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BIOLOGICAL BULLETIN

THE BREEDING BEHAVIOR OF THE SUCKERS AND MINNOWS.

I. THE SUCKERS.[1]

JACOB REIGHARD.

CONTENTS.

1. Introduction to the Series.

This paper is the first of a series on the breeding habits of nine species of suckers and minnows. These fishes form a well-defined group by some systematists united into a single family,

[1] Contributions from the Zoölogical Laboratory of the University of Michigan.

by others separated into the Catostomidæ and Cyprinidæ. To accumulate the field notes and sketches on which the papers are based has been the work of many seasons. If there were no bad weather, no university duties, and no human interference with breeding environment or breeding fish, such work might be carried on with as little interruption as that of the laboratory; but it would not progress as rapidly, for observations need to be many times repeated. The behavior is often so complicated or so rapid that it is only by analyzing it into its elements and observing each of these repeatedly that a degree of certainty is possible. Such attitudes as these shown in Fig. 3 of this paper may be correctly represented only after many observations on the position of each fin and on every other detail. The observations upon which my descriptions are based have been very many for each element of the behavior, often in the neighborhood of a hundred, sometimes probably several hundred, or even thousand. Although the work has been spread over a period of years no one should suppose that the record is complete. I have studied *only the breeding behavior* and that in but few fishes; I have not the life history or the full natural history of any one. Of the breeding behavior I shall try to give for each species a composite picture taken from many fish through many seasons. This I give in such detail as I have, because that seems necessary to clearness. It further affords a better basis for the discussion of theories to be considered in the final papers of the series. The behavior that I describe may be easily seen in suitable places and at the proper season. Yet few are likely to take the trouble. This has seemed to me an added reason for fullness of treatment.

The suckers and minnows that I have studied, with the exception of the blunt-nosed minnow, *Pimephales notatus* (Rafinesque) breed in running water. During the breeding season the males of all the species studied have pearl organs, hard, tough, white, usually conical elevations of the skin which consist of cornified epidermal cells. They occur in many situations. Some of them are so small as to be visible only under a lens; others may be seen with the naked eye at a distance of ten or twelve feet. They commonly make the surface of the breeding males distinctly rough to the touch and are in that case referred to as "effective" pearl organs.

The drawings that show fish in action have been made from field notes and sketches with the help of specimens and in some cases with the aid of models or photographs. I am indebted to Mr. Charles R. Knight for valuable suggestions in connection with the drawings but he is in no way responsible for their obvious shortcomings. To my former student, Professor Norman H. Stewart, of Bucknell University, I am indebted for data from his unpublished manuscript on the distribution of the pearl organs. To the United States Commissioner of Fisheries, Doctor Hugh M. Smith, I am indebted for permission to publish.

2. Breeding Behavior of the Suckers.

A. The White Sucker (*Catostomus commersonii* LeSueur).

1. *The Breeding Grounds and Breeding Season.*—A little way from the State Bass Hatchery, near Grand Rapids, Michigan, Mill Creek is spanned by a bridge of the Pere Marquette Railway. Just below the bridge, where the stream is three or four rods wide, a line of four-inch waterpipe is laid across its bed. An eighth of a mile above the bridge the stream is dammed to furnish water for the hatchery. Between the dam and the pipe-line it is made up of a series of alternating rapids and pools. For a little distance above the bridge and beneath it the water runs six to twelve inches deep, swift and smooth. Below the pipe-line it breaks into ripples. Under the smoother water the bottom is sandy gravel, but under the broken water this gives place to larger stones. Over the gravel in the smoother water a thin brown mantle of silt and algal growth usually stretches without interruption from shore to shore and obscures the bottom.

Rapids of this sort are typical of a drift-covered country and afford the characteristic breeding ground of the white sucker.[1] In the spring patches of the gravel bottom in the upper water of the rapids often look as though they had been scoured by a broom. The silt mantle is absent from these patches and the bright colors of the clean gravel and sand throw them into

[1] The usual name in Southern Michigan; known also as common sucker, fine-scaled sucker, brook sucker.

I am indebted to Professor W. M. Smallwood for permission to use his unpublished notes on this species. He records seeing it in the spawning attitude on stony bottom in Lake Clear in the Adirondacks in late June (*cf.* Reighard, 1915).

sharp contrast with the surrounding silt-covered bottom. The patches show where the suckers have been breeding.

The water of a rapid is rarely so smooth that one can see readily into it. Not often is such a rapid near a bridge or other perch; but under this Mill Creek bridge the surface of the stream is but little broken and on a sunny, cloudless day, when the water is clear and there is no wind to ruffle its surface, it is possible to see in detail what happens on the rapid. With field glasses the fish may be studied almost as readily as though they were in air. From this vantage point I watched the white suckers at intervals.[1]

My work was done by day. It is well known that in the spring suckers ascend small streams in great numbers at night and it is possible that their breeding activities are continued at night. They are often interrupted by colder weather or roily water.

2. *General Activities of Breeding Fish.*—About 2 o'clock on April 23, 1903, I cautiously took my place on the Mill Creek railroad bridge. Numerous white suckers were on the rapids. Although I walked with extreme slowness and made no sudden movements of any part of my body the fish were at once aware of my coming and scurried to the shelter of the banks and nearer pools. I sat quiet and in the course of fifteen minutes they began to reappear in the shallow, swift water. Thereafter, for an hour, any quick movement on my part resulted in the fish starting swiftly up stream, but if the movement was not repeated they dropped slowly down-stream to where they had been. To get the field glasses to the eyes or the hand to the notebook without startling the fish needed a movement so slow that it must have been scarcely perceptible at the distance of twenty-five or thirty feet at which the fish were. It was probably about two inches per second. As time went on the fish became gradually used to my presence and after an hour were no longer disturbed by slow movements. By three o'clock twenty suckers from eight to twelve inches long were on the rapid and were moving slowly up stream in small groups. The fish stopped

[1] From April 23 to May 6, 1903. In Honey Creek near Ann Arbor, I saw them breeding April 27 to May 2, and in Mallet Creek, Ann Arbor, on May 10, 1909. The two creeks last named are only about a third the width of Mill Creek (Grand Rapids) at the point at which the suckers were seen.

here and there in the rapid on the patches of cleaned gravel and were seen to take gravel into the mouth and spit it out. They were presumably in quest of eggs that had been laid in the disturbed gravel areas and their occurrence in small groups is perhaps in part the result of the distribution of such areas. In their search they were accompanied by numerous small minnows doubtless on the same quest. Other suckers were seen crossing the pipe-line. At four o'clock no suckers were to be seen from the bridge, but a dozen were found just below the dam an eighth of a mile further up stream. The fish had apparently covered this distance in an hour. At nine o'clock the same evening the search light showed suckers still crossing the pipe line bound up stream.

In my experience the white sucker is one of the most difficult of our native fishes to approach in the open. Ordinarily it becomes accustomed to the observer with extreme slowness and at no time permits him any great freedom of movement. Confined with other fish in an aquarium it is among the last to become accustomed to the observer or to take food.[1]

[1] I have noted but two exceptions to this general fact. (1) On May 6, in the morning, I placed in an outdoor aquarium at the Mill Creek Hatchery four males and two females that had been captured in a seine on the previous evening. At three o'clock on the same day the fish were moving about and feeding and by four o'clock they were spawning. They did not react to an observer within two or three feet of them. (2) At Douglas Lake in Cheboygan County, Michigan, suckers are found in rather deep water. At night they come into shallower water to feed and are occasionally seen there at dawn. At such times they flee to the deeper water at the first glimpse of a moving object. In late June the log perch (*Percina caprodes*) are laying their eggs in the sand in very shallow water. At that time suckers enter shallow water in the day time and feed on the eggs of the log perch. Each sucker is accompanied by a group of log perch which appear to be feeding on eggs uncovered by him and perhaps on other crumbs from his table. At this time the suckers may be approached with little trouble and I have come close enough to photograph them as they lay at my feet. I have thought this absence of the suckers' usual wariness due to the presence of the log perch. These are breeding and are then unafraid. In deeper water the sucker has probably found freedom from disturbance where they were present. Safety and log perch have been closely linked in his experience. So now, so long as the log perch are on the shallows, he is not easily startled and feeds there undisturbed by sights that would otherwise send him hurrying to shelter.

To these observations may be added one of Smallwood (unpublished notes). At the end of June he found *Catostomus commersonii* and two other species of sucker on stony bottom in the shallow water of Lake Clear in the Adirondacks.

3. *Coloration and Color Changes.*—The mature white sucker, when seen in its native waters or in captivity, is ordinarily uniformly olivaceous on the back and sides and white below. There is no color pattern nor are there color differences between the sexes. The suckers seen from the bridge (Fig. 4) were so different in coloration from all that I had seen before, that I was at first doubtful as to their identity. Each had a broad yellow-white stripe which crossed the occiput and extended thence down the sides. When some of these fish were seined they were found to have the usual uniformly olivaceous color. They were placed in an aquarium, males and females together, and four hours later the males had begun to move about and to feed. Shortly afterward the light stripe appeared across the occiput and down the sides. Beneath the light stripe was a broad dark stripe (Fig. 4) and in one of the males this had a rosy tinge. During the actual pairing described below, the rosy tinge gave place to a brilliant crimson. Later I often saw the light stripe appear in a few seconds on uniformly colored males that were on the rapids. This happens regularly in the breeding season when the sexes are together.

4. *Sexual Differences.*—It is at first difficult to discriminate between males and females. As seen from the bridge the paired fins are transparent white in both and in both the yellowish white stripe crosses the occiput and extends down the sides. But the males are on the average smaller than the females and slenderer. It is soon apparent that the occipito-lateral stripe is whiter in them and that their backs may be flecked with white especially between the dorsal fin and the occiput (Fig. 4, male at right of female). In the region of the occiput the white flecks may form a distinct patch which, seen from a distance, looks like fungus. The white flecks are perhaps not always present in males, but I have never seen them in females. In pairing males, seen in the aquarium, the dorsal half of the eye is lighter colored than in females, but I do not know that this is not the case at other times. The differences so far noted afford

C. commersonii and one of the other species, probably *C. catostomus*, were seen in the spawning attitude. The fish were not disturbed when a boat, in which were two children and a barking dog, was poled about above them so that they were not more than five feet from it.

excellent field characters by which the sexes of the breeding fish may be distinguished in their native waters. Less striking differential characters are the greater length of the caudal and lower fins of the male, the difference in length of his caudal lobes and his possession of pearl organs.

Effective pearl organs (*vide* introduction) occur on the male in the following situations (Fig. 1): (1) Large, sharp-pointed

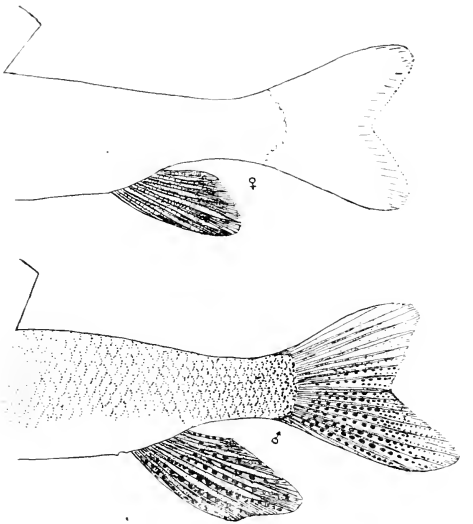

FIG. 1. Lateral view of a part of a male and female of *Catostomus commersonii* drawn to the same scale. The black dots represent pearl organs. The anal fin and lower lobe of the caudal are longer in the male and bear large pearl organs. All the scales of his sides bear small pearls, somewhat larger behind the caudal The female has no pearl organs.

organs are found on the anal fin and on the lower part of the caudal. They may be visible to the unaided eye at a distance of three or four yards. (2) The caudal margins of the scales on the sides bear small hemispherical organs, which are effective behind the dorsal. (3) The upper surfaces of the pectorals and both surfaces of the pelvic fins bear small organs. (4) The rays of the dorsal fin bear small organs. Pearl organs do not occur in the female (Fig. 1).

The difference between the sexes in fin length are shown in Table I.

TABLE I.

SHOWING IN MILLIMETERS THE AVERAGE LENGTH OF FINS IN MALES AND FEMALES OF *Catostomus commersonii* OF EQUAL LENGTH, THE DIFFERENCE IN AVERAGE LENGTH OF FINS AND THE PERCENTAGE DIFFERENCE.

	Length, Tip of Snout to Base of Caudal.	Caudal Lower Lobe, Length.	Anal, Length.	Dorsal, Length.	Pelvic, Length.	Pectoral, Length.
Males, M.	312	62	65.5	47.8	42	57.5
Females, F.	312	54.6	49.8	45.7	37.7	51
Difference, D = M − F.	000	+ 7.4	+15.7	+ 2.1	+ 4.3	+ 6.5
Percentage of Difference, % D = (M − F):F.	000	+13.4 (+31.1)	+31.5	+ 5.0	+11.4	+17.8

By fin length is meant the greatest distance from base of fin to its margin, approximately the length of the longest fin ray. The table is based on nine fish of which six were males. The males ranged in length from 260 to 405 mm. with an average of 312 mm. All had well developed pearls. The females averaged 263 mm. in length (210, 215, 365). In order to compare fish of equal length the female average length has been made equal to that of the male and the average fin lengths of the females as obtained from measurements have been corrected in proportion. The figures in the lower horizontal line therefore show in percentages the sex difference in length of fins in fish of the same length. Since the caudals of two of the females were broken there is added for the caudal a corrected percentage (31.1) obtained by comparison of a single male of 340 mm. length with a female of 365 mm.

From the table it appears that in fish of equal length the anal and caudal (lower lobe) of the male are about 31 per cent. longer than those of the female; the pectoral about 18 per cent. longer; the pelvic about 11 per cent. and the dorsal only 5 per cent. In the female the caudal lobes are of about equal length whereas in the male the lower lobe in two perfect specimens averaged about 10 per cent. longer than the upper. The fins appear to differ in robustness in about the same proportion as in length. This is shown by the width at mid-length of the longest anal ray in a male and female of equal length; in the male 4.5 mm. in the

female 2.6 mm. In smaller individuals there is no great difference between the length of fins in individuals of opposite sexes, but these differences appear with increasing size. A male of 135 mm. when compared to a female of 137 mm., had slightly shorter dorsal, caudal and pectorals, but somewhat longer anals and pelvics.

It is to be noted that in adult specimens those fins of the male (lower caudal lobe, anal) that bear the largest pearl organs also exceed the corresponding fins of the female by the largest percentage. (*Cf.* Table I. and Fig. 1.) Indeed the fins of the male may be divided into three groups on the basis of the percentage by which they exceed the corresponding fins of the female; the anal and caudal with 31 per cent. excess; the pectoral and pelvic with 10 to 20 per cent. and the dorsal with 5 per cent. The size of the pearls borne on the fins of these three groups is roughly proportional to these percentages.

When the fish are seen close at hand, or under favorable conditions with field glasses, the pearl organs of the male and the greater size of his anal fin suffice to distinguish him from the female (Fig. 1). But were the sexes identical in form, size and color, the behavior differences described in another place would differentiate them.

5. *Breeding Activities.*—During the breeding season males are at all times much more numerous on the rapids than females and during the greater part of the time none but males are present. It does not follow that males are actually more numerous than females. On the contrary data that I have collected at other localities and at times when the fish were not breeding indicate that the males and females are equally numerous. (Reighard, 1915.) In the breeding season the females do not mingle with the males on the rapids until ready to lay their eggs. But from time to time a female comes from her retreat in the deeper water above or below the rapid or from beneath the bank and takes her place on the rapid. If no males happen to be near she may lie quiet in one place for a considerable time. But if males are near they at once approach her, sometimes one or two, sometimes as many as ten. Pairing is best seen when but two males are involved and will be first described under these

conditions. As the males approach the resting female she hurries forward as though to escape, but presently stops with her belly on the bottom. As the males again approach she hurries forward a second time, but soon stops as before. Thus she appears to be driven here and there over the spawning ground, too "coy" to allow the males near her. After a varying number of apparent efforts to escape her "coyness" vanishes and she rests quietly on the bottom and permits the males to come near. In what follows I describe the spawning behavior as I saw it very many times from the bridge or from the banks of Mill Creek. Details were observed several times in the aquarium (Fig. 4).

When a male comes within a few inches of the waiting female he is often seen to stop, spread his pectorals, erect his dorsal and protrude his jaws (Fig. 4, second fish from bottom). Then, for perhaps a second, his head trembles with a slight, rapid vibration from side to side. The movement is not unlike the tremor of a palsied hand. It is like the tremor that one may produce in his own head by strong continuous contraction of the muscles of the neck. This tremor may be seen not only when a male approaches a female, but often when he approaches another male on the spawning ground. I have never seen it in a female.

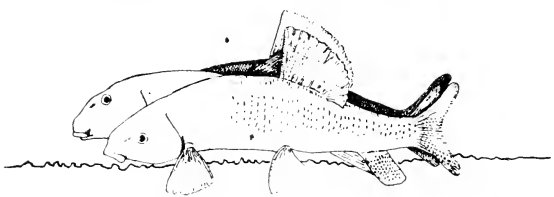

FIG. 2. A female of *Catostomus commersonii* engaged in pairing, with a male on each side of her. The body and tail of one male are shaded. The pearl organs are shown on the anal and caudal of the nearer male, but not elsewhere. The figure represents the pairing act near its end with the head of the female well above the bottom, which is represented by the irregular horizontal line. Drawn with the help of a photograph (see Fig. 4).

It is probably nothing more than a beginning of the tremor of the whole body which accompanies spawning. It necessarily produces a vibration in the water which may be of such a rate as to stimulate other fish through the skin, ear or lateral line

organs (Parker, 1917). In many fishes the spreading of the dorsal at the breeding season results in the display of a conspicuous marking or color pattern (Reeves, 1907 and citations). This is true of some of the minnows to be described in later numbers of this series. *It is noteworthy that in the suckers, although the dorsals are unmarked, the display movement* (Fig. 2) *does not differ from that of those fishes that have a conspicuous dorsal.*

As the male approaches the female another change becomes evident. The dark stripe on his side (Fig. 4), which may have become rosy a little while before, now suddenly turns to a vivid scarlet and remains so during the pairing. At the same time the eye becomes red and continues so while the female is present.

The males with fully extended pectorals and erected dorsals now press close against the female one on either side (Figs. 2, 3).

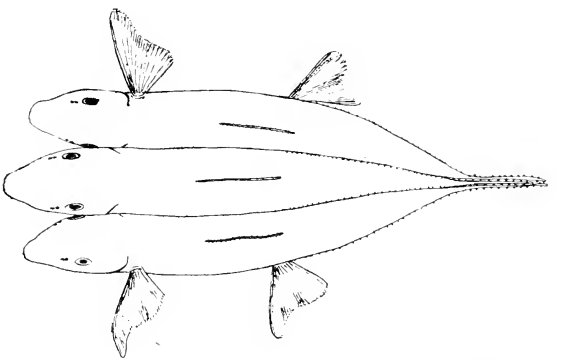

FIG. 3. Diagram showing the pairing of *Catostomus commersonii* as seen from the dorsal side. Compare Fig. 2.

The smallest males that I have seen pairing were about six inches long. The two are usually of the same size and commonly are a good deal smaller than the female. But they are not always of the same size and one or both may be as large as the female.

When the males are in position (Figs. 2, 3) their adjacent pectorals are spread beneath the female. Their caudal fins press on either side against that of the female, but may extend for some distance behind and below it, so that their distal parts press against each other. Their anals are spread and extended

downward so that they press against the sides of the tail and sometimes against the anal of the female. The backs of the males are arched and their dorsal fin-rays, spread like the ribs of an extended fan, stretch the membrane between them (Figs. 2, 3).

The lateral surfaces of the anal and caudal and the sides behind the dorsal, all of which are roughened by pearl organs (Fig. 1) are thus pressed vise-like against the female so that she is firmly held (Figs. 2, 3). But the roughened caudals and anals of the two males also press against each other where no part of the female separates them. Thus the pearl organs aid males to keep their positions with reference to the female as well as to each other.

When the fish have come into position there is a rapid vibration of the whole bodies of all three together. This is wide and vigorous behind the dorsal fin, while in the region of the head it is a little more than a tremor. At this time the fish are often in water so shallow that their backs are exposed. The powerful movement of the tails of the three fish stirs up the gravel and a cloud of sand is released and washed downstream. At this time, in the aquarium, one may see milt spurt from the genital openings of both males and cloud the water. No doubt the eggs are extruded at the same instant and buried in the gravel, but the water is made so turbid by sand and milt that I have not seen the eggs laid. I have estimated the length of the spawning act at a second and a half but have not actually timed it. It is often repeated especially by the larger males. Large males are often taken in which the front edge of the anal fin is raw and worn by rubbing against the gravel. I have seen nothing of the sort in females, but their anal fins are smaller than those of males and are protected by them during the spawning act, while the number of pairings of the average female can be but half that of the average male.

When a pairing act is completed the female moves on, usually upstream, and presently pairs with other males on another part of the breeding ground. Her eggs are thus scattered in small lots over a considerable area, very likely over more than one rapid and are commonly fertilized by many pairs of males. The two

males separate, the red stripe on their sides and the red in their eyes fades, but the white occipito-lateral stripe remains for some time. Each male now moves about and feeds as before. And so they continue until another female appears when one or both may succeed in pairing with her and this may happen on any part of the spawning ground. Thus the eggs fertilized by one male may lie anywhere in the gravel of a rapid or in that of several rapids. The breeding activities are in no way centered about individual males, for the coöperation of two males in pairing makes it impossible to know what eggs are fertilized by an individual male.

I have never collected from the bottom the eggs laid at a single pairing. But the smaller fish lurking in the neighborhood tell one plainly enough where they are. The black-nosed dace (*Rhinichthys atronasus*) and the rainbow darter (*Etheostoma cæruleum*) gather at once in great numbers over the spot where the pairing suckers were. They come in a straight line from down stream attracted, no doubt, by the trail of milt, eggs or bottom materials swept down by the current. They gather in an area six or eight inches across and each burrows in the bottom with its snout as though seeking eggs. The whole little area is soon concealed by their wriggling tails, close-set like threads in the pile of velvet. Some of the eggs may have been swept down stream, but many of them must be buried where the small fish are rooting.

When more than two males follow a female (Fig. 4) it may be difficult to see what happens. When she finally stops the two males nearest or most vigorous in the pursuit attempt to pair with her. But the others at once crowd about and try to force their way between her and her mates. They try either to squeeze in at the sides of the female from above so as to force her mates outward, or to wedge themselves beneath the pairing males from the side so as to force them up and take their places. But once the two males have the female firmly held between them it is difficult to dispossess either and I have never seen this happen. As many as ten males have been seen with a single female during the spawning act, and the act was nevertheless completed; but often, when many crowd about, she interrupts

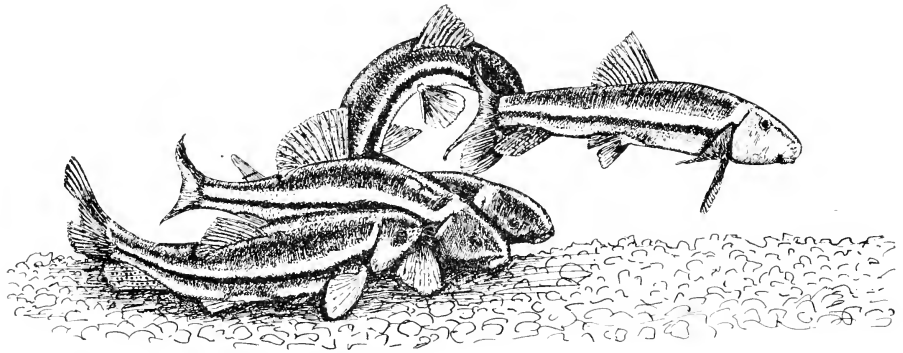

FIG. 4. Group of breeding *Catostomus commersonii*, consisting of one female and four males. The second fish from the right, with head only showing, is the female. One male has taken position on the right side of the female while another with his head hidden by the female's body is coming into position on her left. The males at the right and left are supernumerary. Pearl organs are visible on the anals of two males. Drawn from a photograph of fish in an aquarium.

the pairing and moves upstream as though seeking an opportunity to pair unmolested. This she may secure and the pairing then takes place in the manner already described. Although supernumerary males may be present and may attempt to interfere they take no part in the normal pairing. While they crowd about the female and attempt to gain a place at her side, there are no combats between them. When not at the side of the female they seem to pay no attention to one another.

When the breeding season is over the male suckers lose their pearl organs. They were beginning to shed them in southern Michigan on May 13, 1913. Both sexes become uniformly olivaceous on sides and back. They are no longer commonly seen on their breeding grounds, and in Michigan most of them seek deeper waters.[1]

B. The Common Red-Horse (*Moxostoma aureolum* Le Sueur).

1. *Breeding Grounds and Breeding Season.*—In southern Michigan the red-horse breeds in May. My two dates are May 17, 1904, and May 4, 1905. On these dates the breeding season of the white sucker was nearing its end. While the breeding grounds of the two species are the quieter, upper parts of rapids in shallow water with gravel bottom and, while the two species often breed in the same rapids, I have not found the red-horse in streams as small as those sometimes frequented by the breeding white sucker. The sucker may spawn in brooks so small that one may step across them but I have found the

[1] In Walnut Lake Hankinson (1908) reports the species in water 15–40 ft. in depth in summer and a few were found in a restricted part of the lake in water 80 ft. in depth in April and May. I have found it in Douglas Lake, Cheboygan County, Mich., from July to September in water up to 45 ft. in depth (Reighard, 1915). Smallwood (unpublished notes) reports the return of suckers to Lake Clear after breeding in Sucker Brook. On the other hand Forbes and Richardson (1908) say: "It is with us essentially a species of creeks and smaller rivers, nearly four times as common, according to our data in the former as in the latter. . . . Our collections show that it is much more likely to be abundant on bottoms with more or less rock and sand than on a completely muddy bottom and that it has also a decided preference for clear, swift water." Without a knowledge of the dates at which Forbes and Richardson's collections were made or of the size of the fish taken it is not possible to say to what extent adult suckers collected by them occur on the rapids at other than the breeding season. Certain it is that they are abundant in the deeper water of some inland lakes of Michigan and in the Great Lakes when not breeding.

breeding red-horse in streams not less than thirty or forty feet in width.

2. *Sexual Differences.*—There are no known color characters by which the sexes of the red-horse may be distinguished with certainty at any season but there are structural differences. According to Forbes and Richardson (1908) the lower fins are "longest in the male." Table II. has been made in the same way as Table I. (See p. 8.) The averages were obtained from five males and two females, all breeding fish. It shows that all the fins of the male are longer than those of a female of the same length with the possible exception of the caudal. The caudal appears to be 5 per cent. shorter in the male. But since some of the caudals are imperfect a comparison was made of a single perfect male of 205 mm. with a perfect female of 280 mm. on the basis of equal length. This shows that the upper lobe of the caudal is 12 per cent. longer in the male and the lower lobe 33 per cent. longer. The latter value is included in parentheses in the table. The anal and pectoral are longer in the male by about 15 per cent. The dorsals and pelvics are longer by about 10 per cent. The lower lobe of the caudal of the male is not only longer than that of the female, but about 14 per cent. longer than the upper lobe. In the female the two lobes are of equal length.

TABLE II.

SHOWING IN MILLIMETERS THE AVERAGE LENGTH OF FINS IN MALES AND FEMALES OF *Moxostoma aureolum* OF EQUAL LENGTH, THE DIFFERENCE IN AVERAGE LENGTH OF FINS AND THE PERCENTAGE DIFFERENCE.

	Length, Tip of Snout to Base of Caudal, Mm.	Caudal Lower Lobe, Length.	Anal, Length.	Dorsal, Length.	Pelvic, Length.	Pectoral, Length.
Average for 5 males, M.....	239	56.8	50.4	47	36.2	49.2
Average for 2 females, F.....	239	60	44	43	33	31.5
Difference, D = M − F	000	− 3.2	+ 6.4	+ 4	+ 3.2	+ 7.7
Percentage of Difference, % D = (M − F): F.....	000	− 5.3 (+33)	+14.5	+ 9.3	+ 9.4	+15.8

The breeding males are further distinguished by the possession of conspicious pearl organs. (Figs. 5, 6.) On the end of the snout and sides of the head as far as the caudal margin of the preoperculum are numerous large sharp-pointed organs, more

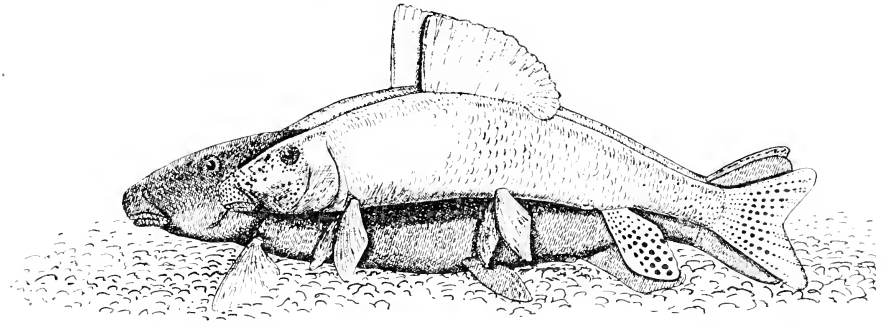

FIG. 5. A female and two males of *Moxostoma aureolum* pairing. The male on the female's left shows the large pearl organs on the caudal and anal, on the sides of the tail and on the head. The smaller pearl organs on the scales of the sides of the male are not represented. The males are not yet in their final position, in which the paired fins are spread, and the pectoral thrust under the female's body. Compare Fig. 6.

than two hundred in a well-developed specimen. On the sides of the anal and caudal fins and on the tail for a little distance in front of the caudal fin are large disc-shaped organs (not pointed). Smaller, pointed organs occur on all the scales of the sides and back, on the dorsal, on the upper surface of the pectoral and on both surfaces of the pelvic. All these organs are effective in proportion to their size. Here again the caudal and anal fins of the male, which exceed those of the female by the largest percentage, bear the largest and most effective pearl organs. It is probable that further data would make possible a more precise statement of the relation between size of pearls and relative size of fins.

This species is one of the few in which pearl organs have been noted in the female. In a single specimen I have found minute organs on the top of the head and on the first few scales of the back behind the head. They were especially numerous about the upper end of the opercular opening. They were too small to be effective and care was needed to see them at all.

