 90—active 5—quiet 60—active 120. Whenever the insects were active they attempted

to turn toward the functional eye, never in the opposite direction. What causes changes from rest to activity, and what is the significance of the fact that the animals attempt continuously to turn toward the functional eye?

During the periods of rest the changes in illumination in the eyes were exceedingly slight for the light remained practically constant, and there was no change in the position of the eyes. Consequently, if the change from rest to activity is due to light at all, which is questionable, since the butterflies do at times move in total darkness, it must be due to stimuli dependent upon the continuous action of light, not upon the time rate of change in intensity. Regarding the nature of the orienting stimulus the results are unfortunately not so conclusive, for the moment the animals become active, and before they attempt to turn, there is a change in the position of the eye owing to the vertical movements of the head, and this, no doubt, results in changes in the luminous intensity on the various ommatidia. Thus, it is evident that the attempt on the part of the animal to turn toward the illuminated eye may be due to stimuli dependent upon the time-rate of change of intensity.

Thus, it is clear that these results do not fully settle the question as to how light acts in producing the orienting stimulus and that the conclusions of Holmes previously stated are not justified.

The method described above of exposing one eye only to light gives opportunity for testing the effect of illuminating different areas of the eye. The results of this experiment are presented in the following section.

2. Effect of illuminating different areas of one eye

We have demonstrated that in *Vanessa* only one eye is necessary in the process of orientation in light. Now the question arises concerning the effect of the illumination of different areas of the eye. If there is any effect then the axial position assumed by the animal ought to bear some specific relation to the area of the eye illuminated.

This was tested by suspending the insects above a disc, as in the previous experiment, and by allowing the horizontal beam

of light to strike the right eye from different directions. In one experiment the beam struck the eye obliquely from the rear as is represented by the arrow (a) in figure 21. Thus the posterior half of the right eye was illuminated while the left eye was in darkness. In another the rays of light struck the right eye obliquely from in front, as is represented by arrow (b) in figure 21.

Under the former conditions, when the posterior half of the right eye was illuminated, eight animals were tested, each for thirty minutes. As in the preceding experiment these insects showed alternate periods of rest and activity. During the periods of activity they all attempted to turn toward the illuminated eye, as was shown by the direction in which they revolved the disc.¹



Fig. 21 Diagram to represent the direction from which beams of light were allowed to strike the right eye (see text).

Under the latter conditions, when the anterior half of the right eye was illuminated, the animals were also alternately quiet and active. When active they varied in their behavior. Some attempted to turn toward the illuminated eye, while others attempted to turn in the opposite direction. They were tested on several successive days, the tests on each day lasting for thirty minutes.

The first animal (*A*) tested attempted to turn continuously toward the shaded eye on the first day. On the second day, during the first part of the test, it attempted to turn in the same direction, but during the last part of the test it attempted to turn in the opposite direction, i.e., toward the illuminated eye.

¹ The disc could not be seen. Its direction of motion was perceived, however, by means of a light paper arrow glued to the bottom of it. The hand of the observer was so held that the arrow struck the hand as the disc revolved.

On the two following days it continued to attempt to turn toward the illuminated eye.

The second animal (*B*) on the first day attempted to turn toward the shaded eye continuously. On the second day its behavior varied. As in the experiments in which one entire eye was illuminated, this animal without any perceptible change

TABLE 9

Showing direction in which eight butterflies attempted to turn on successive days when the anterior surface of the right eye was illuminated obliquely from in front

DESIGNATION OF ANIMALS	FIRST DAY	SECOND DAY	THIRD DAY	FOURTH DAY
A	Away from illuminated eye	Away from illuminated eye	Toward illuminated eye	Toward illuminated eye
B	Away from illuminated eye	Away from illuminated eye and toward this eye	Toward illuminated eye	Not tested
C	Toward illuminated eye	Toward illuminated eye	Toward illuminated eye	Toward illuminated eye
D	Away from illuminated eye	Toward illuminated eye	Toward illuminated eye	Toward illuminated eye
E	Toward illuminated eye	Not tested	Not tested	Not tested
F	Toward illuminated eye (Most cases)	Toward illuminated eye	Toward illuminated eye	Not tested
G	Away from illuminated eye at first; then toward illuminated eye	Away from illuminated eye in most cases	Not tested	Toward illuminated eye
H	Away from illuminated eye	Toward illuminated eye	Toward illuminated eye	Not tested

in the environment exhibited alternate periods of activity and rest. Forty periods of activity were observed during the test. In fifteen of the first twenty of these it attempted to turn away from the illuminated eye, while in fifteen of the last twenty it attempted to turn in the opposite direction. On the third day it attempted to turn toward the illuminated eye uniformly.

In table 9 the results secured with the eight animals used in these tests are summarized.

By referring to this table it will be seen that when the anterior surface of only one eye is illuminated Vanessa usually turns toward the shaded eye when first exposed, but that later it turns consistently toward the illuminated eye. Consequently, since it always turns toward the illuminated eye when the posterior surface is exposed, it is evident that the reactions may depend to some extent upon localization of the photic stimulus in the eye.

The results presented in this section show, moreover, that the tension of the muscles in the legs on either side is not specifically dependent upon chemical changes in either eye, as demanded by the 'continuous action theory,' for without any change in the illumination of a given surface of one eye the animal may turn either to the right or to the left.

GENERAL SUMMARY AND CONCLUSIONS

1. Vanessa antiopa creeps and flies toward a source of light, that is, it is positive in its reactions to light, never negative.
2. Butterflies of this species, in direct sunlight come to rest with the head away from the source of light.
3. When placed in a horizontal beam so as to face the light Vanessa with one eye blackened usually turns toward the functional eye. In some cases it continues to turn in this direction and consequently performs circus movements both in the beam and in the shaded region beyond it; usually, however, it proceeds in a fairly straight course diagonally across the beam until the edge of the beam is reached, where it usually turns toward the covered eye and moves directly toward the source of light. Some-

times the insect does not turn toward the source of light until it has gone a few centimeters beyond the edge of the beam. The angle through which the butterflies turn before they proceed toward the edge of the beam, varies in different individuals and in the same individual under different conditions. Moreover this angle bears no observable relation to the luminous intensity. It was found to be the same in 200 mc. as in 2000 mc.

4. In non-directive light, or before an open window, these insects move in circles toward the *functional* eye, and continue to do so, showing no apparent improvement. This is probably due to the absence of changes of illumination on the surface of the eye. Thus, the performance of circus movements is, in many cases dependent upon the approximately equal illumination of large areas of the functional eye in all the positions assumed by the organism.

5. Circus movements, however, throw no light on the nature of the stimulus effective in orientation, for the slightest change in position of the head may produce changes of intensity on certain ommatidia in the functional eye.

6. Vanessas with one eye blackened do not move in smaller circles in strong light than they do in weak light, unless it is extremely low. On the contrary, the evidence seems to indicate that the stronger the light is the larger the circles are. These results also are not in harmony with those demanded by the 'continuous action theory.'

7. If, however, the intensity of non-directive light is made *very* low, Vanessa with but one functional eye deflects neither to the right nor to the left, and, if it is made still lower, it moves in circles toward the blinded eye.

8. These animals modify their behavior as the result of repeated trials. This modification in reactions is shown in three respects: (1) decrease in the number of circus movements made, (2) decrease in the angle of deflection and (3) increase in the promptness with which they orient on reaching the edge of the beam.

9. If the luminous intensity is suddenly changed when specimens of *Vanessa antiopa* with one eye blackened have oriented in a beam of light, and are moving toward the source of light at a

certain angle with the rays, the response varies. Usually, however, if the luminous intensity is suddenly increased the butterflies increase the angle of deflection, and if the intensity is suddenly decreased, they decrease the angle of deflection. These results are probably dependent upon the time-rate of change and are not due to the difference in the amount of light energy received by the functional eye under the different conditions.

10. *Vanessa antiopa* with one eye blackened can re-orient. If, when the animal is moving toward a source of light, the direction of the rays is changed so that the light strikes the butterfly on the side of the blinded eye, the organism changes its direction of motion by turning directly toward the source of light. If the source of light is moved to the other side of the animal, the butterfly again changes its direction of motion and goes toward the light. Thus, with one eye functional, the animals in orienting may turn either toward the side bearing the functional eye, or toward the side bearing the blinded eye. These results contradict the assumption of the 'continuous action theory,' that orientation is dependent upon the relative amount of light energy received by the two retinas.

11. Specimens of *Vanessa* with one eye blackened move in circles toward the blinded eye when placed in darkness, while in light they tend to turn in the opposite direction. This shows that the covering of the blackened eye produces a stimulus. It also shows that the circus movements toward the functional eye in the presence of light are due to a stimulus produced by light, and are not due to stimuli received by the blinded eye.

12. When suspended above a rotating disc with the head pointing toward the center of the disc, and with only one eye illuminated, *Vanessa* attempts to turn toward the illuminated eye. Under such conditions there are alternate periods of rest and activity. The stimulus initiating a period of activity is not due to change in luminous intensity, and hence it must be due either to internal factors or to the continuous action of light.

13. If, however, the light is so arranged that only the anterior surface of the right eye is illuminated, the animal may turn either to the right or to the left. This indicates that the reac-

tions may depend upon the localization of photic changes within the eyes, and it seems to show conclusively that the tension of the muscles of the legs on either side of the body is not specifically controlled by photo-chemical changes in either eye in accord with the 'continuous action theory.'

14. The following facts: (1) that *Vanessa antiopa* with but one eye functional can orient, (2) that in a beam of light circus movements become less frequent and the angle of deflection decreases with experience, (3) that the degree of deflection is no greater in light of high intensity, than it is in light of low intensity, (4) that *Vanessa* can turn under certain conditions toward either side when only one eye is illuminated, and (5) that these insects can, in the process of orientation, turn either toward the functional or the blinded eye, all, indicate that orientation in *Vanessa* is not wholly dependent upon the relative intensity of light on the two eyes. They show moreover that the path in the nervous system along which the impulses travel is not permanently fixed. Regarding the question as to the nature of the orienting stimulus our evidence is, however, not conclusive.

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A FEMINIZED COCKEREL

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SEVEN FIGURES

The relation of the gonads to the secondary sexual characters of animals has received considerable attention during recent years. The work of Steinach with rats and guinea pigs is of particular interest since he was able to transform males into the simulacrum of females by grafting ovaries into castrated males. A like result has been secured with the individual described in the present paper. This is the only successful result that I have been able to secure from several trials made at intervals during the past five years.¹

The chick, a Brown Leghorn, was hatched May 27, 1914, of eggs purchased from a breeder. On June 19, the bird was castrated by making an incision on each side and carefully removing the testes. Particular care was taken to see that all testicular matter was removed. Just previous to the operation on this bird the ovaries had been removed from two pullets of the same strain belonging to the same brood and placed in moist cotton. They were cut in several pieces and dropped into the abdominal cavity on each side. No attempt was made to suture the pieces in place. The bird was returned to the flock to await developments. August 25 it was mistaken for a castrated female and its plumage was described in notes made at that time as 'fair male juvenile.' It should be remembered in this connection that the juvenile plumage of the male Brown Leghorn has some points of resemblance to the female. Two days later the entire

¹Since this paper went to press, the results of several trials made in the autumn of 1915 have become available. Three individuals externally are essentially like the one described in this paper. This brings the number of successful instances to four.

flock was gone over and checked up with the records of the various operations, which showed that this individual was not a castrated female but had the history given above. At first it was thought that some mistake had been made in banding the chick or recording the number but none could be found. Moreover, the presence of the line of suture on each side of the bird showed that it must have been a male, because in ovariectomizing the female, the left side only is opened.

At this time, August 27, the notes state that the bird had the size and general appearance of a female, was without spurs and had a small comb, in marked contrast to the normal males of the same flock. In general, the color at this age must be described as neither male nor female, but as showing evidences of both. The ventral region was black with buff or brownish red edgings and shadings, according to the section involved. The hackle feathers were black with golden buff centers. The dorsal regions were black with red or reddish brown markings. In the wing bow region the feathers were black with much red, very much like those of a young male. In the remaining dorsal regions there was very little red, and what there was was confined to the margin of the feathers. The secondaries were much like those of the adult male but were sprinkled with fine black spots. The two rows of secondary coverts were red, stippled with black, quite a different sort from those of the normal adult male which are uniformly black. The bird was shown to several experienced poultrymen without knowledge of the history of the case, and they all pronounced it a female. From this time on, it was kept under close observation. It maintained its general feminine appearance, except that it grew somewhat long-legged and rangy, as a cockerel would do. The spurs remained undeveloped a long time. When the adult plumage came in, it lost some of its nondescript character and in most sections was clearly that of the normal female. The chief difference lay in the feathers of the dorsal regions which were black with relatively few minute brown spots instead of the uniform mixtures of minute dull black and brown spots characteristic of the Brown Leghorn female.



Fig. 1 Normal Brown Leghorn male.

Fig. 2 Normal Brown Leghorn female.

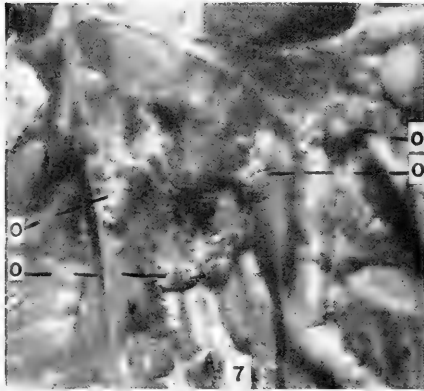
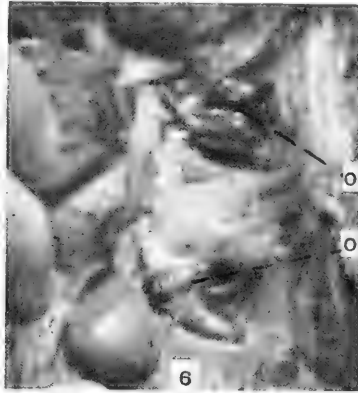
Figs. 3, 4, 5 The feminized cockerel taken at different ages. The photographs were made at varying distances. Figure 3 is proportionately too large. 3, December 14, 1914; 4, September 17, 1914; 5, July 30, 1915.

The bird was placed in one of the laying houses in the fall in order to watch its behavior. In particular it was desired to see if the bird would make visits to the trap nests, not that it was expected that eggs would be laid—a physical impossibility. Normal hens often make visits to the nests without laying, sometimes for periods of several months at a time. The feminized cockerel made only one visit, probably accidental.

During the spring the bird's comb and wattles began to grow and became very red. On several occasions the bird was both seen and heard crowing. It was also observed treading the hens. It is unusual for a hen to tread another even in the absence of the male. Although such cases have been recorded, only one instance has come under my personal observation. Crowing hens are likewise on record but they, too, are rare. I have not seen such a bird crow, though we had a hen at one time that was suspected of crowing. Later in the season the comb and wattles became somewhat shrunken and the bird ceased crowing. Although in fair flesh, the bird did not seem in the best of health. Often it staggered slightly in moving about. This condition continued for more than two months until the bird was killed. This was done July 30. The autopsy showed the following findings: Weight 3 pounds, 7 ounces; oviduct not found, nor were vasa deferentia; spleen hypertrophied; very little body fat; bursa fabricii not found. Ovarian tissue was found in the following positions: On the left side one piece was attached to the body wall, ribs and transverse septum and enclosed in a serum filled sack. The ova were very small, not more than a millimeter in diameter. A second mass lay on the surface of the kidney just lateral to the junction of the iliac with the vena cava. Four of the pieces placed on the right side were found to have become attached, three of them in the form of elongated masses, one attached to the ribs, another to the transverse septum and liver, while the third was attached to the mid-dorsal mesentery at the level of the adrenal. The fourth had adhered to the outer body wall. Some of the ova on this side reached 3 mm. in diameter. There were no evidences of empty follicles. The blood supply of the pieces of ovum on this side was well developed.

EXTERNAL APPEARANCES

The bird is shown in figures 3, 4, 5. Unfortunately, the photographs show nothing of the bird's color. They show, however,



Figs. 6 and 7 Showing the engrafted pieces of ovary (*o*) at autopsy. 6, left; 7, right side.

the shape of the feathers and the general appearance of the bird. The weight of the bird, 3 pounds, 7 ounces, is near that of a hen. The spurs are an inch long. They did not begin to develop

until sometime after those of the normal males of the same age. Spurs of this size, however, are not unusual in perfectly normal hens.

Color. Except for the dorsal regions posterior to the neck, the bird was colored like a female. Ventrally its color duplicated the hen save that the breast feathers were not as bright a salmon as is usual. The wing and tail feathers were dull black. The dorsal body feathers were dull black with a little sheen and a well-developed reddish shafting. Scattered over the web were various small spots of brown. The total area of the brown was very small in proportion to that of the black, whereas in the normal female they are approximately equal and are scattered about evenly over the web of the feather.

Feather shape and length, together with the presence or absence of barbules are good criteria of their male or female character, except in the case of hen-feathered males. In several years' experience with this strain, hen-feathered males have not been encountered. The breeder of the strain confirms my experience so that we can dismiss the possibility of the bird's being constitutionally hen-feathered. In the female, the dorsal feathers have rounded ends, are relatively short and possess barbules along nearly the entire length of the barbs. In the male and capon, the feathers are long and pointed, while about one-third the length of the barbs of the distal two-thirds of the feathers is without barbules, thus producing the glistening appearance, characteristic of the male. All the dorsal feathers of this bird are shaped like those of the normal female. There is no trace of any of the male feather characters described.

THEORETICAL CONSIDERATIONS

A single individual is a very slight foundation from which to draw conclusions. Since Steinach has secured similar results with guinea pigs and rats, it may be assumed that essentially similar results will be obtained whenever a successful graft can be secured. It has already been shown that the removal of the ovaries in birds results in the development of the male plumage,

while castration of the male does not alter the plumage. In other words, the secondary sexual difference of the birds thus far studied can be largely explained on the assumption that an internal secretion of the ovary is responsible for the differences observed. All the differences, however, cannot be explained thus. The size of the comb and wattles in the male depends upon the testes but in the female the relation to the gonads is more complicated. The comb of the young pullet as a rule remains small until about the time egg production begins, when it enlarges rapidly to adult size. With the cessation of laying, the comb shrinks, often to half its former size, but enlarges somewhat with the resumption of laying, though it does not often reach its former size, at least in Leghorns. In old Leghorns, the comb often remains shrunken throughout the laying season. In some castrated females, the comb becomes very large and cock-like, while in others it has remained small. The spurs appear to be independent of any influence of the testes since they develop well in the capon. The ovary appears to exert an inhibitory effect on the development of the spurs, since all castrated females develop spurs. The inhibition may or may not be complete because in certain strains spurs frequently appear on the females, though almost completely absent in other strains. It is possible that the secretion of the ovary is complex, one constituent controlling the spurs or the shape of plumage, another the color, etc. Or, it may be that the general growth potentialities are greater in some individuals than in others, so that characters such as spurs escape from the inhibitory influence of the ovary. The vocal organs and behavior of the birds are also somewhat independent of the sexual organs. Capons frequently crow and sometimes tread the hens. They also brood the chicks. On the other hand, none of the castrated females, even those that develop the habit of the males most perfectly, have ever been seen or even suspected of crowing, nor as far as known do they ever tread the hens, although special attempts have been made with this end in view.

It is evident, I think, that the difference between the secondary sexual characters of the sexes cannot be ascribed solely to the

internal secretions but that the genetic basis of each character must also be taken into consideration. At least four groups of characters can be recognized: Head furnishings, dependent in the male upon the testes, in the female independent of the ovary in certain respects, in other respects, dependent; spurs independent of testes, but on which the ovary exerts an inhibition, often incomplete; voice and behavior, which in the male is partially dependent and partially independent of the testes yet closely correlated with these; and plumage, which is independent of the male organs but on which the ovary exerts a modifying influence.

Since the male may be feminized, it follows that if the ovary be considered an inhibitor merely, then the male must possess both potentialities for the secondary sexual characters and that the ovarian secretion suppresses the male character, allowing the female plumage to develop. Genetically, then, the male secondary sexual characters must be considered dominant to the female. On the other hand, if the ovarian secretion be considered a modifier, transforming the male character into the female, we need not assume that both potentialities exist in the male, but only the one. We may also make a similar assumption for the normal female. At present, it is impossible to determine whether or not the ovarian secretion is an inhibitor or modifier.

THE WATER CURRENT PRODUCED BY ASCIDIA ATRA LESUEUR¹

SELIG HECHT

ONE FIGURE

I

1. *Ascidia atra* is a common tunicate at Bermuda,² living attached to rocks, in most cases well under low water. In specimens that were taken to the laboratory and allowed to remain undisturbed until both siphons had opened, a vigorous current could be demonstrated entering the oral, and leaving the atrial siphon. The outgoing current seems always to be stronger than the incoming current, probably because of the somewhat smaller size of the atrial opening, the volume of water passing through the two apertures being, of course, the same (Wallengren, '05, p. 12). This difference in the velocities of the two currents and the angle formed by the axes of the two expanded, diverging siphons, are concerned with keeping the outgoing water from being drawn in again with the incoming current.

I have always found this current to be present until the closing of one or both of the siphons by some stimulus caused it to stop. The oral siphon is the more irritable and is more likely to be closed first, if the stimulus is not very strong. The siphons therefore act like valves, since they control, but in no way help to produce, the current. This is maintained by the cilia which line the lateral margins of the stigmata in the wall of the branchial sac (Orton '13). In *Ascidia mentula*—a species closely related to *A. atra*—Herdman ('99, p. 17) has estimated that adults of medium size have 192,000 stigmata. Since there is

¹ Contributions from the Bermuda Biological Station for Research, No. 42.

² My best thanks are due to Dr. E. L. Mark, and to the trustees of the Humboldt Fund, for making it possible for me to stay at the Bermuda Station during the summer of 1915.

a row of cilia on each side of these slits, there are in all about 384,000 short rows (ca. 0.2 mm. long) of cilia lashing water through the pharynx of the animal.

Such water currents are not uncommon among marine animals. They have been studied elaborately in Lamellibranchs by Wallengren ('05); and the work of Orton and of Andrews ('93) has shown how similar the mechanism of their production is in Lamellibranchs, Ascidians, and Amphioxus. The details of the maintenance of such currents in the gastrovascular system of *Aurelia* (Widmark '13) have brought to light the long suspected fact that, unlike *Amphioxus*, Tunicates, and Sponges (Parker '10 and '14), muscular movements are an important factor in reinforcing the ciliary current produced by medusae.

2. Respiration (cf. however, Dakin, '09, p. 52; Pütter '07, Tab. V) and the gathering of plankton organisms (Orton, p. 20) are the two functions commonly ascribed to the incoming current. To these, Pütter ('07) has proposed the addition of a third. On the basis of metabolism studies, he believes that plankton food is insufficient for the carbon requirement of a variety of marine animals, "and that sea-water constitutes a very dilute nutrient solution, from which they resorb their food entirely or in part" (Pütter, '14, p. 98). To the water current, then, falls the task of bringing this 'nutrient solution' to the place where it may be resorbed.³

I mention Pütter's conclusions because he, and others who have criticised him (Lipschütz, Moore et al.) make frequent assumptions as to the volume of the current produced by various animals, having, however, almost no accurate quantitative data on which to base their statements (Pütter '07, p. 293).

The velocity measurements made by Wallengren (p. 23) on the anal current in mussels, and the roughly accurate estimation of the volume of water passing through the Gehäuse of an appendicularian, made by Lohmann ('09, p. 220), show that the quantities are rather large. The only values actually determined are those found by Parker ('14) for a Bermuda sponge. A finger

³ An actual case of such a resorption (really a synthesis, too) has just been demonstrated for fats by Churchill ('15) with the fresh water mussel.

of *Spinosella* whose volume was ca. 95 cc. discharged 54 cc. per minute through its osculum, or about 78 liters a day.

II

1. For determining the volume of the current of water generated by *Ascidia atra*, I adopted a simple indirect method. A glass tube whose bore was a few millimeters less than that of the expanded oral opening, was suddenly introduced into that opening, and the siphon allowed to contract upon it. The tube was then securely tied to the siphon. If an attempt is made to force a tube into a siphon which has begun to close, the mantle

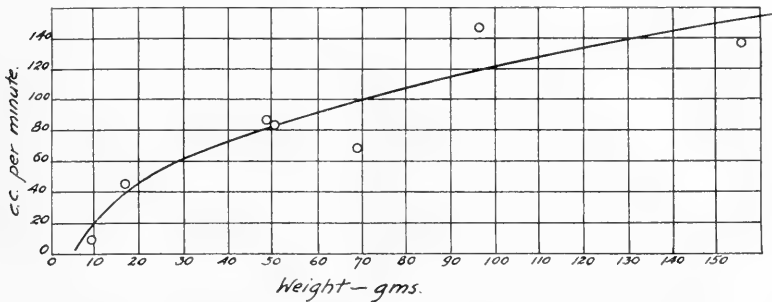


Fig. 1. The volume of sea water passing through different sized specimens of *Ascidia atra*.

tissue is always injured, and the animal never recovers from the injury. The velocity of finely divided carmine particles as they passed through the tube into the animal, was determined at frequent intervals, until a maximum velocity was attained. Ten readings were then made and an average taken. Knowing the diameter of the tube and the velocity of the current, the volume of water passed through the tube may be readily calculated.

A preliminary determination made on an animal ca. 10 cm. long, gave this volume a value of 94.2 cc. per minute. Seven animals of various sizes were then selected, and the volume of the currents measured. The weights of the individuals and the number of cubic centimeters of sea-water passing through in a minute are given in the accompanying figure.

2. According to these results an adult of medium size (ca. 100 gm.) moves about 173 liters of sea-water in a day. Large as this quantity seems to be, I am convinced that, if anything, it is less than the normal accomplishment. *Ascidia atra* is not a good aquarium animal, specimens rarely surviving longer than a few days in the laboratory at the summer temperature. The handling and removal from the water consequent on collection and transportation would hardly improve its condition. A case in point is the following experiment.

Experiment 76, July 30

- 12.30 Fixed the tube into the oral siphon.
 Animal kept out of water 3 minutes.
- 12.45 Average of 10 readings for the time required for carmine particles to travel the length of the glass tube, 3.6 seconds.
- 1.00 Average of 10 readings, etc., 2.4 seconds.
- 3.40 Average of 10 readings, etc., 1.1 seconds.
- 8.25 Average of 10 readings, etc., 0.98 seconds.
- 9.20 Average of 10 readings, etc., 1.3 seconds.
- 10.45 Average of 10 readings, etc., 1.4 seconds.
- Weight of specimen, 69 grams. Visible length of tube, 5.7 cms.
 Diameter of tube, 0.50 cms.

It took nearly eight hours for the velocity of the current to reach a maximum, in other words, for the effect of the manipulation at the beginning of the experiment to disappear. I cannot say that recovery was complete, but judging from the appearance and behavior of the animal, I believe that it was nearly so. Recovery therefore is a very slow process. The only experiment which I made with a specimen in its natural environment was unsuccessful, and the termination of my stay at Bermuda prevented its repetition.

3. The above curve shows that the volume of water produced by the current, and hence the total energy of the ciliary mechanism in the branchial sac, is not quite a linear function of the weight of the animal. In general the energy manifested per unit weight varies inversely as the size. That this is true for many invertebrates has been maintained by Vernon ('95) and by Pütter ('07, p. 301) and clearly proved for *Octopus vulgaris* by Polimanti ('13).

III

Of equal interest with the volumes of water found for the currents in *Ascidia*, is the pressure at which these currents are maintained. This I determined in the following manner.

A glass tube was tied into the atrial siphon and the animal placed so that the tube was vertical. Enough sea-water was added to cover the animal and the free end of the tube. After a few minutes the oral siphon opened, and a disturbance of the surface of the water near the tube showed the presence of a current through the branchial sac. The level of the water in the jar was then lowered very slowly by means of a siphon, until the height in the tube was about a centimeter below the projecting end. The height of the water in the tube above that of the water in the jar was then measured. The level in the jar was again lowered, and again the height in the tube measured. This was done five times for each animal. Care was taken to see that the oral siphon remained open and submerged during the operation.

After the fifth determination, the animal was removed from its attachment to the tube, the latter retaining its position. The jar was filled with sea-water and similarly five measurements were made of the height of the water in the unattached tube. The difference between the two sets of readings gives the current pressure in millimeters of sea-water.

The pressures thus found are slightly lower than those obtained by Parker for most of the sponges. An average of ten determinations on *Ascidia* (specimens of ca. 100 gms. each) gives the pressures as 1.7 mm. of sea-water, the range of variation being from 1 mm. to 2.1 mm.

IV

These observations show that the quantity of energy devoted in *Ascidia atra* toward the maintenance of a sea-water current is distributed, in the light of the functions of such a current, in an efficient manner. A current pressure very much in excess of that necessary to overcome the inertia of the water, would

be useless (Meltzer '07). Obviously more is to be gained by utilizing that energy in the moving of large quantities of water through the organism. Such a partition of the energy is actually present.

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COLOR CHANGES IN THE RHINOCEROS BEETLE, DYNASTES TITYRUS

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FOUR FIGURES

PART 1

The 'rhinoceros' or 'cow' beetle of the Southern United States is noted not only for its great size but for the marked differences between the males and the females. In the male (fig. 1), the head bears a long horn pointing forward and overhung by a still longer horn from the thorax and there are two smaller horns one on each side of the large thoracic horn. In the female (fig. 2) there is but a slight suggestion of a horn on the head and none at all on the thorax. The spots on the wing cases of the female are also smaller than those of the male.

These horns of the male have been cited as good examples of so called secondary sexual characters, the use of which however is entirely problematical.

Some of these beetles found in a dead stump in the Eastern Shore of Maryland were kept in captivity in glass aquaria with moist decayed wood and pieces of apple for more than a month, but no evidence of use was found for these secondary sexual characters though it was noted that the male in throwing back the head may oppose the horn on the head to that on the thorax with force sufficient to suspend its weight from one's finger. The remarkable tuft of setae, like a hair-brush, on the under side of the thoracic horn raises the question whether it may not be sensory and aid the beetle in estimating the size, etc., of objects clasped between the two horns and in the literature is found the suggestion that the male holds the female. However, watching males and females showed that while early in June the male followed the female for days and seized her with

his legs and finally on the fourth was in copulation, just as in other beetles, with no possible use of the horns at this period, and though on the fifth the male was again following the retreating female and endeavoring to copulate, there was no observed use of the horns.



Fig. 1. Photograph of living male *Dynastes tityrus*, showing form of head and thorax and distribution of dark spots on the elytra.

While the significance of these male horns thus remains entirely problematical, certain color changes in both males and females were definitely established and will be here described.

Mr. M. G. Gichner in setting about to make photographs of these beetles observed that the color of the beetle might rapidly change, and as such changes, back and forth from one color to another, seem to be unrecorded in any such insect, or in fact in any animal not having changeable pigment cells or blood vessels so disposed as to make a color change possible,

it seemed important to find out more about this remarkable exception.

As ordinarily seen, the beetles are very dark red or black on all the lower surfaces, and on the limbs and the horns and in fact on all the top of the head and the thorax where the horns occur, but with abundant yellowish 'hairs;' the same is true of both sexes. But the rest of the thorax posterior to the horn



Fig. 2 Photograph of living female *Dynastes tityrus*, showing form of head and thorax and distribution of dark spots on the light colored elytra.

region and the wing covers or elytra are not dark but very light yellowish. This yellowish color is homogeneous on the thorax posterior to the black horns, or horn region, but on the elytra there are many spots of dark red which may be nearly black. These spots are roughly in rows and differ in each specimen in size, number and arrangement, but tend to run in lengthwise rows. The spots are both large and small, the larger compounded of several smaller more or less fused. Many of the spots have a marked concentric arrangement of dark circles about lighter centers.

As stated in text books, the beetles when freshly emerged from the pupa are of a dark chestnut or red color without spots. In our museum we have such a specimen, a female of homogeneous red chestnut color, together with a male of the faded greenish white with black spots as figured and described also in books.

When the live specimens, that were prevailingly light yellow with dark spots, were put into glass dishes with decayed wood that was wet and were left quiet over night, it was observed that they all had become very dark reddish with spots scarcely discernible, so that the background of yellow had changed to about the same color as the spots had been.

When these dark males and females were removed for photography they rapidly returned to the usual light coloration.

The startling nature of this color change is best seen in the actual specimen where the extremes are very diverse; but to indicate the range of color we may make use of the color code of Paul Klinecksieck, Paris, '08. The dark color may be called a red-orange like no. 80 or it may be violet-red like no. 580 or again nearly black. The light color assumed usually may be an orange-yellow like no. 187 or a green-yellow like no. 287 or 297 or a yellow-green like nos. 272 or 292 in the female and somewhat more yellow in the male. Owing to the high polish of the surface, the actual color is difficult to determine. The change of color is the more emphatic since the spots on the elytra remain dark when the background bleaches from dark to light.

The cause of the change of color seemed at first to be complex and to involve internal factors of response of the whole animal. Thus, when the beetles were quiet in dark, moist wood, they were dark; when active in dry light places they were light. When taken from the moist wood they very quickly changed to the light coloration, but did not change back to the dark when restored to the more natural environment till after a long time had elapsed, so that it might be that disturbance of the nervous system from light and from handling caused the bleaching which might persist till the nervous system had been slowly changed in the apparent sleep of the quiet beetle.

It might also be imagined that the change was of significance to the beetle in its normal life, since when in the moist wood it is dark and inconspicuous and when active in the light it is light colored and spotted, so that it might very well be protected from observation and detection by enemies as blending with the lights and shades of usual daylight or moonlight illumination of probable backgrounds in nature.

A number of experiments also suggested that light might act through the eyes in bringing about adaptive coloration. Thus when the beetle was quiet and dark colored, if light from the window shone very long upon one side only, of that animal, it would change to light on that side only, but still later might change to light on the other side also.

It was evident that of the many factors that might be concerned in the color change, there might be not only the external changes from moist to dry, from darkness to light, from quiet to mechanical disturbances, but unknown internal factors associated with the life of the animal.

To eliminate one after the other of these possible factors, a number of trials were made. It was found that mechanical disturbance alone did not induce color fading, unless there was actual translation of the insect from one part of the glass dish to another, as when it was pulled up to the top by a string attached beforehand and operated without changing or opening the closed vessel: and on the other hand, beetles might remain dark colored when very much awake and active; and they might bleach out when quiet as well as when active.

The changes from darkness to light seemed at first to be important, but it soon transpired that the beetle could remain dark in the light as well as in the dark, by day or night, and would remain light colored in the dark also.

The third factor, the change from moist to dry, seemed the decisive one and potent over everything else. Thus beetles kept in a closed glass with dry filter paper, remained light colored day and night but remained dark if the paper was wet. When a beetle was put into a bowl of water it turned dark as far as

it was wet, though it was very active, and as soon as dry it resumed the light color.

This response of the thorax and elytra to water is very pronounced and instantaneous, so that if a drop of water be placed on the shell of these regions the light color at once changes to dark in that round spot where the water wets the shell. This becomes striking in case a piece of wet filter paper be laid for a moment upon the thorax or elytra and then removed leaving a black area of the size and form of the paper.

That changes in moisture are the only cause of changes in color was not at first suspected, but became more patent eventually when the indirect action of light was found out. When, as above stated, a beetle stands long with one side brilliantly illuminated and that side becomes light and the other remains dark, it is strongly suggested that the light acts as such upon either the surface exposed directly, or else upon the eye, and so eventually effects change through the nervous system.

When an electric bulb (having a ground glass, ten inches from the beetle) was allowed to shine its light upon one side of male beetles for three minutes, then the side of the beetle toward the light had changed from red to yellow and the very strict bilateral difference between the two sides was very pronounced. Four minutes afterwards, light color appeared in blotches on the less illuminated side and in ten minutes after the beginning of the illumination both males were light colored on both sides as a result of the illumination.

These beetles were in a small closed glass dish, an elongated trough with glass lid laid on and the bottom was wet with filter paper. Two hours after removal of the light both males were dark again.

Also, when the beetle stands with a shadow across its body so that, say the anterior part is brightly illuminated and the posterior in the shadow though all is in the light of the room, the dark color changes in the most illuminated part, leaving the back of the beetle very strangely and sharply marked cross-wise with light yellow anteriorly, and dark red posteriorly, as if painted.

Such local and rapid changes in the light might be due to some photochemical change. That this is not probable seems to follow from the following. When the beetle is exposed to the light of a naked mazda bulb shining through 7 cm. of water the color bleaching is very much retarded, in fact not till the water becomes decidedly warm does the bleaching effect appear. Again, a beetle exposed to dark rays only, through black paper, may be bleached by the heat. It was therefore thought probable that the action of the light might be explained as due to its heating the surface of the beetle and thus altering the moisture conditions, either by drying off the moisture in the beetle directly, or by increasing the moisture holding power of the air next the beetle and thus making it less saturated and so leading to drying off of the beetle's surface.

In like manner, the results of pulling a beetle to the top of a dish of air saturated at the bottom by wet paper, might be to bleach it from its being thus brought into dryer air, and other experiments in which mechanical disturbances appeared to cause bleaching could also be explained as the result of change of air and consequent loss of moisture.

When the light colored beetle is exposed to wet air, it becomes dark but slowly; often thirty minutes seems to make no change though careful observation may reveal a sooty appearance within five minutes, but if left one hour or two hours, both thorax and elytra may be dark. In some cases, however, the elytra changed noticeably in three minutes, and in six minutes were all dark red.

If the beetle is floating in water it may become dark in one minute, when, however, the beetle crawls about so that the shell is in contact with wet paper, the change from light to dark may be completed in five minutes, and if moist paper is actually pressed upon the thorax or the elytra, the region so moistened may turn dark in a second.

On the other hand, when the dark beetle in a saturated atmosphere is exposed to the ordinary air of the room, the rate of turning light is rapid but differs in different cases according to the

amount of change, apparently, that results in dry or in moist weather.

The change from dark to light may be brought about in several seconds or in several minutes.

The changes are progressive, being first completed usually on the elytra while not yet finished on the thorax. The yellow color comes on in patches or clouds and leaves the shell for a time marbled or clouded with dark that finally vanishes as if drying away.

Thus, in one case when the air was moist after rain, a female beetle removed from wet air began to show color change in fifteen seconds, in thirty seconds the shell was blotched, in forty-five seconds the blotches of yellow were larger, in one minute the dark was reduced more, in two minutes there were still blotches of dark remaining but in three minutes the elytra were all yellow on the background and the thorax was about half yellow and half dark, in five minutes there still remained some sooty clouds on the thorax. The same beetle on two other days showed the beginning of bleaching on removal from wet air within five seconds, and was very light in twenty seconds, while in thirty seconds the elytra were the maximum yellow and the thorax yellow with smoky black shades which had disappeared by the end of sixty seconds.

In various trials with the males the light began to be noticed in ten to fifteen seconds after removal from the wet air, in thirty seconds there was much light on the elytra and some on the thorax, in sixty seconds the elytra were light and only sooty shades remained of the dark on the thorax, and these shades might be gone before two minutes from time of change of air. When exposed to strong sunlight the change of color from dark to light was about the same rate as if the animal had been removed from the wet air, but this was true only when there was a large amount of air and a strong side light that might well have quickly changed the degree of saturation of the air.