3. *Coloration.*—At 2:30 P.M. on May 17, 1904, I found ten to twelve red-horse lying quiet in shallow water near the bank of Mill Creek, near Ann Arbor. The water was smooth and I was able to come within ten feet of them and watch them with field glasses from an elevated position. The fish were from twelve to fourteen inches long from tip of snout to tip of tail.

The red-horse, seen at other seasons, whether in its native waters or in aquaria, has the sides and back in both sexes uniformly olivaceous, but somewhat darker above. The belly is smoky white. The sides show tinges of salmon in front of the dorsals and the lower fins have some orange near the base.

The red-horse before me were of such exceptional coloration that they were at first not recognized. Pectorals, ventrals and anals were bright salmon. Along the sides and running forward above the eye was a white stripe similar to that of the white sucker. It was more prominent in the darker colored individuals but in none of them was a red stripe visible beneath it as in the white sucker. In a few individuals infrequent, elongated white spots were seen above the lateral stripe and running lengthwise of the back. With field glasses pearl organs were visible on

anals and caudal. With good lighting these could be seen with the naked eye at a distance of ten or twelve feet. Subsequent observation showed that all these fish were males.

4. *Breeding Activities.*—The fish were quiet most of the time, but now and then one dropped downstream a few feet and then slowly returned to his original position. Two of them were seen to pick stones from the bottom as though feeding. After an hour and a half a female joined the group of males. She was longer and relatively thicker bodied than the males, not spotted with white on the back and without visible pearl organs. She was at once approached by five males, two of which took position one on either side of her while the other three crowded down from above. One of the upper males was seen to vibrate his tail for a moment but no actual spawning took place at the time. The fish remained grouped for but an instant and then separated. The female went upstream a little way and then dropped back among the males. The group reformed, but immediately broke up again. In these aborted attempts at spawning it was noted that after a single male had placed himself by the side of a female a second male, upon approaching on the same side, turned at once to the unencumbered side. He behaved as though he discriminated between the sexes of the two fish, but by what means this was accomplished I could not tell.

The female again went upstream but this time to a greater distance and followed by two males only. When she had come to rest on the bottom one of the males approached and placed himself by her side. After half a second the other male took his place on her opposite side and spawning occurred very much as in the white sucker (Figs. 5, 6). The backs of the two males were strongly arched so that their dorsal fins were carried well above that of the female and fully spread. Their caudal and anal fins were close pressed against those of the female and against each other as in the white sucker. Their snouts were turned inward and pressed close against the sides of the head of the female below her eyes (Figs. 5, 6).

While the males were in this position with backs bowed and heads straining inward and upward the female was held firmly by the functional pearl organs of their snouts, caudals, anals

and tail. The spawning vibrations lasted two or three seconds. The fish then separated and the female went up stream. Repeatedly after this she dropped down among the males, but when approached by them moved away and did not again spawn. The spawning was not accompanied by any change in color (red stripe) such as was noted in the white sucker.

Except in the attitude of the pairing males the breeding be-

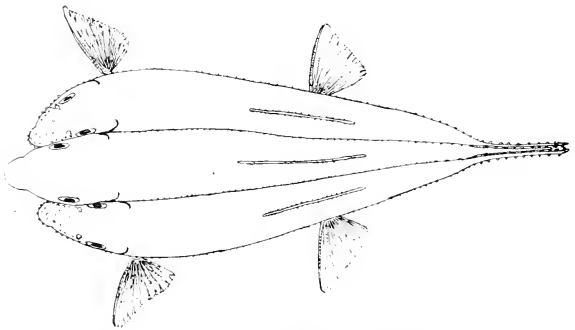

FIG. 6. Dorsal view of a female of *Moxostoma aureolum* pairing with two males. Effective pearl organs are shown. Compare Fig. 5.

havior of the red-horse does not differ essentially from that of the white sucker. The eggs are presumably buried in the bottom as in the white sucker. Those of a single female are scattered in small lots over a considerable bottom area. Those fertilized by a single male are also widely scattered.

C. The Hogsucker (*Catostomus nigricans* Le Sueur).

1. *General Activities.*—*Catostomus* (**Hypentelium**) *nigricans* is known locally in Michigan as the black sucker or pugamoo. Over a wider territory it is known as hogsucker, hogmolly or stoneroller. When not breeding it may often be seen feeding on the rapids of our brooks, creeks and smaller rivers. In feeding, the fish puts its snout under a stone and roots it up or thrusts it sidewise. It then sucks up the slime between the stones and with it obtains immature insects which form its chief diet (Forbes and Richardson, 1908). I have once seen the fish when thus engaged each

accompanied by ten or twelve small shiners (*Notropis*) and by an occasional *Campostoma*. These formed a little school at his sides and below him, and seemed to be waiting for fragments from his feeding.

2. *Sexual Differences.*—In color the sexes appear to be alike at all times. The back and sides are olivaceous, darker above, with dark irregular cross blotches. The belly is satiny white; the lower fins are dull red. Thus the colors of the fish blend with those of the stony bottom on which it is commonly seen. I have determined the following percentage differences in fin lengths (percentage D = (M − F):F) for males and females of the same size based on a single female and the average of two males: caudal − 4.5, anal 17.4, dorsal − 0.2, pelvic 4.2, pectoral − 2.5. These differences are probably within the limits of individual variation except in the case of the anal. The anal of the male thus shows the greatest excess in length over the corresponding fin of the female. The upper and lower caudal lobes are of about equal length in both males and females.

This is one of the few species in which pearl organs are developed in both sexes. In the male (Fig. 7, lower male) they occur on both surfaces of all the fins, on the upper surface of the head, on the opercle and on every scale of the body and tail except those of the ventral surface. The largest organs are those on the anal fin, on the ventral half of the caudal and on the sides of the tail, especially near the caudal fin. Those of the anal reach a diameter of 0.8 mm. and a length of 0.28 mm. in a fish 12 cm. in length, while on the caudal of the same specimen the organs are about 0.5 mm. wide, high and sharp pointed. On the remainder of the fish the organs are small, 0.08 mm. to 0.25 mm. in diameter. All these organs make the surface rough to the touch and are effective in proportion to their size. It is again noteworthy that in the male the anal bears the largest pearls for it is of all the fins the one that shows the greatest percentage of excess length over that of the female. In the female the organs are smaller than those of the male but have a similar distribution. They are absent from the dorsal fin and sides of the body and from the ventral surfaces of pectoral and pelvic. On the anal they are nearly as large as in the male, while on the

caudal they are somewhat smaller, still smaller on the sides of the tail. Those on the anal are distinctly perceptible to touch, those on the caudal and sides of the tail are barely perceptible while the rest are quite imperceptible. Probably only those on the anal are in any degree effective and they are not sharp. It is evident that neither the coloration, the length of the fins, nor the pearl organs afford means of discriminating the sexes in the field. For this purpose one is compelled to rely on the difference in average size and in behavior.

3. *Breeding Activities.*—I have several times seen a single large hogsucker moving upstream in rapids and accompanied or followed by three or four smaller. In one case the large fish was some twelve inches long and the four following her half as long. Occasionally she stopped and one of the smaller fish placed himself by her side. But nothing further occurred and the fish presently moved on.

My only opportunity to observe the actual spawning of this species was on May 4, 1904, in Mill Creek, near Ann Arbor, at the point at which the spawning of the red-horse had been already seen. I was watching the rapids about 4:30 P.M. when a large hogsucker came upstream followed at a short distance by half a dozen others of two thirds her length. Size and behavior indicated the larger fish in this and other cases to be a female. Presently she stopped and remained quiet on the bottom while the males pressed against her three on either side, so close as to hide every part of her except the head and tip of the caudal fin (Fig. 7). The seven fish remained together for several seconds and during this time the female several times made rapidly repeated movements of protrusion and retraction of the mouth. She was not seen to make any other movement nor was any seen in the males. After remaining thus grouped for a fraction of a minute the fish moved on, the female leading. Two somewhat larger males now approached and when the female again stopped these added themselves to the other six, so that the eight of them formed a complete mantle over her back and sides from which only her head and caudal projected. Again the female was seen several times to make rapidly repeated movements of the mouth. The fish then passed out of sight on their way upstream, the female still leading.

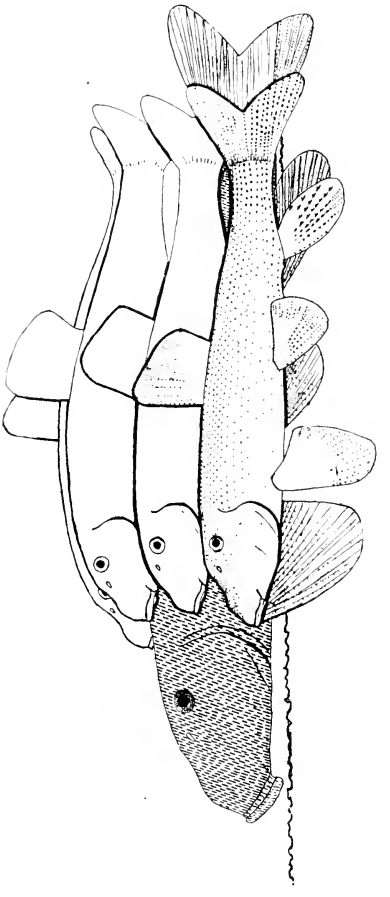

FIG. 7. Lateral view of a female of *Catostomus nigricans* "pairing" with six males, four of which show in the figure. The spots on the lower male and on the fins of the female represent pearl organs. The irregular horizontal line is the bottom.

Although the absence of the vibratory movements characteristic of spawning suggests that the fish whose behavior is described did not actually spawn, the attitudes can be no other than those of spawning fish. These attitudes can be the more readily maintained owing to the general distribution of pearl organs over the surfaces of both sexes. Not only is the occurrence of these organs on the female exceptional, but their distribution in the male is unusual in that they are found on the lower surfaces of the pectoral fins. Males which are above the female presumably have the lower surfaces of the pectorals in contact with her back and sides while those at the sides of the female have the upper surfaces of their pectorals in contact with her belly.

Assuming that the spawning behavior was observed it differs from that of the white sucker in the following particulars. (1) The act is not preceded by change of color; the fish show the same color characters as at other seasons. (2) The act was participated in by more than two males. If it be objected that all but two of the males present were supernumerary, and that the spawning was aborted by their presence as in the case of the white sucker, it may be replied that the fish remained in position long enough to spawn and that those above maintained their positions quietly and did not attempt to displace those below. The whole appearance was that of spawning except for the single feature of lack of vibration.

Summary of Observations.

A. *The White Sucker (Catostomus commersonii).*

1. The white sucker breeds in southern Michigan in April and early May.

2. The fish then congregate in the shallow swift water of small streams where the bottom is gravel and sand.

3. At this time both males and females have a yellow-white occipito-lateral stripe not known to occur at other seasons.

4. This stripe is whiter in males than in females and below it on the sides males have a dark lateral stripe often of rosy tinge. The backs of males are often flecked with white not observed in females.

5. The fins of the males exceed those of females of equal

length by the following average percentages; anal and lower lobe of caudal 31 per cent., pectoral 18 per cent., pelvic 11 per cent., dorsal 5 per cent.

6. Effective pearl organs occur in the male on the anal and lower lobe of the caudal on the upper surface of pectorals, on both surfaces of pelvics and dorsals and on the scales of the sides behind the dorsal (Figs. 1, 2, 3).

7. The size of the pearl organs on the fins of the males is roughly proportional to the percentage by which these fins exceed in length the corresponding fins of the female.

8. Females are without pearl organs.

9. In the field males may be distinguished from females by their coloration, by their larger anal fins, by the possession of pearl organs and by behavior.

10. The smallest males and females observed breeding were about six inches long.

11. The males congregate on the rapids and the females remain apart from them in the immediate neighborhood and come to them one or a few at a time.

12. When a female comes to a rapid she is pursued by two or more males.

13. The female intermittently flees from the males but finally stops and permits their approach.

14. The pairing is preceded by a characteristic tremor of the head of the male and preceded or accompanied by a spreading of the fins and a change in the color of the dark lateral stripe from black to a brilliant red.

15. The fins of the males are without conspicuous colors or patterns; yet they are spread by the pairing males just as in certain other species of teleosts in which the spreading displays conspicuous colors or patterns.

16. The female "pairs" with two males at one time (Figs. 2, 3).

17. The two males may be of unequal size and both may be smaller than the female.

18. In the pairing position, one male lies on either side of the female with his pectoral fin spread beneath her and his dorsal elevated. The pearl organs of his sides and of his caudal and anal fins are then in contact with the sides, caudal and anal of the female (Figs. 2, 3).

19. The caudal and anal fins of the two males may project beyond those of the female so that their distal portions are in contact with each other by their roughened surfaces (Figs. 2, 3).

20. The roughened surfaces of the males aid them to hold the female between them.

21. By their contact with each other the roughened surfaces of their caudals and anals may aid the two males to maintain their positions with reference to each other.

22. Spawning is accomplished during a rapid vibration in unison of the bodies of all three fish. This agitates the bottom material and causes its lighter parts to be swept downstream by the current.

23. During spawning milt is seen to spurt from the genital openings of the males and cloud the water.

24. After spawning the fish separate and each may repeat the act many times in various places and in combination with various individuals.

25. Small fish usually gather at once over the area in which the spawning occurred and there crowd together in a small space.

26. These fish root in the bottom with their snouts and appear to be eating eggs that have just been buried.

27. At each pairing act supernumerary males may be present and may attempt to supplant those actually engaged in pairing (Fig. 4).

28. At the close of the breeding season, the pearl organs are shed and both males and females become uniformly olivaceous in color.

29. Outside the breeding season the mature fish are not seen on the rapids in great numbers but in Michigan are abundant in lakes in deeper water.

B. The Red-Horse (Moxostoma aureoleum).

30. Red-horses congregate on the rapids in southern Michigan in May in situations like those occupied by white suckers, but not in streams as small as some of those in which the white suckers breed.

31. In both sexes at this time there is a white lateral stripe,

not observed at other seasons. It extends forward above the eye.

32. The backs of certain males show elongated white flecks running lengthwise, but they do not show the red lateral stripe characteristic of the white sucker at the moment of pairing.

33. The fins of males are longer than those of females of equal length by the following average percentages; lower lobe of caudal 33, anal 14.5, pectoral 16, pelvic 9, dorsal 9.

34. The lower lobe of the caudal of the male is about 14 per cent. longer than the upper lobe.

35. Effective pearl organs occur in the males on all the scales of the back and sides; on both surfaces of caudal, anal, pelvic and dorsal; on the upper surface of the pectoral and on the sides and top of the head to the end of the snout (Fig. 5).

36. Minute non-effective pearl organs occur occasionally in females on the top of the head and on the first few scales of the back behind the head.

37. Of all the fins of the male, the caudal and anal, which exceed the corresponding fins of the female in length by the largest percentage, bear the largest and most effective pearls.

38. The males may be distinguished from the females on the breeding grounds by the pearl organs, by length of anal fin, by behavior and in some cases by differences in coloration (white flecks on the back).

39. The behavior of the fish on the breeding grounds is like that of the white sucker in the particulars stated in paragraphs 11, 12, 13, 14 (except color change), 15, 16, 17, 18, 19, 20, 21, 22, 24, 27, 28.

40. The attitude of the pairing males during spawning differs from that of the white sucker (par. 18) in that the backs are more arched and the snouts with their covering of pearl organs are pressed against the sides of the head of the female (Figs. 4 and 5).

41. In the position indicated in paragraph 40 the female is held not only between the tails, caudals and anals of the two males but between their heads as well.

C. The Hogsucker (Catostomus nigricans).

42. The hogsucker has been seen to breed in early May on the grounds used by the red-horse.

43. No difference in the coloration of the sexes has been noted at any season.

44. The males observed were on the average much smaller than the females.

45. The anal fins of the male are on the average about 17 per cent. longer than these of females of equal length. The remaining fins do not differ greatly from those of the female.

46. Both sexes are provided with pearl organs which are larger and more widely distributed in males than in females.

47. Effective pearl organs occur in the males on both surfaces of all the fins, on the upper surface of the head, on the opercle and on every scale of body and head except those of the ventral surface (Fig. 7, lower male).

48. Pearl organs occur in the female in the same situations as in the male except that they are lacking on the head, sides of body, dorsal fins and lower surfaces of the paired fins. Those of the anal are effective.

49. Of all the fins the anal of the male exceeds that of the female in length by the largest percentage. It also bears the largest and most effective pearls.

50. In the field the sexes may be distinguished by difference in average size and by behavior.

51. The breeding behavior is like that of the white sucker in the particulars enumerated in paragraphs 11, 12, 13, 15, 20, 24.

52. In the hogsucker not less than six nor more than eight males have been seen to "pair" with a single female at one time.

53. In the spawning position one male lies on each side of the female and others place themselves above these and against the sides and back of the female so as to form a mantle about her, from which her head and tip of her caudal may project (Fig. 7).

54. The distribution of pearl organs generally over body and fins in both sexes is such that in the spawning position the surfaces of males in contact with females and with other males as well as certain of the surfaces of females in contact with males are more or less roughened and the fish are thereby the better able to maintain their positions.

Conclusions.

The breeding behavior of three species of suckers has been studied, the white sucker (*Catostomus commersonii*), the red-horse (*Moxostoma aureoleum*), and the hogsucker (*Catostmus nigricans*). All three make use of similar breeding grounds, the upper parts of rapids, where the water is moderately swift and the bottom gravel and sand. They differ in that some of the streams in which the white sucker breeds are smaller than any frequented by the other two species.

It is possible to discriminate the sexes of all three species on the breeding grounds either by differences in coloration, size of body and fins, pearl organs, behavior, or by some combination of these characters. In all three species the males are seen to congregate on the rapids while the females linger in the neighborhood and enter the rapids at intervals, usually singly, sometimes two or three at once. When a female has come to the rapid she is at once pursued by as many males as happen to be near. She flees, stops, flees as the males again approach, and so continues for some time alternately fleeing and stopping. Sooner or later she comes to rest on the bottom and permits the males to approach. When these have come to her, in the case of the white sucker and the red-horse, two of them pair with her at one time, one on either side. The fins of the males are without conspicuous colors or patterns. Nevertheless during pairing and just before it the males spread their fins after the manner of fish which thereby display conspicuous colors or markings. The display movement occurs, although there is nothing to display. Supernumerary males may approach and by crowding those attempting to pair may interrupt the pairing act. In the hogsucker six or eight males may pair with the female at one time. All appear to take an equal part so that no supernumerary males can be distinguished.

In all three species pearl organs occur on the male and in the hogsucker effective organs occur also on the female. The largest and most effective organs on the fins of the male occur on those fins that exceed the corresponding fins of the female by the greatest percentage of length. In all three species the pairing attitudes are such as to bring into contact with the female those

surfaces of the males roughened by pearl organs. There is at the same time more or less contact with one another of the roughened surfaces of coöperating males and of those of the female of the hogsucker with pairing males. Thus the pearl organs aid the pairing fish to keep their positions with relation to one another in the swift water during the vigorous vibrations which characterize spawning. These vibrations are very pronounced in the white sucker and red-horse but have not been observed in the hogsucker. They continue in the white sucker for about a second and a half and during that time the tails of the fish agitate the bottom and the lighter bottom materials are swept down stream by the current.

When the pairing white suckers have separated, their eggs are left buried in the bottom. This is inferred from the behavior of the numerous minnows which congregate over the spot. The similarity of spawning behavior indicates that the eggs of the other species are buried in similar fashion.

The females of the species studied deposit eggs in various parts of the breeding ground and in doing so each pairs with many males. It results from the breeding activities that the eggs of a single female are widely scattered and are fertilized by many

wAz	*yBz*	*zCw*	*xDz*
wCx	*yDx*	*wAy*	*xBz*
yBx	*yAx*	*zDy*	*wCy*
wDz	*wBx*	*zCy*	*xAz*
xAy	*xDw*	*wBy*	*yCx*
xCz	*zBw*	*xAw*	*wDy*

FIG. 8. Showing the distribution of pairings and their character over the spawning area in the case of suckers in which two males pair with one female. *A*, *B*, *C*, *D*, females; *w*, *x*, *y*, *z*, males.

males. It results further that the sperm of a single male fertilizes the wide-scattered eggs of many females. The coöperation of at least two males in pairing with a single female makes it impossible to know the male parentage of a given embryo. This same coöperation makes it impossible that the eggs deposited in any small, continuous bottom area should be fertilized by one male. They are in fact not fertilized by one pair of males. The rela-

tions of the sexes are as indiscriminate as they well can be. We may represent four females of the white sucker or red-horse by the letters *A*, *B*, *C*, *D*, and four males by the small letters *w*, *x*, *y*, *z*. Six pairs may be formed with the four males, *wx*, *wy*, *wz*, *xy*, *xz*, *yz*. Assuming that each female spawns once with each pair of males and that her spawnings are distributed at random over the breeding ground we should have some such space-distribution of pairings as shown in Fig. 8. In the hogsucker in which one female pairs with more than two males the relations would be still more complicated.

No male occupies any particular locus of the spawning ground and attempts to defend it against other males. On the contrary each male is free to wander over the whole spawning ground. He may "pair" in any part of it, for he does not enter into combat with other males but coöperates with them. The female does not restrict her activities to any part of the spawning ground. Were she to do so she would be finally beset by so many males that normal pairing might be difficult or impossible. She does not actively reject any of the males, but when beset by so many that spawning is difficult, she seeks a new locus. This she continues to do until normal pairing becomes possible. She may be said to try various situations of the spawning ground until she finds one in which the conditions permit spawning. This relation of the sexes is neither polyandry nor polygamy. It is promiscuity, corresponding to the hypothetical communal marriage of primitive man. It occurs along with lack of combat amongst the breeding fish. Where combat occurs, as in minnows, promiscuity gives place as will be shown in later papers, to a sex relation that approaches polygamy.

LITERATURE CITED.

Boulenger, G. A.

'04 Teleostei, systematic part, in The Cambridge Natural History, Vol. VII., pp. 541–727. London.

Forbes, S. A. and Richardson, E. R.

'08 The Fishes of Illinois, pp. cxxxi–357, text and atlas of 103 maps, with 40 colored plates, 15 black and white plates, and 76 figures in the text. Urbana.

Hankinson, T. L.

'08 A Biological Survey of Walnut Lake, Michigan. A Report of the Biological Survey of Michigan, published by the State Board of Geological Survey as a part of the report for 1907, pp. 157–288, pl. XIII.–LXXV., 6 text figs. Lansing.

Parker, G. H.

'17 The Reception of Mechanical Stimuli by the Skin, Lateral-line Organs and Ears in Fishes, Especially in *Amiurus*. *Am. Jour. Phys.*, XLV., 463–489. Published also as Contribution from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, no. 298. Cambridge.

Reeves, Cora D.

'07 The Breeding Habits of the Rainbow Darter (*Etheostoma cæruleum* Storer), a Study in Sexual Selection. BIOL. BULL., XIV., 35–57. 3 text figures Lancaster.

Reighard, Jacob.

'04 Further Observations on the Breeding Habits and on the Function of the Pearl Organs in Several Species of *Eventognathi*. Science, Vol. XIX., p. 212.

'15 An Ecological Reconnoisance of the Fishes of Douglas Lake, Michigan, in Midsummer. Bulletin of the U. S. Bureau of Fisheries, Vol. XXXIII. (for 1913), pp. 219–249, map and ten tables. Document no. 814, U. S. Bureau of Fisheries. Washington.

ROUGHOID, A MUTANT LOCATED TO THE LEFT OF SEPIA IN THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER.

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ORIGIN OF THE CHARACTER, ROUGHOID (ru).

In March, 1919, Dr. A. H. Sturtevant handed over to me a culture of *Drosophila melanogaster*, containing a new mutant, named by him "roughoid," owing to its similarity with the old character "rough."

In December, 1918, he had collected a single pair of wild flies near Columbia, S. C. The F_2 generation from these was found, two months later (February 14, 1919), to contain many roughoids (culture 5570 A.H.S.). Hence one of the wild flies must have been heterozygous for the mutant gene. The point of special interest in connection with roughoid is that its locus is to the left of that of sepia and therefore establishes a new left end of the map of the third chromosome.

DESCRIPTION OF THE ROUGHOID CHARACTER.

This gene produces somatic effects mainly upon the size and texture of the normal eye; (*a*) it materially decreases both the length and width of the eye as well as making it more convex; (*b*) the ommatidia lose their hexagonal shape, and become crowded together irregularly; (*c*) due to this irregularity, the hairs project in all directions from the surface; (*d*) the hairs are somewhat thickened and strikingly longer than in the normal eye. These effects are very constant in appearance and have proven to be favorable for purposes of classification. Associated with these effects there are found sometimes, (*e*) several black ommatidia distributed over the surface of the eye, but more commonly in the posterior ventral region. They tend to darken the normal red eye color. They are more than likely a part of the roughoid character since I have never seen this effect in any

fly other than one that was also roughoid. However, their occurrence is very inconstant, most roughoid eyes being as light red as the normal wild type.

Chromosome Carrying Roughoid.

The first experiment (culture No. 142) consisted in out-crossing a roughoid male to a female showing the second-chromosome recessive vestigial.[1] The F_1 generation flies were all wild type, thus proving roughoid to be a recessive character. By inbreeding the F_1 flies, F_2 was obtained. One quarter of the F_2 vestigials showed the roughoid character (culture No. 142*b*). The presence in F_2 of the double recessive class proves that roughoid cannot be located in the second chromosome. At the same time, I out-crossed a roughoid female to a dichæte hairless male from stock (culture No. 141). These two characters are third-chromosome dominants. By back-crossing the F_1 dichæte hairless females to roughoid males from stock, evidence was obtained that roughoid shows linkage relations with these mutants in the third chromosome. The size relation of the various classes indicated that roughoid was considerably to the left of dichæte. As the back-cross flies were not hatching out as well as expected, I did not complete the count of the cultures of this experiment.

A cross between roughoid and the original rough gave in F_1 only wild-type flies, showing that roughoid was neither rough nor an allelomorph of rough.

Location of Roughoid in the Third Chromosome.

The next step was to make up a stock containing both the new character, roughoid, and sepia (located at the extreme left end of the then known third chromosome). In the third generation from a cross between roughoid and sepia, I obtained such a stock. Since I had obtained roughoid and sepia together in such a short time, I concluded that the two were not very closely linked. By using the back-cross method, I obtained the following results:

[1] This female was from the stock known as "5 ple" and carried in addition to vestigial, the second-chromosome recessives black, purple, arc and speck. These other characters were disregarded in the F_2 classifications.

$$\text{♀} \frac{ru \quad se}{+ \quad +} \times \text{♂} \frac{ru \quad se}{ru \quad se}.$$

July 24, 1919.

Culture No.	ru se.	+	ru.	se.	Total.	Crossovers.	Crossover Value.
601	133	79	31	27	270	58	21.5
602	99	98	38	37	272	75	27.5
606	106	87	33	37	263	70	26.6
607	56	68	26	15	165	41	24.8
608	71	67	22	26	186	48	25.8
Total	465	399	150	142	1156	292	25.2

$$\text{♀} \frac{ru \quad +}{+ \quad se} \times \text{♂} \frac{ru \quad se}{ru \quad se}.$$

May 24, 1919.

Culture No.	ru.	se.	ru se.	+	Total.	Crossovers.	Crossover Value.
405	71	77	16	36	200	52	26.0

The total of these cultures (1,356 flies) shows the cross-over value for roughoid and sepia to be 25.3.

Again, a dichæte female (known to be heterozygous for roughoid) was backcrossed to a roughoid male from stock. From that experiment, I found the cross-over value for roughoid and dichæte to be 35.9, as follows:

$$\text{♀} \frac{ru \quad D}{+ \quad +} \times \text{♂} \frac{ru \quad +}{ru \quad +}.$$

July 10, 1919.

Culture No.	ru D.	+	ru	D.	Total.	Cross-overs.	Cross-over Value.
605	59	64	41	28	192	69	35.9

This indicates that roughoid is situated to the left of sepia, since the normal distance between sepia and dichæte is known to be about 11.7 units.

Three-Point Back-Cross—ru se × D.

In order to place the gene more accurately, a three-point back-cross was undertaken. A sepia roughoid female was crossed to a dichæte male, the F_1 dichæte females were then back-crossed to roughoid sepia males from stock (pair matings being made). Results:

$$\text{♀}\ \frac{ru\ \ se\ \ +}{+\ \ +\ \ D} \times \text{♂}\ \frac{ru\ \ se\ \ +}{ru\ \ se\ \ +}.$$

July 7, 1919.

Culture No.	0.		1.		2.		1, 2.		Total.
	ru se.	D.	ru D.	se.	ru se D.	+	ru.	se D.	
604	82	82	17	30	16	18	1	0	246
611	45	51	15	24	14	12	2	0	163
Total	127	133	32	54	30	30	3	0	409
	260		86		60		3		

The results give a cross-over value for roughoid and sepia of 19.5 and 25.1 respectively, or a mean of 21.7; a cross-over value for sepia and dichæte of 14.2 and 17.2 or a mean of 15.4.

Four-Point Back-Cross—ru se × D H.

One other back-cross experiment was performed, using the four characters roughoid, sepia, dichæte and hairless. From this, 980 flies were counted, giving the cross-over value for roughoid and sepia as 25.6.

$$\text{♀}\ \frac{ru\ \ se\ \ +\ \ +}{+\ \ +\ \ D\ \ H} \times \text{♂}\ \frac{ru\ \ se\ \ +\ \ +}{ru\ \ se\ \ +\ \ +}.$$

August 12, 1919.

Culture No.	0.		1.		2.		3.		1, 2.		1, 3.		2, 3.		1, 2, 3.	
	ru se.	D H.	ru D H.	se.	ru se D H.	+.	ru se H.	D.	ru.	se D H.	ru D.	se H.	ru se D.	H.	ru H.	se D.
609	52	64	23	24	17	14	11	14	2	0	10	6	1	6	2	1
610	53	64	21	18	16	9	22	18	0	0	3	5	4	6	0	2
612	30	54	14	19	10	16	7	18	1	0	6	7	4	3	0	0
Total	135	182	58	61	43	39	40	50	3	0	19	18	9	15	2	3
	317		119		82		90		3		37		24		5	

$$\text{♀}\ \frac{ru\ +\ D\ H}{+\ se\ +\ +} \times \text{♂}\ \frac{ru\ se\ +\ +}{ru\ se\ +\ +}.$$

May 24, 1919.

Culture No.	0.		1.		2.		3.		1, 2.		1, 3.		2, 3.		1, 2, 3.	
	ru D H.	se.	ru se.	D H.	ru.	se D H.	ru D.	se H.	ru se D H.	+ .	ru se H.	D.	ru H.	se D.	ru se D.	H.
404.....	61	75	24	42	25	20	20	9	0	0	4	12	1	4	1	5
	136		66		45		29		0		16		5		6	

Summing up the data in the previous tables:

Culture No.	Total Flies.	ru se Value.	se D Value.	D H Value.
404.....	303	29.0	18.4	18.4
609.....	247	28.3	17.4	20.6
610.....	241	20.3	15.3	24.9
612.....	189	24.8	17.9	23.8
Total.....	980	25.6	17.3	21.6

The value for dichæte hairless (21.6) is approximately the expected result (24.8). Sepia dichæte crossing over is noticeably high, since the average normal value is 11.7 units. This is possibly due to a complicating factor, linked to the new factor, roughoid, and "stretching out" that part of the third chromosome. More work will have to be done before this can be certain. It will be noticed that the double cross-over class (1, 2) is lower than the triple cross-over class (1, 2, 3). This is unexpected and remains unexplained at the present time.

Summary.

(*a*) A new mutation has occurred in the third chromosome of *Drosophila melanogaster* to the left of sepia, which for several years has been the leftmost of the known loci.

(*b*) There was a roughoid sepia cross-over value of 24.9, based on a total of 2,748 flies of which 685 were cross-overs.

(*c*) Associated with roughoid, there is possibly a linkage modifier increasing the crossing over between sepia and dichæte, and perhaps between roughoid and sepia as well.

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BIOLOGICAL BULLETIN

NOTES ON THE BIOLOGY OF SOME COMMON LAMPYRIDÆ.[1]

WALTER N. HESS.

CONTENTS.

INTRODUCTION.

The fireflies (family Lampyridæ) are among the most common of insects, yet because of the larval habits of most species, comparatively little is known regarding them except what has been learned from a study of the adults. The larval forms are rarely seen, as most of them are active only at night, and usually are found on, or in the ground, in damp or marshy regions.

The insects were observed both in the field and in specially prepared large plant-pots at the insectary. These pots were filled about half full of rich mellow earth on which was placed a small amount of moss. Some of the pots were covered with glass, while others were covered with cheese cloth. Since oviposition occurred very readily in captivity, eggs were obtained for a study of the incubation periods of the different species, by confining ripe females in small jars that had been partially filled with earth. For a study of the feeding habits of the larvæ and adults, these insects were confined in glass jars, some of which

[1] Contribution from the Entomological Laboratory of Cornell University.

contained only a small amount of moist filter paper, while others contained earth and moss. Since no reference was found to a published account of the nature of the mouth-parts of any of our native firefly larvæ, these structures were figured in order to better illustrate the method of feeding of these larvæ.

The following species were studied: *Photinus consanguineus* Lec., *Photinus scintillans* Say, *Photurus pennsylvanica* DeGeer and *Pyropyga fenestralis* Mels.

The author is indebted to Dr. James G. Needham, Dr. William A. Riley and Dr. O. A. Johannson, under whose supervision the greater part of this study was made, for their helpful suggestions and criticisms.

History of Biological Work on Lampyrids.

Although many workers, both in this country and in Europe, have studied the light-organs of the fireflies, comparatively little has been done on the biology of this group of insects.

Newport (1857) studied the life-history of the glow-worm, or larva, of *Lampyris noctiluca*. He not only discussed the development and general habits of these larvæ, but he also performed several experiments to determine the nature of their feeding habits.

Hudson (1891) published an interesting account of the habits and life-history of the New Zealand glow-worm.

Barber (1905, 1914) described the egg-laying habits of Phengodes, together with certain habits of this group of fireflies.

Bongardt (1904) published a brief account of the biology of certain European Lampyridæ.

Knab (1905) described the habitat, flight and light-emission of *Photinus scintillans* and *Photurus pennsylvanica*.

McDermott (1910, 1911, 1912, 1914) studied especially the nature of the flashing of fireflies, together with the attraction of the sexes by means of light-emissions.

Olivier (1911) described the distinguishing structural characters of the common species of fireflies. In addition, he discussed their general habitats and distribution.

Mast (1912) studied the sexual attraction of fireflies (*Photinus pyralis ?*) with special reference to their orientation. He found

that the female, in responding to the flash of the opposite sex, always turned the ventral side of her abdomen, so that it would emit light in the direction of the male.

Fabre (1913) maintained that the glow-worm, in feeding on snails, injected a substance in the nature of an anæsthetic which paralyzed its host.

Vogel (1915) did by far the best biological work that has been done on this group of insects. He described the external and internal anatomy of the larva of *Lampyris noctiluca*, together with its life-history. He observed that the larva lived in the ground, and that it fed on snails. By making a careful study of the structure of the mouth-parts, pharynx and gizzard he found that the digestive juices of the mid-intestine were emitted through the hollow mandibles. By this means the larva was able to paralyze its prey, and to digest the tissues before eating them.

Haddon (1915) described also the process of feeding and the nature of the mouth-parts of *Lampyris noctiluca*.

Blair (1915) and *Morse* (1916) reported the interesting phenomenon of the synchronous flashing of fireflies, in which the fireflies in a given locality were found, at times, to flash in unison.

Williams (1917) described the life-history of several of our common Lampyrids. His discussions of the biology of *Photinus consanguineus* and *Photurus pennsylvanica* are especially valuable.

Photinus consanguineus Lec.

The insects of this species are elongate and slender with the head covered by the prothorax. The prothorax is rounded on the anterior and lateral sides, truncate behind with the angles acute. It is light yellow with a black median bar, which is bordered with pink on either side. The elytra have wide side margins and bear two or three sub-obsolete carinæ. The suture and side margins are pale yellow, while the remainder of the elytra is grayish in color. They are granulate and rather pilose. The abdomen in the male is depressed, but in the female it is often rounded, due to being distended with eggs. The eyes of the male are larger and better developed than those of the female. The light-organs of the male cover the entire sternites of the sixth and seventh abdominal segments, while in the

female the organ occupies only a small area on the sixth abdominal segment. They measure from 8 to 12.5 mm.

LeConte reports them from Massachusetts, Pennsylvania and Virginia. Blatchley lists them for Indiana, and Williams found them abundant in Massachusetts.

The biology of this species has been well described by Williams (1917), so an attempt will be made here to discuss only a few of the more important features.

The adults begin to emerge about June 1 and can be found along moist areas until about the first of August. They are frequently found in association with *Photinus scintillans* and *Photurus pennsylvanica*. The males are active fliers, and though the females have well developed wings they were never found in flight. The flight of the males begins about 8:15 P.M. and continues until about 10:00 P.M. (old time). The light of the male is a single bright flash, though at times he emits two or three flashes in rather close succession, but in every case there is a considerable interval between each flash. The female of this species crawls up a stem of grass, or some similar object, and emits a faint flash in response to the flash of the male. Just before emitting the light, however, she turns her abdomen so that the ventral side is in the direction of the male, thus in part, at least, obviating the necessity for larger light-organs.

What was discribed as the synchronous flashing of fireflies was first discussed by Blair (1915), who reported observing fireflies, in a certain locality, flashing in unison. Later his observations were confirmed by other writers. Morse (1916) reported an observation in which the light emitted by these little creatures pulsated in a regular synchronous rhythm, so that at one moment the tree, about which they were flying, would be one blaze of light, while at another the light was dim and uncertain.

According to Blair (1915) and McDermott (1916), this phenomenon does not occur among the American species of *Photinus* and *Photurus*. The writer, however, observed the flashing of fireflies in unison on two very dark evenings during the present summer while collecting eggs and larvæ at Ithaca, New York. Toward the south side of the City Cemetery is a small valley,

and on both occasions the entire valley, for a moment, was a blaze of flashing lights, and then for a moment it was in darkness, except for an occasional flash, which seemed to come from fireflies of different species than those that were flashing in unison. The fireflies in this particular locality were almost entirely of the species *Photinus consanguineus*, and at each period of flashing both males and females were observed to emit light. On both nights this phenomenon occurred shortly after it became dark, at approximately 9:00 P.M. On the first night the phenomenon was observed for approximately fifteen minutes. How much longer it continued after that was not determined. By very careful observation it was discovered that each period of flashing started on the crest of the hill at the south side of the valley, by one, or only a very few flashes, and that the impulse stimulated by these few insects instantly appeared to sweep over the valley, resulting in the great mass of flashing lights. On the second night an experiment was performed in which it was discovered that, by standing on the side of the valley and causing short flashes with a pocket flash-light, the fireflies of the entire valley responded. At first, after estimating the length of the latent period, the flash-light was flashed just before the normal time for the fireflies to flash, with the result that the entire mass of fireflies responded. Then two flashes were emitted from the pocket flash-light with the interval between flashes reduced to about three fourths that of the normal flashing period, and the fireflies responded with apparent equal results. Finally, the period was reduced to approximately one half the normal time. The fireflies as a mass appeared to respond to the first short flash. The second time a large per cent of them responded, but after this second short period, the flashing in unison was so disturbed that each insect flashed independently of the flashing period of the others. Blair (1915), in commenting on the reason for the synchronous flashing of fireflies, states: "The flashing in unison is too regular to be caused by chance puffs of wind. A more probable explanation of the phenomenon is that each flash exhausts the battery, as it were, and a period of recuperation is required before another flash can be emitted. It is then conceivable that the flash of a leader might act as a

stimulus to the discharge of their flashes by the other members of the group, and so bring about the flashing of the whole family." From the observations and experiments performed above, it seems evident that the theory of a leader is the most probable. Although the author has done extensive collecting of these insects during the past four summers, at no time was this phenomenon observed on the part of any of our other native species.

The small, smooth, spherical eggs are laid on the soil at the base of the roots of grass and moss where they hatch in about twenty to twenty-two days. The larvæ are slender, elongate, and of a rather uniform dark grayish color. They were found chiefly a short distance below the surface of the soil, though a few specimens were taken at the surface. It seems, however, that its habitat is subterranean, rather than terrestrial, in contrast to that of *Photurus pennsylvanica*. They are predacious, feeding on snails, etc., similar to the other species studied.

It seems very evident that the insects have a two-year life cycle, as both mature and half-grown larvæ were taken at the time of pupation.

Pupation takes place in the soil near the surface of the ground. Here the mature larvæ excavate a little chamber, in which the period of transformation is spent. After transforming to pupæ they lie on their backs in an arcuate position. In this condition they measure about seven mm., but when straightened out they are about nine mm. long. The pupæ are yellowish white in color with the pleural regions somewhat pinkish. The pupal period lasts from twelve to fifteen days.

Photinus scintillans Say.

The adult beetle of this species is rather elongate and slender, somewhat flattened with the head completely covered by the prothorax. The antennæ are eleven-segmented, the second segment being short and transverse. The prothorax is rounded anteriorly and along the sides, truncate behind with the angles acute. The elytra have wide side margins. The head is black. The prothorax is pale yellow, except the small black median bar on its central posterior half, which is bordered with pink. The elytra are pale yellow, except the side and suture margins which

are a lighter yellow. They are finely granulate and pubescent. The females resemble the males except that they are slightly larger and their abdomens, frequently distended with eggs, project considerably beyond the elytra. The eyes of the male are larger than those of the female. In the male the light-organs cover the entire sternites of the sixth and seventh abdominal

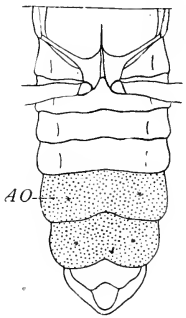

FIG. 1.

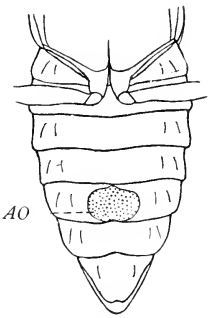

FIG. 2.

FIG. 1. *Photinus scintillans* male, ventral view of abdomen. The shaded portion on the sixth and seventh abdominal segments represents the adult light-organ (*AO*).

FIG. 2. *Photinus scintillans* female, ventral view of abdomen. The shaded area on the sixth abdominal segment represents the adult light-organ (*AO*).

segments, while in the female the organs occupy only a small area at the center of the sixth sternite (Fig. 1 and Fig. 2). The beetle measures from 5.5 to 8 mm.

These insects were found by LeConte in Massachusetts, Pennsylvania and Kansas. Blatchley found them in Indiana.

The natural habitat of this species appears to be very similar to that of *Photurus pennsylvanica*. Yet, however, they are much more widely distributed, and are often found in drier localities than is characteristic of this species. They are very common about central New York, being our most common firefly. They were found very abundant at Ithaca, New York in the City Cemetery on Stewart Avenue, and, in fact, on the campus, and on the lawns near many of the private houses.

The period of emergence for this species varies from about June 1 to July 20, depending on the season.

During the daytime the adults are rarely found, as they remain hidden underneath the leaves of low herbage, or near the ground covered by grass or moss.

Neither sex was observed feeding, so it is not known whether these insects eat as adults or not, but it is probable that they do since they live for two or three weeks.

The flight of these insects begins very early in the evening, considerably earlier than that of the other native luminous species. Almost with the first sign of twilight they are flying about, and because it is comparatively daylight the beetles themselves can be distinctly seen. In fact, when they begin flying, their flashes appear rather faint, due to the daylight obscuring their brilliancy. Before total darkness sets in, the flight of these insects has ceased, unless for an occasional one here and there. These few stragglers, however, soon stop. Among the fireflies there seems to be, to a certain extent, an evening periodosity of flight. This species of fireflies flies only during twilight, after which other species as *Photinus consanguineus* and *Photurus pennsylvanica* take its place. The flight begins shortly after 7:30 P.M. and continues until about 8:30 P.M.

In comparison with most of our luminous species of fireflies, the males of this species fly very low and slowly, and emit one distinct flash at each period of flashing. Females were never taken in flight and it seems probable that they never fly. Instead, they climb to near the top of some projecting blade of grass, or some similar object, and apparently remain quiet until a male comes near. Like the male, the female emits only one flash, but it is much less distinct.

There can be little doubt but that the light-emissions among these insects serve as definite signals between the sexes, by which means the male is able to find the female of his species. Numerous observations confirming this were made, which can be well illustrated by the following example: on the evening of June 30, 1916, a male *Photinus scintillans* was observed flying about two feet above the ground, and a second after he flashed a female that was almost underneath him flashed. He appeared to drop directly to the ground, but his velocity of flight carried

him about a foot beyond where she was resting. He at once quickly ascended the nearest stalk of grass, seemingly expecting to find her near its top. He then began to descend, at which time he emitted a flash of light and she responded. He then rushed up another stalk of grass that was in her direction and, not finding her, he flashed again. She responded, and he flew in her direction but alighted nearly a foot beyond her, and he then immediately ascended the nearest stalk of grass. Flashes were exchanged for a period of twelve minutes, during which time he ascended about twenty stalks of grass, and he flew in her direction five different times before he finally found her. Each time she responded to his flash, and each time she orientated her abdomen so that the light was emitted in his direction. It was 8:13 P.M. when he found her, and at once copulation took place. This lasted until 8:58 P.M. when they separated, and at once crawled down the blade of grass to the ground where they were concealed by the vegetation. On another occasion, while the male was in the grass about a foot from a female in his search to find her, a small pocket flash-light was used, in which it was found that she would readily respond to a very short flash. As soon as the pair was in copulation the flash-light was again flashed several times, at different intervals, but with no response. After the pair separated the flash-light was again flashed, but with no results. After copulation took place neither one of the pair was observed to flash, and after separating they concealed themselves in the grass, without emitting light, all of which seems still further to prove that the light-organs serve to bring the sexes together, and having accomplished this end, they are no longer functional until at some possible later date. In a very few instances flashing was observed on the part of one of the copulating members, but it seemed to occur only when they were disturbed, which was the exception rather than the rule.

The pocket flash-light referred to above was used with good success in collecting females. They usually responded when the light was at a distance of at least eight feet, but rarely responded when it was nearer.

Oviposition usually took place about one week after emergence

and continued for a period of two or three weeks. As the eggs of the females do not all ripen together, they are deposited over a considerable period of time. Like the other species studied the small, whitish eggs were deposited on the ground, at, or near, the base of moss and grass. They were usually laid singly, though in a few instances they were found in masses. When deposited they were covered with an adhesive substance, which caused them to adhere to the object on which they were placed. The period of incubation occupied from eighteen to twenty-one days.

In this species, as in all the others studied, the eggs appeared very slightly luminous at the time of laying, but this faint luminosity disappeared in about a day and there was no more light emitted from the eggs until the larval light-organs became functional shortly before hatching.

The newly hatched larva is whitish, except for the black lateral eyes and brownish mouthparts. It soon becomes pigmented, appearing dull gray in color. At this stage it measures about 2.4 mm.

The mature larva resembles in general shape that of the first instar. It is elongate and narrow, varying from 12 to 13 mm. in length. Its head is small, being about half as wide as the prothorax. Like the other species studied, the head can be withdrawn into the thorax. The body is widest in the region of the thorax and tapers gradually posteriorly. The head is black, the tergites dull gray, and the pleural regions slightly pinkish. The habitat of the larva is largely subterranean, though it is usually found near the surface. Its feeding habits resemble very closely those of *Photurus pennsylvanica*. It is not active during the day.

The larvæ were not reared from eggs to adults, yet while collecting these insects each spring, larvæ of two sizes were found: some that were mature and others that were about half as large, indicating that the insect, probably, has a two-year life cycle.

Pupation usually takes place near the surface, although it sometimes occurs under stones. The pupal period is rather brief, taking from nine to twelve days. The pupa assumes an arcuate position, lying on its back within the pupal cell. It

measures about 8 mm. in this arcuate position, but when straightened out it is about 10 mm. long. The lateral tergites project slightly and each bears a group of short setæ. The body is somewhat flattened, being yellowish white in color except along the lateral sides of the thorax and adbomen, which are slightly pink. The larval light-organs function throughout the pupal period and do not degenerate until shortly after the emergence of the adult. The adult light-organs, which develope independently of the larval organs, become functional shortly before the adults emerge.

Photurus pennsylvanica DeGeer.

This is one of the largest of our native fireflies throughout central New York, and though very common in certain moist localities, it is by no means our most common species.

The adult insect is elongate, somewhat flattened, with the head partially covered by the thorax. The head is rather rounded, and slightly narrowed behind the large convex eyes. The antennæ are eleven-segmented, slender and tapering, extending about half the length of the body. The prothorax is rounded anteriorly and along the sides, and subtruncate posteriorly. It is dull yellow with a central and basal dark stripe, while the disc at each side of the dark area is red. The surface is rather coarsely punctate. The elytra are elongate, extending considerably beyond the end of the abdomen. They are brownish except the lateral margins and a narrow tapering area extending from the anterior part to beyond the center, which is a dull yellow. The body is covered with short yellowish pile. The length ranges from 12 to 15 mm. An illustration of the male is shown on Fig. 3.

The sexes are similar in form except that the female is slightly larger than the male. The light-organs of the male cover the entire sternites of the sixth and seventh abdominal segments (Fig. 4), while these organs occupy only about two-thirds of the corresponding region in the female (Fig. 5). In the male the abdomen ends in a point while that of the female is truncate on the tip.

This insect is widely distributed throughout North America.

According to LeConte (1851) it is abundant in every part of the United States. Blatchley states that it is the most common firefly in Indiana. Williams also makes a similar statement for Massachusetts.

As is characteristic of our luminous fireflies, the adults of this

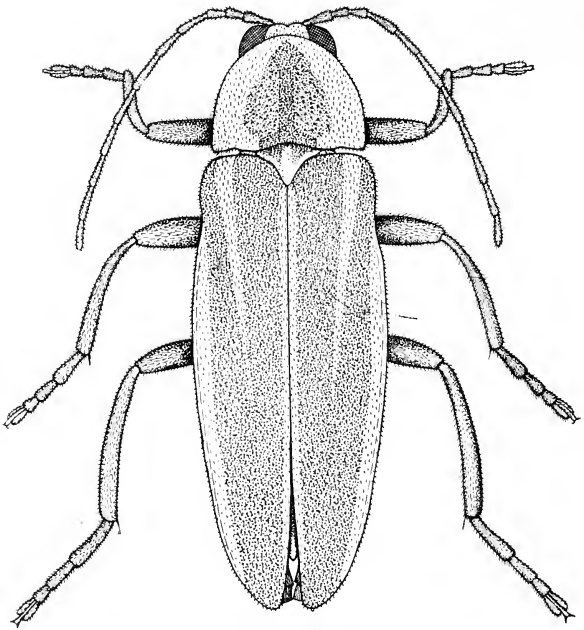

FIG. 3. *Photurus pennsylvanica* male.

insect are usually found active only at night. Like many other insects, this species has well-defined centers of distribution, being rarely found except along marshy or moist localities. This, however, is more characteristic of the larvæ, but as a rule the adults are found comparatively rarely outside of such regions. They were found most abundant in the City Cemetery, on Stewart Avenue, and in the Renwick marshes, at Ithaca, New York. The cemetery, while not in any sense marshy, has been filled in

with dark loamy soil, which is usually moist in the depressions along the walks and between the graves.

The period of emergence for this species, in this locality, extends from June 5 to July 15, depending on weather conditions.

During the daytime the adults remain in seclusion, usually at the base of moss or grass, although occasionally specimens were found clinging to the underside of leaves of low vegetation.

Many insects during their adult life eat little or no food, but the adults of this species, especially the females, are very vora-

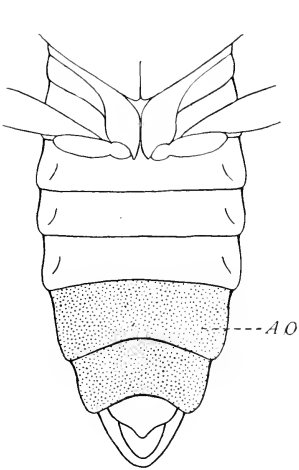

FIG. 4.

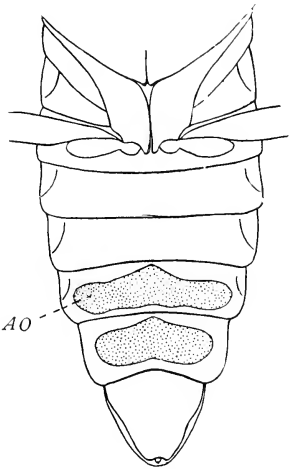

FIG. 5.

FIG. 4. *Photurus pennsylvanica* male, ventral view of abdomen. The shaded portion on the sixth and seventh abdominal segments represents the adult light-organ (*AO*).

FIG. 5. *Photurus pennsylvanica* female, ventral view of abdomen. The shaded portion on the sixth and seventh abdominal segments represents the adult light-organ (*AO*).

cious in their feeding habits. During the evening, while they are active, they are either found flying, or on the ground, usually about the base of grass, actively in search of food. The females were commonly observed devouring other species of fireflies (*Photinus scintillans*, *Photinus marginellus* and *Photinus con-*

sanguineus). In each case, however, the firefly being devoured was a male, which had probably attracted the *Photurus* by means of its frequent flashing, as the females of these species flash very rarely, except in response to the flash of the male. In captivity the female was often found devouring the males of her own species, and occasionally a member of her own sex. The males were not found feeding, though it seems probable that they also are predatory at times.

Though both sexes fly readily, comparatively few females were found on the wing during the early part of the season, although later (about July first to fifteenth), when it seemed evident that there was a less abundance of males, the females were found flying fully as much as those of the opposite sex. The flight began about 8:15 P.M. and continued until after 10:00 P.M., although by that time comparatively few were flying. Some have been observed on the wing as late as 1:00 A.M. Unlike our other native species, these fireflies frequently fly high and their flashes can often be seen in the tops of the highest trees.

The flashing of this firefly is very distinctly different from that of any of our other native species. Like the other fireflies, these have definite periods of flashing. The male flashes three, four, and even five times in rapid succession at each period. The flashes are bright, although they become less distinct at the end of each period. In the case of the female the number of flashes at each period is reduced to three, two or one. There is usually a longer period between the flashes than in the male and they are less distinct. These periods of flashing occur at rather regular intervals of about eight to ten seconds. The male flashes slightly more frequently than the female. When on the ground the brilliancy and regularity of flashing, on the part of both sexes, seems to be the same as when they are flying.

It is agreed by most students of fireflies that the light-emissions serve to bring the two sexes together, although McDermott (1911) and Williams (1917) seem to doubt that they can serve such a function in this species. In the other native luminous species that were studied there is a definite interchange of flashes, in which the female responds to the flash of the male. In *Photurus* the female is an active flier and flashes frequently,

whether in the presence of the male or not. In no case was there observed a definite exchange of flashes between the sexes, yet it seems very evident that the light-emission functions in bringing the sexes together. On several occasions, while holding females in my hand, males flew to them and they would have alighted had my presence not scared them away, and on two occasions, while holding males, females flew and alighted beside the captured males. This would lead one to believe that there is a definite sexual attraction by means of light-emissions between the sexes of this species, and that the female, having become an active flier, is also attracted to the male. In each case observed where the two sexes were attracted to one another, both continued to flash actively, but in no case was there any evidence that one was responding to the flash of the other. In no case did the female of this species assume a vertical position or expose the abdomen so that the light would be flashed in the direction of the male. It is possible that the brilliancy of the light-emissions on the part of the female has obviated the necessity for such exposure. Even while the females were on the ground the flashes were easily perceptible for a considerable distance.

Copulation was observed in the field on three occasions. Unlike our other native fireflies, these beetles were never observed in copula on, or near, the ground, but while clinging to the leaves of trees, often at considerable height. While in this state the flashing of the light-organs apparently ceased, though one female was found emitting a rather dim continuous glow during this period.

Egg laying usually began about one week after emergence and continued at intervals for a period of about two or three weeks. Several females, which were captured early in the season, were dissected to determine their egg laying capacity. On the average, each female contained about fifty mature eggs, with from seventy-five to a hundred smaller ones, indicating that the eggs were at varying stages of maturity.

The characteristic place for the oviposition by the female is at the base of grass or moss in damp loamy soil. Oviposition was not observed in the field, yet on several occasions eggs were found which had been deposited at the base of the roots of grass

and moss. In confinement, at the insectary, oviposition was observed on several occasions. The female walked slowly over the soil, thrusting out her long ovipositor into the depressions in the earth, where the light yellowish eggs were deposited. Eggs laid by these females were usually deposited singly, though sometimes in masses. Some were placed from one eighth to a quarter of an inch underneath the surface, others on the surface, and some were deposited on the roots, basal stems and leaves of moss and grass. The egg (Fig. 6) is small, nearly spherical, about .7 by .8 mm. in diameter. It is without surface markings, though at the time of laying it is covered with an adhesive surface.

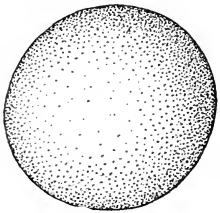

FIG. 6. *Photurus pennsylvanica* egg.

It is frequently stated in literature that the eggs of fireflies are luminous, and Williams (1917) states that the eggs of this species glow when deposited and probably continue to emit light until the time of hatching. The certainty of this statement seems in doubt. At the time of laying, the eggs were found to be very slightly luminescent for a period of two days, but in no case did they, at this period nor until the light-organs of the larva were developed, definitely emit light. Eggs removed from the ovaries of a ripe female showed no evidence of luminosity, though Williams thought he saw a light in some of the eggs that he so removed. Fabre (1913) states that the eggs are luminous even before leaving the body of the mother, but this seems very much in doubt. The so-called luminescence of the eggs at the time of laying is probably due to the substance with which they are covered, rather than to any internal property of their own, and as this becomes dry the slight luminescence disappears. At a period about four days before the eggs hatch the larval light-organs become functional, and from this time until hatching, the eggs emit a distinct light.

In the breeding cages at the insectary where normal outdoor conditions were maintained, the eggs of this species hatched in from twenty-five to twenty-seven days, depending on weather

conditions at different periods. The largest number hatched on the twenty-sixth day. The newly hatched larvæ are not pigmented for a period of a few hours, but they soon resemble the mature larva in shape and appearance, except for their minature size. At this stage they measure about 2.25 mm. in length. As nearly as could be determined the larvæ were in the fourth instar by winter. The first two molts take place rather early in the life of the larva, the first occurring at about the age of two or three weeks. The mature larva (Fig. 7) is elongate, rather narrow, varying from 16–19 mm. in length, and it is about three times as long as it is wide. The head is small, a little less than one third as wide as the prothorax, and it can be withdrawn into a pouch within the thorax. The antennæ are three-jointed, the mandibles are arcuate and notched near the middle. The legs are somewhat spinose and of nearly uniform length. The body is much flattened. The prothorax is rounded on the anterior and lateral sides and subtruncate behind, much the same as in the adult. This is the largest of the body segments. From the metathoraeic segment to the caudal end of the larva the tergites are concave posteriorly especially the caudal ones. Each bears a spine on its caudo-lateral margin. The head, mouth-parts and tergites are colored a dark brown, except for a few irregular pale yellowish areas. The dorsal surface is more or less irregularly coarsely punctate. The last segment of the abdomen is provided with numerous retrac-

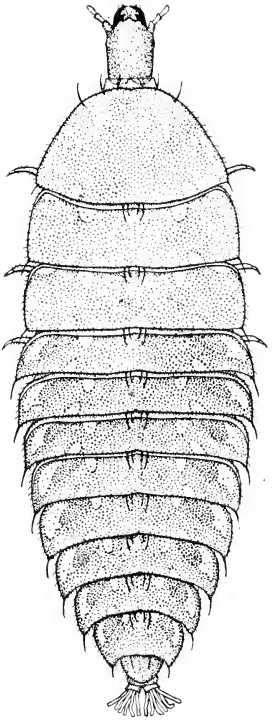

FIG. 7. *Photurus pennsylvanica* larva, full grown, dorsal view.

tile elements, the caudal filaments (Fig. 8, *C*, *F*), which are used in propelling the body forward. On the lateral sides of the eighth abdominal sternite are two luminous areas, the larval light-organs (*LO*).

Resembling to a certain extent the habits of the adults, these larvæ unless disturbed were not found active during the daytime. At this time they are usually underneath stones or concealed in depressions in the ground. At night they become active and their light can be frequently seen as they wander about in their natural habitat. When the ground is smooth a small garden rake is of advantage in collecting them, as they can be easily disturbed by it, thus exposing their light-organs to view. The glow of their light-organs is not visible when the larvæ are lying on the ground, as it can only be seen when the ventral sides of their abdomens are exposed to view. A flash-light is also of assistance in collecting them.