When a live beetle was put into an atmosphere dried by phosphorus pentoxide, the change from dark to light color was, in most cases, more rapid than when put from moist air into the air

of the room. It may be sixty seconds as compared with one hundred and twenty seconds. On another trial the dark red beetle changed to yellow in two minutes when put into artificially dry air, and in four minutes in air of room. When onto the elytra of a dark living male, some phosphorus pentoxide powder was put, that spot changed at once to light yellow with evolution of such heat as to cause a strong reaction from the beetle.

The difference in rate of color change from yellow to red and from red to yellow is well shown when the beetle is held in running water and then put in the air of the room. The running water does not seem to wet it, that is, it glides over its surface without apparently sinking in, so that when the beetle is removed its polished surface seems dry save for a few drops of adhering water, but the color changes slowly from yellow to dark red extending from some regions to entirely embrace all the thorax and the elytra. To complete the change about a minute and a half was needed.

When the same beetle, now all dark, is placed on the table, it begins at once to turn yellow, so that yellow is visible in five seconds and by the end of sixty seconds all the elytra surface and most of the thorax is yellow, with, however, clouds of dark on the thorax still remaining.

The assumption that the material of the thorax and elytra is such that it responds with great delicacy to slight changes in moisture in the air and not merely to actual wetting with large amounts of water is sustained in the next section.

PART 2

Having tentatively reduced the external factors that seemed responsible for changes in color in these beetles, to changes in moisture, the internal factors due to the beetles being alive needed to be considered.

Having in the museum a male specimen mounted by Prof. Otto Lugger some time prior to 1885, it was suggested by Mr. M. G. Giehner that this be tried in comparison with the living

beetles: certainly it may be supposed to have no nervous factors entering into its reactions! The dry color of the long preserved specimen was much lighter than that in the living; in fact, the light color was much like no. 378 A of Klineksieck's scale.

In general, the reactions of the dead beetle were the same as those of the living beetle in all that concerns change of color from dark to light and the reverse. The experiments may be reported as light, heat and moisture experiments, chiefly.

Light

When the dead beetle was left in a moist box with light shining from a window on one side of body, that side eventually turned very light grey while the other side remained very dark red, so that the contrast was most startling. Again, when the dry, light beetle was put into a moist box and one side was exposed to window light, the other side turned dark much sooner than the illuminated side.

When the dark red beetle was left in the moist box one hour with light sunshine from a window on one side but with shadow of opaque object on the posterior part of body, the side exposed to light changed to a light color as far back as the light went, while the part in shadow remained dark as did the opposite side of the body. When the light was removed, the entire beetle turned all dark again within ten minutes.

When, upon the long-dried beetle, wet paper had made a cross-shaped area of dark red across thorax and elytra, exposure to sunshine removed this dark color and removal of light led to restoration of outlines of the cross. Exposing the dead beetle to the light of tungsten bulb, led, in about ten minutes, to change of color from red to light grey, while removal of light was followed by return to dark color within one hour, all in a moist closed glass vessel.

The light from a frosted bulb shining upon the left side of the beetle in moist air was followed by the appearance of cloudy areas of light grey within a few seconds, and these clouds of

light formed both upon thorax and elytron of the left side, which was the one illuminated. In one minute the contrast between right and left was very marked indeed.

Heat

Standing in moist air with one side illuminated from an electric bulb with naked metal filament shining through some 7 cm. of rather turbid distilled water, the dead beetle remained dark colored more than one hour, but a half hour later when direct sunlight shone along with electric light through water, the beetle had turned light colored on the side toward the light. However, by this time the water was hot and the glass dish next to the beetle felt warm. When removed, this beetle turned pale slate-colored in ten seconds. Even the spots on the side illuminated and heated were now still affected by that treatment since they were very pale, while on the side not illuminated they had remained dark red-brown.

In another experiment, light passing from a tungsten bulb through 7 cm. of water changed color of both elytra and thorax on one side in twenty minutes. While in the above it was attempted to cut off heating effects in illumination, in other experiments the dead beetle was exposed from one side to heat rays only, coming from electric bulb enclosed in black paper and with all outside light shut off. After an hour the edge of the elytron nearest to the source of heat had turned white while the rest of the elytra and thorax surfaces were dark and very wet with small drops of water collected on it. Removed from the wet air into that of the room, in five seconds the light grey color had spread from the whitened edge of the elytra up nearly to the middle line of the back and also onto the thorax where the heat had evidently produced some effect. In one minute the elytra were most all white even where not exposed to heat rays.

Moisture

The first experiment made with this long-dried beetle showed its fine response to moisture; that is, when a cross-shaped piece

of filter paper was moistened and placed on the long-dried beetle across both elytra and thorax, there flashed out under it instantly a dark blackish area very striking against the pale whitish background: much as in figure 3. This dark area slowly extended as the moisture spread in the substance of the shell.



Fig. 3 Photograph of dead male *Dynastes tityrus*, showing distribution of dark spots on light colored elytra; and the persistence of a dark colored cross on the thorax, produced by placing a cross of wet filter paper there just before photographing and immediately photographing the dark red cross left when the paper was removed.

Upon removal of the paper the black melted away in one minute like a dissolving cloud, but left after fifteen minutes some faint trace of the outlines of the former dark cross.

Again when the moist breath was blown from a fine pipette upon the side of the elytron for part of a minute, a red spot appeared where the breath struck the shell, growing from the

periphery as a circular area that became all solid red, but vanished in a few seconds when the moist air ceased to be applied.

As to the rapidity with which the dead beetle changes color, a few experiments show that the change from dark to light is much more rapid than the change from light to dark, when the air alone is the factor concerned. Thus, when removed in the dark state from wet air into that of the room, there was marked bleaching in fifteen seconds and in sixty-five seconds nearly all the surface was bleached white, and in one hundred seconds only the natural spots remained dark red. Put back into the wet air it was again all dark red within one hour.

On another occasion, the same dark beetle removed to the air of the room on a dark rainy day, showed visible lighter areas on the elytra in three to five seconds but there were only white clouds in thirty-five seconds and white clouds on the thorax in sixty seconds. The entire surface was chiefly white in eighty seconds. A live female at the same time showed yellow in five seconds and was all light yellow in forty seconds.

When the beetle was held in running water its elytra and thorax turned dark and then when returned to the air of the room it became a noticeable yellow on both thorax and elytra in ten seconds. In sixty seconds, only clouds of sooty black remained on thorax and elytra in place of the universal dark. Then again held in running water, which glides over it as if not wetting the surface but gradually here and there produces a darkening, in one and a half minutes it was again dark. The surface, now dry save for a few small drops that did not run off, in five seconds began to be light colored and in sixty seconds was again light except for a few clouds on the thorax.

PART 3

It being then evident that both dead and living beetles behaved alike in changing color under conditions that might be interpreted as chiefly involving changes in the amount of water presented to the surfaces of the thorax and elytra, being dark red when wet or in saturated air and light yellow or white grey

when in dry air, and rapidly taking on the light color if rapidly dried and slowly turning red if slowly moistened; it seemed that there must be some special peculiarity of the surfaces of the shell of the animal in those regions which caused it to change color according to its dryness or wetness.

Whether this change in the shell was chemical; a change due to hydration; or a change due to some physical effect upon light connected with distribution of light was not at first evident.

The application of other liquids than water and also microscopic observations of the substance of the shell both point to the conclusion that the causes of the color change in the substance of the shell are physical arrangements of the material that allow of distributions of air that lead to the reflection of yellowish light and on the other hand, substitution of liquids for air and resulting passage of red light or partial absence of light.

Experiments with other liquids than water

While a drop of stiff honey on the thorax of a live or a dead beetle neither soaks in nor produces any change in color, at least not for a great many hours, a drop of vaseline acts quite like water. An area covered with vaseline turned black gradually and not uniformly, for some spots remain light longer, so that where vaseline is thickest the shell may remain light, and thin layers of vaseline at the edge of the drop may enter the shell and turn it black. Much later, the black spots so produced were no longer to be seen.

Xylol instantly blackens the shell but in a minute or so, on drying, leaves the shell again light. The xylol dries out first at the center leaving a dark circle for a time.

Ether dropped on the shell instantly produces a black spot that instantly vanishes with a dark ring remaining a little longer.

When absolute alcohol was poured over a beetle, its surfaces turned dark at once and returned to the light color as the alcohol evaporated in a few seconds. On the other hand, a dead beetle that was dark from standing in moist air, when brushed over with 95 per cent alcohol did not bleach but when so treated with

absolute, it instantly changed to mottled white. Also, when the elytra were wet with filter paper and absolute alcohol was brushed on, the dark red color there turned to white in fewer seconds than did other parts of the shell having only water on them.

Under the microscope it is evident that the absolute alcohol enters the shell at certain centers only and thence spreads within the shell substance making circles of dark which rapidly vanish as evaporation takes place. Some areas of the shell are much more resistant to entrance of absolute alcohol than other areas and various results arise.

In the live beetle, also, painting with absolute alcohol causes areas to turn black, especially on the edges of areas treated, and on the other hand, when the shell is dark from washing with water, addition of absolute alcohol causes bleaching, though at the same time extension into dry areas may turn them dark.

While the behavior of the shell toward alcohol and toward the other substances might be explained as the result of hydration, perhaps in all cases, it was found that when well boiled and boiling linseed oil was applied in minute drops to the shell, that it entered into the shell and produced the same dark color though it can not apparently be thought that the oil carried any water with it.

Spots of dark so produced remain permanently in the shell and are very similar to the natural spots of the elytra, though they do not have the sharp cut boundaries, but on the other hand, under the lens are seen to fade out gradually as distributed outward in the substance of the shell.

It is then inferred that any liquid that can enter the shell may cause it to change from light to dark.

Microscopic examination

The shell, under the microscope, is pitted with minute hollows of different sizes which are dark at the bottom and in the larger size bear each a minute bristle or spine rising from the bottom. The colored spots visible to the eye have often a concentric structure, that is, there may be dark rings more or less

filled in with less dark, and in like manner, when moisture dries away there is an intermediate state in which the wet spot is represented by a dark rim and light center which suggests that the material of the shell holds the liquid by some adsorption (?) phenomena. When the spots are made more than normally pale by heating they show more distinctly the concentric structure and formation as if from conglomeration of coalescent spots. Breathing upon these paled spots is sufficient, in a second and a half, to make them turn more dark; to again turn pale in some two seconds.

Though the changes from dark to light are usually quite reversible, there are sometimes changes that remain more or less permanent as above indicated, and seen in the following. After wet paper on the beetle dry for thirty years, had at once made a dark area, the edges of this long remained darkened somewhat, though the area reverted to the light color. Again, after great heating with an electric bulb, under black paper, the edge of the thorax long remained very pale. Gradually the experimented beetle ceased to turn quite light on the right of the thorax and remained more red there, as is the case where linseed oil was applied.

All this is thought to indicate that the phenomena are due to spread of substances through some peculiar structure of the shell, with permanent changes only in so far as the added substance remains permanently, or else changes the structure as by carrying substances from one place to another.

That the water may dissolve substances and so carry them to other regions was suggested by the observation that when the beetle had stood long in wet air, drops of water collected somewhat in rows along the elytra and thorax and that when these were wiped off with filter paper the paper was stained yellow. The beetles also, whether alive or dead, have, when moist, a strong and disagreeable odor that may be associated with some substance soluble in the shell.

Examined under a Spencer Binocular 25 mm. 10 X in direct sunshine, the yellow areas of the elytra show, besides the scattered pits, a uniformly distributed structure of very fine scintil-

lating areas or appearances: minute regions of fiery red and of green in juxtaposition as if from refraction of light due to some minute lack of continuity of the substance of the shell.

These optical appearances seem beneath a surface pellicle. When a needle point is pressed along this surface, it leaves a groove that is mottled with dark and light as if some spongy or powdery structure had been crushed in, and these structural changes remain, so that it is possible to write dark lines on the yellow background by compressing the material near the surface.

When minute water-drops from a single hair of a camels-hair brush are placed on the surface, they sink in and spread in the shell to vanish at once as if they had spread in some dry powder. The edges of the advancing water within the shell are sinuous, that is, the water does not spread entirely with the same speed all along the edge of the drop. Where deep grooves are made by pressure of the needle the water does not enter readily but it goes in elsewhere and spreads thence. A larger area may take thirty seconds to be blotched over with black clouds as the water enters here and there and spreads.

That the light color is due to some way in which air is held within the shell structure seems indicated again by the fact that burnishing the surface with a blunt ivory point produced a permanent dark area, as if the material had been brought together and the air expelled.

Possibly then, many of the minute dark pits on the surface of the animal may have arisen as results of pinching of the surface by legs or horns of other beetles or by other mechanical compressions.

With needle sharpened as a chisel, the material of the shell can be shaved off as yellow shavings that look, under the microscope, to be waxy masses full of minute granules. Digging deeper shows more and more of the same material, and the bottom of the excavation is black with scintillating points as if it were a compounded structure. Water put into these minute excavations sinks down into the black bottom material. The shavings from regions of the shell that are not yellow but are red spots differ from the yellow only in somewhat darker, more

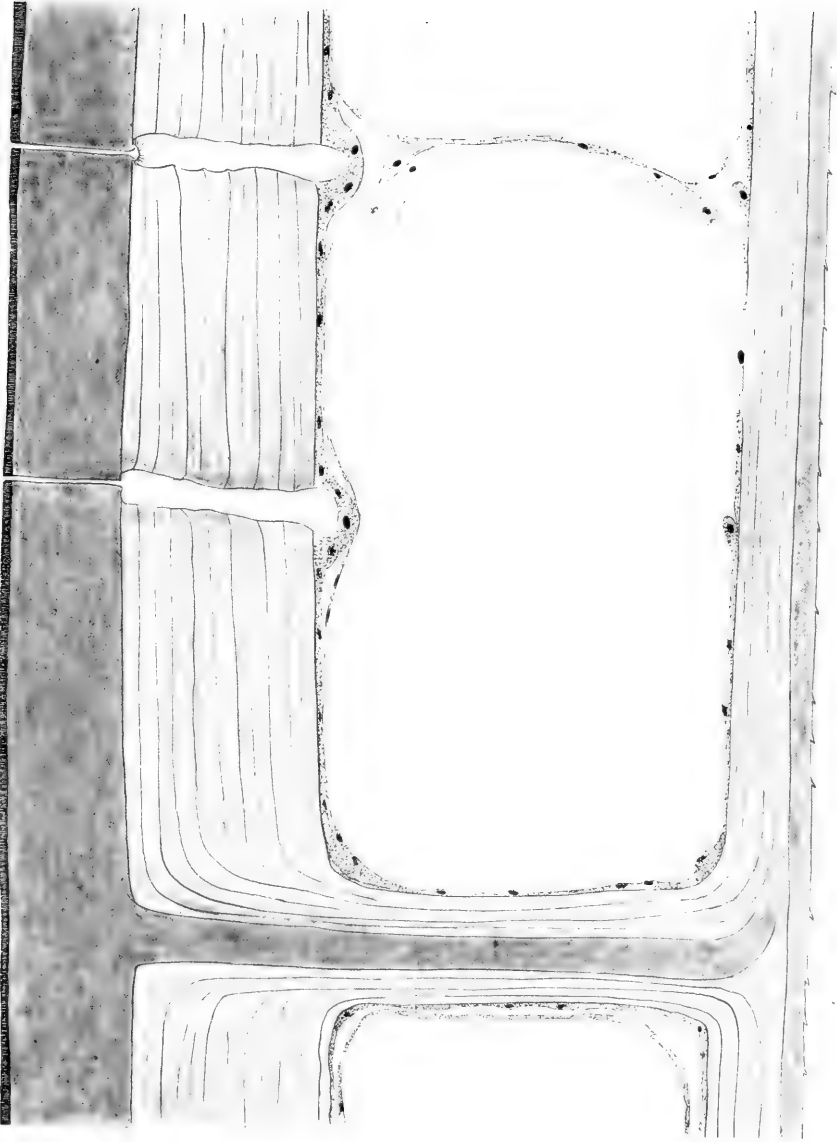


Fig. 4 Part of a vertical section through the elytron of *Dynastes tityrus*, showing large central air sacs, very thick exoskeleton above and thin below, connected by vertical column to the left. The outer or upper exoskeleton is pierced by ducts of glands and is composed of three parts: a laminated, clear inner and thicker portion; a heavily pigmented layer shown as dark; and a thin outermost lamina represented black, the physical nature of which brings about the changes in color as seen from the outside.

orange tint. These minute shavings are very hygroscopic so that when breathed on they quickly curl up and quickly lie down again flat when the moist air passes away.

Under higher powers, Zeiss 4. D, the shavings seem made of parallel pyramids or cones that are subdivided or forked toward the surface and there end in large granules or pieces of the surface. The appearance is as if the surface of the shell were perforated by vertical canals or pores between constituent cones or clubs of yellow material, resembling to some extent, a cheese with regular pores in place of irregular cavities. The edges of pieces of these shavings under Zeiss 4 ocular and 2 mm. seem to be made up largely of granules about 0.6μ in diameter.

Structure of elytra

Raising the elytra they are seen to be all yellow on the under or inner side next the body, but the light coming through the translucent elytra is red. Thus the substance of the elytra, both outside and inside is yellow by reflected light but red by light that passes through. Adding water to the inner face of the elytra does not change its color nor does it soak into the shell. It is only the outer surface of the elytra that is both absorptive and changeable in color.

The long veins on the under side of the elytra mark out areas that are filled with small yellow vesicles or air bags, in some regions crowded together, in others scattered, and seemingly expansions of terminal twigs of the trachea. Where these yellow vesicles are crowded together, that is, toward the median line, they show marked red dots scattered between them.

When pieces of shell are removed for section they absorb the fixing liquid, and though they remain yellow on the inner surface, they turn uniformly red on the outer surface, since the former yellow areas now change to red on the entrance of the liquid.

Vertical sections through the elytra (fig. 4) show that the large central air spaces are lined by a thin epidermis, and that the upper and lower skeletal layers are connected together by fre-

quent vertical columns in which the same cuticular substance is continuous from the upper to the lower shell of the elytron. These columns are the above red dots.

The exoskeleton, or shell of the lower surface, is thin and made up, for the most part, of several parallel layers or laminae, of which the outermost differs but little from the inner ones, but bears numerous fine, oblique spinules, and in some parts of the elytra, long setae.

The upper exoskeleton is very thick, and plainly of two portions, the inner like that of the lower surface, but thicker; and the outer of dark dense material, yellow-red in sections. It is this outer part of the shell that gives the dark red color to the beetle as seen from the outside. This outer layer is not, however, all alike in structure, but is in turn divided into a thicker inner and a very thin outer portion, the latter only 8μ in thickness.

When the section is allowed to dry out, the shell changes to light color but this change is only in this thin outermost lamina. Where the shell has naturally red spots, the dry shell still preserves these spots and the section shows that here, again, the outermost lamina is the only part that is responsible for this difference in color. The outermost lamina does not lose its dark color in the region where natural spots are found.

The substance of this color-determining lamina is seen, under the immersion lens, to be penetrated vertically by pores or slits or some lack of continuity at intervals measured as about 0.6μ . Where the dark red spots of the shell occur, the lamina seems more homogeneous, as if infiltrated with some material that filled its pores or cracks.

As indicated in the figure, the shell is underlaid by the epidermis, and this cellular layer supplies large glands from which tubes perforate the inner part of the shell and then suddenly becoming more narrow, continue on through the outer part to the very surface as only 2.5μ wide.

The laminated inner part of the exoskeleton is evidently the secondary cuticula of Tower,¹ and the colored layer is his pri-

¹ Development of colors and color patterns in Coleoptera. Chicago University Publications, 1903.

mary, or color-containing cuticula, the specialized part of which on the outside may well correspond to such lamellae as he figures for *Chrysobothris*, and which in *Dynastes* has the peculiar light-intercepting and moisture-holding structure. The thickness of the colored layer is about 80 μ and that of the moisture-absorbing lamella about 8 μ .

SUMMARY

In these large beetles there are rapid and striking color changes that may be associated with changes in temperature, changes in illumination, changes in mechanical disturbance and position of the animal.

Analysis of these factors show that it is the changes in moisture which underlie the other conditions that are decisive in bringing about the color changes.

While it might be imagined that the changes of color were connected with internal nervous changes, such complication is not needed to explain the facts.

The material composing the outermost layer of the exoskeleton of the elytra and of the dorsal part of the thorax is such that it readily absorbs and gives off moisture from the air as well as from liquid. When this material absorbs liquids it allows the color of the underlying part of the exoskeleton to be seen as dark red; when the liquid is out of the outermost layer the air that takes its place prevents the color of the underlying part of the shell from being seen and the shell looks light.

The structure of the shell that thus modifies the light is that of a material whose continuity is interrupted at intervals of less than one micron either by pores or by some other physical arrangement not determined, which makes it like a sponge or a dried and cracked colloid.

The natural spots on the shell which do not change to light color in drying seem to be formed by the presence in this layer at such areas, of some matter that clogs the pores or fills its interstices so that it acts as if permanently wet or impregnated with liquid, and so permits the seeing of the color of the underlying layer.

Whether, in nature, the animal changes its colors on leaving the moist wood in which it lives, to assume a lighter color when flying in the day or moonlight, and so is less conspicuous in both environments than otherwise, and whether this purely physical structure of the outermost parts of portions of its exoskeleton is of any use to it, are questions whose answer awaits more thorough study of the natural history of the insect.

SOME EXPERIMENTS ON PROTECTIVE COLORATION

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EIGHT TEXT FIGURES AND THREE PLATES

The theory of protective and aggressive coloration, assuming as it does, the use of such color in protecting its possessors from their enemies, or enabling them to steal unnoticed on their prey, has, as yet, insufficient experimental evidence for its foundation, being based largely upon human experiences of taste, sight, and smell, which are by no means a fair criterion of similar senses in lower animals.

Beddard ('92) in his work on *Animal Coloration* says, page 156:

The instance shows the pitfalls which surround the path of those who wish to deduce theories from experiments of this kind, which are necessarily made in very great ignorance of bird psychology, or even physiology.

On page 166 of the same book he says:

These experiments certainly bring out the fact that the likes and dislikes of insect-eating animals are purely relative. They are further proof of the old saying that one man's meat is another man's poison. . . . But none of these experiments are thoroughly satisfactory. It is difficult to interpret them and they are often contradictory, for a bird will eat one day what it has refused before.

Again on page 196 he says:

It is not reasonable to lay much stress upon the fact of insects possessing qualities which are disagreeable to ourselves, for it by no means follows that these same qualities affect the enemies of the insects. Indeed, there is experimental evidence that the reverse is the case.

There are numerous other difficulties in experiments of this sort, first among which is the fact that they have mostly been

performed upon caged animals, and it is quite possible that confinement materially alters their senses. The space factor also enters into experiments with caged animals, by limiting the distance between them and their prey. I shall refer at greater length to these objections later on.

In addition to the experiments cited by Beddard (l.c.) and Poulton ('90), the most important work I know of is that of Finn ('98), Judd ('99), Pritchett ('03), and Reighard ('09), all dealing chiefly with warning color. Taken as a whole, these experiments are inconclusive, some of them supporting, others refuting, the theory. Their general tendency, however, has been to cast grave doubts upon it. There are a few isolated experiments on protective coloration proper, but they are too few to carry much weight and their results are also contradictory.

Among the criticisms of this theory is the objection that such coloration is not really protective, and that animals possessing it seek refuge in other ways, as in flight and hiding. Thus Werner ('07), sarcastically refers to a certain supporter of this theory as a 'Sonntagsjäger,' and cites evidence supposedly proving the inefficiency of color in protecting its possessors from their enemies.

To put the theory to an experimental test, I have carried out a series of 144 experiments during the past six years, using for the purpose crows, hawks, owls, domestic chickens, prairie chickens, grackles, kingbirds, and martins as preyers, and several kinds of mammals and insects as prey.

The photographs illustrating this article were taken on Cramer ischromatic plates through a ray filter,¹ ² and printed on 'glossy Cyko' paper manufactured by the Ansco Company of Binghamton, New York.

Considerable difficulty attends a representation of color contrasts by photography, as the effect upon the photographic plate is not necessarily the same as upon the human retina. Very different effects may be obtained by manipulating either the exposure or the development of the plate, or both. I have

¹ Ingento,' Series B, Burke and James, Chicago, Illinois.

² Except figures 37, 50, and 52, in making which no filter was used.

endeavored to neither increase or decrease the contrast in any case, but to reproduce as nearly as may be the effect as it appeared to my eye. If I have erred in any direction, it has been in minimizing the effect of the contrast and resemblance. In every case the camera was placed much nearer (from 0.2 to 3.0 m.) the backgrounds than were the birds at the commencement of the experiments,³ thus diminishing the effect of the contrast and resemblance. With the exception of figures 8, 11, 12, 13, 17, 18, 21, 22, 23, 49, 50, 51, 52, and 53, the photographs were made with dry specimens. The color changes, if any, were not, however, great enough to be appreciable in the photographs.

Light and shade moreover in many instances altered the color effect. Thus in figures 25, 27, 41 to 43, 46 the effect of the reflected light has been to make a dark insect (*Gryllus*, *Silpha*) appear light against a dark background. The same effect of course occurs in nature, so that the photographs are probably reasonably true to life in this respect.

SERIES I

Plate 1, figures 12, 17 and 18

In the first series of these experiments, I employed two individuals of the common crow (*Corvus americanus*) and frogs (*Rana cantabrigensis* and *Rana pipiens*). The crows were kept in a cage 0.9 x 0.9 x 1.2 m. in size, on the top of which was a perch 0.7 m. from the floor. On this floor, I prepared two backgrounds, one of mud or fresh grass, the former of which harmonized very closely with the color of *R. cantabrigensis* and the latter with that of *R. pipiens*, and the other of dry sand, with which the color of the frogs made a decided contrast. Upon each of these backgrounds a frog was placed, rendered insensible by sharply tapping the skull. The crows soon learned to drop from the perch and seize the frogs,⁴ and a record was kept of the number of frogs taken from the mud and sand respectively, in the hope

³ In many cases, as I shall explain later, the birds were close to the backgrounds at the time the experiments became effective.

⁴ When first fed frogs, the crows appeared suspicious of them, but soon learned to eat them readily.

of ascertaining whether or not the harmony of the frogs' color with that of their background was really protective to them. I at first designed to keep the crows confined in a closed compartment at the top of the cage in order to prevent their observing the placing of the frogs and the consequent fastening of their attention on one of them. I soon found that this proceeding was unnecessary, however, as the crows delayed for several minutes, in some cases more than half an hour, before dropping to seize their prey, and gave no evidence whatever of having their attention fixed on either of the frogs at the time these were placed upon the backgrounds. In some instances, the frogs would move after being placed in position, thus drawing the attention of the crows. Where this occurred, I have noted it in the account of the experiment. A further difficulty was experienced in the high wind to which the cage was at times exposed during the experiments, which blew the loose sand over the frogs, changing to a considerable extent their color, as well as that of the mud background. A third difficulty was the change of color which the frogs underwent after being stunned, and also after being removed from the dark box, in which they were kept, into the light. Thus their color, in addition to the individual differences, was not constant in all of the experiments. The sum total of these influences, while introducing uncertainty into the results, tended to diminish rather than to increase the protective influence of the frogs' color. Hence my results, tending to prove such influence under these disadvantageous conditions, should be at least accepted at par value, rather than discounted in consequence of such conditions.

Experiments 1 and 2. One specimen of *cantabrigensis* was placed on mud and one on sand in each experiment. In each the frogs were taken from the sand. In the second experiment the frog on the sand moved just as I left the cage, so that the crow's attention was possibly drawn to this frog rather than to the other. This is not probable however as the crow delayed seizing it for several minutes after I left. After seizing this frog, that on the mud background moved, and the crow dropping the first frog, seized the other one.

Experiment 3. One specimen of *pipiens* was placed on grass and one on sand. A few minutes later a crow dropped on to the grass, but took the frog from the sand.

Experiment 4. One specimen of *pipiens* was placed on grass and one on sand. After more than half an hour, a crow dropped and picked the frog from the grass.

Experiment 5. One specimen of *pipiens* was placed on mud and one on sand.⁵ In a few minutes a crow dropped on the mud but took the frog from the sand.

Experiment 6. One *cantabrigensis* was placed on sand and one on mud.⁶ After considerable delay, the frog was taken from the sand. In this experiment particularly it appeared very probable that the crow saw the frog before dropping to seize it, as it turned its head from side to side examining the floor of the cage before doing so.

Experiment 7. One *cantabrigensis* on mud and one on sand.⁶ The former was seized by a crow directly after dropping and with little inspection.

Experiment 8. One *pipiens* on sand and one on grass. Former taken.

Experiment 9. This was a repetition of Experiment 8 and the same results were obtained. The frog was seized by the crow directly on alighting.

Experiment 10. One *pipiens* on sand and one on grass. Former taken.

Experiment 11. Same as Experiment 10.

Experiment 12. One *pipiens* on grass and one on sand. Former taken. In this experiment the frog was apparently not seen until after the crow had alighted, as the latter started to walk to the opposite side of the cage and was very close to the frog on the grass before appearing to see it, when it stopped and seized it.

Summary

Thus in nine of these twelve experiments, or in 75 per cent, the frog was taken from that background with which it formed the better contrast, while in one of the remaining three, the difference in contrast between the two backgrounds was not marked.⁶

SERIES II

Plate 2, figures 39 and 41

In this series also a crow was employed as the preyer and crickets (*Gryllus pennsylvanicus*) as the prey. The crow was confined in a cage 5.0 x 2.4 x 1.5 m. in size, at one end of which was a

⁵ In this experiment the frogs died, changing color from green to dark olive after being stunned.

⁶ A high wind was blowing during this experiment and the sand was driven over onto the mud and altered the color of the latter to a considerable extent.

perch 0.3 m. from the ground. At the opposite end backgrounds were prepared of sifted flour and coal dust mixed with pieces of anthracite coal. The crickets were arranged in similar positions on each background, the number used being indicated in every experiment. Care was taken in placing the crickets to avoid attracting the crow's attention to any of them, the experimenter placing himself between the perch and the background when arranging the experiments. The harmony between the color of the crickets and the coal background was very close, the dull color of the cricket's back and wings agreeing well with that of the coal dust, and the brilliant black of the legs and head of the cricket simulating closely the bits of coal scattered among the dust.

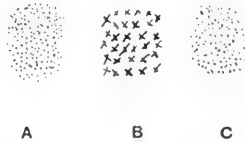


Fig. A A and C—flour, B—coal.

Experiment 1. Four crickets were placed on flour and four on coal. In about one minute the crow dropped to the ground from its perch and went directly to the backgrounds taking a cricket from the flour.

Experiment 2. In this experiment, three crickets were placed on flour and three on coal. The results were similar to those of Experiment 1, except that two crickets were taken from the flour.

Experiment 3. Two crickets were placed on each background. The crow went immediately to the backgrounds, approached a little nearer to the coal than the flour, but took a cricket from the flour.

Experiment 4. In this and the following experiments, three backgrounds were prepared as shown in figure A; A and B being the same as those employed in the previous experiments, and C a new one. Two crickets each were placed on B and C. A minute later the crow dropped to the ground and passed directly to the backgrounds. After hesitating a moment, it took one cricket from C.

Experiment 5. Three crickets were placed on both A and C and six on B. The crow dropped from its perch to the floor on the side of the cage nearest A. It several times ran past all three backgrounds, and finally went to B taking three crickets therefrom. The experiment lasted about nine minutes.

Experiment 6. My notes regarding this experiment are not entirely clear in respect to the number of crickets. Either two or four (probably two) were placed on *A* and *C* and four on *B*. The crow twice dropped from its perch to the floor near the backgrounds, and returned to its perch. A third time it flew to the side of the cage nearest *A*, passed and re-passed the three backgrounds and then returned to its perch. A fourth time it left its perch passing to the side of the cage near *A*, then to a point near *C*. It then returned to *A* and took one cricket therefrom. Time of experiment six minutes.

Experiment 7. Same arrangement of background and prey as in Experiment 6. The crow left its perch almost immediately, passed the backgrounds from side to side twice, and then took one cricket from *B*.

Experiment 8. Ten crickets were placed on both *B* and *C*. The crow left its perch, passed the backgrounds two or three times, and then took one cricket from *B*.

Experiment 9. Same arrangement of background and prey as in Experiment 8. The crow at once left its perch and passed to the side of cage nearest *A*. It then approached the backgrounds, retreated a step or two, approached again and took one cricket from *B*.

Summary

In this series the arrangement showing the greater contrast was chosen five out of nine times, or 55 per cent.

SERIES III

Plate 2, figures 50 and 52

Experiments with the Purple Martin (*Progne subis*) and grasshoppers (*Melanoplus*⁷ and *Xyphidium fasciatum*).

A pair of purple martins were taken from the nest just before learning to fly. They were kept in a cage 2.4 x 2.4 x 0.8 m. in size. At first the birds were very wild, refusing to eat unless forced to do so. They soon became tame enough to eat readily from the hand, however, but it was several days after their capture before they learned to feed themselves.

⁷ I have not attempted to differentiate the spp. of *Melanoplus* used in this and succeeding series of experiments. Both *M. atlantis* and *infantilis* are common in the region and these are probably the two mostly used. The differences in color and markings between different species are so small as to be negligible for the purpose of these experiments.

The experiments to be recorded were performed by arranging two backgrounds, one of straw and one of grass,⁸ on the floor of the cage, on each of which were placed in as nearly their normal position as possible five dead grasshoppers of each species. During the placing of the insects the birds were confined in a small box on the wall of the cage. After being released they seldom flew directly to the floor of the latter, usually alighting first on a perch placed about midway between the box and the floor. They would then drop to the floor and hop back and forth in search of food. They did not apparently espy the insects until coming close to them. The insects were occasionally tumbled over by the birds in alighting, walking, or flying over the backgrounds; thus in some cases bringing the ventral surface uppermost, and in others causing them to drop down between the blades of straw and grass, and changing the conditions of the experiment. I endeavored to obviate this difficulty by replacing the insects in position after each time that the birds fed from either background. As the birds were hungry, however, they would usually pick up several insects before I could drive them away, so that a certain amount of uncertainty is necessarily here involved. The reasonably consistent results of the experiment, however, render this uncertainty practically negligible.

The order of resemblance between the grasshoppers and the background, as it appeared to my eye, was as follows, giving those combinations first in which the resemblance was greatest: *Xyphidium*—grass, *Xyphidium*—straw, *Melanoplus*—grass, and *Melanoplus*—straw. The resemblance between *Xyphidium* and grass was so great as in many cases to cause me great difficulty in finding the grasshoppers on this background a few moments after placing them, and with my eye at a distance of only about 30 cm. above it. The fact that the martins did not approach the insects from above, but from the side, the bird's line of sight striking the body of the insect a little above the middle of the latter, necessitates the comparison of the lateral, rather than the dorsal color of the insect with that of the background in

⁸ In Experiment 2 a single background consisting of a white sheet, and in 5 and 6, backgrounds of mud and sand, instead of grass and straw, were employed.

evaluating the results. In both cases, however, the lateral color is practically the same as the dorsal, while in that of *Xyphidium* the light straw colored wings folded over the back tended on both backgrounds to enhance the protective effect; for on the straw background they closely matched the color of the straw, and on the grass they gave the appearance of blades of dry grass lying among the green.

Experiment 1. The insects were placed on the backgrounds at 7.26 a.m., and the box opened. Previous to 7.45 a.m. the dead insects were not eaten, although the birds passed them several times in hopping about the floor of the cage, and one live and active specimen, which had been left in the cage by mistake, was taken from the grass. From 7.45 to 8.30 the birds were not observed. During this time two *Melanoplus* were taken from the straw and one from the grass. Between 8.30 and 8.45 the three *Melanoplus* remaining on the straw and two of the four on grass, and one of five *Xyphidium*⁹ on straw were taken. Twice during this period a bird passed by the grass without feeding. Summed up the result of this experiment is: 8 *Melanoplus* (5 from straw, 3 from grass), and 1 *Xyphidium* from straw (probably) eaten.

Some uncertainty was introduced in this experiment by the presence of active specimens in the cage, which, dropping upon the backgrounds among the dead insects, attracted the attention of the birds.

Experiment 2. In order to ascertain which species, if either, the martins preferred, I placed five of each on a white cloth where both kinds were plainly visible. If any difference in contrast existed, that between *Melanoplus* and the white cloth was the greater. One bird took five *Xyphidium* and one *Melanoplus* and would probably have eaten the remaining *Melanoplus* if I had not interrupted it at this point. They were eaten shortly after. This shows that for this individual at this time at least there was no preference for *Melanoplus* over *Xyphidium*.

Experiment 3. In this experiment one bird passed by the grass and picked a *Melanoplus* from the straw. Then a bird¹⁰ flew over both the grass and the straw and picked two *Melanoplus* from the latter. I now left three of each species on the straw and five of each on the

⁹ Some uncertainty exists regarding this. At 8.45 there were five *Xyphidium* remaining on the grass. From its position, it is possible, though not probable, that one of these had been thrown from the straw to the grass by the movements of the birds, in which case the record would be one *Xyphidium*, from the grass and none from straw taken. It was of course impossible to watch the birds from a distance and keep individual specimens of the grasshoppers in view.

¹⁰ The identity of each individual of the two birds I could not distinguish.

grass. One bird now picked a *Melanoplus* from the grass and then passed to the straw and picked one of the same from the latter.

Two of each species were now left on the straw and four of each on the grass. A bird went first to the grass where a *Melanoplus* jumped directly before it and fell under a grass blade. The bird then passed to the straw and took one *Melanoplus*.

This experiment was not observed further for some time, when it was found that all of the insects had been eaten except three or four *Xyphidium* on the grass.¹¹ Summed up the results of this experiment are as follows: During the period of observation five¹² *Melanoplus* were taken from the straw, and one from the grass. After this one *Melanoplus* and two *Xyphidium* were taken from the straw and four *Melanoplus* from the grass.

Experiment 4. In this experiment the position of the insects was reversed they being placed ventral side up. A yellow and dark gray surface in the case of *Melanoplus*, and a green and straw colored surface in that of *Xyphidium* was now exposed to the view of the birds. The order of resemblance to my eye in this case was as follows: *Xyphidium*—grass, *Melanoplus*—straw, and *Xyphidium*—straw, *Melanoplus*—grass (the two latter about equal).¹³ One bird passed first to the straw and seized a *Xyphidium*. Four of each species were now left on the straw and five of each on the grass. A bird now passed to the straw first (from the side of cage nearest to the latter) and took a *Melanoplus* therefrom. Five of each species were now placed on straw and grass respectively. A bird passed to the straw (from the side nearest thereto) and took one *Xyphidium* and one *Melanoplus*. Four of each species were now placed on straw and five of each on grass. A bird flew over the straw to the grass and took a *Melanoplus*. Four of each species were now left on both straw and grass. A bird went to the straw and took one of each species. Three of each species were now left on straw and four of each on grass. One bird now passed to the straw and took two *Xyphidium* and one *Melanoplus*. The straw was now removed and five of each species were left on the grass. In three feedings from the grass four *Melanoplus* and no *Xyphidium* were taken, the number of each species being kept constant after each feeding.