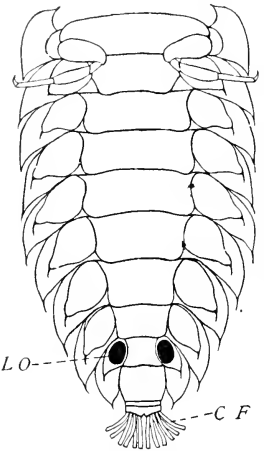

FIG. 8. *Photurus pennsylvanica* larva, ventral view of abdomen. *LO*, larval light-organ; *CF*, caudal filaments.

While in the field, collecting on numerous occasions during the evening, larvæ were found wandering about apparently in search of food. On two occasions they were taken while feeding on snails, which they had evidently killed a short time before being discovered. As the larvæ crawled about, their heads were fully distended, the maxillary palpi and antennæ were constantly in motion, and it appeared as if they were feeling their way by means of these organs. From observations on their movements, even when a snail was very near, it seemed very probable that the larvæ find their food by chance, and having found it they tap it several times with their maxillary palpi and antennæ before beginning to feed. Since newly hatched larvæ and those one-year-old were abundant, efforts were made to determine more in detail the methods of feeding and the nature of their food.

Larvæ taken into the laboratory were placed under as normal conditions as possible, where various experiments were performed to determine the possible nature of their food. In no case were newly hatched larvæ found in the act of killing their prey, though they were observed feeding on bits of snail that was cut up and placed near them. They fed much the same as the older larvæ, and, in fact, there can be little doubt but that their food habits are similar. As the larvæ were not active during the daytime, and as they were disturbed by artificial light, my observations on the feeding habits were largely limited to the larvæ of *Pryopyga fenestralis* which is active during the day. On six different occasions a slug (*Agriolimax campestris* Binney) was placed with six larvæ of *Photurus pennsylvanica* and in every case it had been eaten before morning. A slug (*Agriolimax agrestris* L.) and a snail (*Succinea avery* Say) were put in with six larvæ. The snail was eaten during the first night, but the slug was not killed and eaten until the third night. On two occasions a small earthworm (*Lumbricus terrestris* L.) was placed in a jar, without earth, which contained eight larvæ. One was killed and eaten the second night, and the other on the fifth night. On two occasions a very large specimen of *Limbricus terrestris* was placed with twelve larvæ. In each case the earthworm was not disturbed, though it remained with the firefly larvæ for over a week, and they received no other food during that time. On two occasions a potato-beetle larva (*Leptinotarsa decemlineata* Say) was placed in a jar with six larvæ and each time it was eaten the first night. On two occasions cutworm larvæ (*Paragrotis messoria* Harris, *Paragrotis tessellata* Harris and *Peridroma margaritosa* Haworth) were each placed in jars with six larvæ and in every instance they were eaten the first night. Finally, on four different nights, two second and two third stage squash-bug nymphs (*Anasa tristis* DeGeer) were placed with six larvæ, and in each instance they were eaten before morning. Sowbugs (*Oniscus asellus* Paulmeier), wireworm larvæ (*Agriotes mancus* Say), ants (*Formica* sp.) and coleopterous beetles including the common ground beetles (*Nebia pallipes* Say and *Chælnius pennsylvanicus* Say) were placed in with these larvæ, but they were never eaten, indicating that the

larvæ require a soft-bodied animal into which they can pierce their mandibles and inject the poisonous secretion.

These results, while giving no definite data as to the exact food of these larvæ, lead one to conclude that they probably eat any soft-bodied insect larva, mollusca or annelid, that they happen to find in their nocturnal wanderings. Snails are probably one of their chief foods, and though these animals are not supposed to be very abundant, they were found abundantly at night in the damp regions where these larvæ live. Cutworm larvæ were also abundant. Earthworms, except very small ones, were not eaten until they had been with the larvæ for a considerable length of time, while other food was eaten very readily. This would seem to indicate that probably snails and small insect larvæ, expecially cutworms, are their natural foods.

Among the larvæ of many members of the Lampyridæ, as well as among certain other more or less widely separated groups of insects, digestion takes place entirely, or partially, outside of the body. This is accomplished by the digestive juices being exuded from the mouth upon the food which is later eaten by the larvæ in a more or less completely digested condition.

It is characteristic of most insects that feed in this way to have

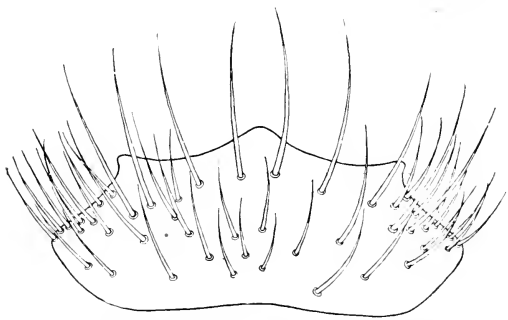

FIG. 9. *Photurus pennsylvanica* larva, labrum, dorsal view.

very small heads, so that the mouth is not sufficiently large to take in only very small pieces of food. These insects, like the larvæ of the Dytiscidæ, are predaceous, feeding on living animal food. The nature of this food is such that it could not be easily

chewed, like vegetable food or decayed animal tissues, and hence it seems that some such method of feeding became necessary. Some have the mandibles grooved, as the larvæ of the Chrysopidæ and Myrmeleonidæ, while others have them pierced by a small canal, such as certain of the larvæ of the Dytiscidæ and Lampyridæ.

The most extensive work on this subject was done by two European workers, Vogel (1912, 1915) and Haddon (1915), although earlier workers made a less detailed study of the problem.

When the head of this insect is withdrawn into the thorax, only the tips of the mandibles and other mouth-parts are visible, but when it is extended the large mandibles surrounded by the other mouth-parts can be distinctly seen.

As the anterior half of the mandibles are exposed on the dorsal side, the labrum lies considerably caudad on the head, extending across the basal portions of these large jaws. It is rounded on the lateral margins, while on its cephalic border are three prominent forward projecting portions with rather acute terminations. Its dorsal side (Fig. 9), shows numerous long projecting bristles while the ventral side is covered with a rather dense mass of small setæ which project forward.

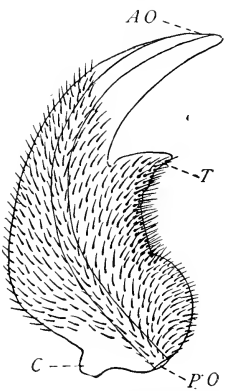

FIG. 10. *Photurus pennsylvanica* larva, left mandible, dorsal view. *AO*, anterior opening of mandibular canal; *C*, condyle; *PO*, posterior opening of mandibular canal; *T*, tooth on inner edge of mandible.

The mandibles (Fig. 10) are very strong. Each has a large curved, anterior tapering tooth and they meet in a median line slightly anterior of the head. On the inner median margin of each mandible is a secondary tooth (*T*). At the base is a knob-like condyle (*C*) by which the mandible articulates with the head. Both mandibles are covered for their entire extent with setæ of varying length, except at the distal end, and around the condyle. About the base is a dense brush of short setæ which project for-

ward. Between this area and the secondary tooth there are larger and stiffer setæ. Those covering the remainder of the surface are rather short and dense. Extending from the base to near its tip is a tubular canal, through which digestive juices

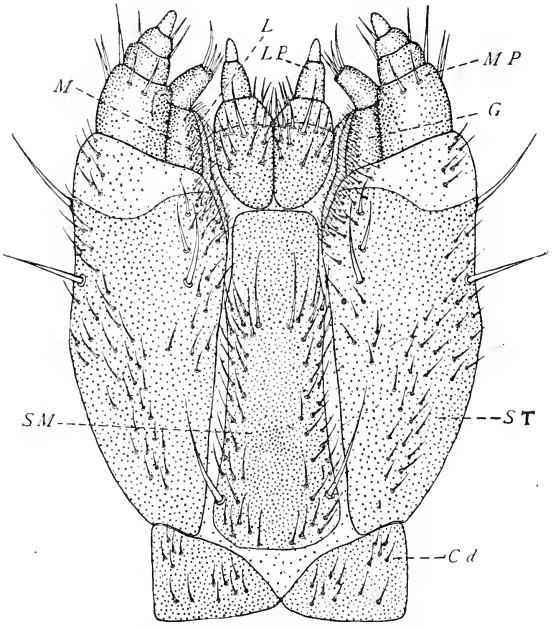

FIG. 11. *Pholurus pennsylvanica* larva, labium, maxillæ and lacinia, ventral view. *LP*, labial palpus of labium; *M*, mentum of labium; *SM*, submentum of labium; *MP*, maxillary palpus; *ST*, stipes of maxilla; *Cd*, cardo of maxilla; *L* lacnia; *G*, galea.

pass, while the larva is feeding. It does not open at the tip but slightly caudad on the outer margin.

The maxillæ, labium and lacinia lie on the ventral side of the head, and in these larvæ they are fused into a flat fleshy plate (Fig. 11). When examined on its ventral side, the maxilla has at its caudal portion a small triangular plate, the cardo (*Cd*), which bears several short setæ. Anterior to it is the large elon-

gated stipes (*St*), which also bears several long bristles and many shorter setæ. Anterior of the stipes on the external side is a stout four-segmented palpus, the maxillary palpus (*MP*), bearing several forward projecting setæ. At the side of the maxillary palpus, internally, is the two-segmented galea (*G*) of the maxilla, which resembles very much in appearance a two-segmented palpus. Beside the galea, internally and also extending a short distance along the stipes, is the rather dense chitinized flattened lacinia (*L*). Both the galea and lacinia bear many setæ, and along the inner margin of the lacinia is a row of rather stiff bristles. The tip of the galea ends in short setæ, which probably function as specialized sense organs.

On the ventral side of the labium, the submentum (*SM*) appears much elongated and it lies in the central region of the mouth-parts. It bears numerous short setæ and a few long bristles. The mentum (*M*) is in the form of a thickened bi-lobed structure, from which project anteriorly the two-segmented labial palpi (*LP*). Both structures bear a considerable number of anteriorly projecting setæ and bristles. On the underside, the sclerites of the maxillæ, labium and lacinia are indistinct, though in this region there are numerous small anteriorly projecting setæ.

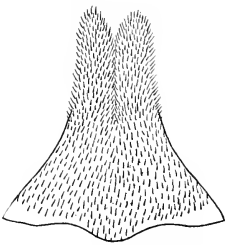

FIG. 12. *Photurus pennsylvanica* larva, hypopharynx, dorsal view.

The hypopharynx (Fig. 12) is triangular in shape with the anterior portion bi-lobed. It is entirely covered with a dense mass of anteriorly projecting setæ, except along its basal portion. The setæ of the anterior half are much denser and longer, and many of them are branched.

From the previous description of the mouth-parts, it is evident that there are numerous setæ projecting forward inside of the mouth region. These setæ, according to Haddon (1915) and Vogel (1915), function as a strainer in preventing all except liquid food from entering the mouth.

In the pharynx region there are several heavily chitinized plates to which numerous muscles are attached, and which,

according to these same authors, function as a suction pump in drawing the liquid food into the mouth. Vogel also described how the mid-intestinal secretions are forced forward, by means of heavy oblique and circular pro-ventricular muscles, into the mouth and out through the mandibular canals.

Though the intestinal juices were not observed passing through the canals of the mandibles, a rather dark-colored liquid was seen suspended from the ends of the long curved teeth and on the bodies of snails and earthworms in the region where the larvæ were feeding. It, therefore, seems very probable that a certain liquid, which, since the larvæ have no salivary glands, must come from the region of the mid-intestine, is exuded through the canals of their mandibles and out through the mouth, and that this liquid functions in paralyzing and digesting the tissues of their prey.

The larvæ were observed, however, to take into their mouths portions of food of considerable size. The fact that they can take up mouthfuls of earth and masticate it in the construction of their pupal-cells is evidence that the larvæ are able to take into their mouths small masses of food before it is completely digested. Yet there can be little doubt but that the greater part of the food is digested outside of the body and taken in through the mouth, in the liquid state. Whether the mandibles function in the intake of food was not determined, but the greater part of it was apparently taken in through the mouth. The portions of undigested food that were taken into the mouth, were no doubt largely digested here before passing on into the intestine, as the larvæ masticated these masses for a considerable time before they disappeared.

The larval light-organs are fully developed at birth, so the larva is luminous from the time that it hatches until it finally enters the pupal state. As stated before, the light is emitted from two elliptical areas on the ventral side of the eighth abdominal segment. The larval light-organs do not emit light in flashes, as the organs of many adults do, but on the other hand, the glow is nearly uniform. While the larvæ were active, the light was found to glow continuously. During the dormant periods, as during the day, but especially during hibernation,

the glow becomes very faint and it frequently is not perceptible even while holding the larvæ ventral side up in a dark room. By moving the insects about so as to agitate them the lights usually become visible. It seems probable that the brilliancy of the glow is in direct proportion to the activity of the larvæ.

The larvæ which were kept in confinement at the insectary were found to go underneath stones, or enter cracks in the soil, late in October, in preparation for hibernation. Some constructed about themselves earthen chambers, while others occupied natural depressions in these protected places. In no instance were they found lying on their dorsal side, such as is characteristic of the pupæ.

During the warm nights of April the larvæ leave their winter quarters and go about in search of food. At this season their little lights can again be seen as they wander about in their natural habitats at night.

Since this species of insect lives as a larva at the base of grass in moist loamy soil, and since it does not enter the ground, or seek other natural means of concealment in which to pass the

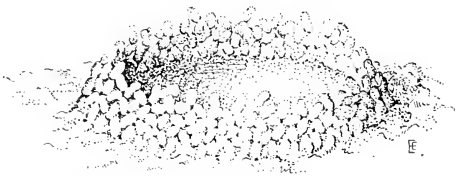

FIG. 13. *Photurus pennsylvanica* partially constructed pupal chamber.

pupal period, as many members of the Coleoptera, as well as some Lampyrids do, it constructs a small earthen chamber in which to pass this period of transformation.

A suitable spot on the surface of the ground, usually at the base of moss or grass is chosen, and at once the larva begins building a lattice work of soft earth over itself, in the shape of a small dome (Fig. 13), by which means it conceals itself, in about a day. In the construction of this cell the larva removes earth from underneath itself by means of its mandibles. This it masticates and mixes in its mouth for a period of about half a minute. It then extends its head to the lattice work of the

dome and regurgitates the moist earth in the form of a short ribbon-like mass, which it applies to the walls of the chamber. By the frequent repetition of this process, the lattice-like framework finally entirely covers the larva (Fig. 14). Even after it forms a complete dome, the larva can be seen for several hours between the meshes, before it is entirely concealed. By repeatedly removing the earth from the bottom of the chamber

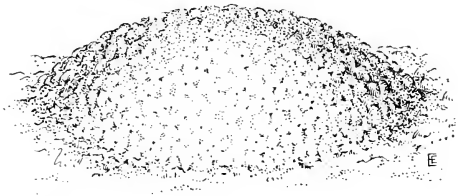

FIG. 14. *Photurus pennsylvanica* pupal chamber completed, lateral view.

and adding it to the inside of the dome-like wall, the chamber is deepened, and its covering is strengthened and made thicker.

The completed chamber is in the form of an elliptical depression in the ground, about one half of an inch in length and about seven sixteenths of an inch in width. Even after the larva conceals itself it continues to add to the walls of the cell until they are from one eighth of an inch, to as much as one half of an inch in thickness.

The time spent in building the pupal-cell is about two days, though larvæ sometimes continue to excavate for three or even four days, making a firmer and thicker covering for their cells.

That the intestinal secretions of the larva are used for moistening the earth, which is used in constructing the cell, there can be little doubt, yet it evidently has no special adhesive content, for the pupal-cells are easily broken, and they seem to offer no more resistance than ordinary earth which has dried, after having been mixed with water. This liquid seems to serve simply as a fluid in which to mix the earth and make it plastic.

From the examination of several of these completed pupal-cells, it seems evident that the method of construction is such as to allow a small amount of air for respiration to pass in and out between their meshes. Some of the domes of the completed

cells, when held against the light, allowed small rays to pass through, giving evidence of their slight porous nature. The pupal-cell, however, makes a sufficiently well-constructed chamber to protect the pupa from drying or other injury. The reason that the larva usually seeks a damp locality previous to pupation is probably for the purpose of choosing a place where excessive drought will not be liable to affect it during transformation.

By the time the pupal-cell is constructed the larva becomes very sluggish, its body becomes distended and in from one to three days the cuticula splits down the anterior half of the back, and the pupa gradually comes forth. From this time,

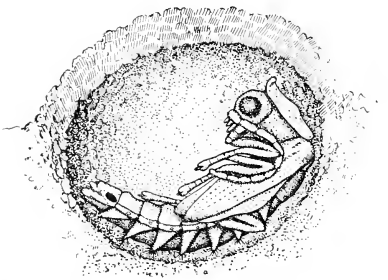

FIG. 15. *Photurus pennsylvanica* pupal chamber completed, internal view with pupa in position.

throughout the entire pupal stage, it lies on its dorsal side within its pupal-cell, and is largely supported by the long lateral setæ, which project from each of the thoracic and abdominal tergites (Fig. 15).

The straightened out pupa measures about twelve mm. and in the arcuate position ten mm. The body is somewhat depressed, with the appendages and wing-pads rather long, and with the lateral tergites drawn out at considerable length. At the end of each of these appendages is a mass of coarse bristles. The color of the pupa is yellowish white. It is quite active and can move about considerably within its pupal-chamber.

Throughout the entire pupal period the light-organs, that were functional in the larva, can be distinctly seen to emit light,

although they do not shine with a bright luminescence unless the pupa moves, or is disturbed (Fig. 16).

The head, thorax, and even the abdomen of the pupa, as well as the newly emerged adult, have been described as luminous (Williams, 1916). It must be admitted that the entire insect appears faintly luminous at these periods, but the cause for it

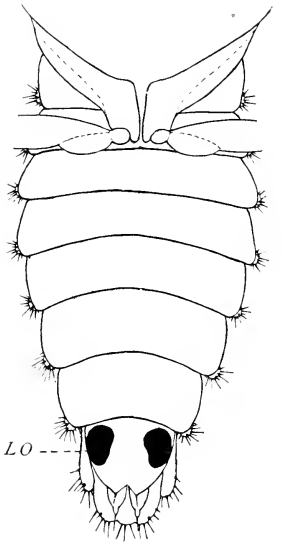

FIG. 16.

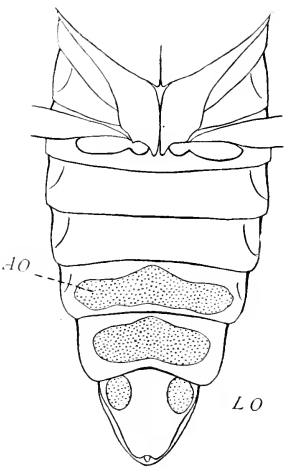

FIG. 17.

FIG. 16. *Photurus pennsylvanica* pupa, ventral view of abdomen. *LO*, larval light-organ.

FIG. 17. Same as Fig. 2, except taken one day after emergence when larval light-organs (*LO*) were still visible. *AO*, adult light-organ.

seems to be due more to the light of the abdominal light-organs shining through the non-pigmented coverings of the insect's body, than to the luminosity of the fat or other internal structures. A freshly molted larva appears much the same as the two stages referred to above.

The date of pupation for this species, at Ithaca, New York, ranges from May 20 to June 15, depending largely on the season.

The extent of the pupal period was found to vary from sixteen to eighteen days under outdoor conditions.

From the fifth of June to about the first of July the pupæ may be found transforming to adults within their pupal-cells. On the first or second night, after transformation, the adult ruptures the pupal-cell and comes forth as a mature insect.

The larval light-organs which are functional during the pupal stage continue to glow until the end of the second day of adult life, when they become fainter and fainter and cease to function.

One species that was reared did not show evidence of the adult organ until after emergence, and then only in the sixth abdominal segment, although the organ in segment seven began to glow a few hours later.

Since the adult light-organs are functional at the time of emergence and since the larval light-organs function for a period of about two days of adult life, there is a brief period during which both organs are luminous (Fig. 17).

Pyropyga fenestralis Mels.

This beetle is elongate, oval and slender. It is entirely black or blackish except for the large pinkish subtriangular space on each side of the black central disc of the prothorax. The head is completely covered by the prothorax. The eyes are small in both sexes. The antennæ are eleven-segmented and slender. The elytra are costate. The sexes are similar except that the female is considerably larger than the male, and her abdomen which is usually distended extends beyond the elytra. The length varies from 6.5 to 10 mm.

LeConte reports these insects from Pennsylvania, Lake Superior Region, Colorado and California. Blatchley found them plentiful in Indiana.

The adults of these fireflies differ from those of the species previously referred to in this paper, in that they are active only during the daytime and not at night. This change in habit can probably be accounted for by the absence of light-organs in the adults. With this one exception, their general habits are similar to those of the other species studied. They are never found in dry localities, being chiefly found in low meadows along streams or marshy areas.

The period of emergence is more prolonged than is characteristic of most species, varying from June 15 to August 10.

During the day these insects can usually be found clinging to grass and weeds by the side of streams. The males are active fliers but the females were not taken in flight, yet it is possible that they fly, for they are sometimes found at a considerable distance from their natural habitat.

The adults were never observed feeding. If they feed at all it is probably very sparingly, for specimens were kept in the laboratory without food from the time of emergence through the period of oviposition.

Copulation was observed to take place from two to ten days after emergence, with frequent repetitions. In the field the copulating pairs were always found about a foot from the ground on a blade of grass or some similar object. The female, early in the season, is usually distended with eggs in varying stages of maturity.

Egg laying starts about four to five days after emergence, and continues for a period of two to three weeks. The females of this species also lay their eggs on the ground at the base of vegetation in damp ground. In every case observed, the eggs were deposited singly.

The egg is small, spherical, whitish in color, measuring about .65 mm. in diameter. It is without surface markings and when first deposited it is covered with an adhesive substance. These eggs, while they are in no sense luminous, give off a slight luminescence when first laid. Under normal conditions they hatch in from nineteen to twenty-one days into little whitish, elongated larvæ.

The larvæ, when first hatched, resemble very closely in appearance those of *Photinus scintillans*, in that they are whitish with dark eyes and brownish mandibles. At this stage they measure about 2.3 mm. in length. On becoming pigmented, however, they appeared dark gray in color.

The mature larva is elongate, narrow, varying from 12 to 14 mm. in length. The blackish head is small, being about half as wide as the prothorax. The body is widest in the region of the thorax and tapers posteriorly. The tergites are dull blackish

in color, while the pleural regions are distinctly pinkish. On the ventro-lateral sternite of the eighth abdominal segment are two luminous areas, the larval light-organs.

Similar to the adults of this species the larvæ are active during the day, though as a rule they are more active at night, when they can be found wandering about on the ground at the edge of streams apparently in search of food. Frequently, however, while searching for them during the day they were found concealed under stones and in the ground to a depth of from one to two inches. On several occasions numbers of them were found assembled together, where they were feeding on a captured snail or earthworm. They were found most abundant at Ithaca, N. Y., on the gravel at the edge of Cascadilla Creek a short distance below the new fish hatchery.

Since these larvæ were active during the day-time, and as they readily took food, it seemed advisable to study more in detail their food habits.

Newport (1857), Meinert (1886), Fabre (1913), Haddon (1915) and Vogel (1915) each studied the food habits of *Lampyris noctiluca*. Newport evidently did not observe closely the mandibles, for he did not mention their hollow nature, which was observed by the other four workers. Newport maintained that the bite of the larva definitely injured the snail upon which it was feeding, while Fabre took a different view, maintaining that the larva injected a substance into its host in the nature of an anæsthetic which paralyzed it, thus making it possible for the larva to feed without being disturbed by the efforts of the snail in trying to escape.

So far as my observations go, I am inclined to believe that the larva does inject a definite substance which serves to paralyze and finally to kill its host. On one occasion a rather small earthworm, about three inches long, was placed with six larvæ. In about two minutes one of the larvæ, as it moved about, feeling its way with its maxillary palpi and antennæ, came in contact with the worm. It touched it several times with these structures, which undoubtedly were supplied with sense organs, and then pierced the worm with its mandibles. The earthworm quickly moved and was evidently slightly injured, but it soon

became quiet. The larva soon released and bit the worm again in the same region. This time the worm moved much less. The process was repeated several times, and each time the worm was less disturbed, until at the sixth or seventh bite the earthworm was not aware of the larva's presence. At about this time another firefly larva bit the worm about an inch away from where the first one was feeding. This part of the earthworm was sensitive, and the worm, though much less active, responded much the same as before. About two minutes later a third larva bit the worm in a still different region, with the result that the worm moved a little but much less than for the other two larvæ. Ten minutes from the time that the first larva bit the worm it apparently was perfectly paralyzed, and so far as could be determined it was dead. Several slugs and snails were observed in the process of being killed by these larvæ. As the reaction of these snails is similar to that of the earthworm, it is sufficient to state that at first the snail appeared to be slightly injured, for in every instance it contracted. In each case, a few minutes after the larva began feeding, the snail was evidently paralyzed for it no longer moved. One of the largest specimens of earthworms obtainable (about seven inches long) was placed in a jar containing moist filter paper with twelve larvæ. Although the worm was left with the larvæ for six days, and though they were given no other food there was no evidence of any effort being made to eat it.

While feeding on its prey, the larva keeps its jaws actively moving back and forth, apparently ejecting digestive juices from its mid-intestine by way of the mandibular canal, and with the two inner teeth on the mandibles it tears the worm to pieces and draws small portions into its mouth. At times the larva would almost bury itself inside the body of its prey, apparently preferring the softer internal tissues to those of the exterior. From an examination of the mouth and intestinal contents of larvæ, which were killed while they were feeding, and from careful observation of their feeding habits, it seems evident that a certain amount of digestive juices are exuded through the mandibular canals into the host, when it is first attacked, which serve to paralyze it. It is also probable that a certain amount of the

digestive juice is constantly being exuded through the mouth as well as by these canals, while the larva is feeding, which serves to break down and partially digest the tissues of the host. Further, it was evident that small portions of undigested flesh, as well as food that was nearly digested, was taken into the mouth, where it was bathed in the digestive juices and worked back and forth by means of the two mandibular teeth, and that liquid food together with very small portions of partially digested food passed into the crop and intestinal region where digestion was completed. As far as could be determined, the mandibular canals did not serve for the intake of foods, as in the Chrysopidæ, but they appeared to function, as far as digestion is concerned, simply as canals for exuding digestive fluids into the host.

At frequent intervals while feeding, certain of the larvæ were observed to extend the caudal filaments, twist the body around, and apply them to the portion of food that was being eaten. It appeared as if the larva was placing some glandular secretion upon the food, or possibly helping to push it into its mouth.

Other experiments were performed to determine whether or not these larvæ limited their food to snails and earthworms. On successive days cutworm larvæ (*Peridroma margaritosa* Haworth, *Paragrotis tessellata* Harris), potato-bettle larvæ (*Leptinotarsa decemlineata* Say), squash-bug nymphs (*Anasa tristis* DeGeer) and wireworms (*Agriotes mancus* Say) were placed in the pots with these firefly larvæ. They readily ate all of the cutworm larvæ, but in no instance were any of the other insects eaten.

From the above experiments it seems evident that these firefly larvæ feed chiefly on snails, cutworms and small earthworms. The other insects that were offered them as food were chosen because they are sometimes found on, or near the ground, and it was desired to see how wide a range of food they would eat.

Although the adults have no light-organs, the larvae have well developed light-organs which resemble very closely, in appearance, those of the other species studied. It, however, appears to be somewhat smaller and emits a less distinct light.

Larvæ were not reared from the egg to the adult, so the length of their life-cycle cannot be given with certainty. Yet, since half-

grown larvæ were found during July and August in association with the mature ones, it seems very probable that the larvæ live for two years before transforming to adults.

Pupation takes place from about June 10 to August 1. The larvæ do not build an elaborate pupal-cell, as the larva of *Photurus pennsylvanica* does, but on the other hand, they crawl back away from the edge of the water, three to ten feet, where they go underneath stones, and there excavate little cells in which the pupal period is spent. The mature larva shortens up slightly and assumes an arcuate position. The cuticula splits down the middle of the dorsal thoracic region and gradually liberates the pupa.

The pupa, except for the yellowish white head, appendages and tip of the abdomen, is of a delicate roseate color. The pleural regions, however, are decidedly pinkish. The abdominal and thoracic tergites are drawn out ventro-laterally into rather acute projections. At the end of each is a mass of setæ. The straightened out pupa measures 8 mm., and in an arcuate position, 6.5 mm.

The pupal period is rather brief, extending for only seven or eight days.

The larval light-organs function throughout the pupal period, but as the time for emergence approaches, the glow usually becomes very faint. In a few specimens the light was observed in the adults for a brief period after emergence.

Purpose of Luminosity.

There can be little doubt but that the chief function of light-emission in insects is to assist in securing the mating of the sexes. It is evident that this has come about as a secondary character when one considers the varying degrees to which the light-organs are developed among Lamyprids. It was suggested by Blair (1915) that possibly the light may be an indication of impalatability. In the case of the adults of *Photinus scintillans*, this does not seem probable, as one frequently finds the females of *Photurus pennsylvanica* and numerous species of spiders feeding upon them. Many adult fireflies when captured emit a pale yellow fluid from between the last coxal joints and from the

pygidium, which it seems may be impalatable to many of their enemies. In the case of luminous larvæ there seems to be little possible use for light-organs, unless it is to warn any possible enemies not to eat them. There are many species of insects outside of the family Lampyridæ that resemble very closely in appearance certain species of fireflies. If the explanation of this mimicry is that of protection, it would seem that the theory of impalatability must have some basis. The exact purpose for the presence of light-organs in larvæ is still indefinitely understood.

Economic Importance.

Practically no work has been done on the possible economic value of these insects, and though the data here given are little more than suggestive, every evidence seems to indicate that these insects are of considerable economic importance. The adults are of little value, as most of them probably eat comparatively little, but the larvæ are voracious little creatures which live on and in the ground, and feed on snails, earthworms, cutworm larvæ, and, in fact, on larvæ of many injurious insects. Most of the soft-bodied animals living on the ground are injurious (unless it is the earthworms), and as the food of firefly larvæ is probably limited to these small animals, they necessarily do much economic good in killing them. The slugs and cutworm larvæ are among our worst economic pests, and it seems evident that they furnish a large part of the food of these larvæ. Since most of the fireflies live two years as larvæ, the number of larvæ that are feeding on the ground during any season is approximately twice that of the adult fireflies. Anyone who has been out during a June or July evening knows that the fireflies are one of our most abundant insects, which, together with the voracious habits of these larvæ leads to the belief that they are of much more economic importance than has been attributed to them heretofore.

Summary.

1. The fireflies studied are luminous both in the larval and adult states, except *Pyropyga fenestralis*, which is luminous only during larval life.

2. In the luminous species the light-organ of the male is

better developed and more brilliant than that of the female. In *Photurus pennsylvanica*, however, the light-organ in the female is nearly as large and as brilliant as that of the male, but in the other luminous species the organ of the female is limited to a small area on the sixth abdominal segment and it emits a rather faint light.

3. The light-organs of the adults undoubtedly function chiefly in bringing the sexes together. The nature of the flash differs for each species, so that members of the same species can readily recognize the flash of the opposite sex. The males and females of certain species, at times, respond to the light of a small flash-light, indicating that this is not always true.

4. In all these luminous species, except *Photurus pennsylvanica*, the female orientates her abdomen so that the light emission is in the direction of the male.

5. In all the species studied the larval light-organ degenerates and, in the luminous species, separate light-organs are developed, which function during adult life.

6. In the case of *Photinus consanguineus*, synchronous flashing was observed in which a few leaders, by flashing, acted as a stimulus to the discharge of the flashes by the others, thus bringing about the flashing in unison of the whole group.

7. The adult fireflies were not observed feeding, except the female of *Photurus pennsylvanica*. She was found to be very voracious, feeding chiefly on the adults of smaller fireflies, but at times she was found to be cannibalistic.

8. The larvæ of all species were found to be predaceous, feeding on snails, earthworms and numerous species of insect larvæ, especially cutworms. They are at times quite voracious, and it seems evident that they are of considerable economic importance.

9. By means of their hollow mandibles, the larvæ eject a portion of the mid-intestinal juices into its host, thereby paralyzing it, and later digesting it so that when the food is taken into the body it is in a digested or nearly digested condition.

10. The larval life, at least, in most of our native fireflies, extends over a period of two years, during which time the larvæ live on or near the surface of the ground.

11. All species that were studied hibernate as larvæ underneath stones, a short distance under the ground or near the surface, often in specially constructed chambers.

12. Pupation takes place in moist earth under stones, or in specially constructed pupal-cells at the surface of the ground. The pupal cell of *Photurus pennsylvanica* is made of short, ribbon-like pieces of earth, which the larva masticates and constructs into a lattice-like dome.

13. The pupæ retain the larval light-organs which function throughout the pupal period. This period was found to be from seven to eighteen days according to the species.

14. The adults live for a period of two to four weeks, during which time they deposit their eggs on the ground about the roots of grass and moss.

BIBLIOGRAPHY.

Annandale, N.

'00 Insect Luminosity. An Aquatic Lampyrid Larva. Proc. Zoöl. Soc. London, pp. 862–865.

Barber, H. S.

'05 Note on Phengodes in Vicinity of Washington, D. C. Proc. Ent. Soc. Wash., VII., pp. 196–197.

'14 On Interspecific Mating in Phengodes and Inbreeding in Eros. Proc. Ent. Soc. Wash., XVI., pp. 32–34.

Bethune, C. J. S.

'68 Luminous Larvæ. Can. Ent., I., pp. 38–39.

Blair, K. G.

'15 Luminous Insects. Nature, XCVI., pp. 411–415.

Bongardt, J.

'04 Zur Biologie unserer Leuchtkäfer. Natw. Wochenschr., Jena XIX., pp. 305–310.

Dahlgren, U.

'17 The Production of Light by Animals—The Fireflies or Lampyrids. Jour. of Franklin Inst., CLXXXIII., pp. 323–348.

Dubois, R.

'86 Contribution a l'etude de la production de la lumiere par les etres vivants. Les Eletreides lumineux. Bull. Soc. Zoöl. France, IX., pp. 1–275, pls. I–IX.

Fabre, Henri.

'13 The Glow-worm. The First User of Anæsthetics. The Century Magazine, LXXXVII., pp. 105–112. (Translated by A. T. de Mattos.)

Haddon, K.

'15 On the Methods of Feeding and the Mouthparts of the Larva of the Glow-worm (*Lampyris noctiluca*). Proc. Zoöl. Soc. London, pp. 77–82, pl. 1.

Hudson, G. V.

'91 The Habits and Life-history of the New Zealand Glow-worm. Trans. and Proc. New Zeal. Inst., XXIII., pp. 43–47, pl. VIII.

Knab, F.

'05 Observations on Lampyridæ. Can. Ent., XXXVII., pp. 238–239.

LeConte, J. L.

'81 Synopsis of the Lampyridæ of the United States. Trans. Am. Ent. Soc., IX., pp. 15–72.

Mast, S. O.

'12 Behavior of the Fireflies (*Photinus pyralis*) ? with Special Reference to the Problem of Orientation. Jour. Anim. Behav., II., pp. 256–272.

McDermott, F. A.

'10 A Note on the Light-emission of some American Lampyridæ. Can. Ent., XLII., pp. 237–363.

'11 Some Further Observations on the Light-emission of American Lampyridæ: the Photogenic Function as a Mating Adaptation in the Photinini. Can. Ent., XLIII., pp. 399–406.

'12 Observations on the Light-emission of American Lampyridæ. Can. Ent., XLIV., pp. 309–312.

'14 Ecologic Relations of Photogenic Function Among Insects. Zeit. f. wiss. Insecten Biol., X., pp. 303–307.

'16 Flashing of Fireflies. Science, XLIV., p. 610.

Morse, E. S.

'16 Firefly Flashing in Unison. Science, N.S., XLIII., pp. 169–170; XLVI., pp. 387–388.

Needham, J. G.

'03 Button-bush Insects. Psyche, X., pp. 22–30.

Newport, G.

'57 On the Natural History of the Glow-worm (*Lampyris noctiluca*). Jour. Proc. Linn. Soc. Zoöl. London, I., pp. 40–71.

Olivier, E.

'11 Contribution a l'histoire des Lampyridæ. Mem. Congr. Internat. Brussels (1910), II., pp. 273–282, 2 cuts.

Vogel, R.

'12 Beiträge zur Anatomie und Biologie der Larva von *Lampyris noctiluca*. Zoöl. Anz., XXXIX., pp. 515–519.

'15 Beiträge zur Kenntniss des Baues und der Lebensweise der Larve von *Lampyris noctiluca*. Zeit. f. wiss. Zoöl., CVII., pp. 291–432. 35 text figures, pl. 4.

Wenzel, H. W.

'96 Notes on Lampyridæ, with the Description of a Female and Larva. Ent. News, VII., pp. 294–296, pl. XI.

Williams, F. X.

'17 Notes on the Life-history of some North American Lampyridæ. Jour. N. Y. Ent. Soc , XXV., pp. 11–33.

AN EXCEPTION TO BATESON'S RULE OF SECONDARY SYMMETRY.

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In the summer of 1917, while employed by the Biological Board of Canada in a Dominion Lobster Hatchery at Bay View, N. S., my attention was called to an abnormal lobster caught by a local fisherman in the adjoining waters of Pictou Harbor. The lobster was a male and measured 7½ inches from rostrum to telson. The abnormality consisted of a double extra claw on the right cheliped, resulting in a condition of incomplete triplication. The presence of the extra parts on the right side did not appear to greatly handicap the animal which, when placed in one of the hatchery tanks, moved about freely. Other abnormalities somewhat similar have been described for the lobster by Faxon ('81), Emmel ('07) and Cole ('10).

DESCRIPTION.

The "triple" chela of the right side consisted of a small "nipper" and a double extra "crusher," while the corresponding appendage of the left side was large, normally developed and of the nipping type. The small "nipper" of the monster claw apparently represented the primary member of the group and, with the exception of the meropodite (*M.*), was normal in all its segments. Arising from the posterior (morphologically ventral) surface of this meropodite was the double extra claw.

The first segment of the right chela, the ischiopodite (*Is.*) was not exceptionally large and presented no evidence of distortion; but in the meropodite, as already noted, the effect of the abnormality in the appendage was very evident. This segment was of normal width proximally but broadened out rapidly distally and terminated in two diverging branches (*M.*, *M.*′ + ″). On *M* was borne the small primary claw while *M.*′ + ″ carried the much larger abnormal structure.

On examining the more posterior prong of the compound meropodite, we find that the next distal segment, *i.e.*, the extra carpopodite ($C.'+''$) is morphologically double. It is more than twice as large as the corresponding segment ($C.$) of the primary claw and bears on it supper surface two groups of spines separated by a shallow longitudinal groove. On the end of the massive carpopodite is the large double protopodite ($P.'+''$). This segment is incompletely divided, with the separation extending only as far as the region on a level with the bases of

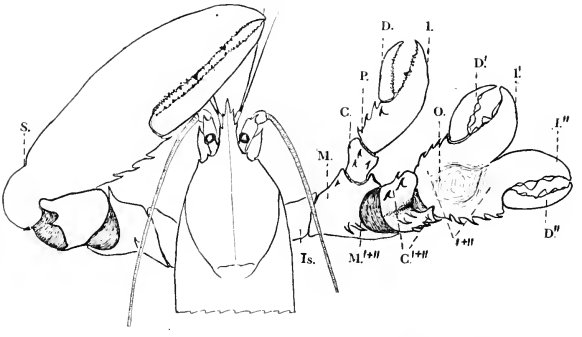

FIG. 1. (One half natural size.) Male lobster possessing an abnormal right chela consisting of a primary nipping claw plus a double extra "crusher." *C.*, primary carpopodite; $C.'+''$, double extra carpopodite; *D.*, primary dactyl; *D.'*, *D.''*, two extra dactyls; *I.*, primary index; *I.'*, *I.''*, two extra indices; *Is.*, ischiopodite; *M.*, primary portion of meropodite; $M.'+''$, extra portion of meropodite; *O.*, conical protuberance on double protopodite; *P.*, primary protopodite; $P'+''$ (*P* omitted in reproduction), double extra protopodite; *S.*, scar on left chela.

the indices ($I.'$, $I.''$). Opposing the indices are two well developed movable dactyls ($D.'$, $D.''$). The two partially separated chelæ are almost exact mirror images of each other. The dactyls and indices are practically identical and the dentition of both consists of heavy tubercle-like teeth. There are no tactile hairs.

Each segment of the double extra claw was decidedly larger than the corresponding portion of the primary claw, but the entire "triple" appendage barely equalled the left nipping claw in weight. The long axes of the double appendage and primary

appendage were in one plane and all three dactyls moved to meet their opposing indices in this plane.

Another point of considerable interest is the color relation of the extra portions. In the double extra protopodite and the two extra dactyls the pigmentation was completely reversed, giving as a result a light colored upper and a densely pigmented lower surface. A peculiar conical protuberance present on the light upper surface of the double protopodite was practically free from pigment.

Theoretical Considerations.

The present example, like most abnormal crustacean appendages, falls into the category established by Bateson ('94), "in which the extra limb or extra parts of a limb are themselves morphologically double," but unlike the others it does not conform to the rules of secondary symmetry formulated by the same author. According to these rules the normal appendage and the extra parts lie in the same plane and "the nearer of the two extra appendages is in structure and position formed as the image of the normal appendage in a plane mirror placed between the normal appendage and the nearer one, at right angles to the plane of the three axes; and the remoter appendage is the image of the nearer in a plane mirror similarly placed between the two extra appendages" (p. 479).

In the case under discussion the pair of extra chelæ are mirror images of each other but the appendage nearer the primary claw is not, as the rules provide, a mirror image of the latter. Moreover, it does not appear possible to explain this exception to the rules of secondary symmetry by a reference to torsion. Emmel ('07) and Cole ('10) were unable to explain apparent exceptions to Bateson's rules by making allowances for changes in position due to possible torsion. It has previously been pointed out that the coloration of the abnormal double structure was completely reversed, but even if the extra segments were rotated so that the dark surface became uppermost the relations of the claws as regards secondary symmetry would remain unchanged since the double extra claw is bilaterally symmetrical.

The specimen here described while exhibiting many points of

resemblance to the abnormal chelipeds figured by Emmel ('07, Pl. 2, Fig. 5) and Cole ('10, Figs. 1, 2) also presents several novel features. The degree of "triplication" of the claw is less than that seen in Emmel's specimen but greater than that described by Cole. In the former, the abnormal processes, consisting of a double carpopodite, two protopodites and two dactyls arose from the meropodite. In the latter, the abnormal structure, two extra indices and a double extra dactyl, was borne on the normal protopodite. In Faxon's specimen ('81) the morphological character of the extra branch which is borne on the forked meropodite is questionable. Faxon himself does not believe that the structure is double but Bateson ('94) is inclined to regard it as being morphologically double. Furthermore both Emmel and Cole found that the conditions in their "triple" claws illustrate the rules of secondary symmetry almost diagrammatically, *i.e.*, when allowance was made for shifting due to torsion.

In the two chelæ described by Emmel and Cole the two extra claws were of the same character as the primary claw, "crushers." In my specimen the primary claw is a "nipper" while the two extra claws are of the crushing type with well-developed tubercle-like teeth. This condition is of special significance, when we recall that the claw of the opposite side is also of the nipping type. In other words, we have a lobster with the "great" claws symmetrical with reference to each other, besides bearing on the meropodite of the right a double extra "crusher."

Emmel ('07) finds the pigmentation reversed on one of the extra claws but is able to explain this abnormal condition by a reference to torsion. The case which I am describing does not admit of such an explanation. The cause of the abnormality is unknown. No scars were to be found on the "triple" claw, but on the protopodite of the appendage of the opposite side a definite scar (*S*) was present. There were no further evidences of mutilation.

The results obtained by Harrison ('17) with transplantation of limb buds in *Amblystoma* larvæ suggest that there may possibly be some direct relation between the reversal of pigmentation and the doubling of the extra appendage. Harrison found that in

transplantations in which the limb buds were inverted, a certain percentage gave rise to double or twin limbs, one being a mirror image of the other. In a few cases there were still further duplications so that more or less complete triple limbs resulted, having approximately the same relations as found by Bateson in the Arthropoda.

In the lobster just described, two facts were noted; first, the extra parts are double and second, their pigmentation is reversed indicating an inversion of the double portion of the triple appendage. Furthermore, it is generally conceded that most abnormal and duplicated appendages among Crustacea are the result of regenerative processes. Both Reed ('04) and Emmel ('07) have found that abnormalities can be produced experimentally by mutilating either the proximal stump or the developing limb bud. It is conceivable then that the triplication found in the present instance may be due to regeneration following injury. In the course of regeneration the growing but may have been also injured so as to cause the development of an extra process (Emmel, '07, pp. 114–115), and this process may have had its dorso-ventral orientation reversed either at the time its development was initiated or at some later date.

Keeping the above statements in mind it therefore becomes possible to elaborate a more or less satisfactory hypothetical explanation of the triple chela. First, there may have been an injury to a normally developed crushing claw, followed by autotomy. Later the developing bud may have been injured, resulting in the appearance of an extra bud on the surface of the primary one. As a consequence of the injury, the primary bud did not develop as a crushing claw but as a small "nipper." (Sufficient evidence has been adduced by Emmel in 1907, Figs. 24 to 31, to prove that abnormal symmetrical claws in lobsters do arise through regeneration following mutilation.) The extra bud, due to mechanical displacement of its tissue at the time of the first injury, or as a result of a subsequent accident, has had its dorso-ventral orientation reversed, causing the development of twin crushing claws which are mirror images of each other.

SUMMARY.

1. An abnormal lobster cheliped is described in this paper. The abnormality consists of a double extra crushing claw arising from the meropodite of a claw of the nipping type.

2. The conditions found here are shown to present an exception to Bateson's rules of secondary symmetry.

3. The primary member of the so-called triple claw was found to be of the same character as the corresponding appendage of the opposite side, furnishing an exception to the normal condition of asymmetry.

4. Pigmentation was reversed in the double extra claw.

5. There is some evidence in favor of the view that there is a definite relation between the reversal of pigmentation and the doubling of the extra appendage.

LITERATURE.

Bateson, W.

'94 Materials for the Study of Variation, Treated with Especial Regard to Discontinuity in the Origin of Species. London.

Cole, L. J.

'10 Description of an Abnormal Lobster Cheliped. BIOLOGICAL BULLETIN, Vol. 18, pp. 252–268. 9 figs.

Emmel, V. E.

'07 Regenerated and Abnormal Appendages in the Lobster. 27th Annual Report Com. of Inland Fisheries, R. I., pp. 99–152, Pl. 1–9.

Faxon, W.

'81 On Some Crustacean Deformities. Bull. of Mus. Comp. Zoöl., Vol. 8, No. 13, pp. 257–275, Pls. 1–2.

Harrison, R. G.

'17 Transplantation of Limbs. Proc. Nat. Acad. Sci., pp. 245–251.

Reed, Margaret A.

'04 The Regeneration of the First Leg of the Crayfish. Arch. Entw.-Mech. Bd. 18, p. 307.

A COMPARATIVE STUDY OF THE CHROMOSOMES OF LACHNOSTERNA (COLEOPTERA).

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A. INTRODUCTION.

The studies presented here were begun in the spring of 1916 at Princeton University and were continued until the spring of 1917. Most of the material used was collected at Cold Spring Harbor during the summer of 1916.[1] The studies were discontinued in the summer of 1917 owing to the war. On returning to Princeton last spring, it was thought advisable to assemble the observations previously made despite the fact that they did not represent as complete a study as had been intended.

I wish to express my sincere thanks to Professor E. G. Conklin for much valuable assistance and encouragement.

B. MATERIALS AND PLAN OF STUDY.

It was originally intended to make a detailed study of the process of synapsis as well as a comparative study of the chromosomes of four selected species of May beetles, genus *Lachnosterna*. While the material was not entirely favorable for these purposes, some interesting facts were brought to light.

The four scarab beetles of the genus *Lachnosterna* which were selected for study were the species *delata*, *fusca*, *gracilis* and *tristis*. Besides these, for comparative purposes, two other scarab beetles were studied, *Pelidonota punctata* and *Cotalpa lanigera*. The form most studied was *L. delata* and since the other forms showed no essential differences from *delata*, the latter will be used as the basis of description in the present paper.

Comparatively little detailed study of spermatogenesis in the Coleoptera has been done. The work of Miss Stevens ('05, '06),

[1] The writer wishes to express his thanks to the Brooklyn Institute of Arts anp Sciences for the privileges of a research fellowship at the Laboratory of the Institute at Cold Spring Harbor, L. I., during the summer of 1916.

while it covered a large number of species, was concerned only with chromosome counts, and especially with reference to the sex chromosomes. In only two species of beetles has there been anything like a detailed study of the chromosomes in synapsis, Voinov (1903) on *Cybister roselii* and Schäfer (1907) on *Dytiscus marginalis*. Both these authors after a detailed study of the growth stages of the spermatocytes describe parasynapsis, while Miss Stevens claims telosynapsis in the forms she studied.

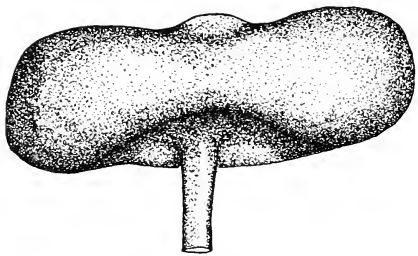

FIG. 1. Single testis of *Lachnosterna* (side-view), with its efferent duct.

The material used in this study was fixed in Flemming's, Hermann's, Bouin's, Gilson's and Carnoy's fixing fluids. In general, the Flemming and the Hermann material was best for the growth stages of the spermatocytes, while the Bouin material was best for the chromosomes. Iron-hæmatoxylin, with and without a counterstain, was employed entirely for staining. Aceto-carmine smears were valuable in checking the observations on the fixed material. All the testes, except those of *L. fusca*, were taken from the adult beetles. The material was gathered in midsummer and showed all stages from spermatogonia to ripe spermatozoa. In the case of *L. fusca*, the adult testes showed few favorable stages and it was necessary to study the larval gonads.

C. Description of Testes and Seriation of Stages.

The testes consist of twelve mushroom-like bodies, three pair in each side of the abdomen. Each testis has its duct (Figs. 1 and 2) and the ducts from each group of testes unite to form two larger ducts; these four larger ducts in turn unite to form the single median vas deferens.

The testes, although of an unusual shape, show the seriation of the stages clearly, being but a modification of the simple straight (orthopteran) type with a linear seriation of the cells. In the species studied, the testes consist of a great many follicles radiating from the center. Fig. 2 represents a diagrammatic section through the center of the testis and perpendicular to its broad surface. In the center of the testis from which the follicles radiate (Fig. 2, *A*), one finds all the spermatogonia and here new cysts are in the process of formation. On each side of this

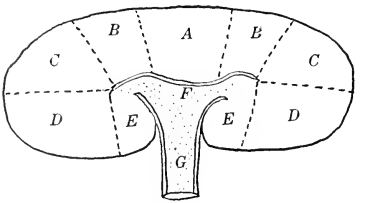

FIG. 2. Diagrammatic section through testis at right angles to its broad surface, to show seriation of stages. *A*, region in which are all spermatogonia; *B*, early growth stages and synezesis; *C*, pacyhtene and later growth stages; *D*, maturation divisions; *E*, spermatids and spermatozoa; *F*, cavity in testis where spermatozoa are retained prior to discharge from testis; *G*, efferent duct.

region (*B*, *B*) are the early synaptic stages (synezesis). In the regions *C*–*C* one finds the later synaptic stages (pachytene and diplotenes), while in regions *D*–*D* show most of the spermatocyte divisions; regions *E*–*E* contain most of the spermatids and spermatozoa. The chamber (*F*) shown in the figure serves as a place where the spermatozoa are collected and stored prior to discharge from the testis; the duct (*G*) leads from the storage chamber. Of course the stages above seriated overlap and there is no sharp delimitation as is diagrammatically shown in the figure. The formation of the cysts in the region A was followed out and my observations confirm those of Wieman ('10) and Hegner ('14) that each testicular cyst is derived from a single spermatogonium. There is, however, no evidence that cell division is by amitosis as Wieman found.

D. Observations.

1. *Spermatogonia and Diploid Chromosome Groups.*

In all of the species of *Lachnosterna* studied, as well as *Pelidonota* and *Cotalpa*, the diploid number of chromosomes as shown in the spermatogonia is twenty, including an unequal (sex) pair (Figs. 1–6). Dividing follicle cells in the ovaries show ten equal pairs of chromosomes (Fig. 7). There are three pairs of J- or U-shaped chromosomes, one pair of which is considerably larger than the others (Figs. 1–5, *AA*). The sex chromosomes are the smallest in the complex, consisting of a very small round chromosome (*y*) and a somewhat larger rod-shaped chromosome (*x*). In comparing the size relations of the chromosomes in the several species studied, one finds no marked differences. In many cases the chromosomes in the diploid complexes are arranged in pairs, homologous chromosomes lying beside each other. In the Diptera, Metz (1916) has found that pairing of chromosomes is not confined to the maturation stages, but at each cell division homologous chromosomes come together. In the Diptera the diploid chromosome number is relatively low; in species where the chromosome number is high, pairing of homologous chromosomes is usually not found to be so complete. It therefore seems that chromosome pairing, outside of the maturation stages, is related to chromosome number.

In the spermatogonial telophases, the chromosomes spin out into fine chromatic threads (Fig. 13) and as the nucleus grows the threads become more and more complex forming a chromatic reticulum or typical resting nucleus. This "resting" nucleus is of relatively short duration, for soon the chromatin begin to condense into heavier threads (Fig. 15), and as condensation continues, all the chromatin of the nucleus becomes confined into large chromatic blocks of a granular nature (Figs. 16, 17). Counts of these chromatic blocks in uncut nuclei always approximate the diploid chromosome number and these blocks may be considered as the anlages of the future spermatogonial chromosomes. The blocks consist of a linin core on which are imbedded the chromatin granules; they are connected to each other by a fine net-work of linin which seems to be continuous with the linin forming the core of the blocks. Most of the cells in the spermato-

gonial area of the testes are in this stage and apparently it is of of much longer duration than the reticular or "resting" stage.

In some respects the chromatic blocks above described correspond to the "prochromosomes" which have been described by Overton ('09) in *Podophyllum*, Arnold ('08) in *Hydrophilus piceus*, Goodrich ('16) in *Ascaris incurva* and other workers. In these cases, however, the chromatic bodies appeared at the beginning of the growth period and, according to the above workers, these bodies arranged themselves in pairs, thereby accomplishing the synaptic process. In *Lachnosterna* there is no such paired arrangement of the chromatic blocks; they merely represent stages in the formation of the spermatogonial chromosome groups and might really be called prophases, except that they are of relatively long duration. In some cases a precocious longitudinal split can be detected, preparing the chromosome for the next cell division.

2. The Synaptic Stages and Maturation Divisions.

Following the telophase of the last spermatogonial division (Fig. 13), the chromosomes spin out in the form of very fine (*leptotene*) threads (Fig. 18, 19) which entirely fill the nucleus and prevent a minute analysis of this stage. The actual pairing of the homologous chromosomes could not be followed in detail, but observations on a few favorable cells (Fig. 19) indicate that the union is side-to-side (*parasynapsis*). Stevens ('06) has described telosynapsis in the Coleoptera, but she did not make a study of the early growth stages.

The leptotene stage gradually merges into a definite contraction stage (synezesis) with all the chromatic threads polarized at one side of the nucleus (Figs. 20, 44). These stages are always found in a definite part of the testes, namely in region B (Fig. 2), and are found nowhere else. McClung ('05) used the word "synezesis" to describe that "condition of the nucleus in which the chromatin is found massed at one side of the vesicle, without regard to whether it is a normal phenomenon or not." McClung and recently some of his students, Whiting ('17) and Hance ('17), have maintained that a unilateral massing of the chromatin or synezesis is an artifact and is due to improper

fixation methods. With this in mind, the writer has sought with most careful technical methods to obtain fixed material which might not show these contraction figures; but without exception cells in the contraction phase were always found in the definite region of the testes mentioned above. It is quite true that poorly fixed material shows an abundance of contraction figures, but in these cases, as will be shown later, they are just as likely to appear in other regions of the testes than in the very definite location above mentioned. There is no doubt that even the best fixation will tend to emphasize the contraction of the chromatin just as it does in the case of the other cell structures, but synezesis is unquestionably a normal process in the beetles studied here. Whiting ('17) has advanced the idea that the chromatic elements during synapsis are in an unstable condition and that "any shock is likely to cause them to clump together." It is questionable whether good fixation is much less of a "shock" than indifferent fixation. It is conceivable that true synezesis may not occur as a normal phase of the maturation possesses in some animals (*e.g.*, Orthoptera), but the fact that it has been described by many workers using a variety of fixing methods supports the fact that it is a normally occurring phase in some cases. In any event it proves that the nuclear condition is peculiar in cases of synezesis.

Following the stage of synezesis, the chromatin threads are released from the polarized bouquet in the form of thick ragged looking *pachytene* threads (Fig. 21). Usually a longitudinal split can be seen in the threads, which marks the point of synapsis of the homologous threads. The chromomeres are imbedded in a linin base, chromomeres of the same size lying opposite each other and being connected with each other by fine linin threads. In the later stages the threads become more widely separated from each other (Fig. 23) assuming the *diplotene* form. In these stages and in still later ones, the threads show a variety of twisting about each other forming rings with and without crossed ends, figures 8, double and even triple crossing-over of the threads. In no case could a secondary split be seen. The *strepsistene* threads continue to become more widely separated and it soon becomes impossible to trace the individual threads (Fig. 22).

This unanalyzable stage is of relatively short duration and is followed by a condensation of the chromatin in the form of heavy threads (Fig. 25). Condensation of the chromatin continues and the definitive maturation tetrads begin to make their appearance. In these early prophases one often finds cells in which all the chromatin is massed at one side of the nucleus (Fig. 26), resembling very much a synezesis figure. Gross ('07) has described a second synezesis in *Pyrrhocoris* and Mottier ('07) believes that in the plants it is a regularly occurring phase in the maturation processes. In *Lachnosterna* these contraction figures are most abundant in material which is poorly fixed and I consider them as artifacts. Fig. 27 represents this stage from well fixed material as contrasted with Fig. 26 from poorly fixed material.

All the first spermatocyte metaphase plates of the four species of *Lachnosterna* studied, as well as *Pelidonota* and *Cotalpa*, show ten bivalent chromosomes the smallest of which represents the sex pair (Figs. 8, 9, 10, 11, 12). These are usually arranged in characteristic groups with fine linin threads connecting the various members of the complex to each other. A comparison of the tetrads of *L. delata* with those of *L. fusca* (Figs. 28, 29) shows no marked differences either in form or in size of the tetrads. Using Miss Carothers' ('17) nomenclature, there are five atelomitic tetrads (non-terminal spindle fiber attachments) and five telomitic tetrads (terminal spindle fiber attachments). The atelomitic tetrads are the largest in the complex and are derived from the three pairs of J-shaped spermatogonial chromosomes and two pairs of the bent rod-shaped ones. In sideview metaphases, the largest of the tetrads (Figs. 28, 29) has a sub-terminal spindle fiber attachment, and is derived from the *AA* pair (Fig. 5) of the diploid chromosome group which also have subterminal fiber attachments. The other atelomitic tetrads consist of two typical crosses and two annular tetrads of the *Stenobothrus* type. The other four autosome tetrads are of the ordinary dumb-bell type while the *x* and *y* elements (sex pair) are fused end to end (Figs. 28, 29).

The types of tetrads above described are found in all four species of *Lachnosterna* studied. On the other hand, in *Cotalpa*

and *Pelidonota* no cross-shaped tetrads and only one ring tetrad are found. The question of reduction division is difficult to analyze here, with the exception of the ring tetrads. The latter are always arranged on the spindle in the direction of the spindle axis and the spindle fiber attachment is median. Consequently the separation of the dyads occurrs at the point of the synaptic union and the division is reductional.