Summation of the results of Experiment 4. Before removal of straw five *Xyphidium* and four *Melanoplus* were taken from it and

¹¹ The exact number left on the grass untouched during this period I am not certain of, as they had all fallen more or less among the blades of grass and one of them may have been concealed by the grass from the first.

¹² One of these was replaced making a total of six instead of five *Melanoplus* placed on straw during this experiment.

¹³ It must be remembered in this, as in the preceding experiments, that the surface exposed to the birds' view from a position on the floor of the cage was somewhat different from that exposed to the view of an observer looking down onto the floor from above.

one *Melanoplus* from the grass. After removal of straw four *Melanoplus* and no *Xyphidium* were taken from the grass.

A comparison of the results of this experiment with those of Experiments 1 and 3 shows a marked difference, due unquestionably to the change in position of the insects and the consequent change in color contrast. In Experiments 1 and 3 the *Melanoplus*—straw combination was the one mostly chosen by the birds, being that one in which the color contrast was greatest; while in 4 the *Melanoplus*—straw and *Xyphidium*—straw combinations were about equally selected, the color contrast being very little different in either case. It is probable that the *Melanoplus*—grass combination would have been oftener selected, had the birds more frequently happened to alight on the side of the cage nearest to the grass.

*Experiment 5.*¹⁴ In this and the following experiment I employed backgrounds of mud and sand instead of grass and straw, placing at the outset of the experiment five *Melanoplus* on each background, those on the sand being (to my eye) the more conspicuous. In arranging these, I placed those on the mud background last, so as to attract, if possible, the birds' attention to these, rather than to those on the sand. A bird passed first to the sand background and took three insects. During the taking of these three the bird left the sand once and passed by the mud; and at another time it inspected the mud closely, but apparently without seeing the insects on it, as it did not take them at that time. Observation was then discontinued for a time and when renewed it was found that all of the remaining insects had been eaten.

Experiment 6. In this experiment five *Xyphidium* were placed on mud and five on sand. One bird flew to the mud past the sand, but without taking any insects from it. It then returned to the sand and ate all five placed on it. In this experiment the comparative resemblance and difference between the insects and their background was not as marked (to my eye) as in Experiment 5, but was still sufficient to influence decidedly the results.

Experiment 7. In this experiment five of each species of insects were placed on grass and five of each on straw. The birds passed the grass (one walking directly over it), and then flew up to their perch. Returning to the floor of the cage, they took two *Melanoplus* from the straw. Three of each species were now left on straw and five of each on grass. A bird came from the side of the cage nearest the grass and took three *Melanoplus* therefrom. In this latter case, as in Experiment 4, the element of chance evidently decidedly influenced the results, the bird feeding from that background to which it first came. It is to be noted however that in this last case that species, *Melanoplus*, was taken which presented the greater contrast to the background (grass).

¹⁴ In this experiment but one bird was employed, instead of two as heretofore.

Summary

A summary of this series of experiments shows that of thirty-seven insects eaten in those experiments (1, 3, 7, and 4, first part,) with four combinations of insects and backgrounds, nineteen or 51 per cent were taken from that background with which they made the greatest contrast, eighteen or 49 per cent from an intermediate background and none from that of least contrast. In those experiments in which there were but two combinations of insect and background (5, 6, and 4, last part) the prey was taken from the background of greater contrast in fourteen cases or 73 per cent and from the background of less contrast in five or 27 per cent.¹⁵ Considering the first choice of the birds rather than the total number of insects eaten, we find that in the four combination experiments, the combination of most contrast was chosen in nine out of thirteen cases or 69 per cent, an intermediate combination in four or 31 per cent and the combination of least contrast in none. In those experiments with but two combinations, we find the first choice of the birds to have been the combination of greater contrast in five cases and that of less contrast in none.

SERIES IV

In this series three young individuals of Krider's hawk (*Buteo borealis krideri*) and dead mice (*Microtus drummondi* and *Mus musculus*) and shrews (*Sorex personatus haydeni* and *Blarina brevicauda*) were used.

The hawks were taken from the nest just before learning to fly and kept in a cage 1.5 x 3.9 x 3.7 m. in size. At one end of this cage backgrounds of different substances were prepared upon which the prey was placed.¹⁶ During the latter operation, the hawks were on a perch at the opposite end of the cage about one meter from the ground, care being taken not to attract

¹⁵ See Experiment 5. The insects were taken from the mud (less contrast) background in this experiment only after at least three (possibly all five) had been eaten from the sand (greater contrast background).

¹⁶ Dorsum uppermost except as otherwise noted.

the notice of the birds more to one background than to another. While the prey in most cases contrasted much more strongly with one background than with the other, it was nevertheless distinct (to my eye) on both at the distance from which the hawks viewed it, the color contrast and resemblance not being very great in any case. In spite of this fact, in a large percentage of the experiments, the prey was taken from the background of greater contrast. In all except Experiments 16 and 17, the direction of the light was either directly or obliquely in the face of the birds. It is noteworthy that in these two experiments, the prey was taken from the background showing less, while in the others it was taken chiefly from the one showing greater contrast. Whether there was any relation between the direction of light and the results of the experiments, or whether the results in Experiments 16 and 17 were merely a coincidence, I cannot say.

Experiment 1. Figure 2. One Blarina was placed on earth and one on lime. One hawk left its perch and after flying about the cage two or three times dropped to the ground and spying the shrew on the lime, ran directly to it and stood watching it for several minutes, but did not seize it. It apparently did not see the other shrew. In this and the following experiments (up to No. 9 inclusive, and 14, 16, and 17) the greater contrast was presented by the prey on the lime background.

Experiment 2. Figure 2. Four alternating backgrounds, two each of lime and earth were prepared in this experiment, and one Microtus placed on each. Three hawks on perch. One immediately left the perch, flew to the ground and took one mouse from the lime.

Experiment 3. Figure 2. Two backgrounds, one of lime, and one of earth, were employed in this experiment, and one Microtus placed on each. Three hawks on perch. For about three-quarters of an hour the mice remained unnoticed by the hawks, although one of the latter flew across the cage once during this interval. I then drove a second hawk from the box, which flew across the cage, dropped to the ground and ran directly to the mice, but apparently did not see them. Then the first hawk flew to the mice and took one from the earth.¹⁷

¹⁷ It is very possible that this mouse had been disturbed by the second hawk which was standing close to it when it was seized by the first, the attention of the latter being thereby attracted to it. I have often noticed that a hawk will be attracted to its prey, which it has previously apparently not seen, by another hawk seizing it.

Experiment 4. Same as Experiment 3. The hawks remained on their perch for five minutes, taking no notice of the mice. I then drove one of the hawks from its perch. It flew across the cage and returned, dropping to the ground near the perch. It then immediately ran across the cage and took the mouse from the lime. After eating this, it took the mouse from the earth.

Experiment 5. Figures 1 and 3. One Mus on lime and one on earth over which were scattered a few scraps of leaves. A hawk left its perch almost immediately after I left the cage and dropped to the ground below. A few seconds later it walked across the cage and took the mouse from the lime.

Experiment 6. The same species of prey and the same backgrounds were employed in this experiment as in Experiment 5. Two hawks left the perch in succession and dropped to the ground, one of them within 0.6 m. of the mice and somewhat nearer the mouse on the earth background. The latter hawk took the mouse from the lime.

Experiment 7. In this experiment the same species of mice and the same backgrounds were employed, but the backgrounds were reversed in position. Two hawks left the perch together and flew to the ground, one of them taking the mouse first from the earth background, and then from the lime.

Experiment 8. The prey and the background arrangement were the same in this experiment as in Experiment 7. One hawk soon left the perch and flew to the ground near the backgrounds, but apparently did not see the mice as it retreated a short distance and remained for several seconds with its attention apparently fixed on something outside of the cage. Then it appeared suddenly to see the mouse on the lime and quickly seized it. The hawk was possibly 6 or 7 cm. nearer the mouse on the lime than that on the earth when it espied the former.

Experiment 9. The same prey and the same backgrounds were employed in this experiment as in the preceding, except that the earth background was moved a few centimeters nearer the perch than the lime background. Three minutes after starting the experiment, a hawk left its perch and dropped to the ground about midway between the perch and the backgrounds. It remained there for a few seconds without apparently seeing the mice. Then it suddenly ran to the lime background from which it took a mouse.

Experiment 10. Figure 10. In this experiment the same prey was employed as in the last experiment, but the position of the mice was reversed, they being placed with the venter uppermost, one on a background of earth and the other on one of dry leaves of corn (*Zea*), the latter background replacing in position that of lime in the last experiment. In this experiment the contrast between the mice and earth was greater than that between the mice and the corn leaves. After four minutes, one hawk flew directly from the perch to the earth background and took the mouse from it.

Experiment 11. Figure 4. In this experiment the same prey was employed as in the last experiment. The prey was placed venter

uppermost. Two backgrounds were employed, one of dry earth mixed with lime and dead leaves, the other of moist earth. The contrast between the mouse and the latter background was much stronger than that between the mouse and the former background. In ten minutes one hawk left its perch alighting near the mice but apparently not seeing them till after alighting. *It alighted with its head turned away from the background of moist earth and facing the mixed background.* After alighting it seized the mouse on the latter.

Experiment 12. Figures 1 and 14. The same prey and backgrounds were employed in this as in the last experiment, but the mouse on moist earth was placed venter uppermost, while that on mixed lime, leaves, and earth, was placed dorsum uppermost, the former presenting the more striking contrast. In six minutes a hawk left the perch and flying directly to the mice seized the one on the moist earth.

Experiment 13. The arrangement of backgrounds and prey employed in this experiment was precisely the same as in the last, except that the position of the backgrounds was reversed. For eighteen minutes the hawks remained on the perch, during which time one hawk looked down several times and appeared undecided whether to fly down or not. It finally flew directly to the backgrounds and took the mouse from the moist earth.

Experiment 14. Figures 1 and 8. In this experiment one background of lime and one of mixed lime, earth, and leaves was employed one Mus being placed on each. After about seventeen minutes, during which time the hawks remained on their perch, one of them circled over the mice and returning lit near the perch. It remained here for a few minutes with its back turned toward the mice, while a second hawk in about three minutes flew directly to the mouse on the lime, which it seized.

Experiment 15. Figures 2 and 13. One light colored Mus and one Blarina were placed on moist earth. After thirteen minutes a hawk left the perch and flew to the ground near the prey, and then returned to the perch. Six minutes later a hawk flew to the background and lit *with its side toward the shrew.* After alighting (apparently not before) it saw the mouse, which it seized, and then took the shrew. In this experiment the greater contrast with the background was presented by the mouse.

Experiment 16. Figures 6 and 11. One background of lime and one of mixed earth, lime, and dry leaves were arranged in this experiment, and on each was placed a Microtus. Three minutes after the mice were placed a hawk left its perch and flew to a point between the backgrounds and close to each. It then retreated a few steps, returned and seized the mouse on the mixed background.

Experiment 17. Figures 1 and 8. The same backgrounds were employed in this experiment as in the last, two Mus being used as prey. In three minutes a hawk left its perch and flying to the ground took the mouse from the mixed background.

Summary

In eleven (Experiments 2, 4, 6, 8 to 10, 12 to 15) of the above seventeen experiments or 65 per cent the combination showing greater contrast was chosen by the birds, and in five (Experiments 3, 7, 11, 16, and 17) or 29 per cent that of less contrast was selected, while in one (Experiment 1) although the prey was not seized by the hawk, the latter was evidently attracted by the combination of greater contrast. Experiments 3, 11, and 15 are doubtful for reasons already stated.¹⁸ Omitting these and Experiment 1, we have the following results:

Combination of greater contrast chosen in ten cases or 77 per cent, that of less contrast in three or 23 per cent.

SERIES V

In this series two¹⁹ young hawks (*Buteo borealis krideri*) and mice (*Microtus drummondi*, *Peromyscus gambeli bairdi*, *Mus musculus*) and rats (*Epimys norvegicus*) were used. The hawks were taken from the nest before learning to fly and confined in a cage 5.0 x 2.4 x 1.5 m. in size, at one end of which were several perches placed from 0.3 to 1.0 m. from the ground. At the opposite end of the cage backgrounds (described under the different experiments) were prepared, upon which was placed the prey.¹⁶ Care was taken while placing the latter to avoid attracting the attention of the hawks. This however was probably not a matter of great importance, as the latter seldom noticed the prey until several minutes after I had left the cage.

Experiment 1. Figure 9. Two backgrounds, one of moist earth, and one of ashes, were employed in this experiment, on each of which a *Microtus* was placed (venter uppermost). The mice were partly imbedded in each background so as to expose only the under surface, legs and tail. The white belly of the mouse on the earth presented a much stronger contrast with its background than that of the mouse on the ashes did with its background. Both hawks were on one of the perches at the opposite end of the cage and remained there for about a half hour evidently not seeing the mice. Then I disturbed

¹⁸ See footnote ¹⁷ and italicized lines in Experiments 11 and 15.

¹⁹ In Experiments 15 to 19 three hawks were used.

one of the two birds, which flew part way across the cage alighting a trifle nearer the earth than the ash background and remained there for another half hour. At the end of this time, one of the birds (which is uncertain, as I was not looking at the moment) took the mouse from the earth and almost immediately afterwards the mouse on the ash background was taken (probably by the same hawk).

Experiment 2. Figure 3. In this experiment two Mus were employed, one being placed on moist earth mixed with dead leaves and the other on ashes. Viewed from a point approximately one meter beyond the hawks' perch, the former mouse was invisible to me in the growing dusk, while the latter was plainly seen. In about two minutes, one hawk left its perch, flew directly to the ground about 0.3 m. distant from the mice, paused for a second and then took the mouse from the ashes.

Experiment 3. This was a repetition of Experiment 2, except that the position of the backgrounds was reversed. In about ten minutes a hawk flew directly from its perch to the ash background and took the mouse from it.

Experiment 4. Figures 4 and 8. Two Mus were placed (venter uppermost), one on ashes and one on moist earth. In this experiment the greater contrast was presented by the latter combination, but the resemblance between the mouse and the ashes was not very close, as I observed it from the opposite end of the cage. Furthermore, in the dusk (6.12 p.m., Sept. 27) objects on ashes are much more conspicuous than those on earth. In one minute a hawk dropped to the ground near its perch, paused for a few moments, and then ran to seize the mouse on the ashes.

Experiment 5. Figures 3 and 15. One Mus was placed on light clay and one on dark earth covered with scattered bits of dead leaves. For thirty-eight minutes the mice were not disturbed. During this time one of the hawks three times left its perch, flew part way across the cage and returned to its perch, while the other one left its perch, flew over the backgrounds and returned. Then the latter left its perch a second time, dropped to the ground and ran almost directly to the clay background from which it took the mouse. The contrast in this experiment was greater between the mouse and the clay than between the mouse and the earth.

Experiment 6. Figure 14. Two Mus were placed venter uppermost, one on light clay and one on dark earth. Both were partly embedded in the background so as to bring the ventral surface flush with it, thus eliminating shadow effects and the contrast between the darker sides of the mouse and the light clay. For one hour and twenty-two minutes the mice were apparently unnoticed by the birds, although during this time both birds left their perches and flew to the ground, and one of them flew over the backgrounds and alighted near them. At the end of this time the other flew over the mice and lit close to them, taking the mouse from the clay. In this experiment the mouse-earth was the combination showing greater contrast.

Experiment 7. Figure 5. In this experiment two *Mus* were placed venter uppermost on dark earth and two on a mixture of dead leaves of the cottonwood tree (*Populus*) and the staminate branches and the styles of corn (*Zea*). As viewed from the opposite end of the cage, the resemblance between the mice and this latter background was close, while the mouse-earth combination presented a strong contrast. In six minutes a hawk flew direct to the backgrounds and picked a mouse from the earth.

Experiment 8. The arrangement of this experiment was the same as Experiment 7, except that only one mouse was placed on each background. In one minute a hawk dropped to the ground from its perch and ran to a point near the backgrounds, but did not feed. One minute later the other flew from its perch direct to the backgrounds and took the mouse on the earth.

Experiment 9. The arrangement here was the same as in Experiment 8, except that the backgrounds were reversed in position, and the venter of the mouse on the earth was brought flush with the earth's surface. It was twilight when the experiment was started (5.54 p.m., September 28). In nine minutes a hawk flew from its perch directly over the mice but apparently did not notice them. In twenty-one minutes the other flew direct to the backgrounds and took the mouse on the earth.

Experiment 10. Figure 3. Two *Microtus* were employed in this experiment, one being placed on ashes and one on a background of dark earth over which were scattered pieces of bark and dead leaves. The mouse on the latter background was plainly visible to my eye from the opposite end of the cage, but the contrast between the mouse and the ash background was much plainer. After looking at the mice for several seconds, a hawk flew direct to the backgrounds and took the mouse from the ashes.

Experiment 11. This experiment was the same as the last but the position of the backgrounds was reversed. In seven minutes a hawk after looking at the backgrounds flew to within several centimeters of them, paused a moment, and then took the mouse from the ashes.

Experiment 12. Figure 3. In this experiment two *Peromyscus* were placed venter uppermost one on ashes mixed with a little earth and one on earth. The venter of each mouse was brought flush with the surface of the background. As seen from the opposite end of the cage, the resemblance between the mouse and the ashes was so close as to render it invisible to my eye. In eight minutes a hawk flew to the ground at the middle of the cage, and walking slowly to the backgrounds took the mouse from the earth. Shortly afterward the other hawk passed very near the other mouse, but did not notice it. Five minutes later, however, the former hawk took it.

Experiment 13. Figure 16. A smaller *Mus* was placed on ashes and a larger one on earth mixed with a little ashes and partly covered with bits of bark, dead leaves and straw. The resemblance here between the mouse and the latter background was close. In twenty-

one minutes a hawk flew to the ground at the middle of the cage and alighted facing the mice, but they were apparently not seen. Four minutes later it ran slowly to the mice and took one from the ashes.

Experiment 14. Figures 1 and 16. Two Mus were placed on ashes, and two on earth covered with dead leaves. The contrast between the mice and the former was greater than between them and the latter background, but the resemblance with the latter was not very striking. About fifteen minutes later one hawk flew to the ground within a short distance of the mice, but apparently did not see them. A few minutes later the other did the same, and fifteen minutes later flew over the mice alighting near them, but taking no notice of them. Then for twenty minutes the experiment was not observed. During this time both mice were taken from the ashes and neither from the leaves, although the latter were not over 22 or 23 cm. from the former.

Experiment 15. Figures 13 and 16. Two Mus were placed on ashes and two on dead leaves. The former combination showed the greater contrast, but the resemblance between the mice and the leaves was only fair. In twenty-eight minutes a hawk ran across the cage to where the mice were, and returned without feeding, twice repeating this performance a few minutes later. About an hour after the experiment started another hawk took one mouse from the leaves.²⁰ I failed to observe whether the hawk went directly to the mice or not.

Experiment 16. Figures 19 and 20. Two Epimys were placed venter uppermost, one on ashes and one on moist earth. Both were partly imbedded in the backgrounds, so as to expose only the ventral surface. As I viewed the backgrounds from the opposite end of the cage the former rat was clearly visible, but the latter was the more conspicuous. Seventy-two minutes after the experiment was started a hawk approached the backgrounds and stood inspecting the rats until another hawk ran from the opposite end of the cage and took the rat from the earth. It was growing dark at this time.

Experiment 17. Figures 22 and 23. Two Epimys were placed on powdered gypsum and two on dead leaves, sticks and straws mixed with a little clay. The resemblance between the rats and the latter background was not very close, as viewed from the opposite end of the cage, but was much closer than that of the rats to the gypsum. For several hours (exact time not recorded) the rats were not taken by the hawks, although the latter frequently looked toward them and one once or twice alighted within about 3 m. of them. I then tied a string to one of the rats and jerked it repeatedly, causing the hawks to look toward the rats, but without any move to take them. I then removed a rat from each background, leaving one on each, and ceased observing the experiment for twenty-five minutes. Returning at the end of this time, I found one of the hawks eating the rat on the gypsum. The hawks were undoubtedly hungry in this experiment as they had had only one rat between them during the two preceding days, and why

²⁰ That one of the two was taken which resembled its background less closely.

they were so slow to feed is a mystery. It cannot be explained as due to unfamiliarity with the rats as food, for they were accustomed to feed upon them. I shall refer to this question later (p. 493).

Experiment 18. This was a repetition of the preceding experiment. For an hour and a half the birds were not observed. At the end of this time I returned to the cage and found a hawk eating the rat on the gypsum.

Experiment 19. Figures 19 and 21. Two *Epimys* were placed, venter uppermost, one on ashes mixed with gypsum, earth and clay, and one on moist earth. Both were partly imbedded in the backgrounds so as to bring their bellies flush with the surface of the latter. The rat-earth combination showed the greater contrast of the two. After an hour and twenty minutes returning to the cage I found two hawks fighting over the rat on the earth.

Summary

In this series of nineteen experiments, the combination of greater contrast was chosen in sixteen or 84 per cent of the cases and that of less contrast in three or 16 per cent. In one of the latter (Experiment 4) the light was such at the time of experiment that objects on a light surface (ashes) were in general much more visible than those on a dark surface (moist earth). In another of these (Experiment 6) it is probable that the hawk did not see the mice until it alighted close to them, at which distance the resemblance between the mouse and the clay could not have had much effect. I shall consider this point further in my discussion of results. In Experiments 1, 2, 3, and 14, also, the weak light enhanced the effect of the white background. If these six experiments (1, 2, 3, 4, 6, and 14), regarding which some uncertainty was introduced by the conditions just mentioned, be removed from the count, the results stand: Combination showing greater contrast chosen in twelve out of thirteen experiments (92 per cent), that of less contrast in one (8 per cent).

SERIES VI

In this series a long-eared owl (*Asio wilsonianus*) and house mice (*Mus musculus*) were employed. The former was taken from the nest in June just before learning to fly and kept in a small cage until October, when the experiments were performed.

For a few days it was fed by hand, but it soon learned to feed itself. The experiments were conducted in the same cage in which Series V was conducted. The backgrounds were arranged at one end of the cage and the prey placed on them while the owl was perched at the opposite end, care being taken, as in the other experiments, to conceal the mice from the view of the owl while they were being placed on the backgrounds. In all of the experiments to be recorded the contrast between the mice and the white background (snow or powdered gypsum) was much greater than between the mice and the other background.²¹ Being performed at dark or later, the effect of the former background in rendering objects placed upon it conspicuous in comparison with those on the other backgrounds was much enhanced. The resemblance between the mice and the latter backgrounds may have been greater to my eye than to that of the owl, since its eyes were adapted to night vision. When the owl was transferred from the small to the large cage and mice first placed on the backgrounds, it left them untouched for about three hours, at the end of which time, observation was suspended. It soon learned however to feed from the backgrounds and did so much more readily than the hawks in Series IV and V.

Experiment 1. Figures 7 and 11. Four mice were placed on snow and four on dead leaves mixed with earth and clay. Fifteen minutes later three mice had been taken from the snow.

Experiment 2. The arrangement of prey and backgrounds was the same in this experiment as in the preceding, except that only two mice were placed on each background. In less than an hour²² one mouse was taken from the snow.

Experiment 3. Figure 14. In this experiment three mice were placed venter uppermost on partly moist clay and three in a similar position on moist earth. The clay and earth backgrounds occupied the same relative position as the snow and leaves in the preceding experiment. At the time of this experiment, the latter combination, as seen from the opposite end of the cage, presented a much greater contrast than the former. Within about five minutes the mouse nearest the clay background was taken from the earth.

Experiment 4. The same as Experiment 3, except that the clay had become more moistened by the underlying damp earth, and the

²¹ Experiments 3 to 5, 9, 10 excepted.

²² Exact time not noted.

resemblance between it and the bellies of the mice was correspondingly lessened. Within ten minutes one mouse was taken from the clay. The mouse taken in this experiment was nearer the baseboard of the cage than the other two on the clay. On this baseboard the owl frequently alighted after flying across the cage from its perch.

Experiment 5. Same as Experiment 4, except that only one mouse was placed on the earth. Within thirteen minutes it was taken from the latter.

Experiment 6. Figures 8 and 13. Six mice were placed on gypsum and six on moist clay mingled with leaves, straw, etc.²³ Almost immediately the owl flew directly to the gypsum and took a mouse therefrom.

Experiment 7. Same as Experiment 6, with same result.

Experiment 8. Same as Experiment 6, except that only four mice were placed on each background. In six minutes, the owl flew directly to the gypsum and took a mouse from it.

Experiment 9. Figure 14. Four mice were placed venter uppermost on moist earth, and four on clay, the backgrounds being sufficiently excavated to bring the bellies of the mice flush with their surfaces. The relative positions of the backgrounds in this experiment was again reversed to the original position. Within twenty-four minutes one mouse was taken from the earth.

Experiment 10. The same as 9, but position of backgrounds reversed. The following morning I found four mice removed from the earth and one from the clay.

Experiment 11. Figures 8 and 11. One mouse was placed on gypsum and one on leaves mixed with moist earth and clay. Within five minutes, the mouse was taken from the former background.

Experiment 12. Same as Experiment 11, except that the backgrounds were reversed in position. The owl flew back and forth across the cage several times, twice alighting on baseboard of cage nearest the background of earth and leaves. In about ten minutes it alighted on the baseboard a third time, remaining there for ten or twelve minutes more. Soon after (exact time not recorded) the mouse was taken from the gypsum. Sometime during the night the mouse was taken from the leaves.

Summary

In eleven out of twelve experiments or 92 per cent the combination of greater contrast was chosen, and in the other experiment (4) the resemblance between the mouse chosen and its background was not great, and the owl probably alighted nearer to it than the other background before feeding.

²³ In this and the two succeeding experiments, the position of the light and dark backgrounds was the reverse of that in the preceding experiments.

SERIES VII AND VIII

Series VII and VIII were conducted simultaneously and the birds (kingbird—*Tyrannus tyrannus* and grackle—*Quiscalus quiscula anaeus*) confined in the same cage.²⁴ The kingbird was taken from the nest before learning to fly while the grackle was an adult which flew into a building and was caught there. In Series VII the latter bird was employed as the prey, and in Series VIII the former. In each series several different species of insects (as recorded for the individual experiments) were used as prey. Different backgrounds (the character of which and the relative contrasts between them and the insects placed thereon being noted in each experiment) were prepared at one end of the cage, while the birds were perched at the opposite end, care being taken in each case to avoid attracting the attention of the birds more to one background than to the other while the prey was being placed upon them. The insects in each case were killed before being placed in position.

Experiment 1. Figures 33 and 37. Five *Melanoplus*⁷ were placed on hay and five on sand. In fifteen minutes the grackle dropped to the ground and walked to the hay from which four grasshoppers were taken followed by four from the sand. The contrast between the grasshoppers and the sand was greater than that between them and the hay, but their resemblance to the latter background was not very great. The bird seemed afraid of those on the sand, drawing back in apparent alarm after seizing one.

Experiment 2. Figures 25 and 44. One *Gryllus pennsylvanicus* was placed on sand and one on moist earth. The former combination presented the greater contrast, but a little sand accidentally mixed with the earth reduced the resemblance between the cricket and the latter background. After three minutes the grackle flew over the backgrounds twice, apparently taking no notice of either cricket. Five minutes later it alighted nearer the earth background, from which it took the cricket.

Experiment 3. Same as Experiment 2. In one minute the grackle flew to the ground alighting nearer the cricket on the earth, which was taken one minute later.

Experiment 4. Figures 37 and 50. One *Melanoplus* was placed on grass and one on sand. The contrast here was greater in the latter than in the former combination. The grackle immediately dropped

²⁴ 3.6 x 1.8 x 1.6 m. in size.

to the ground and then walked to the backgrounds, somewhat nearer the grass, but took the insect from the sand.

Experiment 5. Figures 25 and 58. One *Oecanthus quadripunctatus* was placed on grass and one on moist earth. The grackle at once dropped to the ground, approached the grass first and took the insect therefrom. The contrast here was greater in the insect-earth than in the insect-grass combination.

Experiment 6. Same as Experiment 4. In one minute the grackle flew across the cage to a point nearer the grass than the sand, but walked past the former to take the insect from the latter background, although it was smaller than that on the former.

Experiment 7. Figures 35 and 58. One *Oecanthus* was placed on grass and one on sand, the latter combination presenting the greater contrast. The grackle flew at once to a point nearer the grass background from which the prey was taken.

Experiment 8. Same as Experiment 4. The grackle at once dropped to the ground and in two minutes passed the grass to take the insect on the sand background.

Experiment 9. Same as Experiment 7. The contrast here was only slightly greater between the insect and the sand than between the former and the grass. The grackle almost immediately dropped to the ground, passed the grass and then turned back and took the insect from it. It then picked some crumbs from the sand. Evidently the insects were not seen by the bird before it reached the backgrounds.

Experiment 10. Same as Experiment 5. After twenty minutes the grackle approached the grass from which it took the insect, then taking that on the earth.

Experiment 11. Figures 25 and 37. Three *Melanoplus* were placed on hay mixed with moist earth, and three on sand. The latter combination showed the greater contrast. The grackle passed first to the hay from which it took the insects and then to the sand from which they were next taken.

Experiment 12. Figures 25 and 58. Four *Oecanthus* were placed on moist earth and four on grass, the arrangement being as shown in the accompanying diagram (fig. B). The grackle's line of approach and the order of seizure of the insects are also shown in the diagram. The grackle approached the backgrounds and began feeding in one minute after the start of the experiment. The contrast in this experiment was greater between the insects and the earth than between the former and the grass.

Experiment 13. Figures 40 and 55. One *Gryllus* was placed on a mixture of burnt paper and moist earth and one on ashes. For some time (time not noted) the grackle remained on its perch. It then flew to the ground and began picking up various small scraps of food, finally approaching the backgrounds, but stopping to peck at something when but a few centimeters distant. It then took the cricket from the earth and next that from the ashes. The contrast between

the cricket and the grayish-white ashes was greater than that between the former and the dark background. The accompanying sketch (fig. C) shows the arrangement of the backgrounds and the line of the birds' approach (xy) and the point (x) where it stopped to pick up a particle of food.

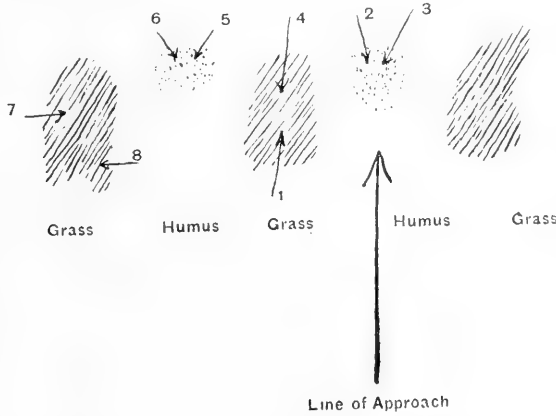


Fig. B The figures indicate the positions of the insects on the backgrounds, and the order of their seizure.

Experiment 14. Figures 40 and 43. One *Gryllus* was placed on scraps of burnt paper and one on ashes. The resemblance between the cricket and the paper was close and the contrast between the former

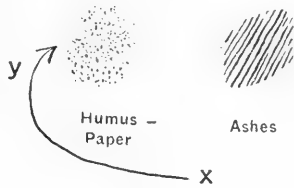


Fig. C

and the ashes was strong. After five minutes the grackle flew to the ground close to the latter background, from which it took the cricket. It then took that on the paper. Although the bird flew directly to the backgrounds in this experiment it apparently did not see the insects until after alighting, for it turned from its first position in order to seize its prey.

Experiment 15. Figures 24 and 30. Two *Melanoplus* were placed on ashes and two on a mixture of dead leaves, straw, and earth, the

former combination presenting the greater contrast. The grackle at first flew to a perch about 2 m. from the backgrounds, from which, three minutes after the start of the experiment it flew to the ground near the latter background, but passed it by and attempted to seize an insect on the former when I interrupted it.

Experiment 16. Same as Experiment 15. In about seven minutes, the grackle flew over the backgrounds alighting nearer the ashes, but seizing an insect from the leaves and straw.

Experiment 17. Figures 30 and 46. Two *Silpha surinamensis* were placed on burnt hay and two on ashes. In twenty minutes the grackle flew to the ground at the middle of the cage and began feeding. It soon approached the backgrounds on the side of the burnt hay background, from which it took a beetle. The contrast here was greater between the beetles and the ashes, but the resemblance between the hay and the beetles was not very close as the latter were a glossy black and the hay dull black.

Experiment 18. Figures 27 and 30. Two *Silpha* were placed on light ashes and two on charred wood, the former combination presenting much the greater contrast. In one minute the grackle dropped to the ground and began feeding. In two minutes it passed the beetles on the black background and took both from the white, taking no apparent notice of the former.

Experiment 19. Same as Experiment 18, except that only one beetle was placed on each background. In one minute the grackle flew to the ground beside the white ashes from which it took the beetle. It then passed the black background once or twice taking no apparent notice of the insect upon it. After fourteen minutes the latter was taken, but I am uncertain whether by the kingbird or the grackle.

Experiment 20. Same as Experiment 19. The grackle immediately flew to the ground and approaching the white background first took the insect from it. It then took the insect from the black background.

Experiment 21. Figures 27 and 45. One *Silpha* on charred wood and one on flour, the latter combination presenting the greater contrast. The grackle at once approached the backgrounds in a fairly direct line, passing nearer the wood, but taking the beetle from the flour. It then took the beetle from the wood.

Experiment 22. Figures 36 and 60. One moth (Noctuid sp.) was placed on a piece of bark which was partly covered with damp ashes producing a background closely resembling the moth, and one on a strip of very light colored wood.²⁵ The body of the insect was inserted in a crack in the bark so as to bring its partly expanded wings close to the surface of the latter, but not in anyway concealing it. The grackle flew from one perch to another and then returned. It then flew to the ground and fed for a few minutes. In ten minutes it approached the backgrounds, passed close to the moth on the bark, and took that from the wood. It was seemingly a little suspicious of the

²⁵ A piece of an ordinary berry box was used.

latter, as it dropped it once before eating it. It then turned back and took the moth from the bark.

Experiment 23. Figures 40 and 42. One *Gryllus* was placed on ashes and one on a mixed background of charred and uncharred wood, the latter combination presenting a close resemblance, and the former a good contrast. The grackle immediately dropped to the ground and began feeding. It soon went to the backgrounds, passing nearer the charred wood, but taking the cricket on the ashes. It then turned and walked over the charred wood, passing directly over the cricket upon it, but apparently not seeing it.

Experiment 24. Same as Experiment 23, except that in the former both crickets were probably in shadow,²⁶ while in this experiment they were in the sun. The grackle immediately dropped from its perch to the ground and approached the backgrounds about midway between them, paused a moment and seized the cricket on the ashes, after which it turned and took that on the wood background. The prompt approach of the grackle to the backgrounds in this experiment apparently indicates that it realized that food had been prepared for it there. Its attention however was not I believe attracted to one background more than to the other in the preparation of the experiment. Its pause for a moment after reaching the backgrounds, and the prompt seizure of both insects suggests that both were seen as it approached, and that it was a matter of chance, or possibly of some individual preference on the part of the bird as to which was taken.

Experiment 25. Figures 51 and 53. One green *Melanoplus* was placed on grass and one on charred wood. For three-quarters of an hour the insects were untouched, although the grackle several times went to within a short distance of the backgrounds. For seven minutes the observations were discontinued. Soon after resuming them, the grackle once again approached to within about 3 cm. of the backgrounds but did not feed. Then it again approached, coming nearer the charred wood, from which it seized the insect, and then immediately took one from the grass. The charred wood combination presented the greater contrast. That the insects were left untouched for so long a time in this experiment, because of the bird not being hungry, is improbable, as it was pecking at objects on the bottom of the cage during this time, and when one insect was finally taken the other was immediately taken also. Further, in the following experiment (26), the grackle took the insects very soon after the experiment was started and within about ten minutes of the last feeding (in Experiment 25).

Experiment 26. Same as Experiment 25. The grackle immediately dropped to the ground and approached the backgrounds. It turned back for a moment and then re-approaching between the two backgrounds, took the insect on the charred wood and immediately after, that on the grass.

Experiment 27. Figures 49 and 51. One green *Melanoplus* was placed on a mixture of grass and straw (lengthwise on a straw so as

²⁶ On this point my notes are uncertain.

to more closely resemble its surroundings), and one on charred wood, the latter combination showing the greater contrast. Before I had left the cage after placing the insects, the grackle crossed from the opposite end of the cage to the backgrounds and took the insect from the charred wood, leaving that on the grass and straw untouched. This was left in position and a few minutes later it too was taken. Comparing Experiment 25 above with Experiment 27, one is impressed with the influence which the attention of the bird exercised on the rapidity with which the results were obtained. In the former experiment, the insects were apparently unseen for over fifty minutes, although during this time the bird several times came near them; while in the latter they were taken immediately, due in all probability, to the fact that the bird realized that food was being prepared for it on the backgrounds. Why its attention was attracted more readily in one experiment than in another is uncertain. I shall refer to this question later (p. 493).

Experiment 28. Figures 27 and 61. One moth (Noctuid sp.) was placed on brown leaves and bits of bark and one on charred wood within about 7 cm. of each other. The former moth closely resembled its background, while the latter combination presented a good contrast. In three minutes the grackle dropped from its perch to the ground and walked directly to the backgrounds, passing by the moth on the leaves. It then paused for a few seconds to inspect the moths, before seizing that on the charred wood, immediately followed by that on the leaves.

Experiment 29. Figures 26 and 27. Same as Experiment 28, except that a background of straw was substituted for the leaves and bark, the moth-wood combination presenting the greater contrast. The grackle immediately dropped to the ground and walked directly towards the backgrounds, but its attention being apparently diverted by some object outside of the cage, it ran past, returning on the side of the straw, from which it seized the moth, and then that on the wood.

Experiment 30. Figures 27 and 59. One moth (Noctuid sp.) was placed on charred wood and one in an angle of a dead leaf so that the wings overlay it, with the head and thorax projecting over the ground, thereby reducing the relief and enhancing the resemblance of the insect to its background. The insects were placed 6 or 7 cm. apart. In ten minutes the grackle dropped to the ground and approached the backgrounds on the side of the latter moth, but passed it by and seized the former, then turning, it apparently was about to seize the latter when I interfered.

Experiment 31. Figures 27 and 40. One Gryllus was placed on charred wood mixed with a little earth, and one on ashes. The former combination presented to my eye a close resemblance. The grackle was about 1.6 m. distant during the arrangement of the experiment. As I was leaving the cage it went directly to the backgrounds and approaching the charred wood first, seized the insect upon it, immediately followed by that on the ashes.

Experiment 32. Same as Experiment 31, except that the grackle was further distant from the backgrounds at the beginning of the experiment. The result was the same as in Experiment 31.

Experiment 33. Figures 27 and 60. One moth (Noctuid sp.) was placed on gray bark and one on charred wood, the former presenting, to my eye, a fairly close resemblance to its background, and the latter a good contrast. After flying across the cage a few times the grackle dropped to the ground and a minute later went directly to the backgrounds, passing the charred wood and taking the insect from the bark, followed by that on the charred wood.

Summary

In 15 out of 33 experiments, or 45 per cent, the combination of less contrast was chosen and in 18 out of 33, or 55 per cent, that of greater contrast. In 15, or 45 per cent, the prey was taken from that background nearest which the bird happened to alight. A further analysis of these apparently inconclusive results will be reserved for later discussion.

SERIES VIII

Experiment 1. Figures 25 and 37. Five *Melanoplus* were placed on sand and five on a mixed background of hay and earth, the former combination presenting the greatest contrast. Almost immediately the kingbird flew direct to the sand from which it took one insect.

Experiment 2. Same as Experiment 1. In three minutes the same result was obtained.

Experiment 3. Same as Experiment 1. In one minute the kingbird flew over the sand alighting on a box about 2.5 cm. from the backgrounds. Here it remained a few moments when the same result was obtained.

Experiment 4. Same as Experiment 1. The kingbird immediately flew to the sand from which it took one insect, and then alit at a point nearer the hay than the sand. It quickly returned and first seized one insect on the hay and then the remaining four on the sand, leaving four on the hay.

The results in the three preceding experiments may have been modified by the memory of the bird's experience in Experiment 1, in which it found insects on the sand. In Experiment 4 however even after it fed from the hay, it left four insects on the latter and took four from the sand, tending to show that this was not the case.

Experiment 5. Figures 35 and 58. One *Ceresa bubalus* was placed on grass and one on sand, the latter combination showing the greater contrast. Before I had time to leave the cage the kingbird flew to the sand, from which it took the insect.

Experiment 6. Same as Experiment 5. In twelve minutes the kingbird flew direct to the sand from which it took the insect. It then looked closely at the insect on the grass for a few seconds and then took it also.

Experiment 7. Figures 25 and 30. Three *Melanoplus* were placed on backgrounds of hay mingled with earth and three on ashes, as shown in the accompanying diagram (fig. D). The latter combination showed the greater contrast. The kingbird flew to the backgrounds, while I was standing nearby, going first to 5, but not feeding. It then took the insect from 1.

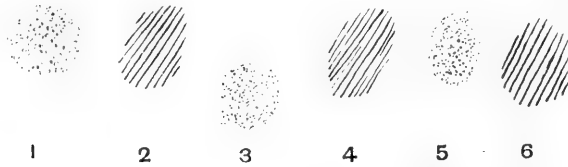


Fig. D 1, 3, and 5—ashes; 2, 4, and 6—hay—earth.

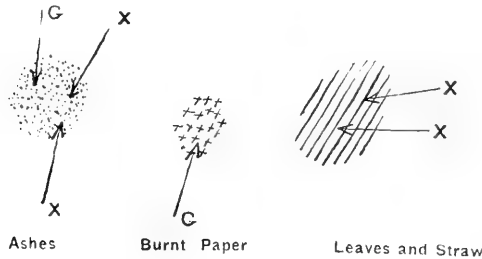


Fig. E g—*Gryllus*, x—*Melanoplus*.

Experiment 8. Same as Experiment 7. The kingbird flew direct to 5, which it took followed by 1. The result in Experiment 8 may have been modified by that in Experiment 7, as both were alike.

Experiment 9. Figures 24, 30, 40 and 43. In this experiment three backgrounds were prepared, the arrangement of which, with the insects placed on each is shown in the accompanying sketch (fig. E). The leaf and straw background was in weak sunlight, while the ashes were in shadow. The insects on the ashes presented the greatest contrast to their background in this experiment. Within two minutes, and while I was still standing near the backgrounds, the kingbird flew direct to the ashes from which it took both *Melanoplus*.

Experiment 10. Same as Experiment 9, except that all the backgrounds were in shadow at the time of experiment. At the commencement of this experiment the kingbird was perched on the side of

the cage.²⁷ In thirty seconds it flew direct to the ashes from which it took one *Melanoplus*, returning to its usual perch. A few seconds later it left its perch and circled over the backgrounds, returning to the perch and then it again flew direct to the ash background, from which it took the other *Melanoplus*. For five minutes it flew back and forth from point to point in the cage, at one time alighting near the backgrounds but not feeding. The observations were then discontinued.

Experiment 11. Figures 25 and 34. Two *Oecanthus* were placed on dark earth and two on a background of mingled sand and ashes over which were distributed grass stems and awns closely resembling in color the insect's wings, the former combination presenting the greater contrast. In five minutes the kingbird flew from the perch to a point on the walls of the cage nearly over the backgrounds which it closely inspected. It then flew across the cage and returned to this point from which one minute later it dropped to the backgrounds and a moment later took one insect from the earth. Two minutes later it again flew to the backgrounds²⁸ and took the other insect from the earth. The two insects were left on the grass and sand background and in about ten minutes the kingbird alit near them twice but took no apparent notice of them. The observations were ended after about thirty-five minutes up to which time they had not been taken.

Summary

In this series of eleven experiments the combination showing the greater contrast was chosen eleven times. This is the only series in which protective coloration was efficient in 100 per cent of the trials.

SERIES IX

In this series the domestic chicken (*Gallus domesticus*) and various species of insects, as recorded in the individual experiments, were employed.

The experiments while few in number cover a series of years (1910, '11, and '12) and were conducted in different cages, the size of which I believe to be immaterial, as the chickens always walked up to the backgrounds, giving them abundant opportunity to see their prey at close quarters. I have therefore not recorded the size of cage in the various experiments. One bird was used in Experiments 1 to 7, another in 8 to 11, and a third

²⁷ Distance from backgrounds not noted.

²⁸ The point from which it flew was not noted.

in Experiment 12. Care was taken in arranging the experiments to avoid drawing the attention of the chicken more to one background than to another.

Experiment 1. Figures 27 and 48. One *Platynus placidus* was placed on charred wood and one on ashes, the latter forming the combination of greater contrast. The chicken was about 2.5 m. distant from the backgrounds, from where it walked toward them, stopping for a few moments when a few centimeters distant from them. It then took the insect from the charred wood.

Experiment 2. Figures 27 and 54. One *Oecanthus quadripunctatus* was placed on charred wood and one on flowers of the golden rod (*Aster*). The light green of the insect's body and the straw color of its wings presented a close resemblance to the latter background, while the contrast of the former combination was good. The chicken slowly approached the backgrounds from a point about 3 m. distant. Approaching from the side of the golden rod background, it passed this and took the insect from the charred wood. Then immediately turning back it took the insect from the former background.

Experiment 3. Same as Experiment 2 and precisely the same results in all details.

Experiment 4. Same as Experiment 2 and same result except that the chicken in passing the golden rod background stepped on it and upset the insect, which it apparently did not see as it did not take it, but moved away after eating that on the charred wood.

Experiment 5. Same as Experiment 2 except position of backgrounds reversed and a larger head of golden rod employed. The chicken approached the charred wood background from a point about 2.5-m. distant. It apparently first saw the insect on the charred wood when but a few centimeters away, for from that point it ran to the wood and took the insect from it. It then paused a moment and turned away, but returned to take the insect from the golden rod.

Experiment 6. Figures 27 and 40. One *Gryllus pennsylvanicus* on ashes and one on charred wood, the former combination showing the greater contrast. The chicken approached the ash background from a point 3.5 m. distant, which it passed to take the cricket on the charred wood. It then moved around the backgrounds, but did not touch the insect on the ashes. After some three minutes I transferred the insect from the ashes to the charred wood and a few minutes later the chicken took it. Here apparently the chicken had some antipathy to the ash background. But note the result in Experiment 7.

Experiment 7. Figures 54 and 57. One *Oecanthus* was placed on ashes and one on flowers of the golden rod (*Aster*), the former combination presenting the greater contrast. The chicken was about 3.5 m. distant. It approached the backgrounds gradually on the side of the golden rod background but took the insect from the ash first and immediately after that from the golden rod.

Experiment 8. Figures 40 and 41. One *Gryllus* was placed on coal dust mixed with small pieces of anthracite coal, the latter shining like the insect's head and thorax, and one on ashes, the latter combination showing much the greater contrast. The chicken was about 1.5-2 m. distant. It approached slowly on the side of the coal background from which it first took the insect and immediately after that on the ashes.

Experiment 9. Same as Experiment 8. The chicken wandered around for about three minutes and then approached the background on the side of the coal, but took the cricket from the ashes, followed immediately by that on the coal.

In both Experiments 8 and 9 the chicken several times passed near the backgrounds (within a half meter) without apparently seeing the insects before it finally took them.

Experiment 10. Figures 30 and 38. One *Melanoplus* was placed on earth over which were scattered bits of dry leaves and one on ashes,

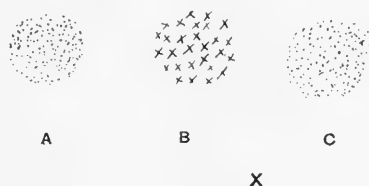


Fig. F

the latter combination showing the greater contrast. The chicken was about 1.5 m. distant. Almost immediately it approached the backgrounds but passed them and then turning back took that on the earth background. During this interval the insect on the latter was apparently unnoticed by the chicken.

Experiment 11. Figures 25 and 40. Three *Gryllus* were placed on each of two backgrounds of moist earth, *A* and *C*, and three on ashes, *B*, arranged as shown in the accompanying sketch (fig. F). The latter combination showed greater contrast. The chicken was about 2 m. distant at the beginning of the experiment. After a minute or two it approached the backgrounds to *X*, within about 0.5 m. of them, without apparently seeing the insects. From here it went to *C* and took three, and then two from *B* and one from *A*.

Experiment 12. Figures 31 and 47. In this experiment several backgrounds, some composed of sifted lime and some of a mixture of earth and straw, were arranged alternately at one end of the cage. Six *Melanoplus* were placed on the lime backgrounds and six on the earth and straw, the former combination forming a strong contrast; the latter a close resemblance. A hen was then turned loose in the cage. She soon approached the backgrounds, taking four insects from the straw-earth backgrounds and then four from the lime.

Summary

In this series the combination showing the greater contrast was chosen in seven out of twelve cases or 58 per cent and the one showing the less contrast in four or 42 per cent. It is noteworthy that in this series, as well as in series II and III, the birds usually approached their prey slowly, giving them opportunity to carefully inspect the backgrounds at close range before making their choice.

Two experiments were also performed with the prairie chicken (*Tympanuchus americanus*) and the grasshopper (*Melanoplus*), one background of lime and one of earth and straw being employed (figs. 31 and 47). Results similar to those of the last series were obtained, the bird in each case walking to the backgrounds and taking the insects from that one which it first approached, in one case the lime, in the other the earth—straw background.

All of the experiments recorded above are open to the objection that they were performed with caged birds (most of them young individuals) which as a result of confinement may not have acquired the normal keenness of vision. So far as I am aware, most of the experiments on protective coloration (including mimicry, warning coloration, etc.) which have been recorded thus far have been performed with caged animals, but it is quite possible that confinement does in many instances render less acute an animal's senses. While none of the birds experimented with became very tame,²⁹ some of them, notably the hawks, *Buteo borealis krideri*, behaved very differently from wild birds. One of these, when removed from the cage, instead of flying off, remained on the ground as long as observed, and when I approached attempted to defend itself by fight, rather than flight. While this condition militates against the value of my experiments, I believe it is far offset in the other direction

²⁹ A short time after removal from the nest the martins (*Progne subis*) became so tame that they would climb up my leg in order to obtain food, while the young kingbirds occasionally fed from forceps held in my hand. As the birds grew older they soon lost this tameness however, and would not permit me to approach them closely when possible to avoid it.

by the smallness of the cages in which the birds were confined. Thus instead of being obliged to seek their prey from a considerable distance as is the case with many wild birds, notably hawks, it was brought close to the caged birds, thereby reducing very materially the protective coloration effect.

I have made several attempts to perform similar experiments with wild birds, but in most instances without success. Wild birds ordinarily have an abundance of food available, so that they will pay no attention to food prepared for them, especially if the food be dead, while their natural food is alive. It is highly probable moreover that movement of their prey plays a large rôle in enabling raptorial and insectivorous birds to secure it. To this latter point I shall refer later.

In a few cases, however, I secured results which will be recorded in the following experiments.

SERIES X

Experiment 1. Figures 29 and 50. In this experiment three backgrounds were arranged as shown in the accompanying diagram (fig. G), 1 and 3 being composed of blades of grass and 2 of flour. Five *Melanoplus* were placed on 1 and 3 and ten on 2. The latter combination presented the greater contrast. About 45 cm. from 1 and 3 to 10. cm. above the level of the backgrounds was a perch, A. Two minutes after arranging the backgrounds two English sparrows (*Passer domesticus*) lit near them and fed from each leaving three insects on 1, seven on 2 and one on 3. Seven minutes later a kingbird (*Tyrannus tyrannus*) lit on the perch and then flew over 1 to 2 where it took one insect. It then took three from 1 and two more from 2. When viewed from the perch then at a distance of 46 cm. the color of the insects was protective, but at closer range this effect was lost.

Experiment 2. Figures 29 and 32. In this experiment two backgrounds were arranged in the same position as in Experiment 1, 1 being composed of a mixture of straw and dry ditch grass (*Ruppia maritima*) and 2 of sifted flour, on each of which were placed seven *Melanoplus*. The latter combination presented the greater contrast. A grackle (*Quiscalus quiscula anaeus*) soon fed from each background feeding from that one (2) which it first approached, from which it took one, and then two from one of the others. A kingbird then flew over both backgrounds and lit on the perch. It then flew over 1 and took an insect from 2.

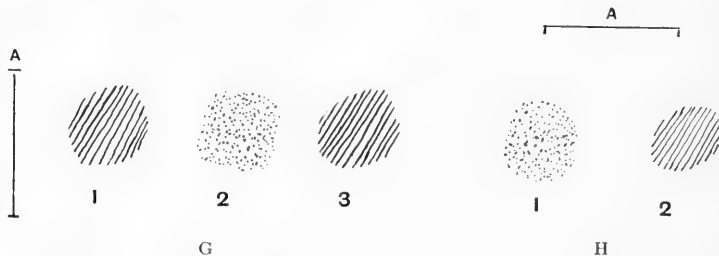
Experiment 3. The same as Experiment 2 except that five insects were placed on each of the two backgrounds. After a sparrow had alighted and fed on 2, a kingbird flew over both backgrounds to alight

on the perch, shortly after returning to 2 and feeding while on the wing. When I examined the backgrounds all had been taken from 2 and none from 1.

Experiment 4. The same as preceding except that nine insects were placed on each background. A sparrow, after alighting on the perch, flew over 1 and fed from 2.

Experiment 5. *Figures 28 and 50.* The backgrounds and a perch were arranged as shown in the accompanying sketch (fig. H), the perch being about 60 cm. away from the backgrounds, and ten *Xyphidium fasciatum* were placed on each background, the insect-flour combination showing the greater contrast. A sparrow lit on the perch and then flew to 1 from which it fed.

Experiment 6. Same as Experiment 5. A sparrow alighted on the perch and then flew to 2 but did not feed, passing instead to 1 from which it took one insect.



Figs. G and H A—perch, 1—flour, 2—grass.

Fourteen other similar experiments were performed but in these cases the birds with one exception hopped up to the backgrounds and fed from the one which they first approached, the exception being that of a kingbird which, flying close to the ground, and approaching the background presenting the less contrast first, fed from it on the wing.

Summary

The results of this series agree closely with the experiments in the preceding series; in those cases in which the birds approached the backgrounds slowly on foot, the similarity in color between the prey and the backgrounds, had less apparent protective value;³⁰ while in those cases on the contrary in which the birds approached the backgrounds on the wing, even from a short distance, the resemblance between the prey and the backgrounds showed an evident protective effect.

³⁰ See pp. 496-7.

In regard to my experiments with the martin and kingbird (Series III and VIII) the further objection may be made that I obliged birds, which normally feed on flying insects, to feed on insects placed on the ground. I can only say in answer to such an objection that I have observed both of these birds feed on the ground, and I have also seen the kingbird and other swallows (*Chelidon* and *Tachycineta*) feeding from the surface of a lake.

In the course of these experiments I have found considerable evidence to show that stillness is of more importance than color in determining the immunity of animals to attack by birds. I have frequently found the prey which I had placed on the backgrounds unnoticed by the birds for several minutes, or even in some instances hours, although the latter were unquestionably hungry, and in many cases were running about the cage, pecking at various objects on its floor. Especially was this delay noticeable in series IV and V with the hawks (*Buteo borealis krideri*).

The failure of hungry birds in many instances to notice the food prepared for them on the backgrounds,³¹ may be attributed to the dulling of their senses by captivity, in the case of those birds which were taken from the nest and reared in captivity. This will hardly, however, explain such failure, in the case of the grackle (*Quiscalus*), which was taken when adult and had been a captive for only a few weeks previous to the experiments. It was kept, moreover, during this time in a large cage,³² freely accessible to light and air and was oftener hungry than the reverse.

If, however, the prey was thrown into the cage so as to attract the notice of the birds it was usually pounced upon immediately. In Series X I saw the kingbird (*Tyrannus tyrannus*) pass the backgrounds three times in one experiment without feeding, although it was feeding on nearby objects (undoubtedly living insects). In Series I, Experiment 2 a crow dropped a frog

³¹ See for example Experiment 3, Series IV, Experiment 17, Series V, and Experiment 25, Series VII.

³² 3.6 x 1.8 x 1.6 m.

which it had seized in order to pursue another frog which moved nearby; and in Series III, Experiment 1 a martin passed dead insects several times without noticing them, but seized a live specimen which I had left in the cage by mistake. In many other instances also, notably in Series VII, Experiments 9, 14, 23, etc., and Series IX, Experiments 8 and 9, the bird passed close to one or other of the backgrounds without apparently seeing the prey upon it.

In order to definitely test this point in the case of the hawks, (*Buteo borealis krideri*) I performed the following experiments:

SERIES XI

Experiment 1. A dead mouse (*Mus musculus*) placed at one end of the hawk's cage, while the bird was at the opposite end 4.5 m. distant, was not noticed by the bird for several minutes, until the latter was driven across the cage to where the mouse lay, when it was taken. A cage containing a living mouse, actively moving about, was now introduced into the hawk's cage, and no sooner was the mouse liberated from its cage than it was seized by the hawk.

Experiment 2. A dead rat (*Epimys norvegicus*) placed on the ground at one end of the cage with the hawk at the opposite end was seized in two minutes. A live rat released in the cage ran toward the hawk and when about one half the distance across the cage it was seized by the latter.

Experiment 3. Two mice (*Mus musculus*) were placed at one end of the cage with the hawk at the opposite end. One of these was dead and the other alive, attached to a small wire by one leg. After seven minutes the hawk seized the live mouse.

Experiment 4. Same as Experiment 3, with same result in five minutes.

Discussion

Birds are frequently given credit for an extremely acute vision and to this faculty is ascribed, by the opponents of the protective coloration theory, their power to discern their prey at long distances regardless of whether the latter is or is not protectively colored.

Thus Entz (1906, p. 136) says:

. . . wo aber ist je eine Beobachtung darüber angestellt worden, ob sich die Schutzfarbe den Tieren des Waldes und der Luft gegenüber wirklich als solche bewährt oder ob etwa Vögel mit schärferen Sinnen

die Raupe und den Schmetterling ebenso sicher erkennen wie der Adler aus gewaltiger Höhe den Hasen oder das Murmeltier auf gleichfarbigem Boden?

This assumption however lacks supporting evidence, and the experiments here cited tend to disprove it. True it is that raptorial birds frequently sail at great heights, but how many instances are on record of their swooping down from these heights to seize their prey? Furthermore anyone who has observed a hawk hovering in the air evidently in pursuit of prey, will realize that it is in all probability the movement of the latter which enables the hawk to follow and finally to seize it. This conclusion is supported by my own observations and experiments just cited.

I have observed numerous cases of ducks flying within gunshot of a hunter lying or sitting motionless upon the ground, without any blind to screen him from their sight. No sooner does the hunter rise to shoot however or the ducks come near enough to see him than they immediately swerve from their course and either turn back, or more often make a wide detour to avoid the threatened danger; proving that it is not fearlessness that brings them into danger, but inability to see a man close to the ground in a motionless posture, or at least to distinguish him from his surroundings.

In this connection the occasional records of birds seizing wooden decoys are of interest. Lawrence ('66, p. 279) says of the duck hawk (*Falco anatum*) "I have a fine specimen which was killed at Rockaway in the act of carrying off one of our wooden snipe decoys, which it had seized."³³

Dr. Frank M. Chapman writes me that his "friend Dr. L. C. Sanford says he has seen a great horned owl attempt to seize a decoy," while Cleaves ('14) cites a case of the osprey (*Pandion haliaetus carolinensis*) seizing a wooden fish decoy.

I have heard indirectly of other instances so that the occurrence can not be very unusual.

³³ This record was kindly given me by Mr. Chas. W. Richmond of the U. S. National Museum.

Now if birds have such keen sight as is frequently attributed to them, keen enough to discern protectively colored animals on their backgrounds, it seems unlikely that they should be unable to distinguish a man in crouching posture until within gunshot of him, or to distinguish between real and decoy birds.

In the case of insectivorous birds those which swoop down upon their prey from a distance, like the Tyrannidae, the Hirundinidae, etc., prey almost exclusively on moving insects, in which case color (apart from mimicry and warning color) can obviously have little or no protective value; while others, like the Icteridae, Sturnellidae, etc., approach their prey so closely before seizing it, that even if it were motionless, it would need to bear an exceedingly close resemblance to its background in order to escape their scrutiny.

Many of the experiments described in this paper, especially those of Series I, II, VII, and IX, are inconclusive for various reasons, some of which have been already stated in connection with the individual experiments. The chief reason however is, as already stated by me in a preliminary communication before the American Society of Zoologists (Young, '15), that in many of the experiments the approach of the birds to the backgrounds was deliberate and gave them ample time to inspect the latter carefully before the prey was seized. In this connection, see Experiments 6, Series V, 28, VII, 1, IX, etc. Under these circumstances it is probable that in many experiments chance determined the bird's choice, that combination being chosen, which was nearest to the point of the bird's approach. In this connection an analysis of the results of Series I, III, VII, and IX is of interest. My notes unfortunately do not indicate in every case the point of the bird's approach. The number of experiments in which this is indicated however is sufficient to enable a fairly definite conclusion to be drawn from them. Of the forty-eight experiments, the combination presenting the greater contrast was chosen in twenty-six instances, and the one showing less contrast in twenty-two. The former may be called positive and the latter negative experiments. In the

former the bird approached from the side of the chosen combination (showing greater contrast) in four cases (15 per cent), from the opposite side in eighteen cases (69 per cent), and took a middle course in four cases (15 per cent).³⁴ In the negative experiments on the other hand, the approach was from the same side in 15 cases (68 per cent), from the opposite side in 5 cases (23 per cent), while a middle course was taken in 2 cases (9 per cent), which *is nearly an exact reversal of the former proportions*. This comparison shows perhaps even more clearly than do the results of the more definite series (III, VI, VIII, and X) the protective effect of color in the animals used in these experiments; for it shows that in spite of a deliberate approach of the birds to the backgrounds, it was possible to so far deceive them that in eighteen out of forty-eight cases they passed by the inconspicuous combination. On the other hand, in only five cases out of the forty-eight, did a bird pass over a conspicuous combination in favor of an inconspicuous one.³⁵ Further in Series III, the martins usually approached the backgrounds on foot, rather than on the wing, giving them also time to inspect the backgrounds more or less closely before feeding. Yet in spite of that fact, the results of this series show clearly the protective value of resemblance to the background in the case of motionless insects.

It is further to be borne in mind that the resemblance between the prey and the background in these experiments, was in many cases not very striking. Had animals with more striking resemblance to their surroundings been available for experiment, I am confident that even in experiments with crows, grackles, chickens, and birds of similar feeding habits still more marked results would have been obtained.

There is another factor also which possibly modified the results in a few cases. Namely, choice of the bird for one background over another, regardless of the relative contrasts presented by the combination of background and prey. This factor, I be-

³⁴ Percentages given to nearest unit only.

³⁵ In this connection, see Experiments 3 and 5, Series I, 3, II, 4, 6, 8, 15, 18, 21, 22, 23, 28, and 30, VII, and 2, 3, 4, 7, and 9, IX.

lieve played a relatively small part in determining the results. That it may have been of some importance however is indicated in Experiment 6, Series IX.

It may be argued from these results that, since the birds find their prey chiefly through the movement of the latter, it is of no consequence in the struggle for existence whether or not an animal resembles its surroundings, provided only that it remains quiet or hidden when exposed to attack. Unfortunately we have as yet no means of knowing the precise rôle which movement plays in animal fatalities. It is obvious even without experimental proof that a moving animal is more liable to attack than a quiet one, but as to what extent moving animals are killed by their enemies we have no definite data. Granting however which is probably true, that moving animals are more frequently killed than motionless ones; it is possible that protective resemblance would have sufficient selective value to become permanent. This of course leads up to the great question as to the efficiency of selection in fixing minor variations upon a race of organisms; a question with which the present paper has nothing to do, the purpose of the experiments being, not to determine this latter point, but rather to ascertain whether or not protective resemblance in the case of motionless animals is really an efficient means of protection to them.

This latter question has I believe been answered in the affirmative by these experiments. I have endeavored in them to put to an experimental test a hitherto practically untested hypothesis. They suggest further lines of desirable observation and experiment as follows:

1. Will close protective resemblances, such as those shown for example by many of the underwing moths, deceive animals of deliberate approach, such as crows, grackles, etc.?
2. Is protective resemblance efficient in the case of moving animals?
3. Is the sight of wild birds as keen as is frequently assumed?
4. Is protective resemblance as efficient with wild as with captive animals?

Many other lines of experiment in the great field of animal color will suggest themselves to every naturalist, but the ones just specified are those which suggest themselves as a logical sequence to those herein described.

SUMMARY

1. Protective resemblance is effective in protecting motionless animals from attacks by caged birds.
2. Stillness is probably a more important factor than color in protecting animals from their foes.

In concluding this paper, it is my pleasure to thank the United States Biological Survey through Mr. Vernon Bailey, and the United States National Museum, through Mr. Richard Rathbun, for the loan of material; and Messrs. Alf. Eastgate, Clark Kelly and Robt. Gray, of Devils Lake, North Dakota, for various courtesies, in connection with my work.

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³⁶ Not seen by me.

PLATES

PLATE 1

EXPLANATION OF FIGURES

- 1 *1*, Mus on earth, leaves and lime; *2*, Microtus and Blarina on moist earth; *3*, Mus and Microtus on dry earth, leaves, and twigs.
- 2 *1*, Microtus and Blarina on moist earth; *2*, the same on lime.
- 3 *1*, Microtus and Mus on ashes; *2*, the same on moist earth, leaves, bark, etc. Peromyscus placed venter uppermost, *1*, on ashes, *2*, on moist earth.
- 4 *1*, Mus on dry earth mixed with lime and leaves; *2*, Mus on moist earth. Both placed venter uppermost.
- 5 *1*, Mus on earth; *2*, Mus on leaves of cotton wood (*Populus*) and styles and staminate flowers of corn (*Zea*). Placed venter uppermost.
- 6 *1*, Microtus on earth mixed with leaves, etc; *2*, Microtus on lime.
- 7 *1*, Mus on earth, leaves, and lime; *2*, Mus on snow.
- 8 *1*, Mus on ashes, venter uppermost; *2*, Mus on lime; *3*, Mus on gypsum.
- 9 *1*, Microtus on moist earth; *2*, Microtus on ashes. Both placed venter uppermost.
- 10 *1*, Mus on earth; *2*, Mus on leaves of corn (*Zea*). Both placed venter uppermost.
- 11 *1*, Microtus on mixed earth, lime, and leaves; *2*, Mus on mixed clay, leaves and earth.
- 12 *1*, *Rana cantabrigensis* on sand; *2*, the same on earth.
- 13 *1*, Mus on moist earth; *2*, Mus on dead leaves; *3*, Mus on wet clay, leaves and straw.
- 14 *1*, Mus on moist earth; *2*, Mus on clay. Both placed venter uppermost.
- 15 *1*, Mus on clay; *2*, Mus on dry earth with scattered bits of leaves and twigs.
- 16 *1*, Mus and Microtus on ashes; *2*, the same on moist earth, leaves, bark, etc.
- 17 *1*, *Rana pipiens* on sand; *2*, *Rana pipiens* on grass.
- 18 *Rana pipiens* on moist earth.
- 19 *Epimys* on moist earth, venter uppermost.
- 20 *Epimys* on ashes, venter uppermost.
- 21 *Epimys* on mixed ashes, gypsum, earth and clay. Venter uppermost.
- 22 *Epimys* on gypsum.
- 23 *Epimys* on leaves, sticks, and straws mixed with clay.

ABBREVIATIONS

<i>b</i> , <i>Blarina brevicauda</i>	<i>mi</i> , <i>Microtus drummondi</i>
<i>m</i> , <i>Mus musculus</i>	<i>p</i> , <i>Peromyscus bairdi</i>

Except as otherwise noted, the animals are in every case placed dorsum uppermost.

¶ For an explanation of the methods employed in photography, see pp. 458-9 in the text.

Figs. 1, 3, 4, and 9—1:9; 2—1:10; 5, 14, 15, and 17—1:8.5; 6—1:9.5; 8—1:7.5; 7, 11, 13, 16, and 18—1:8; 10—1:6.5; 12—1:7.5; 19 to 23—1:20; 28, 29, 31, 32, 55, 57—8:7; 48—6:7; 49, 51, 53—4:3; all others 1:1.5.

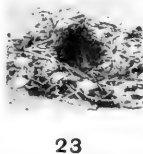
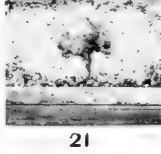
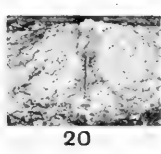
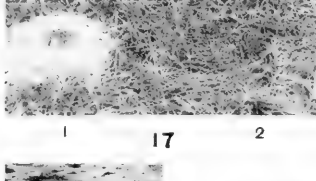
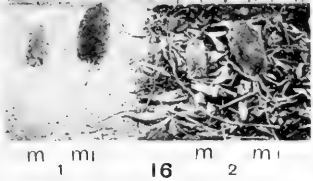
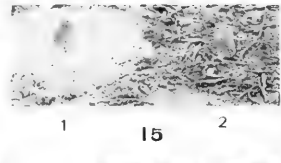
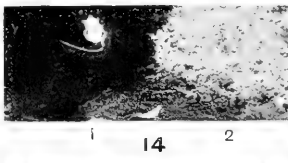
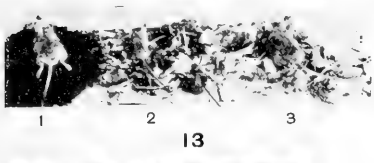
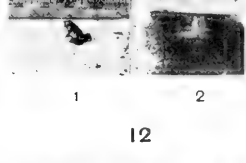
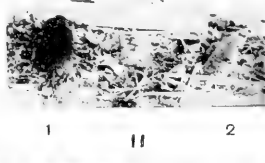
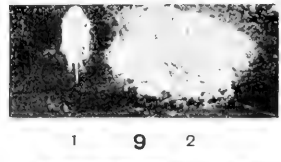
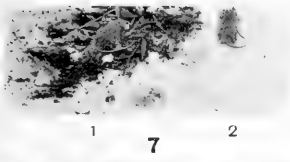
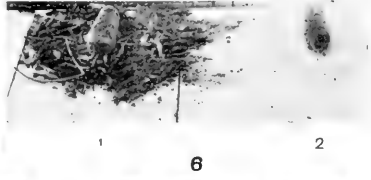
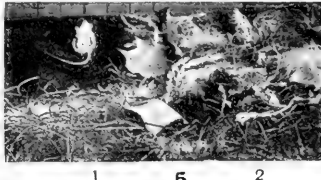
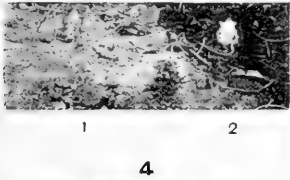
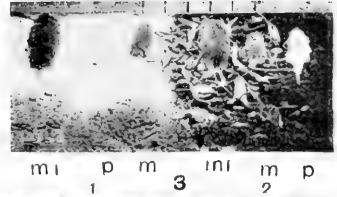
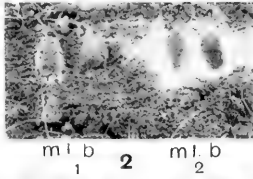
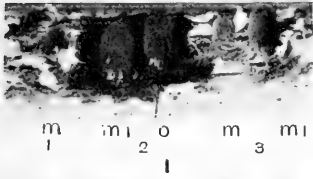


PLATE 2

EXPLANATION OF FIGURES

- 24 Dissosteira on leaves; Melanoplus on leaves, straw, and earth.
25 Melanoplus on hay scattered over moist earth; Gryllus and Oecanthus on moist earth.
26 Noctuid sp. on straw.
27 Noctuid sp., Oecanthus, Gryllus, Platynus, and Silpha on charred wood.
28 Xyphidium on flour.
29 Melanoplus on flour.
30 Dissosteira, Melanoplus, and Silpha on ashes.
31 Melanoplus on lime.
32 Melanoplus on dry Ruppia and straw.
33 Melanoplus on hay.
34 Oecanthus on pale grass stems.
35 Oecanthus and Ceresa on sand.
36 Noctuid sp. on wood.
37 Xyphidium and Melanoplus on sand.
38 Melanoplus on earth and dry leaves.
39 Gryllus on flour.
40 Gryllus on ashes.
41 Gryllus on coal.
42 Gryllus on charred and uncharred wood.
43 Gryllus on burned paper.

ABBREVIATIONS

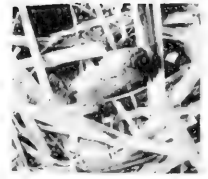
- | | |
|-----------------------------------|--------------------------------------|
| <i>c</i> , Ceresa bubalus | <i>o</i> , Oecanthus quadripunctatus |
| <i>d</i> , Dissosteira carolina | <i>p</i> , Peromyscus bairdi |
| <i>g</i> , Gryllus pennsylvanicus | <i>s</i> , Silpha surinamensis |
| <i>me</i> , Melanoplus spp. | <i>x</i> , Xiphydium fasciatum |
| <i>n</i> , Noctuid moth sp. | |



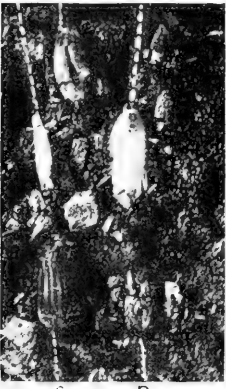
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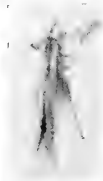
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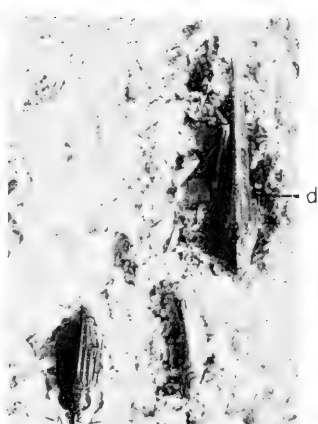
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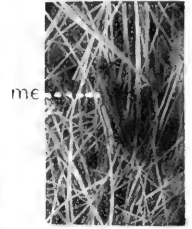
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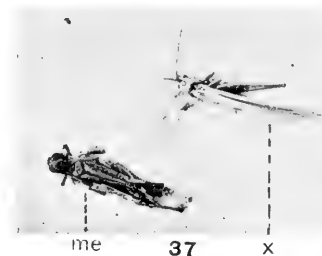
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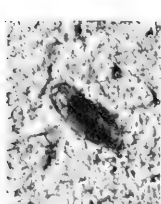
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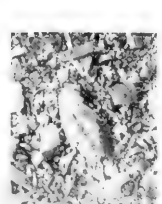
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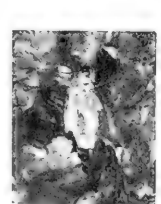
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PLATE 3

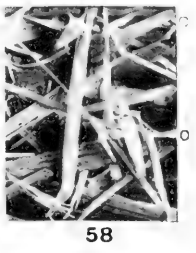
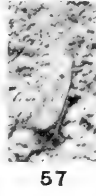
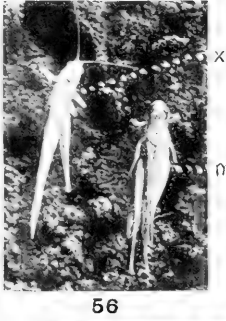
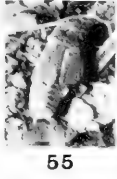
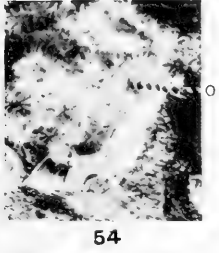
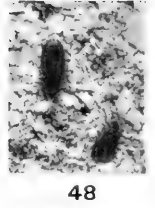
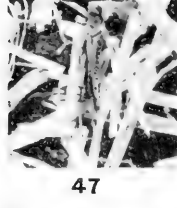
EXPLANATION OF FIGURES

- 44 Gryllus on sand.
- 45 Silpha on flour.
- 46 Silpha on burned hay.
- 47 Melanoplus on straw and earth.
- 48 Platynus on ashes.
- 49 Melanoplus on grass and straw.
- 50 Xyphidium and Melanoplus on grass.
- 51 Melanoplus on charred wood.
- 52 Xyphidium and Melanoplus on straw.
- 53 Melanoplus on grass.
- 54 Oecanthus on flowers of Aster.
- 55 Gryllus on moist earth and burned paper.
- 56 Xyphidium and Melanoplus on moist earth.
- 57 Oecanthus on ashes.
- 58 Oecanthus and Ceresa on grass.
- 59 Noctuid sp. on dry leaf.
- 60 Noctuid sp. on bark.
- 61 Noctuid sp. on earth and dry leaves.

ABBREVIATIONS

c, Ceresa bubalus
me, Melanoplus spp.
n, Noctuid moth sp.

o, Oecanthus quadripunctatus
x, Xyphidium fasciatum



CONTRIBUTIONS TO THE STUDY OF CELL MECHANICS

I. SPIRAL ASTERS

THEOPHILUS S. PAINTER

(Instructor in the Sheffield Scientific School)

From the Osborn Zoological Laboratory, Yale University

SEVEN TEXT FIGURES AND TWO PLATES

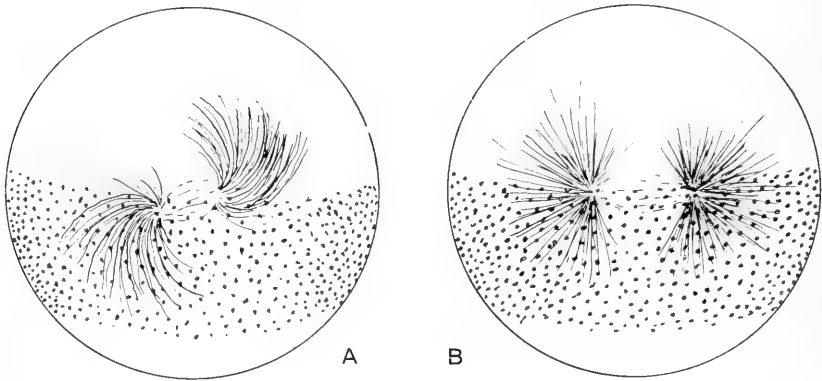
While making an experimental study of sea urchin eggs in Naples, during the spring of 1914, it was the good fortune of the author to observe one case of a 'spiral aster.' The egg in which this was seen had previously been treated with a dilute solution of phenyl urethane shortly after fertilization, and had subsequently been washed free of the narcotic.