3. *Sex Chromosomes.*

The earliest work on the sex chromosomes was done by Miss Stevens ('05, '06) on the Coleoptera. She found the so-called sex chromosomes in over forty species and her work and that of Wilson's on the Hemiptera and McClung's on the Orthoptera have been the basis of the later work correlating sex determination with the chromosomes. In the Coleoptera the sex chromosomes are found as unpaired "accessory" and as unequal elements which separate in one of the maturation divisions and divide equationally in the other maturation division. Arnold ('08) has maintained that in *Hydrophilus piceus* there are no sex chromosomes. There is present in the growth stages a chromatin nucleolus which may even persist up to the first maturation division and may even pass undivided to one cell. However, it disappears and cannot be found in any of the second spermatocytes.

In *Lachnosterna*, *Pelidonota* and *Cotalpa* the sex chromosomes are of the *xy* type the *y* element being the smaller of the unequal pair (Figs. 1–6). There are no marked differences in the size and form of the sex chromosomes in the four species of *Lachnosterna* studied, but in *Pelidonota* the *x* element is considerably larger than in the *Lachnosterna* material. In all cases the sex pair separate in the first maturation division and divide equationally in the second, thus yielding two types of spermatozoa. (Figs. 31, 32, 33, 35, 36). In a single case the sex chromosomes failed to separate in the first maturation division, both chromosomes going into one of the daughter cells. This is undoubtedly a case of non-disjunction similar to that which has been found genetically and cytologically by Bridges ('16) in *Drosophila*.

The sex chromosomes presist throughout the entire growth

period as definite compact chromatic bodies. They are always contained within a chromosomal vesicle such as has been described by Wilson ('12) in *Oncopeltus* and *Lygæus* (Figs. 21, 25). In *Lachnosterna* the sex elements usually remain separate from each other, each enclosed in a separate vesicle. In *Pelidonota* and *Cotalpa*, the sex pair remain fused during the synaptic period, the smaller (*y*) element usually being imbedded along the side of the larger (*x*) element.

E. General Considerations.

1. *Chromosome Number and Species.*

The intensive work of McClung and his students on one family of Orthoptera, the Acrididæ, has shown that the chromosome number in all the species studied of this group is the same, namely 23 in the male. This has led McClung to the generalization that species closely related taxonomically might show similarity in their chromosome groups. It is very evident that this generalization cannot apply to all groups since, in some cases there is a wide divergence in chromosome number among members of the same genus. It is possible that in some cases this difference in chromosome number between closely related species may be due to a fusion of several chromosomes or else a breaking up of one or more chromosomes into several distinct components. In the case of *Hesperotettix*, McClung ('17) has shown that the chromosome number may vary from 17 to 23. He has shown that these variations are due to a fusion of chromosomes resulting in the formation of "multiple chromosomes." In one species, *Hesperotettix viridis*, he has found the haploid or reduced number to vary from 9 to 13. On the other hand, the work of Stevens on the *Diabroticas* (Coleoptera) has shown that the species *vitatta* has 21 chromosomes, while the species *soror* and 12-*punctata* have but 19. However, in the latter two species there may be present from 1 to 4 additional or "supernumary" small chromosomes. It is quite possible that the supernumary chromosomes of the species *soror* and 12-*punctata* represent the fragments of a pair of chromosomes, which would therefore make an agreement in chromosome number between these two species and the species *vitatta*. As McClung ('17, p. 545) has pointed

out, he has confined his idea of this similarity of chromosome number in closely related species, only to the family Acrididæ. It is possible that in other forms correspondence in chromosomes may extend only to the subfamily or genus. In the Hemiptera and Coleoptera certainly there is no such uniformity of chromosome number in the various families as is found in the Acrididæ.

The four species of *Lachnosterna* studied here differ from each other very much as far as taxonomic characters are concerned, nevertheless the chromosome groups show no difference either in form or number. The two other forms studied, *Pelidonota* and *Cotalpa*, differing generically, have the same chromosome number (20 in the diploid groups), but there are some differences in the form of the maturation tetrads. Only one other scarab beetle has been studied, *Euphoria inda* by Stevens ('06), and it corresponds with a diploid group of 20 chromosomes, so that all the species of the family *Scarabidæ* thus far studied correspond in chromosome number. The genus *Lachnosterna* embraces over one hundred species, some very much alike so that it is difficult to separate them taxonomically, others differing markedly from each other. The most constant difference is found in the male copulatory organs, which probably prevents the interbreeding of species in nature. Perhaps further cytological studies in this genus will yield results similar to those in the Acrididæ. Certainly there is a wealth of material for such a comparative study.

2. *Cyst Formation and Cell Polarity.*

Hegner ('14) has studied the formation of spermatogonial cysts in the testes of *Leptinotarsa;* the facts concerning cyst formation in the beetles studied here show results essentially similar to those of Hegner. The primary spermatogonia are not arranged in cysts and are more or less polygonal in shape, with the nucleus usually located in the center. Cyst formation begins by the rapid division of a single primary spermatogonium, together with an adjacent epithelial cell which forms a follicular membrane around the cyst. Consequently we can say that all the cells within any one cyst are the descendants of a single primary spermatogonium. With the formation of the cyst,

the spermatogonia are arranged in the form of a rosette, and are now triangular or wedge-shaped with the nucleus at the base and the rest of the cytoplasm extending toward the cyst cavity. Thus, with the formation of the cyst, there is a polarity established in the spermatogonia which is maintained up to the formation of the ripe spermatozoa, for, the side where the nucleus is located is destined to form the head of the spermatozoon, and the cytoplasmic portion extending toward the cyst cavity is destined to form its tail. Hegner. ('14) has homologized the process of cyst formation with the differential divisions in insect oogenesis which establish nurse cells and oöcytes. It has long been known that the insect egg possesses a remarkable polarity besides being highly organized. Since, as it has been above shown, the polarity of the sperm cells are established at the time of cyst formation, and since this process is homologous to nurse cell-oöcyte differentiation, it is probable that the polarity of the egg may have its origin at the time of the differentiation of nurse cells and oöcytes.

3. *Linin and Chromosome Structure.*

When one studies the history of the chromatin of the nucleus from the resting stage through the synaptic period up to the reconstitution of the definitive maturation chromosomes, one begins to seek for some of the underlying mechanisms concerned in the movements of the chromatin particles. From the diffuse granular state of the chromatin up until the formation of the chromosomes, the linin network of the nucleus plays an active part. Chromatin granules in the nucleus are never isolated as such, but always have linin connections with other granules. The synaptic threads consist of linin threads with the chromomeres embedded along them. As has been before stated, homologous chromomeres have linin connections running between them. Conklin ('17) has shown that the ground-work of the cytoplasm is the relatively stable and elastic spongioplasm, and he attributes to it the maintenance of cytoplasmic organization and the movements and localization of cytoplasmic substances. Similarly in the nucleus it seems that the linin is a relatively elastic substance which forms the ground-work of the nucleus

and maintains the organization of the nuclear elements. Wenrich ('16) has shown how remarkably constant the organization and "architecture" of the chromosomes are. By means of certain structural peculiarities which his "selected" chromosomes presented, he was able to recognize and trace them through all the stages of spermatogenesis. The tendency has been noted in many forms for the chromosomes to appear in the metaphase always in a definite configuration. It is possible that the linin connections between the chromosomes which have often been figured (Figs. 8–12) are responsible for the definite patterns assumed by the chromosomes in the metaphase plate. The uniting in pairs of the homologous leptotene threads may be due to the contractility of the linin connectives running between the homologous chromomeres. In short, the morphological stability of the nuclear elements and the constancy of their form, arrangement and organization is in the last analysis referable to the linin.

F. Summary.

1. The diploid chromosome groups of four species of *Lachnosterna*, namely *delata*, *fusca*, *gracilis* and *tristis*, as well as *Pelidonota punctata* and *Cotalpa lanigera*, show twenty chromosomes, one pair of which is composed of two unequal elements (sex chromosomes).

2. There are no essential differences in the form and arrangement of the chromosomes in the species studied.

3. The growth period of the spermatocytes is marked by the appearance of delicate leptotene threads which are derived from the chromosomes of the last spermatogonial division. These threads become polarized and there is evidence that they are arranged in pairs parasynaptically.

4. There is a definite contraction stage which does not seem to be caused by fixation, but is a normally occurring phase in the growth period.

5. The sex chromosomes persist through the entire growth-period in the form of definite compact bodies, sometimes being contained within chromosomal vesicles. The unequal sex elements separate in the first maturation division and divide equationally in the second maturation division.

6. There are five atelomitic tetrads in the first maturation division and five telomitic tetrads (including the sex pair).

7. Cyst formation in the testis begins by the rapid division of a single primary spermatogonium, so that all the cells within any particular cyst are the descendants of a single cell. The visible polarity of the cells seems to be established at the time of cyst formation.

G. Addenda.

Since the manuscript of the foregoing study was written, the work of Goldsmith[1] has appeared on the chromosomes of the Cicindelidæ. He has studied in all five species of this family and finds that they agree in chromosome number. He has described a double odd-chromosome which passes undivided to one pole of the spindle in the first maturation division and divides in the second maturation division giving rise to spermatozoa with ten and twelve chromosomes respectively. In his study of the growth stages of the spermatocytes he has been unable to find that the leptotene threads actually pair. His figures of the synaptic stages are not clear and he makes no decision as to the method of synapsis (parasynapsis or telosynapsis).

He describes the "early" spermatogonia as being arranged in syncytia without any discernible cell-walls. He describes the appearance within the syncytial cytoplasm of "cytoplasmic fibrillar bridges." "With the increase in age and size of the cells, these bridges become more dense and assume a definite arrangement about a number of cells. This continues until the entire tubule is subdivided into a large number of syncytia—cysts containing cells without perceptible cell walls" (p. 445). Both his descriptions and figures of this peculiar method of cyst formation lack in clarity. From his Fig. 5, I interpret the "cytoplasmic fibrillar bridges" as being probably the persisting spindle remains or mitosome of the previous division, and it is also probable that the deeply staining bodies in the cytoplasm are the mid-bodies (cell-plate) persisting with the mitosome. That these "fibrillar bridges" are really spindle remains is further

[1] A comparative study of the chromosomes of the tiger beetles (Cicindelidæ). *Jour. Morph.*, Vol. 32, No. 3, 1919.

indicated by the fact that they" become more dense and assume a definite arrangement about a number of cells." This is exactly the behavior of the spindle remains which Hegner[1] has described in Leptinotarsa and which I have described in *Passalus*.[2] It is difficult to see how these "fibrillar bridges" are concerned in dividing the syncytia into a number of cysts. Furthermore, it is difficult to believe that a true syncytium of spermatogonia actually does exist, for the later stages certainly do possess cell-walls which must have been preëxisting.

LITERATURE CITED.

Arnold, G.

'08 The Nucleolus and Michrochromosomes in the Spermatogenesis of *Hydrophilus piceus*. Arch. f. Zellforsch., Bd. 2.

Bridges, C. B.

'16 Non-disjunction as a Proof of the Chromosome Theory of Heredity. Genetics, Vol. 1.

Carothers, E. E.

'17 The Segregation and Recombination of Homologous Chromosomes as Found in Two Genera of Acrididæ (Orthoptera). Jour. Morph., Vol. 28, No. 2.

Conklin, E. G.

'17 Effects of Centrifugal Force on the Structure and Development of the Eggs of Crepidula. Jour. Exp. Zoöl., Vol. 22, No. 2.

Goodrich, H. B.

'16 The Germ Cells in *Ascaris incurva*. Jour. Exp. Zoöl., Vol. 21, No. 1.

Gross, J.

'07 Die spermatogenese von Pyrrhocoris apterus L. Zoöl. Jahrb. Abtheil. f. Anat. u. Ontog., Bd. 23.

Hegner, R. W.

'14 The Germ Cell Cycle in Animals. Macmillans.

Metz, C. W.

'16 Chromosomes Studies on Diptera, II. Jour. Exp. Zoöl., Vol. 21, No. 2.

Mottier, D. M.

'07 The Development of Heterotype Chromosomes in Pollen Mother Cells. Ann. of Bot., Vol. XXI.

McClung, C. E.

'05 The Chromosome Complex of Orthopteran Spermatocytes. BIOL. BULL., Vol. IX., No. 5.

'17 The Multiple Chromosomes of *Hesperotettix* and *Mermiria* (Orthoptera). Jour. Morph., Vol. 29.

Overton, J. B.

'09 On the Organization of the Nuclei in the Pollen Mother Cells of Certain Plants with Especial Reference to the Permanence of the Chromosomes. Arch. f. Mikr. Anat., Bd. 70.

[1] Studies on the Germ Cells, I and II. *Jour. Morph.*, Vol. 25, No. 3, 1914.

[2] Mitochondria and Other Cytoplasmic Structures in the Spermatogenesis of *Passulus cornutus*. BIOL. BULL., Vol. XXII., No. 5, 1917.

Schäfer, F.

'07 Spermatogenese von Dytiscus. Zool. Jahrb. Abtheil. f. Anat. u. Ontog., Bd. 23.

Stevens, N. M.

'06 Studies on Spermatogenesis, II. Carnegie Inst. Pub. No. 36.

'05 Studies in Spermatogenesis with Especial Reference to the Accessory Chromosome. Carnegie Inst. Pub. No. 36.

'08 The chromosomes in *Diabrotica vitatta*, *Diabrotica soror* and *Diabrotica 12-punctata*. Jour. Exp. Zoöl., Vol. V., No. 4.

'09 Further Studies on the Chromosomes of the Coleoptera. Jour. Exp. Zoöl., Vol. VI., No. 1.

Voinov, D.

'03 La spermatogénèse d'été chez le Cybister roeselii. Arch. de Zoöl. Exp. et Gen., 4^{e} Series, T. 1.

Wenrich, D. H.

'16 The Spermatogenesis of *Phyronotettix magnus* with Especial Reference to Synapsis and the Individuality of the Chromosomes. Bull. Mus. Comp. Zoöl., Harvard Coll., Vol. IX., No. 3.

Whiting, P. W.

'17 The Chromosomes of the Common House-mosquito, *Culex pipiens*. Jour. Morph., Vol. 28, No. 2.

Wilson, E. B.

'12 Studies on the Chromosomes, VIII. Jour. Exp. Zoöl., Vol. 13, No. 3.

Wieman, H. L.

'10 A Study in the Germ Cells of *Leptinotarsa signaticollis*. Jour. Morph., Vol. 21.

EXPLANATION OF PLATES.

All drawings (except Figs. 28, 29, 30) were made with camera lucida using No. 12 ocular and 1/16 mm. oil immersion objective. Figs. 28, 29, 30 were made using No. 18 ocular and 1/16 mm. oil objective.

PLATE I.

(Figs. 1 to 12.)

FIGS. 1 to 6. Metaphase plates of spermatogonia in the six beetles studied all showing 20 chromosomes including an unequal pair (*XY*).

FIG. 1. *L. delata.*

FIG. 2. *L. fusca.*

FIG. 3. *L. tristis.*

FIG. 4. *L. gracilis.*

FIG. 5. *Pelidonota.*

FIG. 6. *Catalpa.*

FIG. 7. Metaphase plate of follicle cell from ovary of *L. delata* showing ten equal pairs.

FIGS. 8 to 12. Metaphase plates of 1st spermatocytes showing ten bivalent chromosomes.

FIG. 8. *L. delata.*

FIG. 9. *L. fusca.*

FIG. 10. *L. gracilis.*

FIG. 11. *Pelidonota.*

FIG. 12. *Catalpa.*

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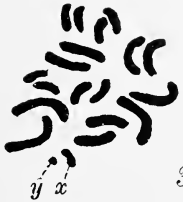

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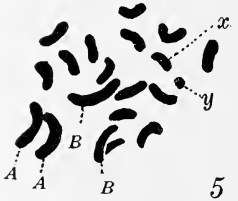

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PLATE II.

(Figs. 13 to 27.)

FIG. 13. Telophase nucleus of spermatogonium, showing chromosomes spinning out into delicate threads.

FIG. 14. Characteristic resting nucleus of spermatogonium.

FIGS. 15, 16, 17. Stages in the condensation of the chromatin from the resting stage to the formation of the chromatic blocks.

FIGS. 18, 19. Early growth stages. Evidences of parallel pairing of leptonete threads.

FIG. 20. Contraction (synezesis) stage.

FIG. 21. Pachytene threads released from synezesis stage.

FIG. 22. Strepsistene nucleus. Chromatin threads unanalyzable.

FIG. 23. Various forms of diplotene and strepsitene threads.

FIG. 25. Early prophase of 1st spermatocyte. Tetrads beginning to form.

FIG. 26. Cell in prophase simulating synezesis; due to faulty fixation.

FIG. 27. Cell in stage similar to Fig. 26 from well-fixed material.

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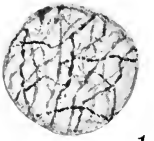

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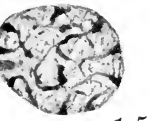

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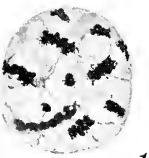

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PLATE III.

(Figs. 28 to 36.)

FIG. 28. Side view of maturation tetrads of *L. delata.*

FIG. 29. Side view of maturation tetrads of *L. fusca.*

FIG. 30. Side view of maturation tetrads of *Pelidonota punctata.*

FIG. 31. Early anaphase of first maturation division in *L. fusca* showing separation of sex pair.

FIG. 32. Anaphase of first maturation in *Pelidonota.*

FIG. 33. Telophase of first maturation in *L. delata.*

FIG. 34. Telophase of first maturation in *L. delata* in which the sex elements have failed to disjoin, and have passed to one daughter cell.

FIGS. 35, 36. Daughter plates of second spermatocyte of *L. delata* and *Pelidonota*, respectively.

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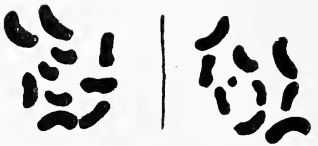

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Vol. XXXVIII. *March, 1920.* *No. 3.*

BIOLOGICAL BULLETIN

CARBON DIOXIDE PRODUCTION IN RELATION TO REGENERATION IN PLANARIA DOROTOCEPHALA.[1]

HARRIET L. ROBBINS AND C. M. CHILD.

Numerous lines of evidence indicate that the body of *Planaria dorotocephala* consists physiologically of more than one individual or "zooid" after a certain limit of size is exceeded in the course of growth. The first or chief zooid of the series includes the region from the head to a level slightly posterior to the mouth, the level at which fission usually occurs, and the region posterior to this consists of one or more short zooids, the limits of which can be distinguished physiologically, but not morphologically (Child, '10, 11*c*, '15, chap. VI). The susceptibility of the body to a large number of chemical and physical agents in concentrations or intensities too high to permit acclimation or tolerance, decreases from the head posteriorly through the length of the first zooid, increases sharply at the level of fission and in long animals shows one or more rises posterior to that level (Child '13*b*). The process of regeneration also shows graded differences at different levels, corresponding to the differences in susceptibility (Child '11*b*).

In the papers referred to, as well as in many others evidence has been presented to that the susceptibility differences in general are in some degree an indicator of quantitative differences in metabolic condition, particularly in rate of oxidations, though susceptibility has never been regarded as an exact quantitative measure of oxidation. According to this point of view the gradations of susceptibility in the body of *Planaria dorotocephala* indicate that the rate of oxidation decreases gradually, at

[1] From the Hull Zoölogical Laboratory, University of Chicago.

least in the ectoderm and body wall, from the head to the level of fission and rises suddenly at the anterior end of the second zooid and of each further zooid, if such are present.

It has also been shown that in isolated pieces of the body of *Planaria*, particularly in those below a certain fraction of the body length, susceptibility is increased during the first few hours following section. This temporary increase of susceptibility, apparently associated with the stimulation of the pieces by section, is least in pieces nearest the head, and increases in successive pieces posteriorly to the level of fission, where it again decreases (Child, '14). In general the regions of highest susceptibility in the intact animal show the least increase in susceptibility in isolated pieces, and vice versa. Within twelve to twenty-four hours after section the temporary increase in susceptibility has disappeared and the susceptibility of each piece is about the same as, or slightly lower than before section. From twenty-four to forty-eight hours after section a gradual rise in susceptibility begins as the regulatory processes leading to the development of a new individual make themselves evident, and the susceptibility after regeneration is completed is much higher than that of the region of the body of the original animal which a given piece represents. This is true not only for the newly developed head and posterior end of the regenerated animal but for the whole body (Child, '15, Chap. V). In the light of various facts, these changes in susceptibility have been interpreted as indicating and in some way associated with changes in rate of oxidation. The present paper constitutes additional evidence for this conclusion. Carbon dioxide production was chosen instead of oxygen consumption as the subject of investigation first because investigation of the temporary changes, immediately following section involves the technical difficulty of preparing very large numbers of pieces within a very short time in order that oxygen consumption may be sufficient for determination in short periods, and second because work on oxygen consumption during the course of regeneration was already in progress in this laboratory. The colorimetric method was used since it is well adapted for obtaining comparative data with small amounts of material.

A few experiments similar to those recorded below were performed by Child from time to time during several years past, and these experiments, although they brought to light certain difficulties as regards technique and were too few in number to constitute conclusive evidence, indicated very clearly that the differences and changes in susceptibility following section and after regeneration are paralleled by differences and changes in rate of carbon dioxide production. A more extensive investigation of the subject by another person was desirable, and this was undertaken by Miss H. L. Robbins in candidacy for the degree of M.S. The data tabulated in the present paper are those obtained in this investigation.

The special technique for the different experiments is described in the following sections, but the general technique used in connection with the colorimetric method is very similar to that employed in the study of CO_2 production during starvation (Child, '19*a*). The weighing of the animals or pieces is the most difficult feature of the preparation, since the presence of superfluous water introduces an error in weight, and since weighing must be done as rapidly as possible to avoid injury or death from drying. After considerable practice, involving repeated weighings of the same lots and determination of the length of time which could be allowed without injury for drainage on filter paper and exposure to the air while weighing, a satisfactory method of procedure was developed, and this was followed in all the tabulated experiments. The container was first weighed and both container and weights were left on the balance pans: next weights equal to the estimated weight of the lot of worms or pieces were placed on the weight pan in order to reduce the time necessary for weighing the animals: the worms were then brought together at the tip of a funnel of well washed filter paper, drained for a certain length of time, transferred on a slightly vaselined scalpel blade to the container and weighed, each of these operations being performed in as nearly as possible the same length of time in each case. Equal or approximately equal weights of the lots to be compared were used (see tables). For the colorimetric estimations the Hynson Westcott and Dunning *H* ion outfit with phenolsulphonephthalein as indicator

was used. After weighing, the worms were returned at once to water and then transferred to pyrex tubes fused at one end and of the same diameter as the standard tubes. After washing twice in aqueous indicator solution of the same concentration as that in the standard tubes, each tube was filled with indicator solution to a 3-c.c. level previously marked. The tubes were then sealed without air bubbles by running in on the surface of the fluid about 1 c.c. of soft paraffin at a temperature just above melting point, the worms being kept at the bottom of the tube to avoid injury from change of temperature. Since leakage past the paraffin plug is difficult to avoid when changes of temperature occur and since it was found desirable to reduce the temperature slightly as a means of keeping the animals quiet (see below), the following method of providing for changes in volume of the fluid was used. A piece of closely fitting soft rubber tubing, previously coated with soft paraffin was drawn over the open end of the pyrex tube, leaving 2 to 3 cm. of the tubing beyond the end of the glass. Indicator solution was then added to fill both the glass tube above the paraffin plug and the rubber tube, and the latter is then closed by a screw clasp. This procedure makes impossible the entrance of air past the paraffin plug when the temperature is lowered. Instead of air a small amount of the indicator solution above the plug may be drawn below, but this occurs within the first five minutes or less of the experiment, and with the changes of temperature involved the amount of fluid passing the plug is negligible, so far as the results are concerned. The indicator solution between the paraffin plug and the rubber tubing is visible and serves as a control for the color change below the plug. Closure by means of the rubber tubing and clamp alone was found to be unsatisfactory because some of the worms creep into the rubber tubing, where they cannot be seen, and it is therefore impossible to determine whether motor activity is going on and whether all are in good condition. Moreover, pieces in the rubber tubing are often overlooked when the lots are removed from the experimental tube and the whole lot becomes valueless for further experiment, unless substitution for the lost pieces is made, but this is at best an undesirable procedure.

The *pH* at the beginning of the experiment was of course the same in all lots to be compared, but the starting point differed somewhat in different experiments, the extreme range being 7.8 to 7.95. Observations were made at least every half hour with few exceptions, but the tables, instead of recording all the *pH* readings, give the times required to reach an arbitrary end point, *Hp* 7.3 being selected as this end point. As each lot approached this point, observation was more or less continuous. A daylight lamp was used for all color comparisons.

A serious difficulty in the earlier experiments, particularly with the pieces after section, was the occurrence of motor activity, which of course increased CO_2 production and introduced a source of error. In these experiments as in earlier work, it was observed that pieces from regions near the head are much more likely to show apparently spontaneous motor activity during the first few hours after section, than pieces from the more posterior levels of the first zooid. After attempting in various ways to eliminate motor activity, it was found that decrease of a few degrees in temperature was usually effective for the length of time necessary. In all the experiments tabulated below the animals were kept at 21° to 22° C in the stocks and during preparation, but as soon as the tubes were sealed they were placed in water at a constant temperature of 18° in very dim diffuse daylight. Under these conditions motor activity occurred only rarely, but its occurrence was always noted in the record of the experiment. The procedure adopted in these experiments has been described at some length because in experimental work with animals of such small size it is extremely easy to go astray, if the various sources of error are not carefully controlled as far as possible.

The Stimulation of Pieces Following Section.

The temporary increase in susceptibility following section and its characteristic relation, both to size of piece and region of body (Child, 14, also p. 104 above) are so clearly shown by the susceptibility method that they are often used as laboratory experiments. Because they are temporary and apparently excitatory in character and so definitely related to size of piece

and region of body these changes in susceptibility are of special interest in connection with the question of the relation between susceptibility and metabolism. If changes in the rate of fundamental metabolism or of certain fundamental reactions are found to parallel these changes in susceptibility, it is evident that the susceptibility method, when properly used, is a rather delicate indicator of at least certain aspects of metabolic condition.

In the earlier experiments of Child, as well as in the preliminary work of Robbins, it was found that the changes and differences in CO_2 production following section appeared more clearly in animals which had been starved for a few days before experiment, than in those which had been more recently fed, although in the former the total CO_2 production and oxygen consumption are less than in the latter (Child, '19*a*, Hyman, '19*b*). It has been pointed out elsewhere (Child, '19*b*, '19*c* and various earlier papers), that the susceptibility method as used in these experiments gives information primarily concerning conditions in ectoderm and body wall, though with certain precautions it may be used to show differences in condition in the alimentary tract of *Planaria* and other forms. The susceptibility data, as well as other facts, indicate that the changes following section are at least in large measure confined to ectoderm and body wall, the alimentary tract not being affected to any great degree. The CO_2 production of the alimentary tract in fed animals constitutes, however, a large proportion of the total CO_2 production, moreover, the volume of the alimentary tract as compared with that of other organs is greater and a larger amount of food and reserves is usually present in regions near the mouth than in regions near the head, therefore it is desirable to decrease this alimentary CO_2 production as far as possible, in order that changes in other parts of the body may appear more clearly. It has been shown (Child, '19*a*, Hyman, '19*b*) that a rapid decrease in both CO_2 production and oxygen consumption occurs in *Planaria dorotocephala* during the first few days of starvation and that an increase in both follows so rapidly after even a single feeding that it cannot be due to oxidation of the food following assimilation, but must be due to stimulation of the alimentary tract by the food. Allen ('19) has recently recorded the occurrence of similar

changes in oxygen consumption in two other species of *Planaria* during the early stages of starvation. It is evident that the rapid decrease in CO_2 production and oxygen consumption during the early stages of starvation is due in large measure, if not wholly, to the decrease in functional activity of the alimentary tract in the absence of food newly ingested. In the light of all these facts the reason for the use in these experiments of animals which have been starved a few days is evident. The length of the period without food is given in each experiment: in no case is it long enough to produce any marked reduction in size or other changes except those in the alimentary tract.

Since the purpose of these experiments is to determine whether differences and changes in susceptibility following section are paralleled by differences and changes in CO_2 production, the size of animals and pieces used and the regions of body included are those which show the most definite and characteristic differences and changes in susceptibility. The experimental material for the data presented in Table I. was prepared as follows: animals sixteen to eighteen mm. were selected from well fed laboratory stock, those which had recently undergone fission being excluded, were kept without food for several days (see Table I.) and were then cut into pieces as indicated in Fig. 1, piece *C* being cut so that the greater part of the pharynx is separated from its attachment and is extracted from the pharyngeal pouch, *i.e.*, piece *C* contains a part of the pharyngeal pouch, but no portion of the pharynx. Pieces *A* and *C* were used in the experiments as representing respectively the most anterior and the most posterior portion of the first or chief member or zooid in animals of this size (Child, '11*b*). In the intact animal the susceptibility of the region corresponding to piece *A* is very much greater than that of the region corresponding to piece *C*. Immediately after section the susceptibility of piece *A* shows either a slight increase or no marked change, while the susceptibility of *C* is increased to such a degree that it is equal to or even greater than that of *A* (Child, '14). The region *B* between *A* and *C* is intermediate both as regards original susceptibility and the changes following section and is not used in these experiments. The increase in susceptibility following section, which is most marked in the *C*-piece is

temporary and after six to eight hours is in course of disappearance and sooner or later (12 to 24 hours under ordinary conditions) the susceptibility of the piece becomes about the same as or a little less than that of the corresponding regions in the intact animal. These temporary changes in susceptibility indicate that the pieces have been stimulated by section, anterior pieces least, posterior pieces most, and that the condition of excitation disappears after a few hours. It has been suggested elsewhere (Child, '14) that the difference in the degree of stimulation in anterior and posterior pieces results from the greater dependence of the more posterior regions upon impulses from regions anterior to them, so that when the paths of these impulses are cut the condition of the more posterior levels is more affected than that of the anterior levels. This is in agreement with the observations of various investigators concerning the difference in intensity of reaction in many animals between levels anterior and those posterior to a transverse cut.

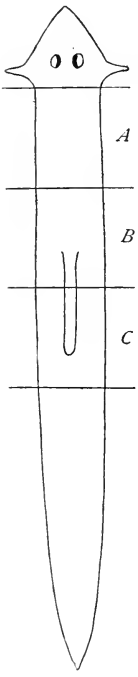

FIG. 1.