Since taking up a cytological study of the sea urchin material, collected at Naples, a number of asters with bent or twisted rays have been observed to occur at a definite period in the development of certain monaster eggs. That these stages are not due to faulty preservation is proved from several considerations. First, the phenomenon has been observed and followed in the living egg. Second, it appears, as we shall see, at definite phases in the development of certain monaster eggs in two separate series of material preserved at different times. Third, one may trace the history of the asters from the time when the first bending appears until it finally disappears.

The facts, in the case of the sea urchin, pointed so clearly to one interpretation only, that I took up a review of the more important papers where spiral asters have been recorded in order to see in how far the explanation arrived at from the study of *Strongylocentrotus* would be applicable to the observations of other workers dealing with widely different forms. The result has been so satisfactory that it seems worth while to describe in detail the phenomena observed in the eggs of *Strongylocen-*

trotus lividus and to show how the interpretation of the facts here throws light on spiral asters; a phase in the history of the centrosome which has been a dark chapter since Mark, in 1881, first described and figures 'spiral asters' for *Limax campestris*.

The monaster eggs, in which the phenomenon has been observed, were produced by shaking violently a few minutes after the fertilization membrane had been formed. Series were preserved at frequent intervals (from 3 to 15 minutes depending



on the stage) either in Boveri's picro-acetic mixture or in sublimate acetic. Sections were cut at 7μ . The stains used were Heidenhain's haematoxylin followed by Lichtgrün.

As previously mentioned the living egg which showed the spiral aster had been treated with phenyl urethane and then washed in pure sea water. When first observed, the majority of the eggs (treated in the same way) were in the 2- and 4-cell stages. This one egg, however, was undivided and judging from the excentric position of the spindle, had passed partially through a monaster cycle. (The treatment of the eggs with this narcotic frequently produces monasters.) Two general centers of radiations were seen, with a very faint spindle between them. The rays were very much bent, as may be seen from figure A, and the spindle itself lay at an angle to the pigment band.¹ As the egg

¹ The eggs of the female upon which this experiment was made showed the pigment ring very clearly. This allowed me to mark the position of the spindle.

was followed under high power, the two asters shifted their positions until the spindle lay in the plane shown in figure B. The bending of the rays soon disappeared and the egg divided.

A short description of the behavior of monaster eggs, produced by shaking, has been given by Boveri ('03), and certain phases of their history has been taken up by the author ('15). From these descriptions, it is clear that while most of the monaster eggs pass through a cycle of changes comparable to the changes going on during normal cleavage, a few shift directly into an amphiaster, a short time after the monaster is formed, and divide. It is from the eggs of this class that spiral asters are derived.

The first sign of the formation of a spiral aster is found in typical monaster eggs at the period when the controls are in the early 2-cell stage. An egg is shown in figure 1 in which a slight bending of the rays may be seen (to the left in the figure). A careful inspection of the figure will show that these few bending rays arise from the edge of the centrosphere, run a trifle forward for perhaps a third of their course, and then bend backwards. In other respects, the egg is a typical monaster with the chromosomes in this case divided forming a half-hemisphere around the aster.

In figure 2 an egg is shown where the bending process has become very pronounced. Here we note that the centers of twisting are localized at two opposite points on the centrosphere. In these regions, the rays arise from the edge of the sphere, run forward for a half of their length, perhaps, and then backwards, that is, opposite to the direction in which the aster seems to be moving. It will be seen, also, that the two centers are bending in the same direction, in this case to the right (clockwise). Those portions of the aster which are not involved in the active movement show the rays bending uniformly backwards. Judging from the position of the chromosomes, the egg is a monaster which has been cut equatorially.² The whole appearance of the figure is what might be expected were there two areas of movement just out-

² This does not refer to the polarity of the egg. In monaster eggs the chromosomes lie on one side of the aster. The plane of section has cut the egg in such a way that we see the aster in the center and the chromosomes on both sides of it.

side of the centrosphere pushing the rays forward in these regions and dragging the whole aster along with it.

Figure 3 shows an egg in which the bending of the rays is still more pronounced. The two areas of twisting are to be made out, less clearly than in figure 2, and a careful inspection shows that the rays in these two regions run forward and then backwards as described for the other cases. This egg is particularly interesting as it shows how the astral rays have been pulled free from their anchorings in the cytoplasm. This egg shows, perhaps, the condition most commonly found.

In figure 4 we see an egg in which the twisting is very clearly localized around two points. The section cuts the aster somewhat obliquely so that only one center shows distinctly. A faint spindle may be seen in the centrosphere connecting the two centers. Again we note that the course of the rays at the two centers of movement is the same as in the other eggs. One further point of interest lies in the position of the chromosomes. It will be noted that in the region of bending two chromosomes have been pulled away from their mates.

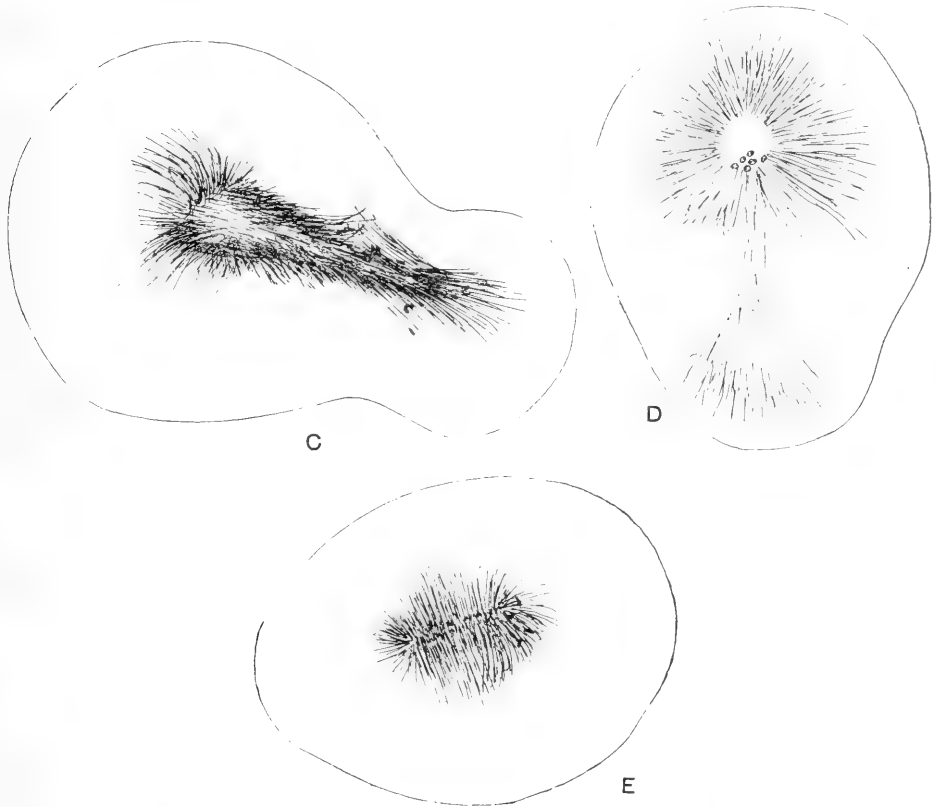
Figure 5 shows an egg in which the two centers have moved far apart. Faint traces of the centrosphere may still be seen connecting them. The chromosomes lie, for the most part, around one center. (This is shown both in this section and in those adjoining it.)

Figures 6 and 7 show later stages of the spiral asters. In figure 6 the chromosomes are scattered, though they lie in the plane of the spindle connecting the two centers. In figure 7, an equatorial plate has been formed. (In the latter case the plane of section is a trifle oblique to that of the spindle.)

In figure 8 we have an egg which appears to be the final stage of the spiral aster. The rays are no longer bent, they appear somewhat finer than those of the preceding figures. The only trace of the bending is seen in the spindle itself. A division of the chromosomes has taken place, though the latter fact is not clear from the figure.

The eggs shown in the figures, with the exception of 3, perhaps, seem to form continuous steps in a process of shifting of the

position of the spindle in the egg. Judging from the large number of cases observed similar to these figures, the usual behavior of spiral aster eggs is that shown. Here and there, however, eggs, which have apparently arisen from those in which the rays were bent, are found which have a different history. Typical



cases are shown in figures C, D, and E. The eggs shown in figures C and D, may be derived, perhaps, from eggs similar to the one shown in figure 5, in which the chromosomes are preponderantly on one side. In this event, a true spindle is not formed, but the one center goes apart from the other dragging with it a few chromosomes, as shown in figure C, and an abortive attempt at division is made. Figure D shows a stage of this. Figure E

shows a type of egg, perhaps some phase in the development of those shown in the foregoing text figures, which is often seen. The centrosphere has collapsed or become greatly flattened and the bending of the rays is seen at one end. What the ultimate fate of these eggs is I am unable to say. At the time the living material was being observed, I was not aware of the occurrence of eggs of this type. Cases were noted here and there in the living material in which the protoplasmic streaming was very severe and it is possible that such eggs had the history shown in figures C and D.

The fate of the spiral aster eggs shown in figures 1, 2, 4, 6, 7, and 8 seems to be a more or less normal cleavage. This conclusion rests on the fact that in the living material those eggs which shifted early from a monaster into an amphiaster divided normally. In one case only have I actually followed the division with high power. That was in the egg treated with phenyl urethane. It might be asked why these monaster eggs dividing early were not followed more closely? It must be admitted that at the time the experiments were being made, the points of greatest interest were centered in those eggs which passed through a complete monaster cycle. In the average experiment, one obtains from 5 to 10 per cent of the egg showing the single aster and of these, perhaps one in twenty shifts early into an amphiaster and divides. These cases were thus so rare that no attempt was made to follow them under higher powers, in order to see the condition of the astral rays.

The stages with the twisted rays are found for a very short time in both series studied. They appear when the controls are shifting from the 2- to the 4-cell stage. No evidence of eggs with bent rays has been found at any other period. That the eggs which show spiral asters come from monaster eggs which shift over into an amphiaster is clear from the following considerations.

When the monaster eggs are first seen in section the centrosphere is round. At a later period one finds the sphere elongated and a careful study of such eggs has shown that a division of the centrioles has taken place. A stage of this is shown in figure 9.

This egg is typical of a number of monasters examined at this time. We see the two centrioles connected by a spindle with new rays arising from the new centers. The chromosomes remain attached to the old fibers which are still very prominent. The centrosphere elongates still more and the new centers can no longer be made out. Up to this time no spiral asters are found in the sectioned material. Following this stage, however, we find the eggs figured in the plates, with the exception, of course, of figure 9.

The figures of the eggs themselves show further that two centers are involved in the spiral aster eggs. Since these stages follow on the division of the centrioles, it can scarcely be doubted that, in the former eggs, the centrioles lie at the two points of the centrosphere where the areas of twisting are greatest, as in figures 2, 3, 4, and 5.

We now have the facts necessary in order to understand what is taking place in the eggs which show spiral asters. In the living egg, owing to the happy circumstance that the pigment ring was prominent, it was possible to see that a shifting of the position of the asters and of the spindle was taking place. From a plane which formed a considerable angle with the pigment band, the spindle shifted until it lay parallel to it. In the preserved material, we find every phase of this shifting; and the majority of the eggs show clearly that two centers are involved, from which we may safely conclude that the division of the centrioles normally precedes the shifting process. There are several phases of the shifting which warrant consideration.

In the foreground stands the question, How is this shifting of the spindle brought about? Do the new centers, the centrosomes, move first, or, are they carried along by the protoplasm lying outside of the centrosphere? The answer to this question is given by a close study of the astral rays. In all of the figures the point has been emphasized that, in the region of the greatest bending, the rays run forward for a part of their length and then backwards. It is clear from the considerations given above that these areas of twisting are associated with the new division centers. Now, were these centers themselves the first points to

move, we should expect that the rays would bend more or less uniformly backwards from these points, certainly, under no circumstances would they bend forward and then backwards. The latter conditions could only come about when some region outside of the centrosphere was the first to move. And, if we may judge from the rays in the spiral aster eggs, the areas of movement are here situated about a third of the ray's length from the centrosphere. It is only by assuming this that we can understand the reason that the rays run forward and then backwards. With two areas in the protoplasm moving, the rays would be pushed forward in these regions and the centrosphere would be dragged along, carried simply by the protoplasm. In the regions where the protoplasm was not so actively moving the rays would bend uniformly backwards. That this is the true state of affairs, a careful examination of the figures will show.

What part the new division centers play in this shifting process, we are unable to say. That the movement is coincident with the division of the centrioles is clear, but whether the one is the cause and the other the effect, or whether they are both the effect of some common cause, is a question upon which the present work throws no light.

A second point of interest lies in the relation between the new and the old rays, for, with the division of the centriole in the monaster we have the formation of new rays, as figure 9 will show. It is in this respect that the conditions in the monaster and in the normal eggs are different. In the normal eggs at the time when the centrioles divide and go apart, the radiations from the old aster have become very faint or have disappeared. In the monaster egg, on the contrary, the centrioles may divide at the time when the radiations from the aster have reached their maximum intensity. It is for this reason, I think, that we find spiral asters in these eggs. Following the division of the centrosome new rays appear. They are concealed from view by the highly developed rays of the old aster. When the new centers shift their positions, the old rays tend to retard the movement and thus we get the peculiar figures described above.

A further question of interest is, what significance has the shifting of the spindle in the monaster eggs? The answer is

given by a consideration of several facts. Boveri ('05) was the first to suggest that the cytoplasm in the sea urchin egg undergoes a series of changes—a sort of development—independent of the nuclear elements, and I reached the conclusion, after studying eggs treated with narcotics that “at the time of fertilization progressive changes, which go on independently of the nucleus and of cleavage, are initiated in the cytoplasm of the eggs and that these changes determine the position of the spindles in the egg and consequently in its blastomeres,” p. 299 (l. c.).

In monaster eggs the division mechanism is delayed through the failure of the centrosome to divide, but the cytoplasmic development is not materially interfered with. This is indicated by the fact that if the aster recovers and divides into an amphia-ster, the spindle tends to take up the same relative position as the normal controls have taken and as a consequence we have the early production of the micromeres as Boveri ('05), and I myself have shown.

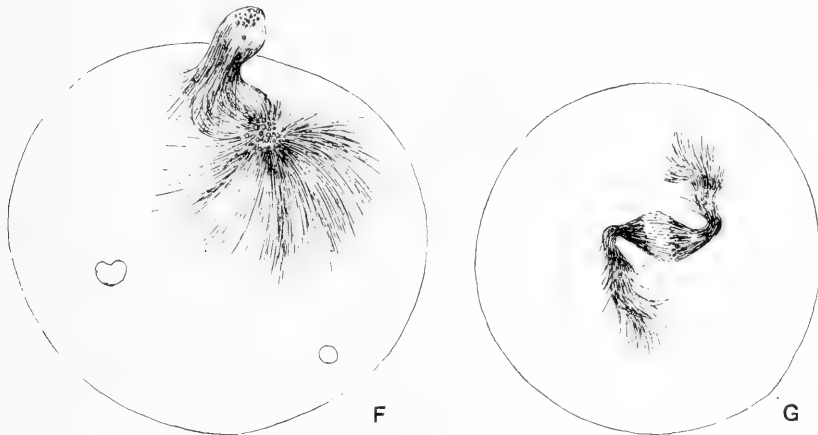
The single aster, during its early history takes up a position at or near the center of the egg. The egg cytoplasm, after fertilization, begins its development which normally, of course, goes hand in hand with certain changes in the nucleus and of the division mechanism. By the production of monasters we are able to separate, for a time, the division mechanism and the cytoplasmic development. When now the division mechanism is released, as through the division of the centriole or the completion of the monaster cycle, the new spindle tends to take up the same position which the control eggs have. Our observations show that the spiral asters arise from monaster eggs which have recovered very early from the effects of the shaking, when the controls are in the 2- and 4-cell stages. From this we should expect that the young spindle will attempt to take up the position which the controls possess, that is, either in the plane of the first or of the second cleavage. This expectation is fulfilled by the single living egg which was observed. The young spindle, resulting from the division of a monaster, lay at an angle with the pigment band, at a time when the controls were in and 2- and 4-cell stages. Boveri ('01) showed that the first two divisions in *Strongylocentrotus*

were meridional, that is, cut the pigment band at right angles. In the living egg the spindle did shift until it occupied the position in which the pigment band would be cut, either in the first or second cleavage plane. That the eggs found in a study of the sections show phases of this shifting process is scarcely to be doubted in the light of all the facts.

A number of authors have recorded the appearance of spiral asters in the eggs of widely separate groups; Nemerteans, Molluscs, Annelids, and in at least one vertebrate, Axolotl (Fick, '93). The phenomenon has been more frequently described for the molluscs, and it seems confined to the gastropods. Mark ('81) was the first to describe and figure spiral asters, in *Limax campestris*. In this form they appeared, somewhat inconstantly, at the time of the second polar body formation. At least four other authors have recorded spiral asters in various snails at this period of the maturation. Kostanecki and Wierzejski ('96) mention it for *Physa*. MacFarland ('96) observed and has given excellent figures of it in *Pleurophyllidia*; Byrnes ('99) describes it for *Limax agrestis*; and Linville ('00) notes its occurrence in *Limax maximus*.

None of these authors have attempted to explain the cause of the spiral aster. Conklin ('02) and ('05), however, has described in detail the movements taking place in the protoplasm of the eggs of *Crepidula*, and the Ascidian *Cynthia* during maturation and cleavage. From the observations of this author it is clear that materials are constantly being shifted from one part of the egg to another in both of these widely separated forms. Kostanecki and Wierzejski have noted that the same was true of *Physa*. Conklin points out, in discussing these movements "that the movements within the cell substance of the unsegmented egg are, in certain cases at least, of a vortical character is indicated by the spiral asters, first described by Mark for *Limax*, and since observed by several other investigators in other animals, and also by my observation that the first cleavage in *Crepidula* is a spiral one, being oblique to the right or dextrotropic" (p. 79, *Karyokinesis and Cytokinesis*).

That the peculiar bent fibers are not caused by the movement of the division center itself, in the case of the gastropods, is shown by the figures of both Mark and MacFarland. I reproduce figure 66 from Mark's paper in figure F. Here it will be noted, first that the egg aster does not lie in a normal plane, but has been shifted to one side. And secondly, that the rays themselves run forward and then backwards. The figures of MacFarland show the same thing for the rays in the eggs of Pleurophyllidia. I have explained how such a course of the fibers, in the case of the sea urchin, is incompatible with the view that the asters themselves move first. It is clear that, as Conklin has shown, the



movements of the protoplasm are responsible for the bending of the rays. The reason that the twisting of the rays has been described so often for gastropods doubtless lies in the fact, that as Linville has pointed out, the egg aster persists for an unusually long time after the second polar body is given off. Byrnes has given figures which show very plainly the persistence of the rays until the pronuclei are uniting.

Coe ('99) in working on the maturation and fertilization of *Cerebratulus* observed many cases where the fibers running from the centrosphere were bent. The most interesting case, perhaps is that shown by the author in figure 37, plate 21, which I have copied in figure G. A glance at this figure will show that

the two division centers are being carried away from the positions which they occupied when the spindle was first formed. In the light of the facts recorded above, the explanation of this condition seems clear. We are dealing here, I believe, with a case of delayed cleavage. The first two cleavage planes, in *Cerebratulus* stand at right angles to each other, just as in the case of the sea urchin. This egg was delayed for some reason in its division, after the spindle was formed, and we see the asters being carried by protoplasmic currents to the new plane where the division of the protoplasm should occur.

Among certain of the leeches, the spiral aster seems to be a normal feature in the development of definite blastomeres and in the polar body formation. Iijima ('81) figures it in eggs before the polar bodies have been given off, but his description is too meagre to allow us to draw any conclusions as to the cause. Later than this, we have the works of Sukatschhoff ('03) on the development of *Nephelis*. This author figures spiral asters as a constant feature in the development of certain blastomeres and advances, tentatively, an explanation for this. After pointing out that we frequently have a shifting of blastomeres during development he suggests that the two cells where he has constantly found the spiral asters rotate on each other. "Nehmen wir nun ferner an,—was a priori nicht ausgeschlossen erscheint—, dass die äusserste plasmatische Schicht der Zelle in sich eine grössere Kohäsion besitzt, als das innere Plasma, so müssten natürlich die Vorher gerade verlaufenden strahlen in der Richtung der Drehung spiralig gedreht werden (p. 333, l.c.).

This author admits, however, that his explanation will not apply to those cases where the spiral asters appear during polar body formation.

A glance at the figures given by Sukatschhoff shows that the rays of the asters run in the same way that I have described for the sea urchin, that is, forward for a part of their length and then backwards. It is scarcely to be doubted that the cause of the bent rays lies in the protoplasmic movement of certain parts of the cell outside of the asters themselves. Since the turning is a constant feature for certain blastomeres, it probably has some

significance and is not due simply to a rearrangement of materials, as Conklin found, for example, in *Crepidula*. It may be possible that it is a reminiscence of a former cleavage which has now been omitted from the ontogeny of *Nephelis*. This explanation does not seem improbable. However this may be, the main point for us is that since the rays run forward and then backwards, the cause of the bending can not lie in the asters themselves but must be in the protoplasm outside of these division areas.

This short review of a few of the papers dealing with spiral asters (I have made no effort to give a complete bibliography) is sufficient to show that the conclusion reached, after a study of the sea urchin egg, is valid for a number of other forms.

There are several points of general interest which should be mentioned here, as our observations throw some light upon them.³

That spindles shift during the development of eggs, especially during polar body formation, has been observed by a great number of workers. It has been generally accepted, that this shifting was brought about by the protoplasm and not by the asters themselves, but this has been founded on indirect evidence only. The observations of the sea urchin egg allow us, I think, to understand the time and the nature of the movement a little more clearly. The conditions in eggs, in which there is a rotation of the spindle of the second polar body, and eggs which shift from a monaster to an amphiaser are similar in this respect, that we have spindles which must shift to take up new positions. Ordinarily, this shifting takes place without any bending of the rays of the young asters in the egg and we have no indication of the cause of the movement. But in monaster eggs, the high development of the old rays allows us to see where the movement begins. An examination of the figures given will show that the areas of movement lie well outside of the centrosphere in the cytoplasm. Were not old fibers present, there would be nothing to

³ I have not touched upon the nature of the fibers in the sea urchin egg, in this paper, though the spiral asters throw much light upon this question. In a work shortly to be published, a complete analysis of monaster eggs will be given. I propose to take up in this the nature of the rays.

indicate that a shifting of the cytoplasm was taking place, and the spindle would probably rotate until the proper plane was reached and then the egg would divide.

In its broader aspect, the present contribution adds just one more bit of evidence to show how very important is the rôle which the cytoplasm plays in development and how very complex it is in its organization. The sea urchin egg, was at first considered as one of the simplest types; it seemed certain that, if it was possible anywhere to reduce certain phases of development to simple mechanical terms, it could be done here. But a mass of experimental data has gone to prove that, in many respects, this apparently simple egg is fully as highly organized as seemingly complex eggs such as the ascidian, for example. The cytoplasm can no longer be looked upon as so much passive material which the nucleus elaborates during development, but it has a complex organization and a development which may be synchronous with certain changes in the nucleus and the division mechanism, and yet is independent of these.

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PLATE 1

EXPLANATION OF FIGURES

All drawings were made under oil immersions with the aid of a camera lucida, by the laboratory artist, Miss Krause.

1. Monaster egg showing the first signs of the bending of the rays.
2. A monaster egg showing that there are two centers involved in the twisting process.
3. A later stage of the same
4. An egg to show the two areas of movement and the faint spindle in the centrosphere.

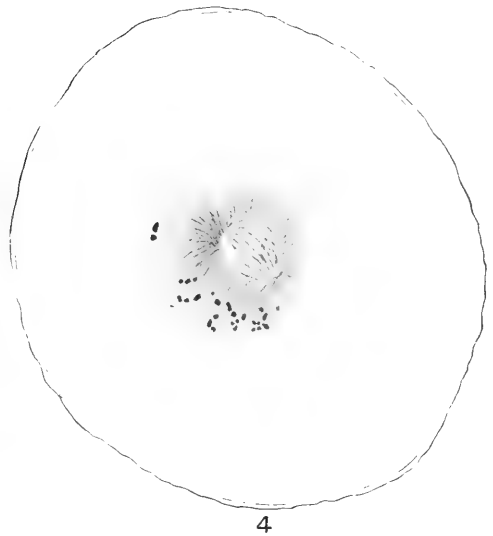
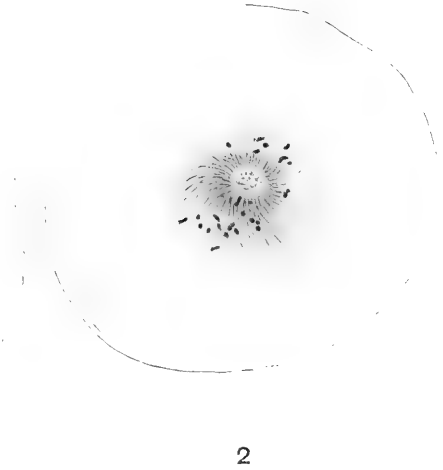
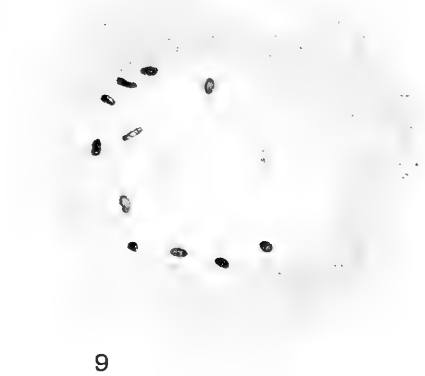
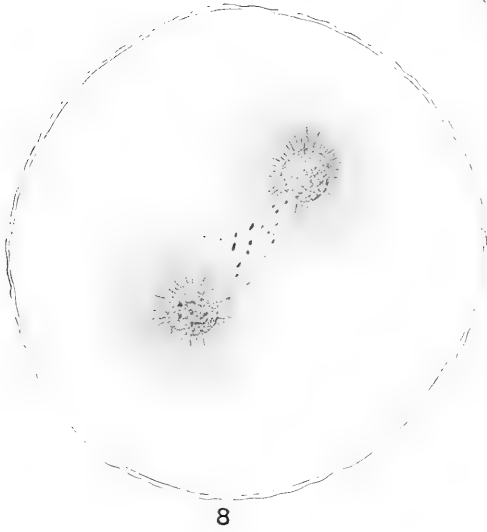
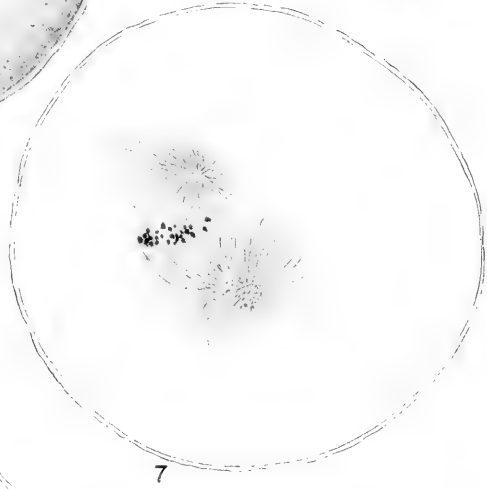
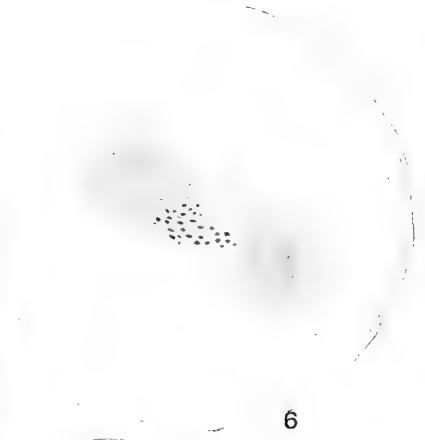


PLATE 2

EXPLANATION OF FIGURES

5. A later stage showing the two points of movement well separated.
 - 6, 7, and 8 show three successive stages in the formation of a new spindle.
 9. Showing the division of the centrioles in the monaster egg.
- Fig. F Showing second polar body for motion in *Limax campestris*. After Mark.
- Fig. G A first cleavage spindle in *Cerebratulus*. After Coe.



ON THE FEEDING HABITS OF AMEBA¹

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SIX PLATES

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¹ The spelling of ameba was adopted after some deliberation; it is not used as a substitute for the word *Amoeba* of scientific nomenclature, as has been done inadvisably, to my mind, by some writers; but it is used as a common name in the same way that the Germans employ *Amöba*, the French *amibe*, and the Italians *ameba*, as common names.

INTRODUCTION

The diet of most of the higher animals includes a large variety of materials. Many of the vertebrates extend or restrict their diets on the basis of past experience; that is to say, they learn that some of the materials which they have been eating are not suitable for food, while some which they have been avoiding may be eaten. The most striking example of the influence of experience on the selection of food is found, of course, in man, who utilizes an almost limitless variety of materials for food purposes. Next to man in this respect comes perhaps the dog, who has learned to eat many of the foods prepared by man. As we pass down the scale of animals, we observe that learning or experience plays a decreasing part in choice of food, and it appears that the sensations received by the organs of smell, taste and touch determine the feeding reactions of the animal more and more. Following out logically this line of thought there is suggested the probability that the unicellular animals, which stand at the bottom of the scale, select their food exclusively by smell or taste or touch, and that learning or previous experience plays no part in selection. It would be expected, therefore, that the problems of choice of food would be reduced to its simplest terms in the protozoa; and it was in the hope of throwing some light on the relation existing between ameba and the materials which it eats, that the experiments recorded in the following pages were carried out.

It is difficult to overestimate the importance of the relation between a free living animal and its food. Every animal must eat. And every animal must either choose its food, eat everything it can get, or eat objects without regard to their chemical and physical qualities. Now it happens that so far as known, all animals choose their food, and the problem of food selection is therefore one of fundamental importance. And yet in spite of its importance, this problem has remained almost wholly uninvestigated. But, even though no extended studies have been made on the relation between animals and the food which they eat, we have in zoological literature a large number of 'notes' on

the feeding habits of animals. These notes, though they are fragmentary and incidental in character, nevertheless lead to certain general conclusions, which point the way for investigation. We may observe, then, from the mass of these fragmentary references, on the one hand, that some of the members of all the great groups of animals have restricted diets; only certain definite substances are eaten. Thus the rhizopod *Vampyrella spirogyrae* 'selects' only spirogyra filaments to feed upon; many insects lay their eggs on certain definite species of plants which serve as a special food for the larvae; frogs eat only moving objects; some birds eat only certain kinds of seeds, others feed only on fish; and so on. In contrast to these animals may be named, on the other hand, those which are omnivorous, representatives of which are likewise found in all the great subdivisions of the animal kingdom. This general comparison is interesting because it illustrates well how variable the basis is upon which food substances are selected. Evidently discrimination cannot be made upon the same basis by all animals. Perhaps, in general, none but the most closely related species agree in having similar bases of food selection, for it is well known that in at least some of the higher forms there are definite individual differences in this respect.

It has been thought worth while to emphasize this fact of diverse bases of food selection among animals, for the impression has become quite general that an animal eats certain substances because they contain certain chemical compounds possessing food value, such as proteins, carbohydrates, or fats. But this is true only if the end results of feeding—digestion—is considered. With the selection of food per se it has nothing to do. For how can a frog in any way be sensible of the proteins in a hard shelled beetle which it snaps up and swallows immediately? Or how can a bird sense the food materials in hard seeds?

With soluble or volatile substances the case is of course different. Meat extractives, some proteins, peptones, some carbohydrates and some fats, may be selected by the senses of taste and smell. If the assumption that there is a specific chemical relation between the substances and the end organ of taste or smell,

is correct, then selection of food could be made in such cases upon a chemical basis. Such (assumed) discrimination is, however, much more restricted in its actual occurrence than might be supposed; for many of the commonest proteins (the albumins) and nearly all the common carbohydrates (the starches) are very nearly or quite tasteless and odorless, whether in the soluble or in the insoluble condition. If animals depended solely, therefore, upon the senses of taste and smell in each case where discrimination occurs, these latter substances would never be eaten, if they existed in the pure form. But since these substances never exist in the pure form, since some tasting or smelling substance is always found associated with them, they find their way into the stomachs of animals because they are supposed by the animal to have tasting qualities which they do not actually possess.

The taste of a mass of food is thus almost invariably made to extend, in the experience of the animal, to every particle of it; while as a matter of fact, the tasting or smelling substance, such as, for example, a meat extractive, or an essential oil, is usually only a very small part of the food mass, and frequently its food value is negligible. In by far the larger number of cases where taste or smell function, an omnivorous animal reacts only to these small quantities of substances which the animal has learned are always associated with substances of real food value.

In contrast to the discrimination in food which an animal as a whole exercises, may be distinguished another form of selection which obtains between the various tissues of an animal and the food substances which come into contact with them. From the same blood stream, as Verworn ('09, p. 175) has pointed out, the muscle cells take certain substances, the cartilage cells others, the ganglion cells still others, and so on. Even among the lowest forms, the protozoa, there is a difference between the reactions of the internal and the external protoplasm with regard to solid objects. Metalnikow ('11) has shown that while a large number of substances are readily eaten by paramecium, some of these are quickly excreted, while others remain for a considerable length of time in the body. Many similar observations are

also recorded in the following pages. This kind of discrimination which is exercised by the external protoplasm may be called, for convenience in referring to it, histonic, in contradistinction to organismal selection, in which the animal as a whole is concerned, and which takes place before the substance is swallowed or rejected. As will appear later, it is very necessary that this distinction be clearly recognized, for so far as known, in no free living omnivorous animal are the two terms synonymous.

These few introductory remarks serve to make clear the problem of choice of food in animals generally; the following pages are presented as a first study of this problem in ameba.

MATERIAL

The Amebas

After a considerable amount of experimental work had been done, it was discovered that the amebas described as *Amoeba proteus* by Leidy ('79) and by Penard ('02) may be separated into two groups as far as their behavior is concerned, and also according to their general morphological aspect, as follows:

The granular type. As the group name implies, these amebas appear more or less densely granular, which appearance is caused by the presence of large numbers of very small ovoid, crystalloid bodies. Pseudopods are usually few in number and cylindrical in shape. The direction of movement is not frequently changed. A 'main' pseudopod may always be distinguished. They readily eat dead organisms and small fragments of isolated proteins and carmine. Small flagellates, such as *chilomonas*, are also frequently eaten; indeed it is not improbable that this organism forms the chief food supply of this type of ameba. Diatoms, desmids, etc., are eaten very seldom. These amebas respond only very slightly, in most cases not at all, to mild mechanical stimulation, such as 'tickling' with a fine glass needle.

Specimens of this type of ameba were obtained at various places around Knoxville. During the summer and autumn, cultures of this form were usually successful. They were made as follows: Six or eight liters of dead leaves, water plants, etc.,

taken carefully from the bottom of a shallow pond where cattails grow, were brought into the laboratory and then thoroughly shaken up with three or four liters of pond water. The water was then decanted and poured into flat-bottomed glass dishes, about twenty centimeters in diameter, to a depth of two or three centimeters. Enough of the dead leaves were placed in each dish so that if they were uniformly spread out and gently pressed down they would form a layer one or two millimeters thick. The cultures were exposed to full north light, but not to direct sunlight. The dishes were covered with glass plates, leaving a small opening for ventilation. Of every dozen cultures set up in this way, at least two or three proved successful.

During the winter and early spring when my own cultures sometimes ran out, and for comparison with my own at other times of the year, granular amebas were obtained from Powers and Powers, Lincoln, Nebraska.

Raptorial amebas. This type of ameba contains only a very few granules which are much larger than those in the granular amebas, and are very irregular in shape. These amebas appear much clearer than those of the granular type. The pseudopods are numerous when moving on a flat surface. They are dorso-ventrally flattened. Frequently there is no 'main' pseudopod, and the direction of movement is, therefore, somewhat uncertain. Pseudopods seem to be formed with ease, and the whole ameba seems to be of a thinner consistency than those of the granular type. Dead and immobile organisms are eaten very seldom. Carmine, globulin, etc., are only rarely eaten. Slowly moving organisms of all sizes, from small flagellates to paramecia and rotifers, and especially diatoms and desmids are the frequent prey of these animals. If these food organisms do not move, frequently no attempt is made to eat them; but if such organisms are mechanically agitated the feeding reaction at once sets in. These amebas are very responsive to mild mechanical stimulation with a glass needle. With careful manipulation they can be led about in any desired direction. They will ingest any small non-poisonous object if it is properly agitated.

If these amebas are disturbed, as when transferring them from the culture vessel to the slide or the watch glass for observation, they sometimes assume a clavate or a spatulate form; but when undisturbed in their culture they are generally of a shape that might best be described as stellate.

These amebas thrive best in cultures where diatoms, desmids, and oscillaria are growing. On account of their peculiar feeding habits, these amebas are referred to in the following pages as raptorial amebas, to distinguish them from the granular type.

The raptorial amebas are easily reared in cultures if slowly decaying leaves only are placed in the culture vessels; but otherwise the cultures may be made up as for the granular type. The chances of success are improved if some diatoms and oscillaria are added.

I have found little difficulty in obtaining amebas from ponds around Knoxville, during every month of the year. Shallow ponds not subject to floods, in which cat-tails grow, may be considered as almost sure to yield amebas if cultures are made as outlined above; or they may be obtained directly if the decanted water is poured into a deep vessel and allowed to settle over night, and the sediment then drawn off with a pipette and placed into a petri dish for examination under the binocular microscope.

The raptorial and the granular types are seldom found together, in numbers. Raptorial amebas have been seldom met with in granular cultures, but a few straggling, sick looking, granular amebas have nearly always been found in the cultures of the raptorial type.

Whether these two types represent two or more species, or only different stages in the life cycle of a single species has not yet been determined. A considerable number of the experiments had been performed before the difference in the behavior between these two types became convincing. But this point is now under investigation and I hope soon to publish a paper which shall clear up this matter.

It is almost needless to say that it is of fundamental importance in physiological studies to know just what animals were used for observation and experiment. It is easy to see that the

conclusions would be different from what they are if all the experiments in this paper, for instance, had been performed on amebas of the granular type, or of the raptorial type, exclusively. Unfortunately, however, it is impossible at this time to give a more accurate description of the amebas I used than is given above.

The needles used in manipulating the test substances were of steel and of glass. Those made of glass were superior to those of steel because of their greater elasticity and resistance to cleaning agents. The glass needles were made by drawing out thin-walled test tubes to a point. The diameter of the needle half a millimeter back from the point was one or two microns. Occasionally needles can be made having thinner points, so thin in fact that they wave about in the water. Such needles were used in the 'tickling' experiments. The most delicate mechanical stimuli could be administered by means of them. The needles were frequently freed from colloidal materials which readily collect on them while working with them in the culture fluid, by immersing them alternately in sulphuric acid and a strong solution of sodium hydroxide, a number of times, and then washing in distilled water and finally in ether. It was assumed that they were sufficiently cleaned in this way to prevent contamination of the test substances.

The microscope used was a long arm Zeiss binocular with a_3 objectives and number 4 eyepieces. The camera lucida drawings were made in the ordinary way.

METHODS

The method of work was very simple. A small quantity of the powdered substance which it was desired to use, was poured out from the container on a clean glass slide lying on the stage. The microscope arm was then swung over it. A particle of the desired size was then selected and transferred with the glass needle to the slide or petri dish containing the ameba whose behavior was to be tested. Frequently some difficulty was encountered in pushing particles through the surface film. But once through the film, the particles were placed wherever they were

wanted without provoking negative reactions on the part of the ameba.

A single ameba was employed for as many experiments as was possible, for in most cases the results of such experiments gain added value on account of the comparisons which may be made. But there is a limit to the value of such procedure in studying feeding habits of ameba since an individual changes its behavior to some extent on account of the food eaten and on account of other previous reactions. Nevertheless, as many as twenty or thirty experiments may be made in succession upon a single specimen under the most favorable conditions.

All the work was done while facing a north window to insure constancy in the direction of light. As will be seen in a later paper, amebas are very sensitive to light, particularly if there are sharp gradations in intensity; and it will be desirable, therefore, to bear this fact in mind while examining the figures. It does not appear, however, that the light had any marked effect in their general movements, for the amebas moved in all directions on the slide, and they changed their direction of movement in almost every conceivable way, just as if the light came from all possible directions with uniform intensity. The explanation of this apparent contradiction regarding the effect of light on ameba lies in the fact, experimentally determined, that amebas readily become accustomed to light of relatively strong intensity; and that they do not react to light of moderate intensity unless sharp gradations of intensity come within sensing range. However desirable it would be to have the light under controlled conditions while testing feeding reactions, the experimental difficulties involved in bringing such conditions about would result in greatly limiting experimental latitude in other respects, for there must always be sufficient light merely to see what is going on. In all the experiments where north light was used I believe we may disregard light as a factor affecting discrimination, as far as the conclusions in this paper are concerned.

Hitherto, however, the effect of continuous daylight has been assumed to play a very important part in ameban behavior. Thus Rhumbler ('98) expressed the opinion that light serves to

retard or prevent the feeding process in ameba, and that the explanation for the small number of observed cases of feeding lies in the probability that ameba feeds mostly during the night.

Es ist eine alte Erfahrung, dass man nur sehr selten die Nahrungsaufnahme der Amöben unter dem Mikroskop zu beobachten Gelegenheit hat. Das ist in so fern merkwürdig, als der umgekehrte Vorgang, die Ausstossung unverdauter Reste aus dem Amöbenkörper gerade sehr häufig beobachtet werden kann. Eine Amöbe kann doch unmöglich mehr Fäkalien nach aussen absetzen, als sie vorher an Nahrung in sich aufgenommen hat. In Gegentheil, sie wird mehr Substanz an Nahrung in sich aufnehmen müssen als sie später wieder ausstösst, weil der Amöbenleib Einiges von der aufgenommenen Substanz zu Wachstum und Ernährung zurückhalten muss. Man sollte als eher erwarten, dass die Nahrungsaufnahme häufiger zur Beobachtung käme als die Defäkation.

Dieses merkwürdige Missverhältniss zwischen dem aprioristisch Wahrscheinlichen und dem thatsächlich Vorhandenen könnte seine Erklärung darin finden, dass die Amöben ihre Nahrung hauptsächlich des Nachts aufnehmen, also zu einer Zeit, in welcher in der Regel nicht beobachtet wird, dass die Defäkation dagegen des Tags über stattfindet und dass sie desshalb bei der meist am Tage stattfinden den Beobachtung leichter und öfter beobachtet werden kann. Dass dieser Unterschied zwischen Nacht und Tag in dem Amöbenleben bis zu einem gewissen Grade vorliegt, halte ich nicht für unwahrscheinlich, principiell, durchgreifend ist er auf keinen Fall.

Hier und da glückt es, auch am Tage die Nahrungsaufnahme zu beobachten; und sehr viel häufiger noch sieht man wenigstens die Amöbe einen vergeblichen Versuch zur Nahrungsaufnahme machen. Eine Alge oder auch ein tochter Fremdkörper, der schon halb von der Amöbe umflossen war, springt plötzlich wieder aus dem umfliessenden Armen der Pseudopodien heraus, um vielleicht mehrmals mit demselben Misserfolg von der Amöbe wieder attackirt zu werden. Es ist wie aus dem Folgenden hervorgehen wird, ganz offenbar das grelle zur Beobachtung nothwendige Licht (Beleuchtungsspiegel), das die Nahrungsaufnahme erschwert, unsicher oder unmöglich macht, und einen ähnlichen hemmenden Einfluss mag auch das Tageslicht auf einige Amöbenarten wenigstens ausüben. Das Tageslicht wird aber nicht so unvermittelt und deshalb auch nicht so störend auf die Thiere einwirken wie das Beobachtungslicht, weil es durch die Dämmerung mit der Nacht verbunden ist, und weil man weiss, dass die lebende Substanz leicht durch allmähliche Übergänge gegen äussere Reize unempfindlicher wird als gegen schnell auftretende äussere Reizveränderungen. Es ist also durchaus nicht gesagt, dass die Amöben in der gewöhnlichen Beleuchtung des Tages in gewohnter Umgebung ebenso selten Nahrung aufnehmen, wie sie das erfahrungsgemäss unter dem Mikroskope thun (Rhumbler, '98, p. 202).²

² Rhumbler seems not to have known of Duncan's paper where feeding was repeatedly observed. See my quotation from Duncan's paper on p. 538.

But shortly after Rhumbler published the paper from which the above extract was taken, Harrington and Leaming ('00) showed that amebas quickly become accustomed to even strong light; that after they had been exposed to strong white or monochromatic light for a few seconds, which causes retardation or cessation of movement, they begin to move normally again as if the light was without further effect. Ameba seems therefore to be affected by light only when its intensity suddenly changes; uniform moderate intensity is without effect in changing behavior.

The explanation of Rhumbler's observations where an *Amoeba verrucosa* ejected a partly ingested alga filament when strong light was turned on lies in the sudden change in light intensity. It is more than likely that no ejection would have occurred if the change had been made very gradually.

Regarding the notion that amebas eat only or chiefly at night, owing to the assumed preventive effect of strong light in the daytime, it may be said that previous observations of ameban behavior have not been protracted enough. This may readily be granted in view of the large number of successful cases of feeding recorded in this paper.

GENERAL DESCRIPTION OF THE FEEDING PROCESS

Before taking up the detailed behavior of ameba toward the various test substances, it may be advisable to insert here a general description of the usual feeding process together with some previous observations by other investigators, in order that the detailed account which is to follow may be clear from the outset.

One of the first students of ameban behavior to describe the feeding habits of this animal was the geologist-naturalist P. M. Duncan, who gives in his *Studies amongst Amoebae* ('77) a very interesting, if anthropomorphic, interpretation of the general behavior of amebas. With regard to their feeding habits, Duncan leaves the impression that food is taken only at the posterior end of the ameba.

The large end ever in advance moves over every obstacle around and under it, but the most tempting food never sinks in or is caught by it (p. 230).

A little watching will show that the spot where things are taken in is close to the small extremity, and that very often one or more pseudopodia are projected there, so as to encircle a diatom or a green animalcule or a piece of alga, which is slowly pressed by them against the Amoeba and then sinks in (p. 230).

The prey becomes environed by a vacuole, or is tumbled about by the endosarc amidst the jumble of things there (p. 230).

. . . . long ridges appear on its surface (p. 230).³
 The pseudopodia closed on the granular mass and brought it to the part where the neck of the head joined the main body, and the prey sank into the endosarc. I have repeatedly seen these Amoebae take in food, and it has always been at this particular spot (p. 230).

. . . . the vesicle is in its very common place, the food entering end (p. 231).

As these young forms were watched, it became evident that occasionally a minute spore or small navicula like diatom coming into contact with the spot where the sticky (hinder) end joined the non-adhering protoplasm of the diaphne, sank into the body, and was soon seen streaming along inside, environed by other prey, and a multitude of granules, granular spheres, and masses of protoplasm (p. 234).

It will be evident from these extracts that Duncan did not observe the essential details of the feeding process. The definite conclusion that food is caught only at the posterior end and not at the anterior end has remained unconfirmed except in a very small number of cases. I am unable to suggest how Duncan might have come to this conclusion.

As far as I have been able to find, Leidy ('79) first described and figured the essential features concerned in the capture of a living organism by an *Amoeba proteus*.

I have had but few opportunities of seeing *Amoeba proteus* capture living animals. In one instance I saw an individual, as represented in figure 5, plate 1, containing, within a large vacuole, an active Infusorian, a Urocentrum, and having a second victim of the same kind included in the fork of a pair of pseudopods, the ends of which were brought into contact, so as to imprison the animalcule within a circle. The latter moved restlessly about within its prison, but after a time became motionless, and shortly after the ends of the pseudopods which enclosed it fused together, as seen at *c* in the figure just indicated. Films of ectosarc extended from the body of the Amoeba towards the fused ends of the pseudopods, and finally the Urocentrum was enclosed

³ The amebas seem to have been of the species described by Penard as *Amoeba nitida*, and of the variety figured by Leidy ('79) in plate 1, figure 7.

in a vacuole like that in the interior of the body of the Amoeba. Having carefully watched the latter for some time, the two vacuoles containing the captured Urocentrums were seen gradually to diminish in size, the contents were reduced to the usual size of ordinary food-balls of the endosarc, and all trace of the previous character of the victims was completely lost (p. 45).

An individual of the kind just described (an *A. proteus*) I had the opportunity of seeing swallow and digest one of another species, the *Amoeba verrucosa*. The steps of the process I have attempted to represent in figures 13–19, plate VII, and they occurred as follows:

In a drop of water squeezed from mud adhering to the roots of the plant *Ludwigia*, collected in a half dried marsh, in the month of August, I noticed an active Amoeba, as seen in figure 13. It was of elongated triangular, snail-like form, with the anterior broader extremity extended into a number of conical antenna-like pseudopods. The posterior end was somewhat coarsely papillose, and from the left side projected two conical pseudopods like those in front. Observing an *Amoeba verrucosa*, figure 12, in its usual sluggish condition, lying almost motionless, directly in the path of the former, I was led to watch whether the two would come into contact and what would be the result.

The *A. proteus* contained a number of large food- and water-vacuoles, together with a single diatom. The contractile vesicle occupied the usual position and exhibited the usual changes. The *A. verrucosa*, besides the granular protoplasm, appeared to contain nothing but a conspicuous contractile vesicle, and this remained unchanged.

The snail-like Amoeba reached the *A. verrucosa*, and turning with tail end towards the right, the body shortened, and a pair of digitate pseudopods extended from the head and embraced the latter in the manner represented in figure 14. The conjoined ends of the pseudopods fused together, and the animal reversed its direction of movement, while the *A. verrucosa* gradually sank deeply within its body, and assumed the appearance of a large sphere, still retaining its contractile vesicle unchanged, as represented in figure 15. The snail-like *A. proteus* assuming nearly the original shape, as first noticed, then moved about after a while and presented the appearance seen in figure 16. The tail end of the body was elongated and papillose, and the swallowed Amoeba, reduced in size, had lost its contractile vesicle and become oval in shape. Later, the *A. proteus* appeared more slug like, while its victim had become pyriform and striate, and was then included within a large water-vacuole, as represented in figure 17. Subsequently, the *A. proteus* was observed to discharge the diatom, previously noticed in the endosarc, from the side of the posterior narrow end; and the *A. verrucosa*, within its now globular water-vacuole, had become bent upon itself, as seen in figure 18. Still later, the body of the *A. verrucosa* appeared to have become broken up into five spherical, granular balls, as seen in figure 19, which rolled about among the other constituents of the endosarc of the *A. proteus*. These observations

were conducted through about seven hours. What finally became of the five balls resulting from the destruction of the *A. verrucosa* I did not ascertain, but supposed that they were digested, to contribute to the nutrition of the *Amoeba proteus* (p. 49).

But with regard to the location on the body at which amebas take food, Leidy was, curiously enough, led to agree with Duncan, but from observations made upon another genus.

Mr. Duncan, in a recent publication (*Popular Science Review*, 1877, p. 217), intimates that the usual position in which the *Amoebae* take their food is at the posterior extremity of the body, which I am inclined to think, from some later observations I have made on the allied genus *Dinamoeba*, is correct (p. 45).

Since Leidy's studies on rhizopods, little or no work was done on the feeding habits of ameba until Rhumbler ('98) attempted to explain their feeding processes, as well as their movements, on the basis of physical and mechanical laws.

Rhumbler described in detail three ways in which solid particles are taken into the interior. In a later paper (Rhumbler, '10), these three methods of feeding are conveniently summarized as follows:

1) Nahrungsaufnahme durch "Import." Hierbei rückt der Nahrungskörper in den Plasmaleib der Amöbe hinein, nachdem er mit deren Oberfläche in Kontakt gebracht worden ist, ohne dass die Amöbe dabei selbst irgendwelche nennenswerte Bewegungen auszuführen braucht, aber auch ohne dass ihr solche an sich beliebige Bewegungen verwehrt wären.

2) Nahrungsaufnahme durch Umfließung ("Circumfluenz"). Auch hierbei wird der aufzunehmende Körper von dem Amöbenkörper direkt berührt; das Protoplasma fließt aber um den Nahrungskörper in enger Anschmiegung herum, indem es sich auf dessen Oberfläche ausbreitet, was selbstverständlich eine entsprechende Gestaltveränderung der Amöbe zur Folge hat, eine Gestaltveränderung aber, die der Amöbe von dem Fremdkörper aus induziert ist; sie verläuft in direkter Abhängigkeit von der Oberflächenform des Fremdkörpers, richtet sich also ganz nach ihr, ohne dass die Amöbe selbst den Verlauf der die Circumfluenz vermittelnden Strömungsrichtungen zu determinieren braucht.

3) Die dritte Art, das "Einfangen der Nahrungskörper" oder auch, wie ich sie benennen möchte, die "Circumvallation," ist besonders merkwürdig, da sie ganz den Eindruck einer berechnenden Handlung-

sweise der Amöbe hervorzurufen geeignet ist. Das Amöbenplasma schiebt nämlich an beiden Seiten der Beute vorbei Pseudopodien, die sich jenseits der Beute miteinander vereinigen, nach ihrer Verschmelzung einen vollständigen Wall um sie herum bilden, und sich bald darauf auch auf der Ober- und Unterseite der Amöbe zusammenschliessen, so dass die Beute vollständig eingekerkert wird, ohne dass das Plasma selbst bis dahin mit ihr irgendwo in direkten Kontakt gekommen zu sein braucht (Fig. 1). Soweit es sich hierbei umlebende Beutekörper handelt, sieht diese Art des Nahrungserwerbes einer Überlistung der Beute zum Verwechseln ähnlich. Die direkte Berührung der Beute scheint vermieden zu werden, um sie nicht wegzuschrecken, wenn sie dann allwärts umstellt ist, vermag sie nicht mehr den verdauenden Einwirkungen ihres Kerkers zu entrinnen (p. 194).

In 1904 Jennings published an extensive paper on the general behavior of ameba, in which he showed that differences in surface tension and the action of other mechanical principles do not explain satisfactorily some of the more important features of ameban behavior. Several interesting observations on feeding are also recorded. Some of these will be discussed further on (pp. 563, 566).

Kepner, Taliaferro et al. ('13) recorded some interesting variations of food cup formation in amebas. These observers concluded that amebas react by the method of trial and error and that "in each reaction there is evidence of purposiveness" (p. 421). This point will be discussed in a later paper.

Character and formation of food cups

The larger amount of feeding in the granular amebas, and practically all the feeding of the raptorial, is accomplished by enveloping the food object in a cup of protoplasm which does not come into contact with the food object until the cup is practically completed. Such a cup is not formed except over food, or in the vicinity of objects which possess qualities that have become associated by the ameba with food. The term 'food cup' is therefore employed to designate this extemporized structure by means of which the ameba eats solid substances.

As observed under the microscope, a food cup is formed in several ways, depending upon the character, size, and movement of

the food object; upon its nearness to the ameba; upon what part of the ameba—posterior or anterior—is near the object; upon the 'condition' of the ameba, that is, its state of hunger, its just previous feeding experiences, etc. As described by several observers, two pseudopods are sometimes sent out toward the food object, one on either side, and not touching it. As these encircle the food, a thin sheet of protoplasm arising from the base and dorsal sides of the pseudopods, is thrown out over the object. The pseudopods and the sheet of protoplasm then fuse all around, forming an inverted cup of protoplasm over the surface of the glass dish or slide, with the food object in the centre. A food cup is frequently formed in this manner over an active organism such as a urocentrum, paramecium, coleps, chilomonas, etc., if such an organism lies fifty microns or less from the ameba.

The two encircling pseudopods are sometimes omitted and only a broad thin sheet thrown out over the prey, forming finally a cup of the same appearance as when the encircling pseudopods are also present. Food cups of this character may be formed at any part of the ameba. Such a food cup may involve the whole ameba, or only the tip of a small pseudopod. In the latter case the main stream of protoplasm is not affected visibly, and the cup may be regarded therefore as a side issue. In nearly all cases the food cups are formed at the anterior part of the ameba for the simple reason that edible prey is encountered here first. Both raptorial and granular amebas form food cups of this kind.

Food cups formed in this way may be of enormous size among the raptorial amebas, if the stimulating object is large, and a number of pseudopods—as many as five or more—may coalesce to form a thin sheet which is sent out over the object.

Food cups formed in any of these ways are usually bounded on the under side by the glass bottom of the object holder. When this is the case the ectoplasm of the rim of the cup adheres to the glass firmly. The captured organism becomes more or less inactive while the glass is still bounding the food vacuole, as may be shown by breaking the ameba loose sometime after the food cup is formed. This might indicate that digestive sub-

stances are poured into the vacuole before it is quite surrounded by protoplasm.

Most of the cases of feeding described in this paper were accomplished through food cups that were formed against the glass surface of the object holder. But food cups may readily be formed free, out of contact with any solid object excepting the food object. In a case where a small flagellate became entangled in the upper surface of the ectoplasm a circular ridge of protoplasm appeared with the flagellate in the center. The ridge was extended upwards until well above the flagellate, when it gradually closed in upon the flagellate and a considerable quantity of water. It is possible also to produce such a free food cup experimentally, by stimulating a part of the ameba mechanically with a very fine glass needle.

Size of food cups

The size of food cups varies very greatly among the different types of amebas. Among the raptorial forms the maximum limit is determined only by the amount of protoplasm in the ameba. In fact, in numerous instances, a whole ameba may be engaged in the starting of a food cup which, to complete, of the size projected, would take the protoplasm of several amebas. The minimum size of a food cup among raptorial amebas is just large enough to take in a small diatom.

With the granular amebas the size of the food cups is less variable. They very seldom involve more than three-fourths of the protoplasm, and even then the walls of the cup are thicker than in the raptorial form. It is difficult to say what the minimum size of a food cup is. A small organism like the flagellate *Chilomonas* is ingested in a food cup nearly always large enough to accommodate easily the ciliate *Coleps*. But particles of isolated proteins are frequently ingested in food cups scarcely larger than the particle itself.

The size of the food cups in all amebas roughly corresponds to the size of the stimulating object. Especially is this the case if only moving organisms, or if the various kinds of lifeless food,

are considered. The relation between the size of the food cup and that of the object is greatly influenced by the condition of the ameba, such as hunger, rate of reaction, etc. The higher the rate of reaction and the greater the hunger, the larger, within limits, will be the food cups.

Number of food cups

In the granular type usually only one food cup is observed at a time but occasionally, when food is plentiful, two or even three may be started at exactly the same time. Whenever a food cup is formed the whole ameba is apparently affected even though the food cup is only a small one, for the main stream of protoplasm is interrupted for a longer or a shorter time.

The number of food cups which a raptorial ameba may form is apparently limited only by the amount of protoplasm. Three may be formed side by side simultaneously or nearly so. Three or four are frequently observed starting at the same time. Sometimes two or more cups coalesce forming a larger one in which the stimulating object or objects are finally ingested. The success or failure of a food cup has no influence on the formation of new cups either in the same vicinity or elsewhere, if the ameba is hungry.

Modifications in the formation of food cups

The range of variation in the shape, size, and mode of formation, of food cups is very great. Indeed it can almost be said that no two are alike in all respects. When it is remembered that among other factors the character of the food object, the condition of hunger, the rate of reaction, the distance between the object and the ameba, the part of the ameba stimulated, the movement of the food object, and the previous experience of the ameba, have each their effect in modifying food cup formation, it is evident that the same constellation of conditions seldom occurs twice. The process is therefore not a stereotyped one, but is adjustable to meet varying conditions.

As a rule food cups continue forming as long as the stimulus acts; when the stimulus ceases to act the food cup is withdrawn. A temporarily immobile *Phacus triqueter* was agitated with a glass needle near a raptorial ameba. A food cup was at once formed about it, but when the needle was removed and agitation ceased, the food cup was retracted slowly, although it was about three-fourths completed. This experiment was repeated twice with the same result. It frequently happens that when a food cup is partly formed over an organism, the organism suddenly escapes. In such cases the formation of the food cup is arrested. But in two or three cases where a raptorial ameba was 'tickled' with *oscillaria* threads and a food cup formed and nearly closed, the alga threads were then quickly removed and the food cup closed up completely.

On the other hand, even though the stimulus remains exactly the same, food cups formed over the inciting object are occasionally not completed; they may be arrested at almost any stage. In these instances a period of quiet of a few seconds usually precedes the withdrawal of the partly formed food cup.

The amount of water enclosed in a food cup with the food object varies greatly. The type and condition of the ameba and the nature of the stimulating object seem to be the essential factors concerned. In general raptorial amebas include more water in the food cups than the granular amebas; hungry amebas and those not having eaten for some time form larger food cups over objects of the same size than those replete with food; actively moving objects, such as organisms, and mechanically agitated particles are eaten with more water than quiet objects. Some objects such as isolated proteins are sometimes ingested apparently without any water; sometimes with just a slight amount of water. On other occasions again, the same kinds of objects are ingested in food cups with larger amounts of water.

All the instances of feeding that have been observed can be arranged in a uniformly graded series, at one end of which would be placed those cases of feeding where very little water is taken in, and at the other end those in which a large amount of water is ingested with the food. In those cases which stand at the middle

of the series moderate quantities of water are taken in with the food object and the protoplasm flows around it at a greater distance and touches it at perhaps only one or two points. Those cases which come at the end of the series where the formation of food cups finds its highest expression, are characterized by the taking in of a very large quantity of water with the food, and the almost complete formation of the food cup before the particle of food is touched. Now the same object may cause feeding reactions falling anywhere in the series as above described. A food cup may be formed touching the object at many points and containing a minimal quantity of water; or a food cup may be formed at a considerable distance from the object and containing a large amount of water. These differences in the size of food cups as compared with the size of the object are not always referable to the object around which the food cup is formed but in some cases exclusively to the ameba, that is to say, to its degree of hunger. There is therefore no essential difference to be observed between Rhumbler's terms 'circumfluenz' and 'circumvallation' excepting such as is brought about by a varying degree of hunger; and the exact form of the circumfluenz is not any more directly conditioned by the shape of the object than is the shape of one's hand conditioned by the pen in writing. In other words, there is no relation whatever between the shape of the object and that of the food cup which encloses it. For these reasons I prefer the one term, food cup, in describing feeding reactions.

No clear case of ingestion by the 'import' method, which is characteristic of amebas encased in pellicles, has come under my observation (see Rhumbler, '10, p. 194).

When it was said above that in some cases of feeding no water seems to be taken in with the food object, it was said designedly. Several amebas that had apparently not taken in any water with the food were examined under a compound microscope immediately after eating, and in each case a thin film of clear liquid, which may have been water taken in with the food, was seen surrounding the food. This is worth noting, as it throws doubt on whether any food is ever ingested without any water whatever.

The food vacuole

After the food cup closes up completely it is known as a food vacuole. If the food particle is an active organism the food vacuole ordinarily remains undiminished or decreases but very slowly in size until the organism stops moving. At this time or soon thereafter, the water rapidly disappears while small irregular projections are thrown out from the inside wall of the vacuole toward the food. This process may be regarded as a change of the ectoplasm lining the vacuole, to endoplasm, which now fills up most of the space occupied by the water, according to the suggestion first advanced by Wallich ('63). The time required by amebas to kill living prey varies greatly. A hungry ameba kills a *chilomonas* in a minute or less and a *coleps* in two to five minutes while a very granular ameba was observed to contain a live flagellate about six microns in diameter in a vacuole for over an hour. I suspect that this ameba was sick, and that its digestive apparatus was disordered. If there is no solid object in the vacuole the water may remain much longer, an hour or more, without any marked diminution in size of the vacuole.

When the water of a food vacuole containing solid food disappears, the food particle is usually carried near the posterior part of the animal, especially if the object is ten microns or more in diameter. After some time, depending perhaps upon its nature, the food particle may be carried along by the main stream of protoplasm. Foods which are only slowly broken up by the digestive powers of the ameba, such as globulin, grain gluten, etc., may be carried for three or four days. A fragment of *aelosoma* meat may likewise be carried for several days, undergoing the while gradual reduction in size.

On the other hand, diatoms and desmids have been observed to be thrown out occasionally, a few hours after eating, without apparent reference to the degree of digestion. If the ingested substances are indigestible, such as carmine or glass, they are usually thrown out within a few minutes after they are taken in; but in a few cases carmine was carried around for over four hours.

Comparatively few observations of this kind have been made for the reason that they did not seem to throw much light on the main problem of feeding; but sufficient attention was paid to the way in which food is handled inside of the ameba to see that it depends at least as much upon the condition and nature of the ameba as upon the character of the food object.

From this brief description of the usual feeding habits of the ameba, it is evident that very great variation is to be expected, that eating is not a stereotyped process, and that no safe conclusions can be drawn from the observation of a few isolated cases. The recording of a large number of experiments seems to be one of the best methods of arriving at the truth in cases where the coefficient of variation is high.

We shall now discuss in some detail one or two typical illustrations of the behavior of amebas toward each test substance.

EXPERIMENTAL RESULTS

Reactions of ameba to whole organisms and to parts of organisms

Ameba is essentially a beast of prey. By far the largest portion of its food consists of living moving organisms, both plant and animal. In a general way it may be said that a hungry ameba eats any organism it can get hold of. Protozoa of any size ranging from a small flagellate such as chilomonas, to a paramecium; rotifers; small entomostraca; diatoms and desmids, etc.; all are eaten providing only that they move slowly enough to allow of the formation of a food cup over them. Observations bearing upon the variety of organisms eaten by ameba have been made and recorded by practically all investigators of this animal, but especially numerous are such observations in the writings of Leidy ('79), Penard ('02), and Gibbs and Dellinger ('08).

Notwithstanding the wide range of diet, small flagellates such as chilomonas appear to form the larger part of the food of granular amebas; while diatoms and desmids constitute the greater part of the food of raptorial amebas. This does not indicate a preference for these particular kinds of food; it may be that these

organisms, flagellates and diatoms, are generally more numerous and therefore easier to obtain, than other classes of protozoa and protophyta.

As far as my observations go, it happens very seldom that one ameba attempts to eat another when two come near each other. Nor do the small amebas of the *radiosa* type call forth the feeding reaction except in very few cases. Other food: coleps, *chilomonas*, *spathidium*, etc., is taken readily while ameban forms are refused. If however an ameba is cut into several pieces, and a fragment placed in the path of an ameba, the feeding reaction is frequently started, but very seldom is the fragment ingested unless it is agitated by means of a glass needle.

Another organism that is much less readily eaten than might be expected, is the motile *Euglena viridis*. In a number of cases these were passed by without any attempt at ingestion, although their rate of movement was slow enough to permit of the formation of a food cup over them. In one instance a euglena was ingested by a raptorial ameba but the euglena could not be kept inside of the ameba's body. After the food cup was closed up the euglena made its way into the endoplasm which was quite thoroughly churned up by the vigorous movements of the euglena as it moved from one part of the ameba to another. The ameba nevertheless kept on moving forward slowly, apparently not much disturbed by the wriggings of the euglena within its body. The ameba's digestive juices did not seem to have any effect on the euglena except perhaps to stimulate it to greater activity. Just fifteen minutes after ingestion the ectoplasm broke and the euglena wriggled out of the ameba as an earthworm might wriggle out of a lump of soft clay. The water taken in with the euglena disappeared in this case, in less than a minute.

From the general acceptance of chemotaxis as the most important factor in the general movement and feeding reactions of the lower organisms, it might be assumed that a quiet organism would be eaten as readily as a moving one, but this is not the case. I shall therefore speak first of

Reactions to moving organisms

When a hungry ameba comes within 100 microns or less of an 'anchored' flagellate or a creeping diatom, the first visible reaction toward the moving object will be a more rapid streaming of the protoplasm in the stimulated region, providing the object is straight ahead of the pseudopod; but if the object lies to the side, there occurs a sudden slowing of streaming followed by the projection of a side pseudopod toward the food organism. The pseudopod flows rapidly forward until almost in contact with the prey, then it stops and forks, the two prongs advancing at the same rate and encircling the prey. Meantime, the covering sheet of protoplasm is sent out from the bases and dorsal sides of the prongs. The encircling pseudopods and the covering sheet of protoplasm then fuse all around, and the ameba attaches itself closely to the substrate. If stimulation is intense, the whole mass of the ameba travels toward the food cup. Usually a few seconds after the complete closure of the food cup the captured organism becomes very active in its movements, and in darting about comes, usually for the first time, into physical contact with the ameba. If the main stream of protoplasm has not thus far been directed toward the captured prey, it is turned in this direction now, especially if the organism is of some size. The ameba usually remains almost motionless for a very variable length of time. In from one to sixty minutes or longer, depending upon the character of the object and upon the condition of the ameba, the captured prey becomes quiet, and very soon thereafter the water in the food vacuole begins to disappear. While the water is being absorbed the inside wall of the food cup sends out irregular projections toward the prey, which seem to be rough and granular on their edges. If the prey is an animal not having a hard exoskeleton, such as a spathidium or a chilomonas, it becomes spherical at about this stage, and is carried along by the protoplasmic stream. Locomotion is usually resumed when the prey ceases its active movements, but there is very great variation in this matter.

The behavior toward moving organisms varies greatly. For instance, the pseudopod may be transformed into a covering sheet only and then thrown over the organism. Very slight movements on the part of the food organism, a mild state of hunger on the part of the ameba, or stimulation on the side of the pseudopod, is likely to produce a food cup of this sort. Under such conditions eating seems to be a side issue, for the main stream is frequently not interrupted or changed in its direction of flow. Sometimes a food organism is excreted undigested thirty or more minutes after ingestion. This is especially true of large objects such as desmids, when there is already a large amount of undigested food in the body. The state of hunger is therefore not directly determined by the digestive abilities of an ameba at any given time.

A small organism like *chilomonas* is apparently as acceptable a food object as can be offered to an ameba, for it has been frequently noted that when globulin or grain gluten or egg albumen, or other substance, was entirely and repeatedly disregarded, a *chilomonas* would be ingested at once.

Reactions to dead and to motionless organisms and to parts of organisms

A motionless organism whether living or dead does not usually call forth a food cup before the ameba has come into physical contact with it. This behavior is in strong contrast to that observed when a living organism is eaten, where, as has been described, a food cup is nearly always formed before the ameba has come into contact with the prey. With the granular amebas ingestion of immobile organisms or parts of organisms may be frequently observed. Pieces of crabs or of worms are frequently eaten. In many cases, however, they are avoided or left behind when coming into contact with them the first time; but if the pieces are shifted so as to lie again in the path of the ameba, they may be eaten.

Just what takes place in the ameba in such a case between the first and second tests cannot be stated. When a higher animal

behaves in this way it is commonly said that the appetite becomes more intense upon repeated stimulation, and finally becomes strong enough to induce eating. As far as this may be translated into physiological terms, the statement also applies to ameba; that is, that repeated stimulation is frequently necessary to induce an ameba to eat. This is not merely an interpretation of what takes place but a plain statement of observation. The ameba has been changed in some way so that at the second stimulation the food object, refused at first, is eaten. The only demonstration of this invisible change that is at present possible is the result following a second stimulation.

The reactions toward motionless organisms and toward parts of organisms, may be readily observed only among granular amebas. Those of the raptorial type have not with certainty been seen to eat an entirely motionless organism; but judging from their behavior toward carmine, it seems likely that they may occasionally do so. One may observe a raptorial ameba move over motionless diatoms again and again without any reaction toward them, but if the ameba comes near an actively moving diatom, it is usually ingested. A number of similar observations have been made. Inactive living diatoms and also a *Phacus triqueter* in the same condition, were very slightly agitated with a very fine glass needle, with the result that a food cup was formed over the agitated organism but was arrested in its further development when agitation ceased. In the case of the phacus, agitation was continued until the food cup was completed almost down to the surface of the glass. Further agitation was of course impossible and the food cup, in consequence, was developed no further. The sides of the food cup did not become attached to the glass, nor was a layer of protoplasm thrown out under the phacus. The ameba finally moved on over the phacus without further reaction.

It is evident from these experiments and observations that a food particle, if not in motion, is not a sufficient stimulus in many cases, among the raptorial amebas at least, to bring about the feeding reaction; and that if a moving particle of food, partly enclosed in a food cup, then stops moving, the feeding process is

interrupted. As far then as the raptorial amebas are concerned, movement is by far the most important quality in food organisms. With those of the granular type, movement of food objects is not so important, but even here it is probably more important than any other single quality.

Reactions to very slightly soluble substances

This group of test substances includes carmine, well dried old egg white, india ink, and uric acid. The behavior of ameba toward these substances is taken up first for the reason that carmine, the chief representative of this group has been used more widely in experiments upon one-celled animals than any other substance. Carmine is indeed the classic substance for this purpose, having been used by Count v. Gleichen Russwurm in feeding protozoa as far back as 1777.

Carmine. The carmine used in these experiments was Grüber's Carmin rubr. II. In some cases it was used pure; in other cases it was mixed with a slight amount of egg white (as it comes from the hen's egg) and dried, to prevent the particles from falling apart when moved around in the water. The mixtures of carmine and egg white were of several grades. Grade 2 was made by adding just enough egg white to the carmine to form a very thick paste. Grade 4 was made by mixing one part of carmine with five of egg white. These mixtures were thoroughly dried with heat but without coagulating the egg white.

As thus made up the carmine-egg white mixture is a complex substance, and is somewhat objectionable as a test substance on this account; for if a change in behavior is brought about by its use, it is not certain just what part of the substance is responsible for the change. But in this case it happens that the objection carries little weight, for in a number of instances pure carmine was employed and the reactions were very similar to those where the mixture was employed. Pure carmine was used at the beginning of the investigation of the feeding habits of the ameba before the camera lucida was affixed to the microscope, but an examination of the laboratory notes shows that pure car-

mine produces about the same reactions as the mixture of carmine and egg white. Pure carmine is ingested readily, although after a few minutes' sojourn in the ameba's body, it is excreted.

Pure carmine is usually regarded as a waste substance as far as its nutritive value is concerned. But there are no studies on record that show this as far as I have been able to find. Chemical analyses have been made (Liebermann, *Jour. Soc. Dyers*, 1885, I, 269) from which it appears that some free tyrosin is probably present in some grades of carmine, but Millon's and Piria's tests for tyrosin did not show its presence in the carmine I used. When carmine is thoroughly shaken up with tap water, examination of the filtrate showed the presence of a considerable quantity of some organic acid and some salt of aluminium.

One of the properties of carmine which doubtless affects the reactions of ameba is solubility. If some carmine is placed in clean tap water enough of it will go into solution in a few days to color the water a very decided crimson. Such a solution of carmine does not seem to have a deleterious effect on ciliates or rhizopods, if these are immersed in the solution for days and weeks, as numerous observers have testified. Nor does the continual eating of carmine for days and weeks appreciably injure paramecium or stentor (Metalnikow; '07, Schaeffer, '10). The short stay of carmine in the ameba's body cannot be due, therefore, to the injurious action of the carmine.

As stated above, carmine is eaten readily whether in the pure form or mixed with a slight amount of egg white. But it is eaten readily only by the granular amebas; the raptorial forms eat carmine relatively very seldom, and then not with readiness.

Let us now take up the reactions of a granular ameba toward carmine as portrayed in figures 1 to 152. A grain of carmine of grade 2 was placed to the right of the ameba's path—1. Although the ameba moved forward in a straight path, the tip of the ameba turned directly toward the carmine grain. The protoplasmic stream then forked, one stream resuming movement straight ahead, while the other moved toward the carmine until it came into contact with it. All the protoplasm then began streaming toward the test object. A food cup was formed by

the mere spreading out of the newly formed pseudopod, and the carmine was then speedily ingested with a considerable amount of water. In about a minute the water was absorbed, and the ameba flowed off in the opposite direction. Three minutes later the carmine was excreted.

One of the most interesting features of this behavior, not hitherto described, is the sensing of the carmine at a distance and the movement toward it. The explanation of the reaction at a distance that at once suggests itself is based, of course, upon the diffusion of the dissolving carmine. Another item of behavior that calls for an explanation is this: Why did the main pseudopod resume forward movement after turning toward the carmine, and after the pseudopod was projected toward it, but was retracted after the ameba came into contact with the carmine? Still another feature of behavior should be noticed at this time, for it will recur again and again. Why should the ameba reverse its direction of movement after eating the carmine? Why did it not move on in the original direction? The direction of movement after eating an object as compared with the previous direction of movement, is not a matter of chance but is fairly well resolvable into two or three factors. If an object is eaten which seems not to be wanted after it has been eaten (which may be inferred from its hasty excretion) the ameba is liable to move away in such a direction as will bring the object soonest near the new posterior end. This experiment also brings out strikingly the contrast between the evacuation of the carmine by the ameba before ingestion and after it has come into contact with the internal protoplasm; or between reactions of ectoplasm and endoplasm to the same object; or between organismal and histonic selection of food. It is at present impossible to state the case satisfactorily because not all the factors determining 'selection' are known. But it seems that an ameba sometimes eats what it does not want.

About eleven minutes after excreting the grain of carmine as above described, another grain of carmine of grade 4 (containing a considerable amount of egg white) was placed slightly to the right of the ameba's path—8. The ameba moved into contact

with the carmine-egg white and then passed on without any indication of a feeding reaction, and this in spite of the fact that there was present much more egg white in this particle than in the previous one.

The carmine particle that had been eaten and excreted was again laid in the path of the ameba—13. The ameba moved on past in contact with the carmine without a sign of a feeding reaction. Why should the same particle not be eaten the second time? Had something been taken out of the particle or had something been added to it? Or had the ameba been changed by its first experience with the carmine in some way?