The region of the body posterior to *C* in Fig. 1 is not used in the tabulated experiments because it consists of one or more zooids indicated by differences in physiological condition (Child, '10, '11*c*). Since the number of these posterior zooids differs in different animals and no morphological boundaries are visible, pieces from the same levels of this region in different animals are not always strictly comparable physiologically and therefore merely complicate the experimental data.

Each experiment on CO_2 production in the pieces *A* and *C* consists essentially in determining the rate of change in *pH*, once as soon as possible after section and again several hours later, of one lot of *A*-pieces and one of *C*-pieces, consisting of thirty to fifty pieces each and of as nearly as possible equal weight, in equal volumes of indicator solution at a constant temperature

of 18° C. As soon as *pH* 7.3 is reached in the first determination, the tubes are opened, the pieces are returned to water and left undisturbed for the desired number of hours, *i.e.*, at least long enough as determined by the susceptibility method for disappearance in large measure of the temporary changes following section. At the end of this time the rate of *pH* change is again determined for each lot under as nearly as possible the same experimental conditions as the first determination.

Fifteen such experiments were performed before the technique used in the experiments recorded in Table I. was fully worked out. In eight of these fifteen experiments the *A*-pieces showed marked motor activity and a more rapid change in *pH* than the *C*-pieces immediately after section, in three there was either death of some of the pieces from drying or an error in weighing, and in four none of these sources of error was involved and the result were similar to those in Table I.

Table I. gives the data for thirteen experiments, in all of which the technique was as nearly as possible the same. The only feature of the table which requires explanation is the column "Time after section." The times given in this column are the times of sealing of the tubes containing the animals in indicator solution. The first time for each lot, "Immed.," *i.e.*, immediately, means simply that the *pH* determination was begun as soon as possible after the pieces were sectioned. Since it requires a half hour or more to cut lots consisting of forty to fifty *A* and *C*-pieces, the time between section and sealing may be as much as an hour for the pieces cut first and only a few minutes for those cut latest.

In eleven of the thirteen experiments recorded, 84 per cent., the CO_2 production of the *C*-pieces immediately after section is about equal to, or greater than that of the *A*-pieces. As regards the other two experiments, Nos. 7 and 8, in No. 7 three *C*-pieces died during the first determination, and in No. 8, motor activity occurred in the *A*-pieces, perhaps because of a slight rise in temperature of the water in which the tubes were kept. The second *pH* determination, begun at various lengths of time ranging from nine to forty-two hours after section, shows in every case a lower rate of CO_2 production in *C* than in *A*.

TABLE I.

A COMPARISON OF ANTERIOR AND POSTERIOR PIECES IMMEDIATELY AFTER SECTION AND A NUMBER OF HOURS LATER.

Expt. No.	No. of Pieces.	Wt. in Grams.	No. of Days Without Food.	Temperature in Degrees C.	pH Starting Point.	Time After Section in Hrs. and Min.	Time in Hrs and Min. to Reach pH 7.3.
1	A 37	0.023	11	17.5	7.8	immed.	5:40
						22:30	5:40
	C 31	0.0231	11	17.5	7.8	immed.	5:25
						22:30	5:55
2	A 49	0.0255	24	18	7.9	immed.	6:25
						9:00	6:30
	C 45	0.0257	24	18	7.9	immed.	6:15
						9:00	6:45
3	A 56	0.0245	21	18	7.82	immed.	5:18
						19:40	6:05
	C 48	0.0243	21	18	7.82	immed.	5:05
						19:40	6:23
4	A 20	0.0182	8	18	7.83	immed.	6:10
						18:40	6:22
	C 23	0.0185	8	18	7.83	immed.	6:00
						18:40	6:45
5	A 51	0.0208	9	18	7.83	immed.	5:30
						20:15	5:35
	C 44	0.0207	9	18	7.83	immed.	5:05
						20:15	5:55
6	A 37	0.0174	12	18	7.83	immed.	6:30
						18:00	6:35
	C 42	0.0176	12	18	7.83	immed.	6:10
						18:00	6:45
7[1]	A 37	0.0143	13	18	7.83	immed.	7:20
						21:30	7:45
	C 37	0.0142	13	18	7.83	immed.	7:35
						21:30	8:00
8[2]	A 40	0.0216	7	18	7.83	immed.	4:50
						17:45	5:05
	C 41	0.0213	7	18	7.83	immed.	4:55
						17:45	5:10
9[3]	A 36	0.0238	8	18	7.83	immed.	4:50
				18.5		19:00	4:38
	C 36	0.0239	8	18	7.83	immed.	4:35
				18.5		19:00	4:50
10	A 37	0.0242	9	18	7.83	immed.	4:40
						22:00	4:55
	C 38	0.0241	9	18	7.83	immed.	4:15
						22:00	5:15
11	A 44	0.0242	7	18	7.95	immed.	5:30
						19:00	5:35
	C 45	0.024	7	18	7.95	immed.	5:10
						19:00	5:50
12	A 44	0.0306	8	18	7.95	immed.	4:45
						42:00	4:40
	C 50	0.0305	8	18	7.95	immed.	4:15
						42:00	4:50
13	A 29	0.021	13	18	7.8	immed.	4:05
						25:00	4:10
	C 21	0.0208	13	18	7.8	immed.	3:50
						25:00	4:25

As regards the two records for the same lot, the *A*-pieces show in general a slightly lower CO_2 production in the second period than in the first, or in some cases about the same. Experiment 9, however, shows a slightly higher rate in *A* during the second period, this being due probably to the slightly higher water temperature during the second period. In the *C*-pieces the rate is distinctly lower, in many cases much lower in the second period than in the first. Differences in time of five minutes between the records of two lots made at the same time mean only that the two were barely distinguishable as different. Differences of this mangitude between the two records of the same lot can mean no more than that the rate is essentially the same in the two periods. Differences of ten minutes or more are however, unquestionably significant and differences of much greater magnitude appear in the table.

The table shows then that immediately after section there is in some cases a slight temporary increase in CO_2 production in the *A*-pieces and a very marked increase in the *C*-pieces, that is to say the differences and changes in CO_2 production following section are in general parallel to the differences and changes in susceptibility. The objection may be raised that the differences in CO_2 production are less than would be expected from the stimulation by section, but when it is remembered that there is no reason to believe that the alimentary tract shares in this stimulation except locally at the cut surface and that the pieces do not undergo motor activity during the determination this objection has little weight. As a matter of fact the close parallelism between the data on CO_2 production and those on susceptibility indicates that even as regards the temporary changes following section of pieces, susceptibility is in some degree

[1] During the first determination in No. 7 three *C*-pieces died. When the determination was repeated the following day, two *C*-pieces, which had been cut with the others but not used before, were added to lot *C* and one piece was removed from lot *A* to make weights as nearly as possible equal. Only two extra worms had been sectioned, therefore three pieces could not be added to take the place of those dead.

[2] Slight motor activity occurred in the *A*-pieces during the first determination.

[3] Here the temperature was half a degree higher in the second determination than in the first, and the *A*-pieces show a slightly increased rate in the second determination. Footnotes for Table 1.

a measure of physiological and particularly of respiratory condition.

Carbon Dioxide Production after Regeneration.

In these experiments stocks of pieces of the size of *A*, *B*, *C*, Fig. 1, were cut from animals 16–18 mm. and allowed to undergo regeneration until the development of the new individuals was essentially complete, usually about two weeks. From these stocks the experimental lots were selected. These consisted only of normal individuals, or in some cases where the number of normal animals in the regenerated stock was not sufficient, mostly of normal with a few teratophthalmic individuals (Child, '11*a*) added. Since the pieces undergoing regulatory development cannot feed until they attain an advanced stage, the animals representing the condition before regulation, with which the regenerated individuals are to be compared, are kept without food for the same length of time as the pieces undergoing regeneration. Usually a stock of intact worms of the same size, 16–18 mm., as those from which the pieces were taken is isolated at

Table II.

A Comparison of Small Animals Regenerated from Pieces with Large Animals that Have Not Recently Undergone Regeneration or Fission, of the same size as those from which the pieces were taken. Both Unfed. Determinations of pH at + 18° C.

Expt. No.	No. of Worms.	Wt. in Grams.	Period of Regeneration or Starvation in Days.	pH Starting Point.	Time in Hrs. and Min. to Reach pH 7.3.
1	3 large	0.0114	10	7.89	10:25
	16 regen.	0.0113	10	7.89	9:00
2	4 large	0.0163	11	7.89	8:55
	22 regen.	0.016	11	7.89	6:45
3	4 large	0.0174	10	7.89	8:00
	27 regen.	0.0176	10	7.89	6:15
4	4 large	0.0117	14	7.9	14:35
	19 regen.	0.0115	14	7.9	12:35
5	5 large	0.0205	14	7.9	9:35
	34 regen.	0.0204	14	7.9	6:50
6	3 large	0.0101	21	7.8	10:00
			7 starved		
	26 regen.	0.099	14 regen.	7.8	7:45
7	3 large	0.0109	20	7.8	8:30
			7 starved		
	19 regen.	0.011	13 regen.	7.8	7:50
8	3 large	0.0166	10	7.8	6:30
	28 regen.	0.0164	10	7.8	3:50

the same time the pieces are cut. This stock merely undergoes a slight degree of starvation, while the pieces undergo starvation for the same period and in addition the regulatory changes. This is the procedure in experiments 1–5 and 8 in Table II., but in experiments 6 and 7 the stock from which both pieces and whole animals were obtained was starved seven days before the pieces were cut.

Each experiment in Table II., includes one lot of worms about 5 mm. in length which have developed from pieces cut ten to fourteen days earlier ("regen." in table), and one lot of as nearly as possible the same weight of worms 16–18 mm. in length, the same size and from the same general stock as the worms from which the pieces were cut, and kept without food for the same length of time ("large" in table). In all cases the *pH* determinations are made before feeding is resumed.

Examination of the last column of the table shows that the rate of CO_2 production is much higher in the small regenerated, than in the large old animals, *i.e.*, the regulatory processes have been accompanied by an increase in rate of CO_2 production. Moreover the rate is in general higher in the regenerated animals

TABLE III.

A COMPARISON OF SMALL ANIMALS REGENERATED FROM PIECES WITH LARGE ANIMALS OF THE SAME SIZE AS THOSE FROM WHICH THE PIECES WERE TAKEN. Fed three times. Determinations of pH at 18° C.

Expt. No.	No. of Worms.	Wt. in Grams.	pH Starting Point.	Time in Hours and Minutes to Reach pH 7.3.
1	3 large	0.0127	7.82	5:00
	10 regen.	0.0125	7.82	4:30
2	4 large	0.0193	7.82	3:45
	15 regen.	0.0191	7.82	2:55
3	4 large	0.0154	7.82	4:50
	13 regen.	0.0155	7.82	3:35
4	4 large	0.0135	7.9	7:15
	18 regen.	0.0134	7.9	5:50
5	5 large	0.0233	7.9	5:05
	28 regen.	0.023	7.9	3:45
6	2 large heads off	0.0155	7.8	8:15
	18 regen.	0.0113	7.8	4:25

than in the pieces of Table I. although the period without food is in most cases longer in the latter than in the former and CO_2 production decreases during the early stages of starvation.

Table III. records experiments similar to those of Table II.

except for the fact that both lots were fed with beef liver three times before weighing and *pH* determination, the first two feedings being on successive days, the third after an interval of one day. In the first five experiments of Table III. worms from the first five experiments of Table II. were used, but in smaller numbers because of the increased weights after feeding, particularly in the regenerated animals. In Table III., as in Table II. the "large" animals are those which have not undergone regeneration and represent as nearly as possible the animals from which the pieces were taken, and the "regenerated" animals are those which have developed from the pieces. Table III. agrees with earlier work (Child, '19*a*) in showing that the rate of change in *pH* is increased in all animals by feeding after a period of starvation, but it also shows that the difference in rate between the regenerated and the large animals persists after feeding. Here again the data on CO_2 production agree with the results of the susceptibility method (Child, '15, Chap. IV.). Data on oxygen consumption recently published by Allen ('19) and by Hyman ('19*b*) also agree with these results.

Additional Data.

Table IV. includes a number of miscellaneous experiments of some interest.

In the course of regulatory development an outgrowth of new tissue occurs at anterior and posterior ends of each piece. All the facts indicate that this tissue, which forms the new head and posterior end, is more or less embryonic in character when it arises and possesses, at least at first, a higher rate of metabolism than the remainder of the piece. In order to determine whether the higher rate of CO_2 production in regenerated animals is due solely to the more intense activity of this new tissue or whether the rate is also increased in other parts, the new heads and posterior ends were removed from regenerated animals leaving only the so-called old or less extremely altered tissue of the middle regions. Lots of such pieces were then compared with lots of equal weight of freshly cut *A*-pieces (Fig. 1) and of animals 16–18 mm. like those from which the pieces were taken, the heads being removed from these large animals in order to

make them more nearly comparable in condition with the *A*-pieces and the headless regenerated animals.

Experiment 1 of Table IV. includes one lot of each of the three groups and it is seen that the "old" parts of the regenerated

TABLE IV.

MISCELLANEOUS DATA.

Regenerated animals from which anterior and posterior new tissue has been removed, compared with *A*-pieces and with large headless animals: whole regenerated animals compared with *A*-pieces and with growing worms of same size from stock. Fed or unfed. Determinations of pH at 18° C.; pH at beginning of experiment 7.8.

Expt. No.	No. and Condition of Animals.	Wt. in Grams.	Nutrition.	Time in Hours and Minutes to Reach pH 7.3.
1	3 large, heads off.	0.093	Starved 19 days.	8:25
	12 *A*-pieces.	0.094	Starved 19 days.	7:15
	11 regen. new tissue off.	0.095	Starved 18 days.	6:25
2	9 *A*-pieces.	0.08	Fed three times.	8:15
	22 regen. new tissue off.	0.082	Fed three times.	7:30
3	3 large.	0.0111	Fed three times.	7:35
	15 regen. new tissue off.	0.0108	Fed three times.	6:05
4	22 *A*-pieces.	0.0167	Starved 10 days.	4:10
	28 regen.	0.0164	Starved 10 days.	3:50
5	11 small.	0.0096	Fed three times.	6:25
	18 regen.	0.0095	Fed three times.	5:25

animals show a higher rate of change than the *A*-pieces, while the headless large animals show the lowest rate of all.

In Experiment 2 the "old" parts of regenerated animals are compared with *A*-pieces, both lots being fed three times before sectioning. The result is the same as in experiment 1, the rate being distinctly higher in the parts of regenerated animals than in the *A*-pieces.

In experiment 3 the "old" parts of regenerated animals are compared with large old animals from which the heads have not been removed, both lots being fed three times after a starvation period of about two weeks. The result is the same as in Experiment 1, the parts of regenerated animals showing the higher rate.

Experiment 4 is a comparison of *A*-pieces with entire regenerated animals, *i.e.*, including the new heads and posterior

ends. Here the difference in rate is proportionally about the same as in Experiment 2, but somewhat less than in Experiment 1.

In Experiment 5 regenerated animals are compared with stock animals of slightly larger size (the smallest in the stock at the time) which were kept without food while the pieces were regenerating, both lots being fed three times before the experiment, and both consisting of entire animals. Here again the regenerated animals show a higher rate of CO_2 production than the slightly larger stock animals.

A few other experiments performed by one of us and in some cases also by students in the laboratory, are briefly mentioned here without tabulation of the data. It has been found, for example, that the degree of increase in both susceptibility and CO_2 production occurring in regeneration depends upon the degree of reorganization which occurs. Consequently the smaller the piece in relation to the size of the body from which it is taken, the greater the amount of increase in susceptibility and CO_2. Similarly in natural fission the posterior piece is not only smaller than the anterior but develops a new head at the anterior end and a prepharyngeal and pharyngeal region by reorganization and redifferentiation within the piece, while the anterior fission piece develops merely a new posterior end. In the animal developed from the posterior piece susceptibility and CO_2 production show a marked increase while in the anterior animal the only marked change in susceptibility is a slight increase in the posterior region, where reorganization and growth have occurred and the increase in CO_2 production is either slight or inappreciable. Allen ('19) has recently recorded somewhat similar results as regards oxygen consumption, an increase occurring in the posterior, but not in the anterior product of fission.

It has also been determined by one of us that susceptibility to lack of oxygen increases during the development of a new individual from a piece, the susceptibility of the new individual about two weeks after section of the piece, being distinctly higher than that of well fed animals of the same size and about the same as, or slightly than that of animals of the same size, kept without food for the same length of time as the regenerating

pieces. In other words, the new individuals developed from pieces show a susceptibility to lack of oxygen equal to or greater than that of much smaller younger animals than those from which the pieces were taken. These observations concern primarily the susceptibility of ectoderm and body wall.

Conclusion.

These experiments constitute a new test of the validity of the susceptibility method as a rough comparative means of determining physiological or metabolic condition and at every point the differences and changes in susceptibility, as determined by KNC and in many cases by various other agents also, are paralleled by differences and changes in rate of CO_2 production. Even the temporary stimulation of the pieces after section, which is slight or absent in the *A*-pieces and very marked in the *C*-pieces, appears in the data on CO_2 production and the increase in rate, at least of respiratory metabolism associated with regulation, is evident in the marked increase in rate of CO_2 production even in the "old" parts of the regenerated animal. This work may perhaps be regarded as in some respects the most delicate test of the relation between susceptibility and respiration which has been made up to the present.

As has been repeatedly stated, the susceptibility method is not an exact quantitative method, but a rather crude means of indicating differences of some sort in physiological condition and the differential susceptibility of different regions of the same individual affords a means of modifying and controlling various developmental and other processes. The facts at hand concerning susceptibility, *e.g.*, the lack of specificity, the close relation between susceptibility and physiological activity in development, growth and function as well as the positive evidence already obtained concerning the parallelism between susceptibility, oxygen consumption and CO_2 production indicate very clearly that a more or less definite relation exists between the susceptibility of living protoplasm, to at least many external agents and conditions within certain ranges of concentration or intensity, and the rate or intensity of certain fundamental physiological processes, particularly those which liberate energy.

This is all that is meant when susceptibility is interpreted in terms of metabolism or oxidation and the exact nature, degree and extent of this relation of course remains to be determined. This interpretation does not involve the assumption that susceptibility must always be parallel or even proportional to total oxidation or even to total oxygen consumption or CO_2 production.

The relation between susceptibility and oxidation is undoubtedly indirect in at least most cases and it is conceivable that susceptibility may be related only or primarily to certain oxidative reactions or to conditions associated with them. Moreover, it is certain that in many cases susceptibility as determined by death and disintegration is dependent primarily upon conditions or reactions in particular regions of the body, *e.g.*, in ciliate infusoria the ectoplasm, in *Planaria* the ectoderm and body-wall. Moreover, as many investigators have pointed out, it is by no means certain that oxygen consumption and CO_2 production are exact quantitative measures of oxidation at any given time. It is to be expected that susceptibility will not always be proportional to total oxygen consumption or CO_2 production, but even then susceptibility may prove in the long run to be a better indicator or comparative measure of physiological condition than the respiratory data.

From what has been said above and in earlier papers (*e.g.*, Child, '19*c*) it is evident that the criticisms of the susceptibility method recently advanced by Lund ('18*a*, *b*) and Allen ('18, '19) need no discussion here, since they are largely beside the point and result from failure to grasp the conception of susceptibility, which has developed from many different lines of investigation, not from one alone. Even if we grant the correctness of certain of their conclusions from experimental data which are or appear at present to be in conflict with conclusions reached in this laboratory (Child, '19*a*, *b*, *c*, Hyman, '19*a*, *b*) on the basis of more extensive investigation, with more satisfactory technique and several different methods instead of one they do not constitute adequate grounds for denying the physiological significance of susceptibility, but rather merely a starting point for the further analysis of the particular cases in question.

As regards the real significance of susceptibility, it makes

little difference whether or not it shall be found to run exactly parallel to the other indices of total respiration in any particular case. It cannot, however be denied that a wide range of facts determined by many different lines of investigation do indicate clearly the existence of a more or less definite relation between susceptibility and oxidation in at least many cases, and it is of interest to determine range, degree and nature of this relation. The present paper like several others which have recently appeared from this laboratory is a contribution to this problem, but it must be remembered that data such as these are not the only criteria of the physiological significance of susceptibility and its relation to the energy-liberating reactions in the metabolic complex.

Summary.

1. The colorimetric estimation of CO_2 production shows that the changes in CO_2 production following section in pieces of the body of *Planaria dorotocephala* run parallel with changes in susceptibility. Immediately following section CO_2 production is markedly increased in pieces cut from near the mouth region, while in pieces from regions near the head it is only slightly if at all increased. These changes are temporary excitations following section and disappear after a number of hours.

2. The development of a new individual from a piece is accompanied by a very considerable increase in CO_2 production which involves not only the new outgrowths at the two ends of the new animal but the "old" parts as well. This increase in CO_2 production is found both before and after feeding is resumed following the development of the piece. In these respects also the changes in CO_2 production parallel changes in susceptibility, both series of data indicating that the animal developing from an isolated piece becomes in the course of this development, physiologically younger than the animal from which the piece originated.

References.

Allen, G. D.

'18 Quantitative Studies of the Rate of Respiratory Metabolism in *Planaria*, I. Amer. Jour. Physiol., XLVIII.

'19 Quantitative Studies, etc., II. Amer. Jour. Physiol., XLIX.

Child, C. M.

'10 Physiological Isolation of Parts and Fission in *Planaria*. Arch. f. Entwickelungsmech., XXX., II. Teil.

'11a Experimental Control of Morphogenesis in the Regulation of *Planaria*, BIOL. BULL., XX.

'11b Studies on the Dynamics of Morphogenesis, etc., I. Jour. Exp. Zool., X.

'11c Studies, III. Jour. Exp. Zool., XI.

'13a Studies, etc., V. Jour. Exp. Zool., XIV.

'13b Studies, etc., VI. Arch. f. Entwickelungsmech., XXXVII.

'14 Studies, etc., VII. Jour. Exp. Zool., XVI.

'15 Senescence and Rejuvenescence. Chicago.

'19a A Comparative Study of Carbon Dioxde Production During Starvation. in *Planaria*. Amer. Jour. Physiol., XLVIII.

'19b The Effect of Cyanides on Carbon Dioxide Production in *Planaria dorotocephala*. Amer. Jour. Physiol., XLVII.

'19c Susceptibility to Lack of Oxygen During Starvation in *Planaria*. Amer. Jour. Physiol., XLIX.

Hyman, L. H.

'19a On the Action of Certain Substances on Oxygen Consumption, II. Amer. Jour. Physiol., XLVIII.

'19b Physiological Studies on *Planaria*, I. Amer. Jour. Physiol., XLIX.

'19c Physiological Studies on *Planaria*, II. Amer. Jour. Physiol., L.

Lund, E. J.

'18a Quantitative Studies on Intracellular Respiration, II. Amer. Jour. Physiol., XLV.

'18b Quantitative Studies, etc., III. Amer. Jour. Physiol., XLVII.

SUSCEPTIBLE AND RESISTANT PHASES OF THE DIVIDING SEA-URCHIN EGG WHEN SUBJECTED TO VARIOUS CONCENTRATIONS OF LIPOID-SOLUBLE SUBSTANCES, ESPECIALLY THE HIGHER ALCOHOLS.

FRANCIS MARSH BALDWIN.

Introduction.

That dividing *Arbacia* eggs show periods of varying susceptibility and resistance when exposed to chemical substances and to various physical conditions has been proved by numerous investigations. When eggs, from a single fertilized lot were placed at regular successive intervals after fertilization in cyanide-containing sea-water (*m*/100 to *m*/200), Lyon[2] found that they were highly resistant to poisoning fifteen or twenty minutes after fertilization, while eggs exposed to the same solution at the time of cytoplasmic division were promptly killed. Later, after the first division had been completed, the resistance to poisoning again returned, followed by a second susceptible period at the second cleavage. Loeb[3] later noted that the unfertilized eggs show greater resistance to cyanide poisoning than the fertilized eggs, and Mathews[4] indicated that in dividing eggs, the period of maximum susceptibility is "immediately before and during segmentation," and that just after segmentation the egg becomes relatively highly resistant. Similar results were obtained by Spaulding[5] in experiments with weak solutions of ether (1/64 per cent. in sea-water). The period of high resistance continued up to the beginning of the first cleavage, and then fell during cleavage to zero, with a sharp rise immediately afterwards. There was a short period of susceptibility immediately following

[1] From the Marine Biological Laboratory, Woods Hole, Mass., and the Department of Zoölogy, Iowa State College, Ames, Iowa.

[2] E. P. Lyon, *Amer. Jour. Physiol.*, 1902, Vol. 7, p. 56.

[3] J. Loeb, *Biochem. Zeitschr.*, 1906, Vol. 1, p. 200.

[4] A. P. Mathews, Biol. Bull., 1906, Vol. 11, p. 137.

[5] E. G. Spaulding, Biol. Bull., 1904, Vol. 6, p. 224.

fertilization. He found also in acid and salt solutions (pure isotonic KCL and NaCL) a similar but less clearly defined rhythm of susceptibility. Eggs subjected to heat, electrical stimulation and hypertonic sea-water behave in a similar manner. Thus, Lyon,[1] observed that the eggs were most resistant to heat at a time previous to the first cleavage, and were most readily injured at the time of division. A. R. Moore[2] finds that the resistance to hypertonic sea-water is least "immediately before and during each cytoplasmic division, and that the maximal resistance is shown 35 to 45 minutes after fertilization and just after each division." More recently Lillie[3] (1916), has made an extensive study of the rhythmical changes in the resistance of the dividing sea-urchin egg to hypotonic sea-water, and has discussed the physiological significance of this rhythm. His experiments show clearly that at or about the time of formation of the cleavage furrow, a marked decline takes place in the resistance of the egg to hypotony, and cytolysis is then rapid and complete. After the cleavage furrow is fully formed the original resistance returns. A similar reversible decline of resistance takes place at the second and third cleavage, and is probably general for mitotic cell-division. The minimum of resistance is found during the formation of the furrow. Both the decline and the return of resistance are rapid, the greater part of each phase occupying four to five minutes. Some increase of susceptibility is apparent ten or twelve minutes before the first appearance of the furrow. Similar observations have been made by Herlant[4] in the egg of *Paracentrotus lividus*.

From such experiments it appears that the resistance of the eggs to a variety of injurious agencies is least at the time when they are undergoing rapid change of form. To account for these rhythmical changes in the physiological state of the egg, Lillie[4] (1909) puts forward the hypothesis that they are essentially the result of variations in the physical condition, especially the permeability, of the surface-film of plasma-membrane, the latter

[1] E. P. Lyon, *Amer. Jour. Physiol.*, 1904, Vol. 11, p. 52.

[2] A. R. Moore, Biol. Bull., 1915, Vol. 28, p. 257.

[3] R. S. Lillie, *Jour. Exper. Zoöl.*, 1916, Vol. 21, No. 3, p. 401.

[4] M. Herlant, *Comptes rendus d. l. Societe d. Biologie*, 1918, Vol. 81, p. 151.

[5] R. S. Lillie, Biol. Bull., 1909, Vol. 17, p. 207.

undergoing a reversible increase in permeability at the time of cleavage. If a rhythm of alternate increase and decrease of permeability accompanies the rhythm of the mitotic process, it seems logical to infer that the entrance of solutes into the cell would occur most readily when there is a loss of semi-permeability. Accompanying this change would be a decrease of the electrical surface-polarization, and this in turn probably would alter the metabolic processes, especially oxidations within the cell. Cell metabolism then is inseparably bound up with cell-permeability; and the plasma-membrane, or semi-permeable surface-layer is something more than a haptogen membrane (to which it has frequently been compared). In discussing this subject in a later paper, Lillie[1] makes it especially clear that this "general characteristic of semi-permeability (the all-essential insulating and diffusing-preventing property) is not merely the result of a special chemical composition and structural density, such as determine the semi-permeability of a precipitation-membrane, but is inseparable from the living condition, *i.e.*, is actively maintained by a continual process of metabolism. The proof of this is that death—the cessation of metabolism—however caused, is invariably followed by a loss of semi-permeability, *i.e.*, the normal state of the membrane then ceases to be maintained and the unhindered processes of diffusion lead to the disintegration of the cell. Hence destruction of the surface-layer by artificial means—cytolytic substances, heat, extensive mechanical injury—is quickly fatal to all cells."

In the experiments about to be described, I have studied the behavior of fertilized Arbacia eggs when subjected for definite brief lengths of time to various concentrations of some of the higher alcohols—anyl, hexyl, heptyl, octyl and capryl—at different periods of the cell-division cycle. This work was undertaken at the Marine Biological Laboratory, at Woods Hole, Mass., during the past summer at the suggestion of Professor Ralph Lillie, to whom the writer expresses his hearty thanks for many kind suggestions and directions during its prosecution.

[1] R. S. Lillie, *Amer. Journ. Physiol.*, 1918, Vol. 45, No. 4, p. 406.

Experimentation.

In order to procure a sufficient number of eggs for each series of experiments, between one and two dozen large females were opened, and their eggs collected into finger bowls. By successive washing and settling, a uniform mass of mature eggs was obtained, which could be inseminated and divided into two parts; one to be used for the control, and the other for the experiments. It was found early in the work that the success of the experiments depended upon having batches of eggs which were sufficiently mature and uniform, so that all eggs reached successive stages in their development at practically the same time. It was also found that great exactness in the time-relations of the operations was absolutely essential, and that any variation once entered upon was sufficient to make the results worthless from a comparative standpoint. Usually two series of experiments were started in a day; one in the morning to be carried over to the gastrula stage by the following morning, and one in the afternoon, to be examined the following afternoon. After extended preliminary experimentation, it was found convenient, in any one series, to keep the time of exposure constant and to vary the concentration of substance used, although in a considerable number of experiments the opposite procedure was adopted, *i.e.*, the time was varied and the concentration kept constant.