The same grain of carmine was again laid to the right of the tip of the main pseudopod—16. There was sent out a pseudopod on the right into contact with the carmine, showing that the test object was sensed at a distance. The carmine grain was rolled around in various directions for four minutes, when the ameba moved off leaving the carmine behind. There was at no time any indication of the beginning of a feeding reaction. The partial surrounding of the carmine just before it was left behind—25, 26—was incidental, for the pseudopod behind the carmine was being rapidly retracted.

Four minutes later the carmine grain was laid to the left of the ameba's path—28. The ameba moved on straight forward without a visible change of reaction until it was one-fourth past the carmine. Then a pseudopod was thrown out directly toward, and into contact with, the carmine grain. The side pseudopod remained in contact with the carmine until the ameba had passed by. There was no attempt to roll the carmine around. The main direction of movement was not even changed. Why should there be such a change of reaction toward the same carmine grain? When first presented it was speedily ingested. When next presented—13—it was passed by without reaction. On the third trial the carmine was rolled around for several minutes. On the fourth trial a pseudopod was thrown out into contact with the carmine, only to be retracted later as the ameba continued in its straight path forward. It is clear that the change does not lie altogether within the ameba nor entirely in the carmine, and it is equally

certain that the change is not due to fatigue either of the sensing mechanism or of the eating mechanism. The difference in the behavior cannot be due only to the difference between the stimuli in these experiments.

But observe this ameba's further behavior. A piece of well dried and nearly insoluble egg white was definitely avoided by the ameba after having come into contact with it—30. A piece of debris was also avoided, but before coming into contact with it—32. But when a fresh grain of carmine of grade 2 was laid to the left of the ameba's path, it was promptly ingested—35. The ameba became relatively quiet for about three minutes, then it moved off in the original direction, excreting the carmine fifteen minutes after ingestion. The grain of carmine seems to have been regarded as possessing food qualities in a higher degree than the first grain eaten; for the water in the food vacuole persisted for a longer time; a period of rest followed ingestion; the ameba moved off in its previous direction; and the carmine remained five times as long in the ameba's body.

Two minutes after it was excreted, the carmine grain was laid directly in front of the ameba—44. The carmine grain was rolled around for four minutes and then left behind without any attempt at ingestion. When laid in the ameba's path again—57—side pseudopods were sent out under the carmine, but the ameba did not change its direction of movement until the carmine lay at the posterior end of the ameba. Then the tip of the ameba broke up into several pseudopods, the one on the left becoming the main one through which the ameba moved away. The ameba again reacted less strongly in each of the successive tests with this grain of carmine.

A new grain of carmine of grade 2, laid on the ameba's right, was again promptly ingested—64. Six minutes after eating it the carmine was egested, the ameba flowing off without a period of rest intervening, 65° to the left, so that the carmine at once occupied a position near the posterior end. Compared with the first experiment where the direction of flow was reversed after ingestion, it may be noted that the carmine was retained twice as long in this as in the first experiment; the stimulus causing excretion

may therefore have been milder. The turning to the left may have been the resultant of two tendencies: moving forward, and getting away from the carmine as quickly as possible.

The ameba moved over the same carmine grain when placed in its path a few minutes later—70. When placed in front of the ameba for the third time—72—the tip of the main pseudopod forked, one prong flowing under the carmine, while the other became the main pseudopod through which the ameba moved away. The carmine grain which had been used in the first experiment was now placed in front of the ameba—78. A pseudopod thrown out on the left indicated a tendency to avoid the carmine, but another pseudopod thrown out under the carmine showed a slight positive reaction. The ameba finally moved off through the middle pseudopod leaving the carmine behind.

A piece of carmine of grade 4 which was placed in the ameba's path, produced a peculiar change of behavior—84. A pseudopod was sent out on the side on which the carmine lay, but anterior to it. The pseudopod flowed straight on carrying the ameba away. The cause of the formation of the pseudopod was undoubtedly some quality the carmine possessed. Another grain of carmine of grade 4 was then definitely avoided—87.

The same piece of carmine which had been used in the beginning of the experiments was laid in the ameba's path for the sixth time—89—but the subsequent behavior was so irregular that it is difficult to say just what part was produced by the carmine. What seemed to be a spathidium was then quickly ingested in a food cup containing a considerable amount of water—92. This shows that the ameba was actually hungry at this time, and that the preceding behavior where carmine was refused was not due to lack of hunger through fatigue or any other cause.

A grain of almost insoluble egg white was then placed in the ameba's path three times in succession—95. It was reacted to with more or less indifference.

A fresh grain of carmine of grain 2 was then laid in front of the ameba—107. A food cup was at once formed over the carmine but only about three-fourths of it was included within the cup. The ameba then remained almost motionless for about

four minutes. Then the ameba gradually withdrew from the carmine, the food cup remaining uncompleted. The ameba moved only slightly for about ten minutes thereafter, then two flagellates were caught in a large food cup. Two minutes later a pseudopod was thrown out on either side of the carmine for a short distance, but these were withdrawn as a spathidium (?) was encountered and ingested in a large food cup. The spathidium moved about in the food vacuole for about eleven minutes. This unusually long time of activity of the captured prey may have been due to a partial exhaustion of the ameba's digestive juices, as a result of its reactions to carmine in the experiment where the carmine grain was only three-fourths surrounded, but held in the food cup for about six minutes—108—111.

Two days later a new grain of carmine of grade 2 was then laid on the surface film near the ameba, which was at this time crawling along the surface film—122. The carmine grain was rolled around for eight minutes. The ameba then moved away. About three hours later a fresh piece of carmine of grade 2 was laid into contact with the ameba, now on the bottom of the dish—129. A food cup was at once formed in which half of the carmine was enclosed. After the ameba had remained quiet for several minutes, the food cup was retracted and the carmine carried to the back of the ameba. A few minutes later the carmine rolled off and the ameba moved away.

Next day a small piece of a worm, aelosoma, was laid in the ameba's path, but no definite change of behavior resulted. A fresh piece of carmine of grade 2 produced a positive reaction, a pseudopod being sent out toward it—139. There was no indication of a food cup, and soon the ameba moved away. The same carmine grain was then shifted so that it lay in the ameba's path—143. Again there was a slight positive reaction, after which the ameba moved off.

Two days later a piece of Knox Sparkling Gelatine, which was placed in the ameba's path—148—produced a slight positive reaction, after which the ameba moved on.

The behavior of this ameba toward carmine may be summarized as follows: Fresh grains of carmine containing only a slight

amount of egg white were readily ingested the first time each one was presented, for the first few trials. But on later trials the ingesting process, though initiated in typical fashion, was not completed. The ameba seemed to discover its mistake too late to completely avoid the carmine, even after a few trials had been made. On the last few trials, however, no indications of a food cup were seen when fresh pieces of carmine of the same grade were laid near the ameba. In contrast to the behavior of the ameba toward carmine grains adulterated with a slight amount of egg white, is that toward carmine grains containing a considerable amount of egg white; for no food cup was started over carmine grains of the latter kind. This is a point worth noting, for the food value of the latter grains is much greater than that of the former; and the solubility is also greater. There is sufficient evidence already at hand to make it clear that it is not merely the presence of a small quantity of food that induces the feeding reaction, but that there are other factors concerned. This conclusion is strengthened by the fact that neither gelatin nor aelosoma meat were eaten, gelatin being presumably a food substance and aelosoma being actually digestible as later experiments will show. That the behavior may be regarded as that of a normal ameba seems quite proper because of the ingestion of various food organisms during the course of the experiment.

One of the puzzling features of the behavior toward carmine, is that a carmine grain that has once been eaten will not be eaten again. This looks as if the carmine grain had undergone a change in passing through the ameba's body. But further discussion of this point will be postponed until the experiments of the next ameba have been described.

All the carmine grains that were eaten were ejected a few minutes thereafter. In the first case the carmine was ejected so soon after eating that one is inclined to think that the carmine acted as a disagreeable stimulus, and was not regarded merely as an indifferent body, such as the indigestible remains of a food mass. This is rendered the more probable by reason of the fact that the ameba, immediately after ingestion, reversed its direction of locomotion and moved away so that the carmine should lie at

once as near the posterior edge as possible. The reaction of the endoplasm toward carmine is therefore different from that of the ectoplasm.

Those cases where the ameba rolled the carmine grain along in various directions in front of it, cannot be described as attempts to ingest the grain in view of the fact that all the grains of carmine which were swallowed at all, were swallowed at once without rolling them. We shall meet with a number of instances of the same sort in the following experiments. The cause of such behavior may now be labeled as a mild state of hunger, a little too strong to leave the test object alone and too mild to produce ingestion. It is not due to a partially or wholly unsuccessful attempt at ingestion as Jennings ('04, p. 196) has suggested.

For the sake of comparison the behavior of an ameba from another dish toward carmine may now be taken up. The external appearance of this ameba was similar in all respects to the preceding one. Both the cultures from which these amebas were taken were inoculated at the same time with material taken from the same pond. The ameba belonged to the granular type.

A grain of carmine of grade 2 was laid near the main pseudopod of the ameba—153. The ameba moved into contact with it, then formed a large food cup with the carmine at its mouth. The carmine was finally completely enclosed and the ameba quieted down for about two minutes. A pseudopod was then thrown out 90° to the right of the ameba's original direction of movement. Through this pseudopod the ameba moved away. The carmine was excreted seven minutes after it was ingested. The direction in which the ameba moved away was the resultant of the tendency to move straight forward and the tendency to move directly away from the carmine, both tendencies being of about equal strength.

Five minutes later the carmine grain was placed to the left of the ameba—163. (A very small fragment broke off in shifting it.) It happened that a side pseudopod came nearer the carmine than did the main one, and the ameba then flowed into the side pseudopod while the main one was retracted. The carmine grain was rolled to the right slightly when another pseudopod was thrown

out toward the test object. Both the main and the side pseudopods rolled the carmine around for three minutes. Then what was previously the side pseudopod became the main one—169. The ameba then ingested the carmine in a typical food cup but with a very small quantity of water. The food cup was formed by throwing out two small pseudopods around the carmine, followed by a sheet of protoplasm thrown over the carmine. There was no period of rest, the ameba continuing forward in the same direction in which it moved before ingestion. Seven minutes after eating it the carmine was excreted.

Six minutes later the same grain of carmine was laid near the forward end of the ameba—176. A pseudopod directed toward it enlarged and finally came into contact with it. A food cup was at once formed and the carmine ingested for the third time with a moderate amount of water. The ameba did not become quiet after eating the carmine, but continued moving forward in the same direction. About seven minutes after eating the carmine it was excreted and left behind.

A few minutes later the carmine grain was placed near the ameba for the fourth time—187—but there was no definite reaction toward it. When laid near the ameba for the fifth time a positive reaction was produced, but after coming into contact with the carmine, the ameba resumed its original direction of movement. A flagellate then happened to hover near the carmine 198—which induced the prompt formation of a food cup over it and partly over the carmine. The flagellate was taken into the protoplasm but the carmine, having been at no instant entirely surrounded, was left outside.

The sixth presentation of the carmine grain produced very slight changes of behavior—202. Slight but definite reactions were brought about but the ameba finally moved on without change of direction, leaving the carmine behind. The seventh trial produced results similar to those of the sixth—211. In the eighth trial, with the same carmine grain, only a very slight positive reaction of short duration was produced—218.

A piece of carmine of grade 2 was then laid slightly to the left of the advancing tip of the ameba—221. The ameba moved for-

ward a short distance then sent out a pseudopod toward the carmine. This pseudopod became the main one. The ameba rolled the carmine about in all directions for twenty-two minutes, at the end of which time it was in clavate form. The ameba then speedily ingested the carmine in a large food cup with a considerable amount of water. The ameba remained motionless for several minutes, then moved off 70° to the left of the direction in which the ameba moved previous to ingesting the carmine. The final direction of movement was again the resultant of the tendency to move forward in the original direction and the tendency to move away from the carmine. About seven minutes after ingesting it, the carmine was excreted.

A piece of meat from the crustacean cypris was then laid twice successively in the ameba's path, but only very mild positive reactions were called forth—246.

The same piece of carmine used just previously was, for the second time, laid near the ameba—262. The ameba reacted definitely positively to the carmine, then moved on. When placed near the ameba for the third time, a definite positive reaction was again observed with a resultant change of direction of motion.

A fresh grain of carmine was then presented—277. The ameba flowed into contact with it and then ingested it in a typical food cup with a considerable amount of water. Five minutes after the carmine was ingested and after the ameba had begun to continue movement in the same direction, a flagellate became entangled in some way on the upper surface of the ameba, causing the formation of a food cup extending upwards in the water, in which the flagellate was ingested. Three minutes after ingestion the flagellate ceased movement and at about the same time the ameba moved off in the same direction in which it moved while ingesting the carmine. The carmine was excreted fourteen minutes after it was eaten.

The most striking feature of the behavior of this ameba as compared with that of the first ameba, is that at the beginning of the experiment the same carmine grain was ingested three times in rapid succession, while the first ameba ate any one

carminic grain only once. But in the five following trials with the same carminic grain, there was not only no attempt at ingestion, but the positive reactions toward it gradually became weaker. But when the behavior toward the second piece of carminic is considered it seems to be strikingly similar to the behavior of the first ameba. On the first presentation the carminic was promptly ingested. On the two subsequent trials, separated from the first by two tests with cypris meat, the carminic was not ingested, although positive reactions were observed. The third carminic grain was again ingested. It is therefore quite evident that some change was wrought in the carminic grain that passed through the ameba. What the change was it is impossible to say. But whatever the change, it is apparently not very marked, or a given particle would not be eaten three times in succession. On the other hand the change seems to be a definite and a constant one and its effects on the later behavior of the ameba seem to depend on the condition of the ameba, possibly on the degree of hunger. The first ameba, which ate each carminic particle only once, may thus be regarded as having been in a state of milder hunger than the second ameba, which ate one particle three times in succession, but later, when it may be supposed the degree of hunger was less keen, a given particle was eaten only once. All the particles that were eaten by this ameba were excreted seven minutes after being eaten, excepting one—282-283. In this later case, in which the carminic was held for fourteen minutes, there is a disturbing factor in that a flagellate was ingested five minutes after the carminic was eaten. There can be no question but that the carminic would have been egested sooner if the flagellate had not been previously caught. It is interesting to know that the ingesting process can arrest, for a considerable time, the excreting process.

The normality of the ameba is shown by the ingestion of the flagellate.

The long time during which the ameba rolled about the second carminic grain, when first presented, is again not to be regarded as an attempt to eat it, but as indicating a mild degree of hunger. The ameba's state of hunger may have been rendered less keen

by ingesting previously another carmine grain three times, and coming into contact with it five times thereafter. The ameba's appetite seems, however, to have improved from contact with the carmine, and finally ingestion took place as a consequence.

So much for the behavior of granular amebas toward carmine; let us now take up a few experiments in which raptorial amebas were tested with this substance.

A small clear raptorial ameba which had partly eaten a grain of aleuronat and then rejected it, reacted faintly positively to a grain of carmine of grade 2 which had been placed near it—1782. The grain of carmine was then placed in contact with the ameba—1786. A decided positive reaction followed but no attempt at ingestion was observed. Subsequent tests with pure carmine brought about reactions essentially similar to these.

Another raptorial ameba that had eaten a piece of grain gluten and later avoided a solution of carmine in a capillary tube, avoided a grain of pure carmine at first, but later reacted positively—2188. No attempt at ingestion was observed.

In the path of another raptorial ameba which had reacted only mildly positively toward a solution of egg albumen, and toward globulin and aleuronat, but which had ingested a flagellate, was placed a grain of carmine of grade 2—2250. The ameba at first seemed to avoid the carmine but presently a pseudopod was sent out toward it, and a few seconds later a food cup was formed and the carmine ingested with a considerable amount of water. The direction in which the ameba moved off almost immediately after ingestion was such that the carmine lay at once in the posterior part of the ameba.

A grain of aleuronat was then placed in the ameba's path. There was only a mild positive reaction. The aleuronat was followed by the carmine grain—2260. First there was observed an avoiding reaction but presently a side pseudopod was thrown out anterior to the carmine, which remained in contact with it while the ameba moved on.

An interesting variation of the reaction to carmine, when once inside the body, was disclosed by an experiment upon the ameba shown in figure 1513. This was a raptorial ameba and was

raised in a culture rich in diatoms, desmids and oscillaria. The amebas in this culture were very seldom observed to eat a motionless object. A small grain of pure carmine, about twenty microns in diameter, was agitated with a glass needle. It was promptly ingested by means of a typical food cup at twelve o'clock. It soon came to lie near a mass of empty diatom shells. At 1.53 two diatoms, experimentally agitated, were eaten. One escaped at 1.57. At 2.07 a diatom shell, with a small dark brown mass inside, was actively excreted. At 2.37 no shrinkage of the soft parts of the diatom eaten at 1.53 was observed. The diatom was then lost from view. At 2.46 the ameba ate a desmid which was mechanically agitated. At 3.11 an active *Euglena viridis* was ingested. The euglena thrashed around in the endoplasm for fifteen minutes, then it crawled out of the ameba as an earthworm might wriggle out of a lump of soft clay. At 4.08 the desmid was three-quarters liberated, and at 4.15 it was entirely freed. The observations were terminated at 4.40, when the carmine grain was still inside the ameba. The carmine had thus remained at least four hours and forty minutes in the ameba.

Thirteen days before the last experiment was performed, another ameba from the same culture as the preceding was presented with a piece of globulin. It was partly enclosed then rejected. A piece of pure carmine was then eaten in the normal manner with the formation of a food cup at 11.20. Then a flagellate was eaten. A fresh piece of globulin called forth a mild positive reaction, then the ameba moved on over it without any feeding reactions being called forth. At 3.15 another piece of pure carmine was presented but it was not eaten. Several times thereafter carmine was presented but in no case was any attempt at ingestion observed. This ameba responded very readily to mild stimulation with the needle. At one time three food cups were formed at the same time over two oscillaria threads lying near each other, and over a motionless but living *Phacus triqueter*, which I mechanically agitated. The food cup over the phacus was almost completed down to the glass, when agitation of the phacus was stopped. The food cup was then retracted although the phacus was still inside. The observations were terminated

shortly after 3.15, with the carmine still inside the ameba, having been ingested about four hours before.

These experiments indicate that the endoplasm of at least some strains of raptorial amebas differ strikingly from the endoplasm of granular amebas in their sensitiveness to carmine.

Summary of experiments with carmine. Carmine, whether pure or mixed with a slight amount of egg white, is readily eaten by amebas, especially by those of the granular type. Raptorial amebas ingest carmine much less readily than the granular.

The granular amebas do not keep carmine in their bodies for anything like the length of time food substances are kept, but it is excreted in from three to fifteen minutes after eating it, the average length of time being about seven minutes. The raptorial amebas on the other hand may retain the ingested carmine for four hours or more. The reason for this difference is not clear but it is possible that the character of their usual food may have something to do with it. The food of the granular type consists mostly of soft bodied organisms, like flagellates and ciliates, which break up quickly after ingestion. The food of the raptorial amebas, which kept carmine in their bodies for several hours, consists of organisms with hard shells—diatoms and desmids—which resist for a considerable time (over forty-four minutes in the case of one diatom) the action of the digestive juices. It is not impossible, therefore, that some rhythm or habit may have become established in the protoplasm by many and frequent repetitions, so that the ingested particles of different composition tend to be treated as if they had the qualities (in this case, slow of digestion) of the usual food substances, and consequently kept for a similar length of time in the body. On a purely chemical basis no explanation of this observation suggests itself.

The excretion of a particle of carmine may be delayed by the ingestion of a food particle.

A piece of carmine is eaten only once if the ameba is in a state of mild hunger; but the same piece may be eaten several times in succession if the ameba is very hungry. The ameba reacts less and less strongly to the same carmine grain if presented a num-

ber of times in succession. The same is true if a number of carmine grains are presented in succession, each grain being presented only once. The change in behavior is not due to fatigue of the food cup forming mechanism.

The carmine grains are egested by the raptorial amebas because they are actually disagreeable, and not merely because they are (presumably) indigestible. This is indicated by the fact that the ameba starts to move away after ingestion so that the carmine shall at once occupy a position in the posterior part of the body. Generally the direction of movement after ingestion is the resultant of the tendency to resume movement in the original direction and the tendency to move away so that the carmine is at once in the posterior region.

From the ameba's standpoint carmine induces ingestion more readily than pieces of crustacean or annelid meat. The latter substances are digestible as is shown in later experiments. Pure carmine, or carmine mixed with but a slight amount of egg white, induces ingestion more readily than carmine containing a considerable amount of egg white, or than particles of nearly insoluble egg white.

India ink. The stick form of india (or chinese) ink which was used in these experiments is a mixture of several substances, the chief of which are carbon, a gum that is added in its manufacture to make the carbon particles stick to each other, and a substance resembling lecithin that may be extracted with hot ether or chloroform. The heterogeneity of the india ink makes it an undesirable test substance, for it is impossible to tell which of the ingredients causes the changes in behavior which the ink produces. For this reason only a few experiments were made in which this substance was employed. One of these, made on an irregularly moving ameba, is figured—1627. There was at first some vacillation, neither positive nor negative behavior being observed. Finally, however, the ameba moved toward the india ink, and extended a pseudopod on either side of it. The pseudopods lifted the ink up. The ameba then resumed its original direction of motion and moved away, the ink being carried to the back of the ameba, from which it rolled a few minutes later. Although there

was no sign of the formation of a food cup the behavior of the ameba must nevertheless be considered as the first part of a feeding reaction, which remained uncompleted because of insufficient stimulation from the test substance.

The general result of the experiments with india ink was changes in behavior such as are produced by carmine, but they were less intense and less definite. India ink, like carmine, was sensed at a considerable distance, and in nearly every case provoked a positive reaction.

Uric acid. The principal properties of this substance, as far as relates to the purposes of this paper, are these: it is only slightly soluble, about one part in 40,000 of water at 18°C.; it is a definite chemical compound; it exerts very slight, if any, injurious effect on protoplasm by lying in contact with it. Merck's product was used.

A small grain of uric acid which was placed directly ahead in a granular ameba's path, produced a positive reaction—1023. After the ameba came into contact with it a food cup was formed over the uric acid and a flagellate which was hovering near it. The ameba then quieted down for about two minutes, after which it moved off in the previous direction. About six minutes after ingestion the uric acid was excreted. A few minutes later another grain of uric acid was laid in the ameba's path—1034. The ameba at once moved into contact with it and ingested it with the formation of a large food cup. About six minutes later the uric acid was excreted.

In another experiment—1115—in which another ameba from the same culture was used, uncertain behavior was observed; that is, there was first a slight negative reaction, then a positive, then a negative, then again a positive, all these reactions resulting in partial encircling of the uric acid granule. The ameba finally moved off through a pseudopod on the right, but there was considerable uncertainty into which of the two pseudopods on the right of the encircling pseudopod the protoplasmic current was to flow. When the uric acid granule was presented for the second time—1123—the reaction was negative, there being only two

small pseudopods thrown out on the side where the uric acid lay—1125.

In the path of a large clear raptorial binucleate ameba was then placed a grain of uric acid—1142. There was first a tendency to move away but presently positive reactions set in and the ameba flowed into contact with the acid and then surrounded it. The acid was partly enclosed in the protoplasm. The whole behavior seemed like a modified feeding reaction. But the uric acid had apparently not been completely surrounded by the protoplasm, for about six minutes later it was left behind. A grain of globulin was then treated in a similar manner. Although a food cup was formed over it, the globulin was not ingested. This ameba responded very readily to 'tickling.'

These experiments show that the stimulating power of uric acid is feebler than that of globulin or carmine, but it seems to be strong enough, nevertheless, to produce ingestion under certain conditions. As in all cases where dissociation is incomplete it is difficult to decide whether the stimulating element is the undissociated molecule, the ions, or both together. It is certain, however, that the acid in solution possesses the power of stimulation, for such behavior as is observed in figures 1120, 1121, 1143 could hardly be explained if the uric acid in solution was without effect.

Uric acid does not seem to be regarded as possessing food properties after it is eaten. This is shown by the short interval of time, only a few minutes, that elapses between eating and excretion. Uric acid does not act as an injurious substance when once inside the body, for in one case a grain of uric acid which was ingested with a grain of globulin remained at least thirty-five minutes in the ameba's body. Whether uric acid can be broken up to any extent by digestive action is not known, and it is consequently impossible to say whether uric acid possesses food value or not.

Solid egg white. The white of several hen's eggs that had been in an incubator for forty-eight hours was poured into an open glass dish and then placed in an incubator where the temperature ranged from 30° to 38°C. In the course of a day or two the

water had evaporated. The egg white was then broken up into small granules and kept in a glass stoppered bottle. About a year after it had been thus prepared it was used in these experiments. The egg white swelled up and softened in water, but it was only slightly soluble.

In only a very few cases was egg white of this sort eaten. One such case is figured—1378. In the path of an ameba which had eaten a piece of aleuronat ten minutes before, was placed a grain of the egg white. Before the ameba came into contact with the test substance two preexisting pseudopods began forming themselves into a food cup. The egg white was eaten in a typical manner and retained as a food mass is retained. This ameba had also previously eaten a piece of cholesterin which had been experimentally agitated, and which was carried around by the ameba for at least ninety-two minutes. That the eating instinct was strong, even exceptionally strong, is shown by later behavior where it tried to eat another ameba as large as itself. This cannibalistic instinct is relatively rare among amebas. I have noticed it only a few times in all my observations. The behavior of this ameba shows, then, that egg white as above prepared, is a very efficient stimulus for inducing the feeding reaction among gluttonous amebas.

But for amebas not in this condition egg white does not possess stimuli strong enough, as a rule, to induce ingestion. Thus the piece of egg white in figure 30 caused a negative reaction. This piece of egg white was too large for the best results. The general result of the second trial with egg white on the same ameba—95—was negative behavior. On the third trial with the same piece of egg white, the general result was again negative behavior, but—102—there is some indication of a positive reaction. The ameba ingested carmine and a flagellate, both before the trials with egg white and after, showing that the ameba was normal and that carmine possessed stronger food qualities than egg white.

To summarize: Egg white as prepared above induces the feeding reaction only in those exceptional amebas in which the feeding process is easily set into operation. The average ameba does

not react definitely toward egg white. Its stimulating qualities are considerably weaker than those of carmine. It also probably ranks below uric acid in this respect.

SUMMARY

1. Experiments upon the feeding behavior of ameba may readily be made. The method of investigation consisted in laying a particle of some substance in the ameba's probable path, and then making camera lucida drawings of the outlines of the ameba, at short intervals, as long as the particle was thought to influence the behavior. Time records were also kept.

2. Two kinds of amebas were tested. One kind was full of granules, formed few pseudopods, and was relatively slow in its movements. The other type contained but few granules in its body, formed many pseudopods, moved actively and ate voraciously. The granular amebas ate carmine readily and retained it for only a few minutes, while the raptorial amebas refused carmine frequently, but when eaten it was retained usually for hours.

3. Ordinary daylight, acting continuously, has no appreciable effect on feeding.

4. Ameba feeds by means of food cups which may be of very variable size and construction depending upon the character of the object to be eaten, upon the general degree of hunger of the ameba, upon the shape and position of the ameba with respect to the stimuli, and so on. The shape and size of the food cup cannot be predicted from the character of the stimulating object alone.

5. Movement of an object is a very important factor in determining whether or not it shall be eaten.

6. Food cups with considerable quantities of water in them are usually formed only over moving animals; dead animals or parts of animals are surrounded by food cups only very slightly larger than these objects themselves.

Carmine is readily eaten by ameba although it possesses no food value. A hungry ameba will eat the same carmine grain

several times in succession, but with each eating the grain becomes less attractive, until finally it is refused. If a new grain is then presented it is usually eaten.

8. The ameba can sense carmine grains twenty microns in diameter at a distance of at least 100 microns, and if the ameba reacts positively at all, it goes unerringly toward the carmine grain.

9. A few minutes after the carmine is eaten the ameba changes its direction of movement in such a way as to get rid of the carmine as soon as possible. The new direction of movement, if not a complete reversal, is a resultant of the tendency to move in the original direction and the tendency to move away so that the carmine shall at once come to lie in the new posterior end.

10. The ectoplasm (of the granular amebas) finds the carmine attractive, the endoplasm repulsive; that is, ectoplasm and endoplasm react to carmine in opposite ways.

11. Ameba reacts to india ink in essentially the same way as to carmine, but india ink is less attractive than carmine.

12. Uric acid grains are eaten less readily than carmine.

13. Solid egg white that is almost insoluble is eaten much less readily than carmine. Only very hungry amebas eat solid egg white. Amebas in a condition of mild hunger usually react negatively to this substance. Solid egg white, as well as uric acid, india ink, and carmine, is sensed at a distance.

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EXPLANATION OF PLATES

The figures are camera lucida drawings taken from the laboratory notes without alterations. The camera lucida was attached to the right hand tube of a long arm Zeiss binocular microscope. Eyepiece 4 and objective a_3 were used, giving a magnification of 65 diameters. A scale by means of which the size of amebas and of test objects can be estimated is shown on plate 3.

The figures are numbered serially from 1 on, for reference. The numbers are placed inside the figures. They are to be looked upon as labels only. They have no other significance. An x following a number, as 7x, indicates the end of the experiment, illustrated by figures 1 to 7x inclusive. A new experiment starts with figure 8 and ends with figure 13x, and so on. If a number is followed by xx, it means that the next experiment was performed upon a different ameba. Thus figures 1 to 152xx represent the results of a number of experiments upon the same ameba. With figure 153 a new ameba was employed, and so on. The order in which the figures were drawn is represented by the serial numbers for all the figures in any one experiment, and in nearly every case for all the experiments performed on any one ameba. The figures were drawn in vertical columns whenever possible. The work on the various amebas is not arranged in strict chronological order. The given arrangement was decided upon in order that the experiments on a given problem could be presented together.

The time of the beginning and the end of each experiment is given in hours and minutes. In many cases the time of drawing of each figure is also given, and where it is not given it may easily be computed.

The arrows show the direction of active protoplasmic streaming. The arrows in the last figure of each experiment denote the direction the ameba took in moving away from the test object.

The test objects are labeled in abbreviated form. See table of abbreviations below. For quick and correct reference the test objects are connected with the proper ameba by leader lines. These lines have no other significance.

All the work was done facing a north window. All the figures were drawn in the same position in the laboratory and on the plates. The top of each plate therefore points toward the North. This is worth noting from the point of view of the possible influence of light on the behavior of ameba.

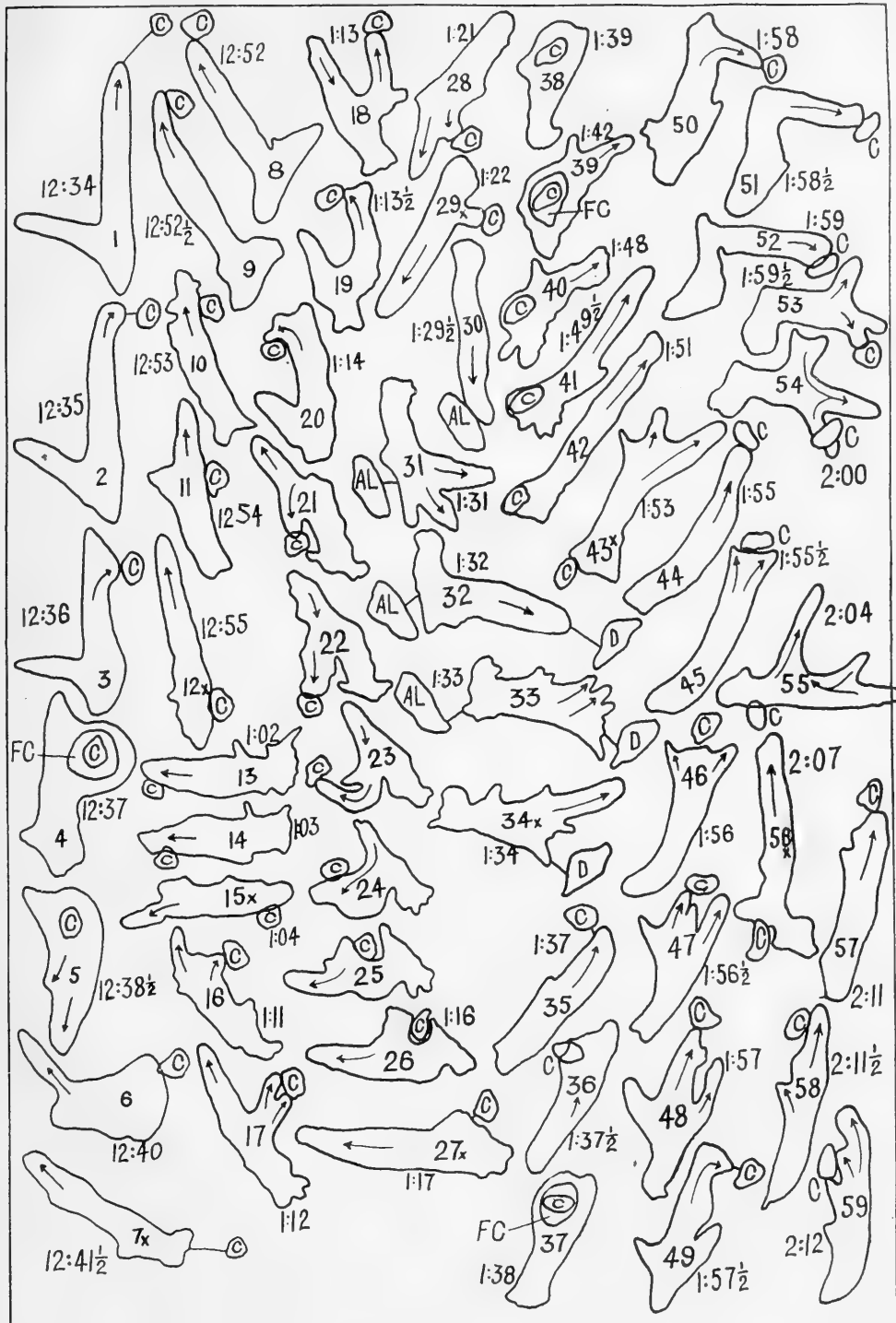
It will be noted that there are slight differences in the size and shape of the same test object as drawn in the figures of any single experiment, even if the object was not rolled around by the ameba. The explanation for this difference lies in the speed with which the drawings had to be made in order to catch important items of behavior. As a rule the parts of the ameba lying nearest the test object received the most careful attention and were drawn first; the posterior parts of the ameba and the test object were drawn last.

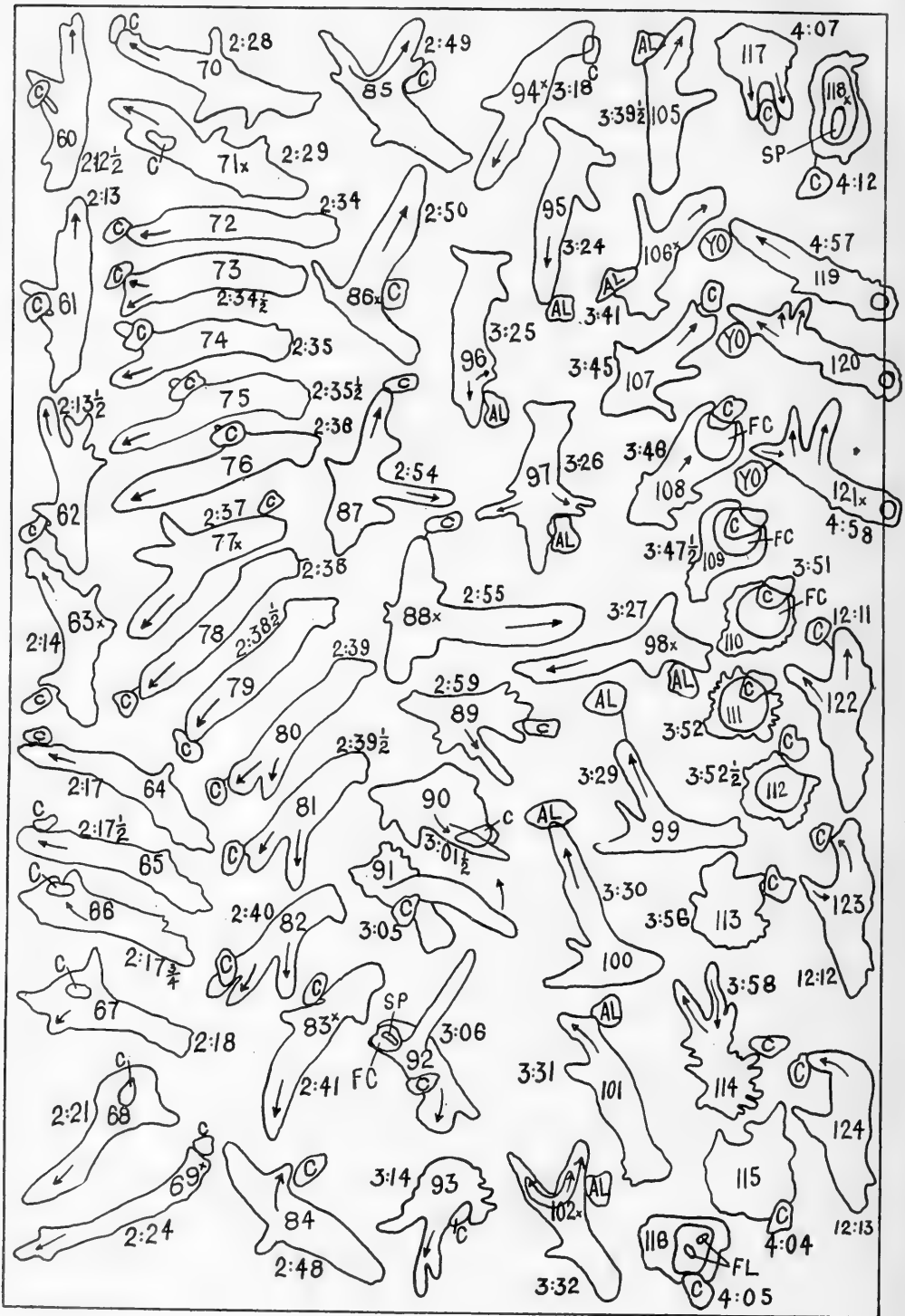
For detailed explanation of figures see pages 556 to 573 of the text.

ABBREVIATIONS

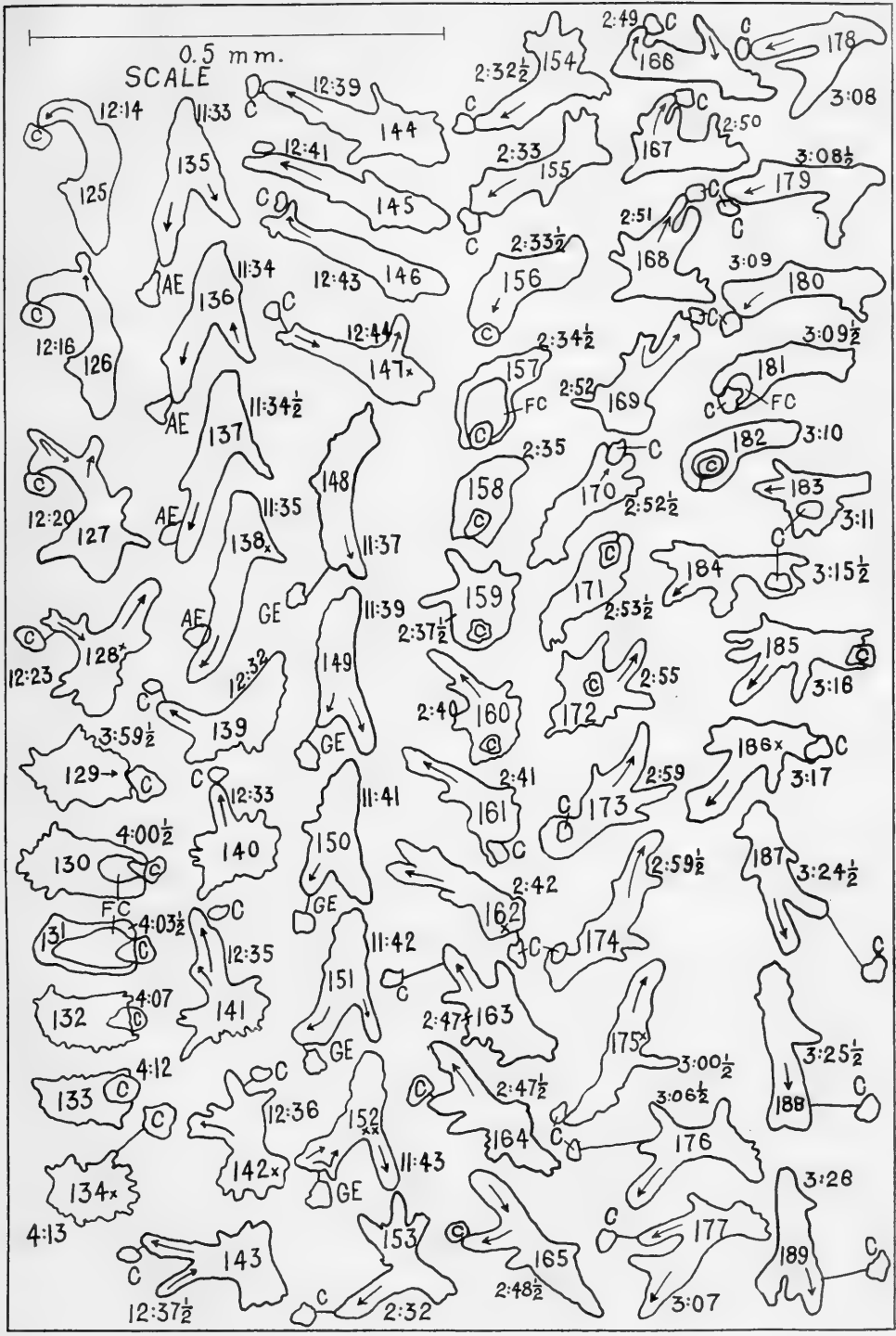
A, Aleuronat
AE, Acolosoma
AL, Egg white
C, Carmine
CH, Cholesterin
CR, Crustacean (Cypris)
DE, Debris
FC, Food cup

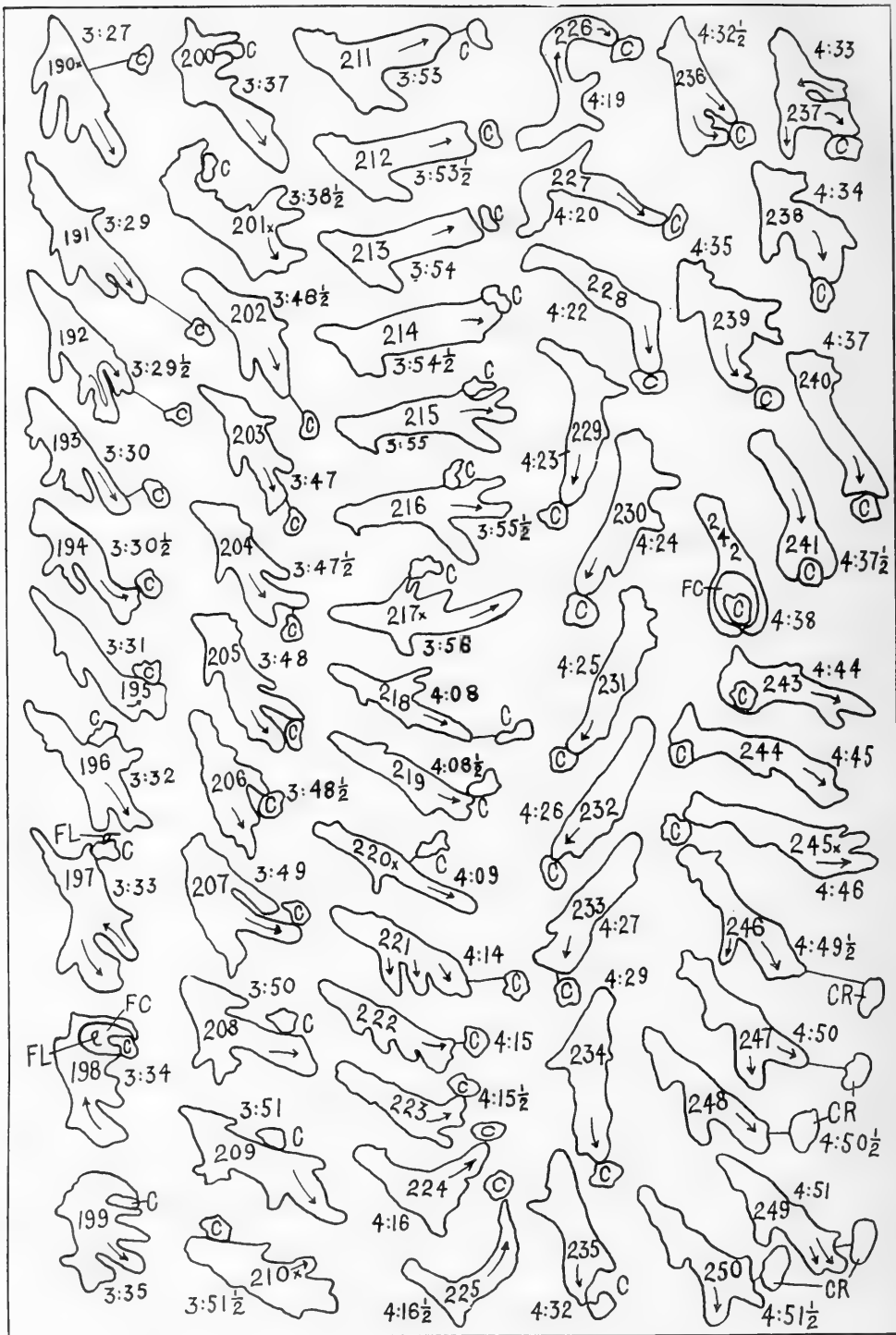
FL, Flagellates
GE, Gelatin
I, India ink
OS, Oscillaria
SP, Spathidium
U, Uric acid
YO, Yolk of hen's egg

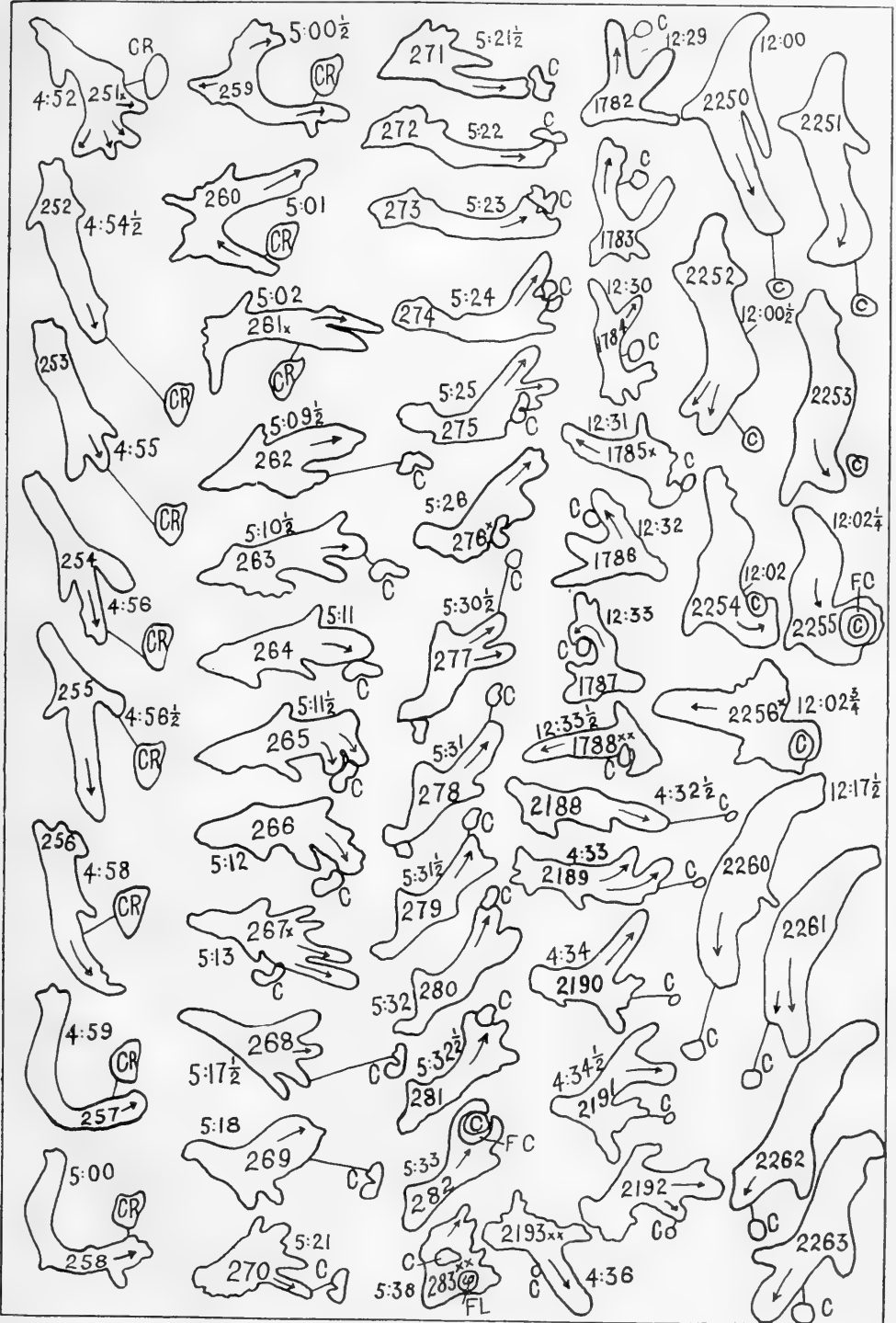


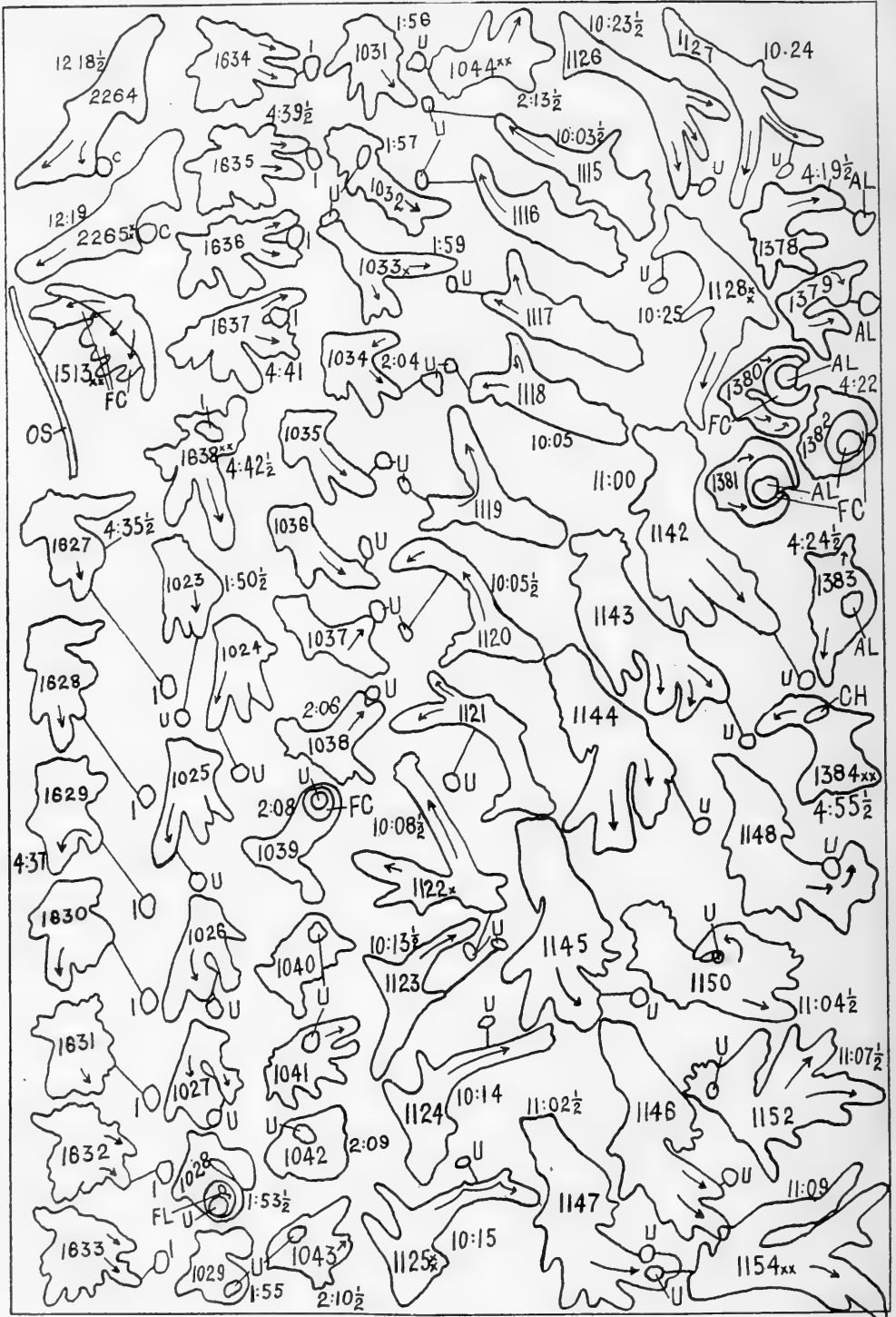


ASA A. SCHAEFFER









THE CHANGES OF THE BLOWFLY LARVA'S PHOTOSENSITIVITY WITH AGE

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FOUR FIGURES

INTRODUCTION

In previous work on the reactions of blowfly larvae (Patten, B. M., '14), it was noticed that the sharpness with which orientation to a change of light direction was accomplished varied markedly in different individuals. By observations incidental to the experiments then in hand, I became convinced that at least a certain amount of this variability in reaction was due to differences in the age of the maggots.

A search through the literature showed that others had observed changes in the blowfly larva's accuracy of orientation at different ages. Pouchet ('72) states that their negative responses become more marked as the animals advance in age, but that even newly hatched maggots show a tendency to avoid strongly lighted areas. Unfortunately the species on which he worked is not in this case recorded. He speaks of them simply as the 'vers' or 'asticots' of the 'mouche à viande.'

Herms has noted that changes take place with advancing age in the photic reactions of larvae of *Lucilia caesar* Linné. One of his tables ('11, p. 190) which gives the percentage of negative and positive responses for a group of ten individuals at daily intervals through their life history, indicates clearly that their negative reaction becomes more pronounced as the larvae grow older.

Working with larvae of *Calliphora erythrocephala* Meigen, Gross observed that they too reacted differently at different ages. He says ('13, p. 476),

When the blow-fly larva first emerges from the egg, it is either indifferent, [or] only slightly negative. . . . As it grows, it becomes more and more responsive to directive light and by the time the feeding period is ended, it is very strongly negative in its response to light.

Other than these general statements I was unable to find any data on changes in the blowfly larva's photosensitivity. There has, I believe, been no attempt made to determine accurately for the larvae of any species of blowfly, the relative sensitiveness at various ages. It was to obtain information on this point that the experiments described in this paper were devised. The problem may be formulated as follows:

STATEMENT OF PROBLEM

What, if any, changes take place in the sign or degree of the blowfly's larva's reaction to light during its life from hatching until pupation?

METHOD

Blowfly larvae during the age when they are most frequently used for experimental purposes respond to a single horizontal beam of light by crawling away from the source in the direction of the rays. Changes in the direction from which the light acts, induce corresponding changes in the larva's direction of locomotion. There is, however, considerable variation in the directness and accuracy with which individual larvae move along the path of the rays (Mast, '11, pp. 177, 178). This variability of reaction is more apparent when the larvae are subjected to sudden changes in the direction from which the light operates.

Without attempting to ascertain the relation of this variability in sensitiveness to the age of the larvae, a method has been devised by means of which larvae can be standardized as to their reactivity to light (Patten, '14, '15). The test employed consisted in subjecting larvae to an instantaneous change of 90° in the direction of a horizontal beam of light. The abruptness with which they came into orientation to the new direction of the rays, was regarded as an index of their photoreactivity. In the experiments referred to, this test was used merely to select, for further

experiments, larvae of uniform 'sensitiveness. The variability in the photosensitivity of different individuals, made apparent by it, indicated that a test of the same nature would serve to show very accurately any changes taking place with advancing age in the larva's reactivity to light.

For carrying out this test three 220 volt, 25 watt Mazda lamps were used. A light-proof case was made for each bulb. In one face of each case was cut a horizontal rectangular aperture, 1.0

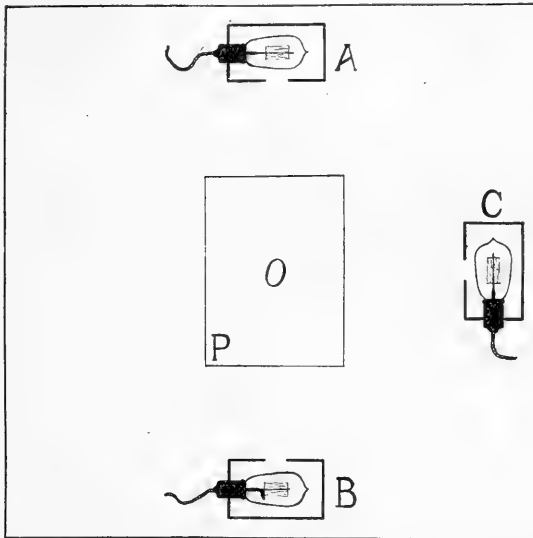


Fig. 1 Diagram to show arrangement of lights used in testing the blowfly's larva's photosensitivity. For explanation see text.

x 3.5 cms. The lights were then fixed to the top of a table, equidistant (23 cms.) from its central point; figure 1 shows diagrammatically their arrangement. Two of the lights (A and B, fig. 1) were directly opposed, their central rays meeting in the same straight line but from opposite directions. These two lights we may call the 'starting lights.' The third light (fig. 1, C) was so placed that its central ray intersected the line connecting the starting lights perpendicularly at its midpoint.

Under the influence of one of the starting lights (the other lights being shut off), larvae were made to crawl until they

reached the central point (fig. 1, O). Then the starting light was turned off and the lateral light thrown on, thus changing the direction of the incident light through 90° without changing its intensity. The course of a larva during this procedure was recorded by placing a drop of dilute methylene blue on its posterior end and allowing the animal to trace its own trail on a sheet of paper (fig. 1, P). Each larva was made to crawl through two tests. One trail was begun under the influence of the starting light near the observer, subjecting the maggot to the 90° change in the direction of illumination from its right side, and one under the influence of the starting light away from the observer, subjecting the animal to a similar change from its left side. The deflection of the trails was measured in degrees by means of a protractor. The average deflection of the responses to right and left stimulation was taken as an index of the sensitiveness of the individual.

The age of the maggots as given in the following records was calculated from the time of their emergence as larvae. The eggs were laid on the afternoon of August 26th, and the larvae hatched early on the morning of the 28th. The 'first day' reaction-measurements were made between 9 a.m. and 2 p.m. of the day of hatching, and therefore represent the responses of animals during the first day of larval life, rather than the responses of larvae 24 hours old. Throughout the remainder of the experiments, the measurements were made at the same time of day, so the changes in reaction shown represent changes which have taken place in 24 hour intervals.

Since temperature affects their rate of development, a record was kept of the temperature of the room in which the larvae were raised (table 1). If larvae were reared under markedly different temperature conditions, the maximum sensitiveness would be likely to appear on a different day. It would in all probability, however, be at the same relative age at which it was found in these experiments.

A group of 50 animals was isolated from a culture of *Calliphora Erythrocephala* larvae, and this group divided into two parts: (1) fifteen larvae each kept isolated in a separate culture jar so

TABLE 1

Temperatures of room in which cultures were kept during the series of experiments

AGE OF LARVAE	EGGS	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	6TH DAY	7TH DAY	8TH DAY	9TH DAY
Time of Temp. . . .	11 a.m.	9.30 a.m.	9.30 a.m.	9.30 a.m.	9.30 a.m.	9.00 a.m.	9.30 a.m.	9.00 a.m.	10.30 a.m.	10.30 a.m.
Temperature	19.0° C.	17.5° C.	19.5° C.	20.5° C.	20.5° C.	18.5° C.	19.0° C.	21.5° C.	23.5° C.	23.5° C.

that the records of the same individual could be compared from day to day; and (2) a group of 35 larvae kept in a common culture and tested each day in the manner described, but without keeping separate the records of individuals. All the larvae were kept in the dark throughout the experiments. By daily observations on these two sets of larvae it was possible to ascertain both the changes taking place in the photosensitivity of individual animals, and by averaging the reactions of all fifty maggots the changes taking place in the photosensitivity of the culture as a whole.

RESULTS

The results of these experiments might be expressed either graphically or by means of tables. The tabular method has the advantage of being more complete, but where the results are as simple as they are here, it is hardly necessary to give a complete tabular statement of all the measurements. Therefore only a single condensed table is given showing the average responses of the fifteen individuals kept isolated throughout the series of experiments (table 2). There were a few casualties among the larvae due probably to excessive handling in the very young stages. In all cases the dead larvae were replaced by fresh individuals of the same age from a gross culture kept for the purpose under precisely the same temperature and moisture conditions as the experimental cultures. Replacements are marked on the table by an asterisk.

A very clear idea of the changes in sharpness of reaction which take place with age, is given by the series of actual trails photographed in figure 2. The trails were selected to show as nearly as possible the characteristic reaction for the age they represent.

TABLE 2

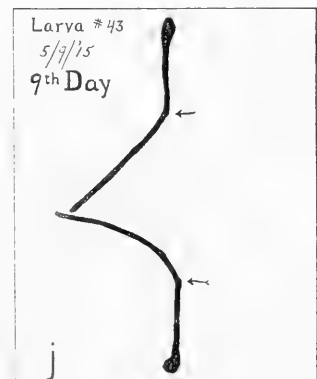
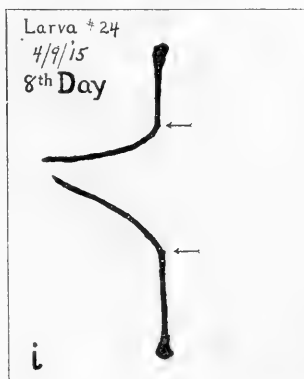
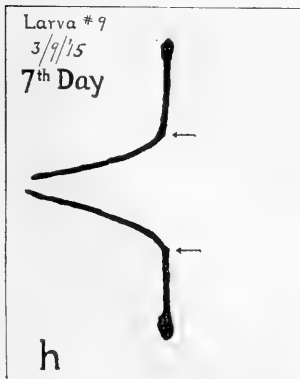
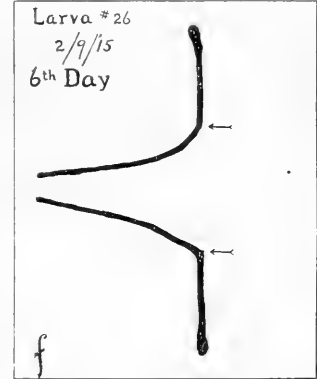
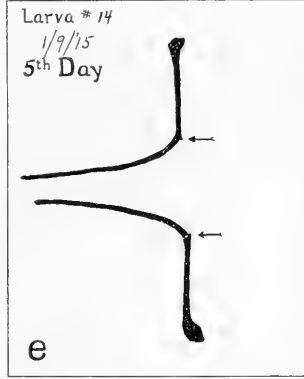
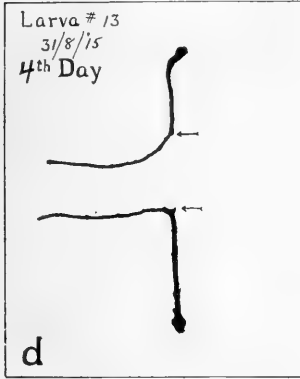
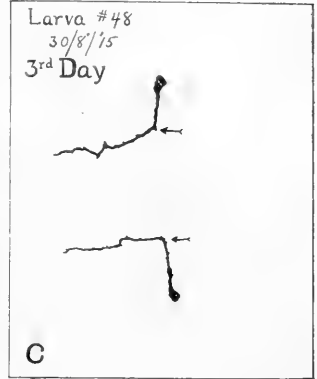
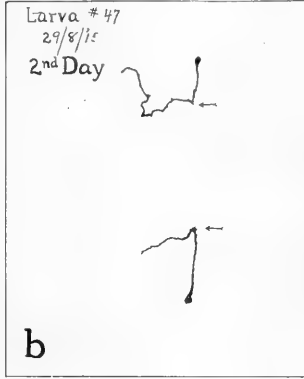
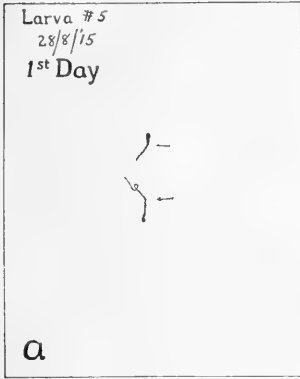
A table showing the deflections of the 15 larvae kept isolated throughout the experiments. The measurements given represent the average deflection of a "right and left pair of trails" such as that shown in figure 2, c. R indicates that it was not possible to make the larva move at all. This rarely happens except immediately before pupation. * indicates the replacement of a dead larva by a fresh individual of the same age. P indicates the pupation of the larva. p, number 12 pupated the following day.

NUMBER OF LARVA	AVERAGE DEFLECTION IN DEGREES MEASURED AT INTERVALS OF ONE DAY										
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day
	deg.	deg.	deg.	deg.	deg.	deg.	deg.	deg.	deg.	deg.	deg.
1	-11.0	-20.0	-62.0	-78.0	-89.0	-68.0	-51.0	-30.0	P		
2	-16.0	-35.0	-66.0	-81.0	-74.0	-63.0	-65.0	-66.0	P		
3	-16.0	-31.5	-45.0	-80.0	R	-43.0	*-74.0	-80.0	P		
4	-15.0	-62.5	-78.0	-88.0	-78.0	-77.0	-74.0	-82.0	P		
5	-36.0	-70.0	-77.0	-75.0	-70.0	*-83.0	-48.0	-78.0	P		
6	-21.0	-82.5	-66.0	-74.0	-76.0	-65.0	-79.0	-36.0	-63.0	P	
7	-23.0	-45.0	-59.0	-81.0	-64.0	-45.0	-65.0	P			
8	-20.0	-57.5	-80.0	-85.0	-80.0	-73.0	-75.0	P			
9	0	-47.5	-82.0	-85.0	*-86.0	-75.0	-67.0	-60.0	P		
10	-6.0	-67.5	-75.0	-86.0	-89.0	-71.0	-51.0	P			
11	-16.0	-50.0	-79.0	-85.0	*-68.0	-68.0	-67.0	P			
12	-9.0	-45.0	-47.5	-85.0	-86.0	-83.0	-71.0	-77.0	-64.0	-59.0	-41p.
13	+5.0	-45.0	-86.0	-81.0	-87.0	-76.0	-55.0	-80.0	P		
14	-15.0	-65.0	-75.0	-87.0	*-72.0	-71.0	-37.0	P			
15	+11.0	-65.0	-63.0	-86.0	R	R	R	-55.0	P		
Average deflection.....	-12.5	-52.6	-68.0	-82.5	-78.4	-68.6	-62.8	-64.4	-63.5	-59.0	-41.0

By computing the average deflection of the 100 trails made daily by the 50 larvae, the sensitiveness of the culture as a whole could be accurately determined. These measurements were plotted graphically in the form of an age-sensitiveness curve. The curve thus obtained is shown in figure 3. Deflection in degrees is plotted along the vertical axis, and age in days along the horizontal axis.

It is interesting to compare with this smooth curve compiled from the average reactions of the culture as a whole, a series of

Fig. 2 Photograph of trails showing the characteristic reactions of larvae at various ages. Each pair of trails represents the reactions of a larva to changes of 90° in the direction of incident light made first on its right side (lower trail of pair) and then on its left side (upper trail of pair). The sharpness with which orientation to the new light direction is accomplished is regarded as an index of the degree of photosensitivity.



similar curves plotted from measurements made on larvae kept isolated through the experiments. For the sake of simplicity only six individual graphs have been plotted, but these have been selected to show the range of variation in the group (fig. 4).

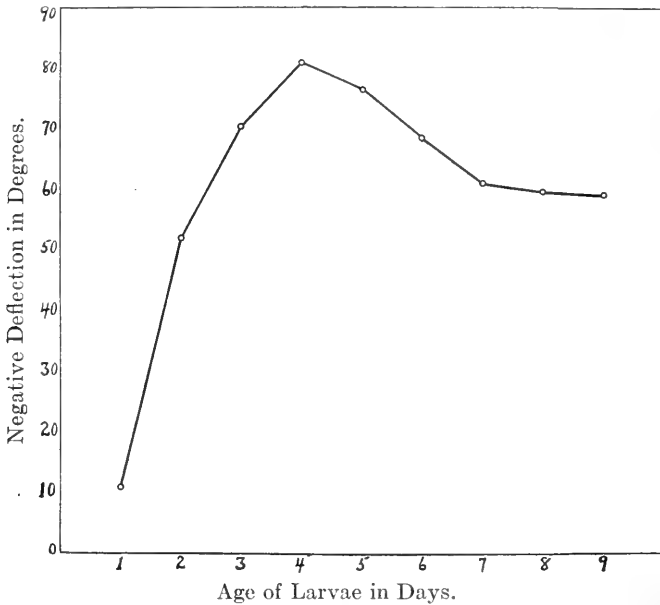


Fig. 3 Curve showing the average degree of photosensitivity from hatching until pupation of a group of 50 *C. erythrocephala* larvae.

DISCUSSION

In working with the larvae from day to day certain differences were observed in their reactions which were sufficiently characteristic and constant to deserve mention.

Larvae when just hatched were found extremely difficult to handle without injury. Moreover, they were so small and their muscular reactions were so weak that it was not always possible to make them give a readily measurable trail. Quantitatively, therefore, the first day measurements must be regarded as only approximate. The trails shown in figure 2 A, are unusually clear cut. In most cases it was necessary to watch the larva

carefully and estimate as nearly as possible its direction of crawling. Even then there were several individuals which gave no perceptible directive reaction. A few larvae gave positive responses, but the preponderance of movements away from the light in the great majority of cases could leave no doubt that the general reaction was negative in character.

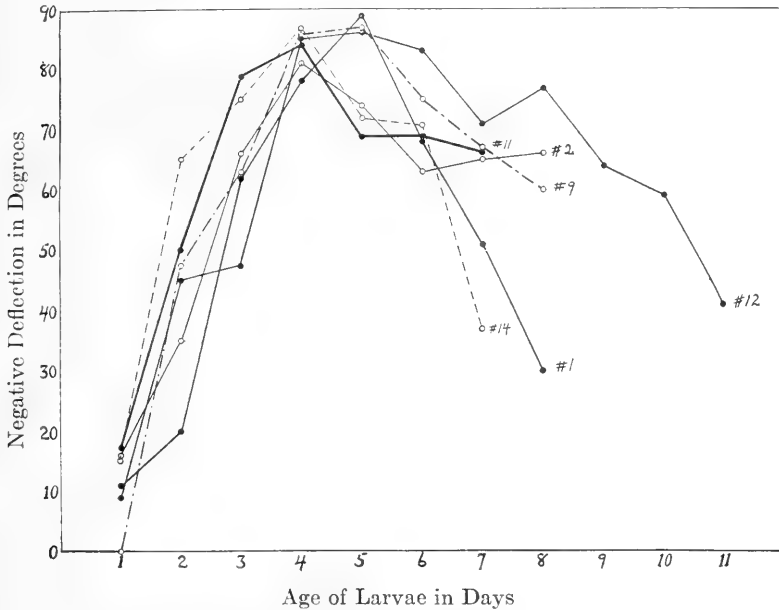


Fig. 4 Curves showing the degree of photosensitivity from hatching until pupation of individual *C. erythrocephala* larvae.

By the second day the larvae had grown sufficiently so there was little difficulty in handling them. All the measurements except those of the first day may, I believe, be regarded as expressing accurately the reactivity of the larvae. There were almost no positive responses obtained on the second day, or later, and these were for the most part due to asymmetry of sensitiveness, for a positive response in one trail was almost invariably associated with a strongly negative response in the trail made when the same animal was stimulated from the opposite side (Patten, B. M., '14, pp. 230-233 and 259). In comparison with

maturer individuals, larvae two days old gave extremely vacillating trails (fig. 2, B). 'Wig-wag' movements of the anterior end were frequent and very pronounced. The larvae at this age were also noticeably more sensitive than fully grown larvae in their reaction to moisture. The tendency to follow moist trails was not infrequently strong enough to throw them out of orientation to the light. In making the records of their light reactions, precautions were taken that this disturbing factor should be eliminated.

On the third day the trails were made with less hesitation than on the second day, though the larvae still made noticeably more 'wig-wag' movements and more changes of course than when fully grown (fig. 2, C).

By the fourth day the maggots had for the most part attained their full growth. Their reactions were more rapid and much more decisive. To put it anthropomorphically, "they seemed to have very definite ideas as to the direction in which they meant to travel" (fig. 2, D).

After the fourth day the wig-wag movements were still further decreased in frequency and extent, as is shown by the smoothness of the trails photographed in figure 2. There was also a noticeable tendency to move more deliberately and to respond less quickly to the change in light direction. The curves of figures 3 and 4 indicate quantitatively the decline in photoreactivity taking place between the fourth or fifth day and pupation.

Just how much of the rise in the curve of reaction (fig. 3) during the first four days is due to increased facility of crawling or better coördination between the photoreceptors and muscular system, and how much is due to actual increase of sensitivity, it is impossible to say with certainty. I believe, however, that after the first day the muscular movements are discharged with enough apparent facility to justify the interpretation that the sensitivity of the larvae to light is really increased between the second and fourth day. There seems to be still more reason to believe that the drop in reactivity after the fourth day is due to decrease in sensitivity. The reactivity falls off during the so-called migratory period, when the larvae leave food and wander

about, as if seeking a favorable spot for pupation. If the muscular system is more effective at one time than at another, we should certainly expect it to be during this period of its greatest activity. It is therefore most probable that the decline in reactivity is not due to decrease in muscular efficiency. It is equally improbable that the coördination between receptor and effector is lowered during a period of increased effector activity. Though this does not constitute proof that the drop in reactivity is due to a decrease of photosensitivity in the receptors, it points strongly toward that interpretation.

The decline in photosensitivity has been said to occur in the migratory period. We may be more specific. Figure 3 shows that reactivity begins to drop between the fourth and the fifth day and continues to drop steadily until the seventh day, thereafter remaining about constant till pupation. On the fourth day all the larvae were feeding. Between the fourth and the fifth day 15 per cent of the larvae had migrated, by the sixth day 60 per cent had migrated and by the seventh day practically all the larvae had left the food. The coincidence of the drop in the reaction curve with the onset of migration in the cultures is most striking; whether or not there is any correlation between the two I cannot say. One is tempted to suggest that there may be, upon the cessation of feeding, a reduction in the rate of metabolism of some photolysable substance. Such a suggestion is, however, of little value except as a hypothesis for further work.

It would be interesting, also, in view of the change in the sign of phototaxis taking place in the blowfly during the pupal stage, to ascertain if the drop in the curve of negative photosensitivity exhibited during the last stage of larval life, were carried on by an increase in positive photosensitivity during the early life of the imago.

Whatever may be the underlying cause, there can be no doubt that larvae of *C. erythrocephala* react to light very differently at different ages. Observations which I carried out on a few larvae of *Lucilia sericata* showed that they exhibited changes in photosensitivity similar to those demonstrated for *C. erythrocephala*. Although the number of individuals handled was not sufficiently

large to justify any quantitative statements for *L. sericata*, it was clear, nevertheless, that age differences greatly modified their photic reactions. Herms ('11) has already shown that larvae of *Lucilia caesar* become more strongly negative to light as they grow older. He did not observe any drop in sensitivity during the migratory period, but this might possibly have been due to the fact that he did not use quantitative methods rather than to the absence of the phenomenon in this species. These observations all indicate clearly the necessity of taking into consideration the developmental stage of blowfly larvae used in photic reaction experiments.

Herms ('11) has also shown that there are differences in the photosensitivity of the larvae of *L. caesar* and *C. vomitoria*. In all probability more extended observations and more refined methods, would show differences in the reactions of the larvae of other common species of blowflies. It would seem superfluous to call attention to the necessity of recording the species of the animal worked on, were it not for the fact that the literature on photic reactions abounds in references to the reactions of 'blowfly larvae (sp. ?)'.

A study of table 2 and figure 4 will make it apparent that for accurate quantitative work it is not sufficient that all the individuals used should be of the same species and the same age. It is necessary to employ in addition some preliminary standardizing test to exclude extreme individual variants. Only by such means may data on photic reactions secured under different experimental conditions be relied on for comparisons. Neglect of species determination, failure to take into consideration the developmental stage of the animals studied, and insufficient attention to the factor of individual variability have undoubtedly been responsible for much unnecessary controversy concerning the reactions of 'blowfly larvae.'

SUMMARY

Larvae of the blowfly, *Calliphora erythrocephala* Meigen, were tested each day from hatching until pupation to determine what, if any, changes take place in the sign or the degree of their reaction to light.

The test employed consisted in subjecting a maggot crawling under the influence of a horizontal beam of light to an instantaneous change of 90° in the direction of the beam. The resulting change in the direction of the animal's locomotion was measured in degrees by means of a protractor. This procedure gave a quantitative index of the reactivity of the larva, for the more sensitive the individual was, the closer did its deflection approach 90° .

Using the same larvae throughout the experiments, one hundred trails were run each day. The average deflection of each of these sets of 100 trails was used to locate a point on an 'age-sensitiveness curve.'

The curve of photosensitivity thus obtained shows that the reactions are negative throughout, with a rapid increase in amplitude during the first days of larval life, reaching a maximum on the fourth day with an average deflection of 81° , and then dropping steadily till the seventh day, remaining thereafter almost constant until the time of pupation.

The decrease in sensitivity occurs coincidentally with the beginning of the migratory period.

The changes in photosensitivity shown to take place with advancing age indicate the necessity of determining and recording either the developmental stage or better, the degree of sensitiveness, of all individuals used for quantitative experiments.

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