Practically the same procedure was observed throughout the entire experimentation. At each of the successive intervals after fertilization, usually ten minute intervals, about one half of medicine pipette containing a suspension of the inseminated eggs was placed in a small corked Erlenmeyer flask, containing 50 c.c. of the solution of the alcohol in sea-water, and allowed to remain for the time of exposure chosen (usually five minutes). After the given time had nearly elapsed, the excess liquid was poured off, and the eggs with a little of the liquid were placed in a watch glass and the immediate results of the treatment were observed under the low power of the microscope. At the termination of the time of exposure, the watch glass containing the eggs was carefully immersed in a large volume of sea-water in a finger bowl and the water was changed several times to rid it of the excess substance. Finally the eggs were very carefully

washed with a stream of water from the medicine-dropper, and set aside to undergo development. The proportion proceeding with development to the free-swimming larval stage was subsequently determined. It was found that the estimate of the proportion surviving to the blastula stage was more readily and exactly made, if the watch glass containing the eggs was removed from the bowl of sea-water just before the free-swimming larval stage was reached. Thus all survivors could be confined within a small volume, and the count or estimate easily made. As a rule, the experiments were carried only up to about the time of second cleavage; since the evidence indicates that the same variation of susceptibility occurs in each cell division cycle; moreover divergencies between the different eggs in any lot become more pronounced as time elapses, and it is important that all eggs of a lot should be in the same physiological state at the time of treatment.

At first several preliminary experiments were necessary in order to determine the most suitable range of concentrations to be used, since the time of exposures determined upon were brief, the longest being ten minutes; in some cases of exposures only three minutes were used. In this connection, the tables given by Lillie[1] in his paper on the action of various anæsthetics in suppressing cell-division in sea-urchin eggs, were exceedingly helpful. For i-Amyl[2] alcohol, he finds 0.45 to 0.4 vol. per cent. a favorable anæsthetic concentration for eggs subjected for two and one half hours, while 0.5 vol. per cent. and above are somewhat rapidly toxic. For Capryl[3] alcohol he finds the anæsthetizing concentrations to range between 0.012 and 0.02, and notes that even in sub-anæsthetic concentrations this alcohol exhibits a relatively high specific toxicity. With the help of these data, and also Fühner's[4] observations showing that in a series of monohydric aliphatic alcohols each member of the group is from three to four times as effective (for equimolecular concentrations)[5] as its immediate predecessor, it became a compara-

[1] R. S. Lillie, *Journ. Biolog. Chem.*, 1914, Vol. 17, No. 2, pp. 129–139.

[2] Cf. reference just cited; Table VIII., p. 135.

[3] Cf. reference just cited; Table IX., p. 137.

[4] H. Fühner, *Arch. f. exp. Path. u. Pharm.*, 1904, LII., p. 69.

[5] Capryl alcohol used in exposures of five minutes seemed not to obey this general rule, since in practically all experiments it was used in concentrations nearly three times its computed strength. (See p. 137.)

tively easy matter to approximate the most suitable concentration of each alcohol after the first had been determined.

Amyl Alcohol.

Summarizing briefly the results of preliminary observations it was found that the most satisfactory concentration of i-Amyl alcohol when used with exposures of three to eight minutes, was between 0.7 and 0.9 vol. per cent. Solutions of this strength are sufficiently toxic to prevent many but not all of the eggs thus treated from developing to a larval stage. Solutions weaker than 0.7 vol. per cent. permit practically all eggs to proceed to

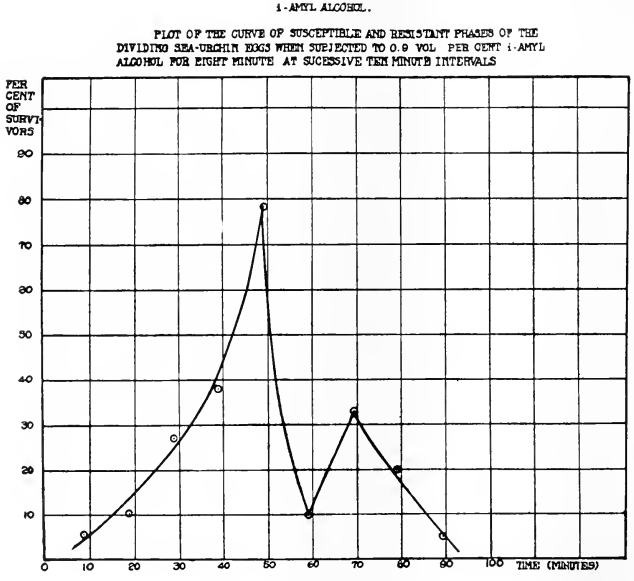

Fig. 1.

the blastula stage with these exposures, with little appreciable difference. At 1.0 vol. per cent. not more than 20 per cent. of eggs form free-swimming blastulæ even when exposed at the period of highest resistance; (*e.g.*, 30 to 40 minutes after fertili-

zation) and above this concentration the toxicity is such that the decline in survivals is very rapid. In 1.25 vol. per cent. solutions, all eggs are killed in all stages with exposures of nine minutes.

Table I. summarizes the results of a typical series of experiments with i-amyl alcohol. This particular series of experiments was started in the afternoon of July 16, and the observations noted in the third column were carried over into the morning of

TABLE I.

I-AMYL ALCOHOL.

July 16, 1:45 P.M. The fertilized eggs were placed at the intervals after fertilization noted in column 1 in 50 cc. of 0.9 vol. per cent i-amyl alcohol. Al exposures except the first (1) (6 minutes) were of eight minutes duration.

Intervals After Fertilization.	Observed Condition of the Eggs at the Time of Removal from Sol.	Proportion Forming Blastulæ and Condition of Remaining Eggs Next Day.
(1) 3– 9 m.	Fertilization-membranes well formed. No marked cytolytic change noted.	About 5 per cent. form free swimming blastulæ. Considerable numbers cytolyzed.
(2) 10–18 m.	No marked change in appearance. Uniform.	About 10 per cent. form blastulæ. Not so badly cytolyzed; most cells intact.
(3) 20–28 m.	Membranes markedly swollen in some cases. Slight fading of pigment.	Nearly 30 per cent. free-swimming blastulæ. Most eggs intact.
(4) 30–38 m.	A few cells plasmolyzed, other membranes markedly swollen.	Between 30 and 40 per cent. free-swimming blastulæ. Most others intact but swollen.
(5) 40–48 m.	No marked change. Faint indication of cleavage furrow in a few scattered cells.	Large majority (75–80 per cent.) form swimming blastulæ.
(6) 50–58 m.	About 65 per cent. have entered the two-celled stage. Some show shrinkage.	Relatively few (less than 10 per cent.) form surviving blastulæ.
(7) 60–68 m.	About 90 per cent. in two-celled stage. Others intact.	Between 30–35 per cent. form blastulæ.
(8) 70–78 m.	Practically all in two-celled stage.	Few (15–20 per cent.) form blastulæ. Others intact.
(9) 80–88 m.	A few are starting second cleavage furrow.	3–5 per cent. form blastulæ. Others intact.

the following day. A similar series of experiments performed at about the same time with the same alcohol in somewhat lower concentration (0.8 vol. per cent.), but with slightly longer (10-minute) exposures yielded substantially the same results. On the following day experiments were carried out on eggs subjected to 1.1 vol. per cent. solutions with only brief (3-, 4- and 5-minute) exposures with the results noted above.

In the controls about one half of the eggs were in the two-celled stage at fifty-three minutes after fertilization, and at sixty-five minutes between 85 and 90 per cent. were divided. There is a definite period of well-marked susceptibility immediately following fertilization; the susceptibility then gradually and progressively declines up to the end of forty-eight minutes (just before the first cleavage). There then follows a very susceptible period just at the time of cleavage. Later the resistant phase reappears until about the time of second cleavage. If the time intervals are plotted as abscissæ, and the percentage of surviving blastulæ as ordinates, the relationships may be represented in the curve shown in Fig. 1.

Hexyl Alcohol.

In exploring the range of suitable concentrations for hexyl alcohol, the next higher member of the series, assuming that it should be approximately three times as effective as i-amyl alcohol, three preliminary experiments were performed. For these, solutions of 0.1, 0.25 and 0.30 vol. per cent. were used respectively. The time of exposure was shortened to five minutes, for the reason that it was thought the concentrations were, if anything, a little above the optimum. The results clearly showed that the solutions of 0.1 vol. per cent. was not sufficiently toxic to demonstrate any variation of susceptibility in the eggs, since at whatever period they were exposed practically all eggs survived to the free-swimming blastula stage. On the other hand, the two higher concentrations proved too toxic, so that practically none of the eggs continued their development after subjection to these solutions at any period. The 0.25 vol. per cent. solution, although it suppressed further development, was not quite intense enough in its action to cause cytolysis in the eggs, with few exceptions. The 0.30 vol. per cent. concentration caused very evident cytolysis, and rupture was almost universal. Accordingly, series of experiments were carried out to test the various concentrations between 0.1 vol. per cent. and 0.25 vol. per cent. Two of these experiments are summarized in Table II., and may be regarded as typical.

These results show a much less definite evidence of a rhythm of

TABLE II.

HEXYL ALCOHOL.

August 11, 10:15 A.M. Fertilized Arbacia eggs were placed at intervals noted in 50 cc. of 0.13 and 0.17 vol. per cent. of Hexyl alcohol respectively, and allowed to remain in them for five minutes. They were then placed in watch glasses, quickly observed, and treated as described in the previous experiment.

Intervals After Fertilization.	(A) 0.13 Vol. Per Cent.		(B) 0.17 Vol. Per Cent.	
	Observed Condition.	Proportion Forming Blastulæ.	Observed Condition.	Proportion Forming Blastulæ.
(1) 15–20m.	Fert. membrane well formed. No marked cytolysis noted.	Majority (90 per cent.) form blastulæ.	No marked cytolysis. Uniform batch of fertile eggs.	Between 70 and 80 per cent. form blastulæ. Other cytolyzed but intact.
(2) 25–30m.	No marked change.	Between 85–90 per cent. form swimming blastulæ	Fert. membrane in most eggs swollen; no great change otherwise.	70–75 per cent. blastulæ. Few ruptured.
(3) 35–40m.	Membrane swollen, few show slight plasmolysis.	Practically all (90 per cent. or over) form blastulæ. Others intact.	No marked change noted.	90 per cent. form blastulæ.
(4) 45–50m.	Slight loss in pigmentation. No marked cytolysis however.	90 per cent. form blastulæ. Few ruptured.	Slight fading of pigment. No other marked change.	Between 65–70 per cent. form blastulæ. Few scattered cells ruptured.
(5) 55–60m.	About half in two-celled stage. No marked change.	80–85 per cent. form blastulæ. Others mostly intact.	All intact, 50 per cent. or over in two-celled stage.	Nearly 60 per cent. form blastulæ.
(6) 65–70m.	Fully 85 per cent. in two-celled stage; no marked change from preceding.	About 76 per cent. form swimming blastulæ.	No change.	Not more than 50 per cent. form blastulæ. Others badly cytolyzed but mostly intact.
(7) 75–80m.	No marked change.	Between 65–70 per cent. swimming blastulæ; others badly cytolyzed.	Slight loss of pigment. No marked change.	About 60 per cent. blastulæ. Two-celled eggs conspicuous. Most others intact.
(8) 85–90m.	Few cells (ca. 5–7 per cent.) show second cleavage. No marked change.	About 60 per cent. form swimming blastulæ. Most others intact.	Few cells in second cleavage. No marked cytolysis.	Nearly 85 per cent. swimming blastulæ.

susceptibility than those just described for i-amyl alcohol. The eggs apparently maintain throughout the cycle a relatively high resistance to the concentrations of hexyl alcohol here used, with only a slight increase of susceptibility at the time of

first cleavage; in the one case (0.17 vol. per cent.) there is evidence of a slight return of resistance just afterwards, and in the other (0.13 vol. per cent.) there is not. Why there should be this difference in the behavior of the two alcohols is difficult to explain. The exposures to hexyl alcohol were perhaps insufficiently

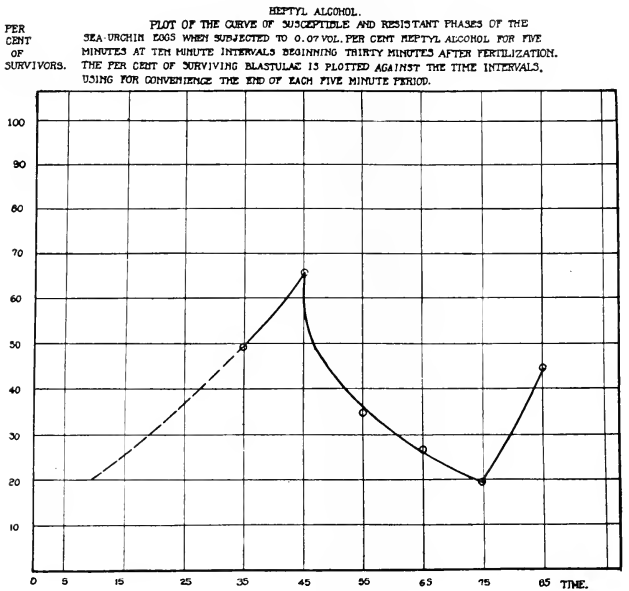

FIG. 2.

prolonged to bring out a well marked differential effect at the different stages of the cycle. Again, it is well known that certain anæsthetics are less effective than others in suppressing the cell-division process; also many neuromuscular responses react differently to a given anæsthetic in different animals, and in the same animal at different ages. Thus Lillie[1] found chloretone much less effective than chloral hydrate in suppressing cleavage in Arbacia eggs. As regards the alcohols that he tried, he lists propyl, butyl, amyl in the order of increasing favorability, while

[1] R. S. Lillie, *Jour. Biol. Chem.*, 1914, Vol. 17, No. 2, p. 130.

ethyl and capryl show a higher toxicity than the others.[1] It may be that differences,—both qualitative and quantitative—in the lipoid elements of the tissues, and hence in the plasmamembrane, form the basis of the observed physiological difference.

In the controls which were running parallel to the two experiments just described, a majority of the eggs entered the two-celled stage at about fifty-eight minutes after fertilization. At sixty-five minutes after fertilization, between 85 and 90 per cent. had cleaved. Practically all eggs in the controls had reached the blastula stage the following day.

Heptyl Alcohol.

A series of six experiments were performed with this alcohol to determine the limits of suitable concentration. Reasoning from the data in the proceeding, it was thought that the optimum concentration would be in the proximity of between 0.04 and 0.07 vol. per cent., but to make sure, concentrations as low as 0.02 and as high as 0.08 vol. per cent. were used. As a matter of fact, solutions of 0.06 and 0.07 vol. per cent. were found to be the best suited to the experiments, although it is interesting to note that even weaker solutions showed marked toxic action on eggs at the time of formation of the first cleavage furrow, while during the stages preceding and succeeding division, they had relatively little influence. Table III. summarizes three experiments with this alcohol, and is fairly typical of results obtained in other experiments. By some unavoidable oversight in technical procedure, records for the first two periods (*i.e.*, respectively 10 and 20 minutes after fertilization) were not obtained, but from the results of other series it is evident that the eggs maintain a rather high resistance at these times. The results given in Table III. show that 0.07 vol. per cent. approximates the favorable concentration for this alcohol, while the solutions on either side are slightly hypo- and hyper-toxic respectively; *i.e.*, in the one case practically all eggs survive to the blastula stage, and in the other nearly all die. Recovery of resistance after the first division appears to be relatively slow. When the percentage of surviving blastulæ is plotted against the time intervals, regarding

[2] See *ibid.*, p. 133.

TABLE III.

HEPTYL ALCOHOL.

Fertilized *Arbacia* eggs were placed in 50 cc.. of the following solutions of heptyl alcohol at ten minute intervals, and allowed to remain in each solution (0.06, 0.07 and 0.08 vol. per cent.) for five minutes. They were then treated as previously described.

Intervals.	(A) 0.06.		(B) 0.07.		(C) 0.08.	
	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.
(1) 30–35 m.	No marked cytolysis. Membranes slightly swollen.	70–75 per cent. free-swimming blastulæ. Few ruptured.	Membranes slightly swollen. No appreciable loss of pigment.	About 48–50 per cent. swimming blastulæ. Other cells ruptured in many cases.	Noticeable loss of pigment. Membranes markedly swollen, cytoplasm shows slight cretination.	Not more than 1 per cent. swimming blastulæ Majority intact.
(2) 40–45 m.	No marked change.	About 82 per cent. swimming blastulæ. Others intact.	Slight loss pigment. No other marked change.	Nearly 65 per cent. swimming blastulæ. Few ruptured.	About as noted above.	Practically no swimming blastulæ. Most badly cytolyzed.
(3) 50–55 m.	Between 25–30 per cent. show furrow. Some show loss of pigment.	88–90 per cent. active. Some ruptured but still active.	No marked change.	Relatively few (33–35) per cent. active. Whole masses of cells ruptured. None of two-celled stage stage seen in field. seen in field.	Marked loss of pigment. All intact however.	Less than 1 per cent. active. Others badly cytolyzed but mostly intact.
(4) 60–65 m.	About 80 per cent. in or entering two-celled stage. No great changes noted.	About 75 per cent. active. Considerable number remain in two-celled stage but are intact.	Nearly 80 per cent. in cleavage. Rather noticeable loss of pigment. All intact.	Between 24–28 per cent. active blastulæ. Marked cytolysis, with all stages of rupturing. Rather striking condition.	Marked loss of pigment. Majority cleaving.	Only a few survivors. Others ruptured, and none of the two-celled eggs intact.

TABLE III.—*Continued.*

Intervals.	(A) 0.06.		(B) 0.07.		(C) 0.08.	
	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.
(5) 70–75 m.	Majority have cleaved, no marked cytolysis noted.	Between 85–90 per cent. active. A few cells are ruptured. Others rather badly cytolyzed.	90 per cent. two-celled. Slight loss of pigment noted.	Not over 20 per cent. active. Most others badly cytolyzed, a few ruptured.	Majority as before in two-celled stage. Pigment faded.	All badly cytolyzed, over half ruptured.
(6) 80–85 m.	Considerable number show second furrow.	Few active blastulæ. Large number ruptured but most two-celled eggs intact.	No marked change.	Between 40–50 per cent. active glastulæ. Others intact although badly cytolyzed.	Pigment loss characteristic. No other marked cytolytic change.	About 5 per cent. active blastulæ. Others ruptured.

as typical the data of the 0.07 vol. per cent. solution, an interesting curve is obtained (Fig. 2) which is fairly comparable with the one shown for i-amyl alcohol. There is a gradual rise in resistance up to the period of first cleavage, with a sharp drop during cell-division followed by a slow recoverry.

Octyl Alcohol.

Normal octyl alcohol is apparently considerably more toxic than its isomere capryl alcohol. In a series of five experiments with octyl alcohol in concentrations ranging from 0.010 to 0.030 vol. per cent., the best concentration for five minute times of exposure was found to be in the neighborhood of 0.015 vol. per cent. On the other hand the outcome of fourteen experiments with capryl alcohol showed the optimum concentration for the same time of exposure to be between 0.035 and 0.045 vol. per cent., which is between two and three times the favorable concentration of normal octyl alcohol. Table IV. summaries a

Table IV.

Normal Octyl Alcohol.

Fertilized eggs were subjected for five minutes to 0.013 vol. per cent. of normal octyl alcohol at intervals of ten minutes.

Intervals After Fertilization.	Observed Condition on Removal from Fluid.	Observed Condition the Following Day.
(1) 15–20 m.	Fertilization membrane well formed. Slight loss of pigment.	Nearly 50 per cent. active blastulæ. Others badly cytolyzed, few ruptured.
(2) 25–30 m.	No noticeable cytolysis although very marked loss of pigment.	About 65 per cent. active blastulæ. Others cytolyzed.
(3) 35–40 m.	Decided loss of pigment. No marked change in membrane or cytoplasm.	Nearly 80 per cent. active. Others intact.
(4) 45–50 m.	About 2 per cent. show first furrow. Slight loss pigment. No cytolysis.	Practically all active blastulæ.
(5) 55–60 m.	Over half in first cleavage.	About 85 per cent. active.
(6) 65–70 m.	About 90 per cent. in two-celled stage.	Almost 60 per cent. active blastulæ, numbers of two-celled egg present and mostly intact. Some badly cytolyzed and ruptured.
(7) 75–80 m.	Aside from loss of pigment no noticeable change.	Between 65 and 70 per cent. active blastulæ. Most others cytolyzed but intact.
(8) 85–90 m.	A few (1 per cent.) just begin to show second cleavage furrow. No marked change in appearance.	Between 85 and 90 per cent. active blastulæ. Others cytolyzed but intact.

typical experiment using normal octyl alcohol of 0.013 vol. per cent. concentration. For exposures of five minutes duration, this concentration gave the best results, and showed very clearly the resistant and susceptible phases.

Capryl Alcohol.

As mentioned before, experiments with various concentrations of capryl alcohol showed that for brief exposures, the most favorable concentration was nearly three times that of normal octyl alcohol. This may perhaps be accounted for in some measure by the fact that not all samples of capryl alcohol are

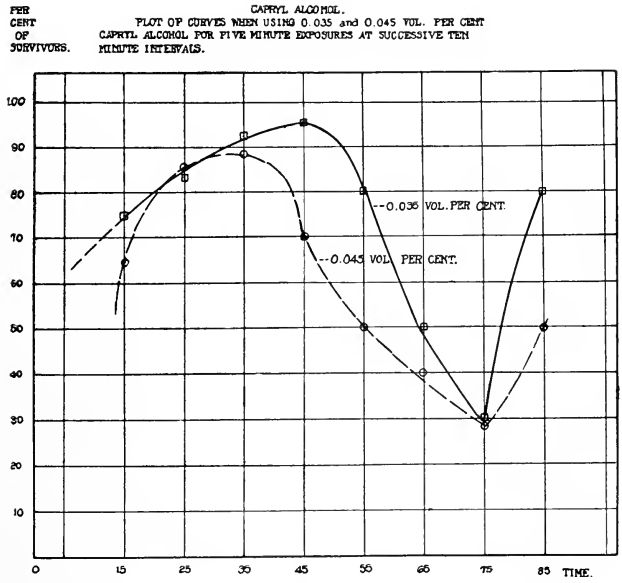

Fig. 3.

uniform in chemical composition and purity; a slight difference in this respect is known to make a decided difference in its chemical and physiological activity. In suitable concentrations, this alcohol is without doubt one of the most satisfactory for

showing susceptible and resistant phases in dividing eggs. Several experiments were tried with this alcohol in which the concentration was kept constant and the time of exposure was varied; and from the data thus gathered, it seems probable that of the two factors, concentration is the more important. In other words, if the concentration is such that it gives the best

TABLE V.

Fertilized eggs were subjected for five minutes at ten minute intervals to 0.035 and 0.045 vol. per cent. capryl alcohol.

Intervals.	(A) 0.035 Vol. Per Cent.		(B) 0.045 Vol. Per Cent.	
	Observed Condition.	Condition Following Day.	Observed Condition.	Condition Following Day.
(1) 10–15m.	Membranes well formed. No great difference from normal eggs.	75–80 per cent. active. Others cytolyzed but mostly intact.	No marked difference from normal eggs.	About 65 per cent active. Others badly cytolyzed.
(2) 20–25m.	Membrane slightly swollen. No marked cytolysis.	About 83 per cent. active. Others badly cytolyzed.	No marked change.	Nearly 85 per cent. active. Others intact.
(3) 30–35m.	Few show loss of pigment, cytoplasm shrunken in some cases.	About 92 per cent. active. Others intact.	Marked fading of pigment. No marked cytolysis.	Between 85–90 per cent. active. Some ruptured eggs motile.
(4) 40–45m.	No marked change. None show cleavage furrow.	About 95 per cent. active blastulæ. Others intact.	No marked change, slight loss of pigment.	About 70 per cent active. Others mostly intact.
(5) 50–55m.	Nearly half show first cleavage furrow No marked change.	80 per cent. active blastulæ. Few ruptured.	No marked change.	About 50 per cent. active blastulæ. Most others badly cytolyzed, many ruptured.
(6) 60–65m.	Nearly all show first cleavage furrow. No marked cytolysis.	About 50 per cent. active blastulæ. Most others ruptured.	Practically all in two cell stage. Some loss pigment.	About 40 per cent. active. All others badly ruptured. Some still in two cells.
(7) 70–75m.	No marked change. Few scattered cells show second furrow.	About 30 per cent. active blastulæ. Others badly cytolyzed.	No marked change, except loss of pigment.	Nearly 30 per cent. active. Others badly cytolyzed. Few persist in two cells.
(8) 80–85m.	No marked cytolysis.	Nearly 80 per cent. active blastulæ. Others mostly intact.	No marked change.	Nearly 50 per cent. active blastulæ. Others cytolyzed but mostly intact.

When plotted the data gives interesting curves as shown in Fig. 3.

results with a five-minute exposure, when the exposure is prolonged to eight minutes, very little or no difference is detected. This generalization, however, could probably be applied only within narrow limits.

The data from two experiments using capryl alcohol in 0.035 and 0.045 vol. per cent. concentrations respectively are given in Table V. These records are fairly typical of results of other experiments.

Summary.

1. The developing sea-urchin egg when subjected to suitable concentrations of various lipoid-soluble substances—i-amyl, hexyl, heptyl, octyl and capryl alcohols—shows unmistakable rhythms of susceptible and resistant phases, which when taken in connection with the earlier observations of Lyon, Herlant, Mathews, Spaulding, Lillie and others, constitute additional evidence that a very intimate relation exists between the general physiological condition of the egg, and the physical state of its plasma-membrane.

2. During the first ten or fifteen minutes after fertilization the eggs are more susceptible than at any other time until the period just preceding division. A comparatively resistant phase gradually becomes more and more marked up to just before the first cell-division (about 45 or 48 minutes after fertilization). This is followed by a period of decidedly increased susceptibility which lasts for about 15 or 20 minutes, during which time marked cytological effects are noted. Subsequently the resistant phase is largely recovered, and maintained up to the time of the second cleavage.

3. The most favorable concentrations of the various alcohols for demonstrating the rhythm of susceptibility range as follows: i-amyl, between 0.7 and 0.9 vol. per cent.; hexyl, between 0.13 and 0.17 vol. per cent.; heptyl, between 0.06 and 0.07 vol. per cent.; normal octyl, about 0.015; while capryl was considerably above its isomere (normal octyl) between 0.035 and 0.045 vol. per cent. The best records were obtained in experiments using i-amyl and capryl alcohols, possibly indicating a higher specific toxicity of these when compared to the others.

4. When suitable concentrations were used, no marked

differences could be detected by varying slightly the durations of exposure. Eggs exposed for five, eight or even ten minutes to the same concentration gave similar results. This, however, would probably apply only within narrow limits.

LIGHT PRODUCTION IN CEPHALOPODS, I.

An Introductory Survey.

S. STILLMAN BERRY,

Redlands, California.

Contents.

1. Introduction.

Recent interest in the subject of biophotogenesis has been so great, and bids so fair to continue at high ebb, until at least the problem of the economical artificial production of chemical light has been solved, that for the use of the many classes of investigators, most of whom are not zoölogists and can scarcely be expected to possess accurate taxonomic knowledge of the group with which they may chance to wish to work, it would be exceedingly desirable if there could be placed on record in compact form a summary of all the species of each principal division of plants and animals which are known or thought to possess photogenic properties. The writer's desire to see this service performed on behalf of the cephalopods, animals which must always stand well up with the highest in the estimation of the student of organic light, furnished the initial stimulus which has finally broadened into the production of the present paper. As a taxonomist, however, and, in so far as this particular group of

animals is concerned, one of that despised species, the "closet naturalist"—he can only go a certain way with his subject, and by the same token, his remarks must perforce have only a very limited value. Yet the effort seems worth while spending, and he can fairly plead in extenuation of his temerity, if not of his own limitations, that cephalopods are such active, delicately balanced creatures, and so exquisitely adjusted to an environment in which it is next to impossible to observe them accurately, and which it is even more vain to attempt to establish, even partially, under artificial conditions, that the difficulties of subjecting the details of their life history and ecology to that searching examination required by the standards of modern biological investigation have proven practically insurmountable. Therefore the unfortunate circumstance that we have no specialist in this branch,—no authoritative student of the bionomics of cephalopods, and that such halting summarization as can be done must be handled by the systematist or general student of the group, if at all. Admitting then the largely pragmatic and temporary rather than permanently intrinsic value of the present dissertation we may proceed with it, for even so small a contribution as this can pretend to be should prove helpful.

Amid the wealth of remarkable features, structural and physiological, with which the entire group of the Cephalopoda entices the student, the variety and multiplication of those which in an earlier day would have been as unquestioningly as delightedly hailed as *adaptive* are supremely conspicuous. These are special for the most part to the conditions and vicissitudes brought about by an exceptionally active manner of life in an environment full of actual or potential diversity. Not even the fishes are better swimmers, nor, with all their aristocratic vertebrate organization, lead a more complicated struggle for existence. It is perhaps concomitantly both a result and cause of all this that the group so fairly teems with bizarre cells, tissues, organs, complexes of organs, which, as we may as well admit without further parley, can scarcely be interpreted otherwise than as marvelously exquisite adaptations, each to its own definite end. Such knowledge of most of these as we possess has been amassed almost wholly since the time of Darwin, else the pages of the

"Origin" might have been enriched by many examples as startling in their way as any of the classical ones. The complex cephalopod chromatophore, the inter-playing system of exactly balanced musculature with scarcely any hard skeletal parts to give it support and leverage which goes to make up the arms and each single sucker, the delicate adjustment between eye, sucker and chromatophore through the mediation of the nervous system to result in one of our most perfect demonstrations of concealing coloration, the innumerable types of hectocotylus often involving the most astonishing modifications in sexual behavior, the amazing and still insufficiently understood mechanism of the spermatophore, the eyes, and, without attempting to prolong the list further, the photogenic organs,—each is in its own way a triumph of adaptive development, how much so we may perhaps infer to some extent from the widespread occurrence of these structures in one form or another among nearly all the now-surviving cephalopods. Continuing with the structures last named, for instance, I think it can be truly said that no other class of animals can compare with the cephalopods in the complexity, diversity, beauty, brilliancy,—in brief, the high specialization of organs devoted to the production and utilization of that form of energy which to our human faculties finds expression as light.

It has been said with considerable show of truth that the generation of light by the plasm of animals and plants is really far less to be marveled at than the transformation of their energy into motion. But motion is practically a general property of protoplasm in all its forms, without which it could scarcely exist as living substance at all. The reason why the production of organic light appears so remarkable to most of us is more special: it is partly of course because of the apparent economy of this light so far as the dissipation of heat energy is concerned, but mainly to the average observer because of its evident highly specialized adaptation to certain particular ends.

Dubois wrote in 1895:[1] "The most resplendent of all animals are *insects*, of which class the glowworm, beloved of the poets, is one of the most brilliant examples." Cephalopods were scarcely

[1] Smithsonian Report, 1895, p. 418.

noticed as being luminous at that period, but now we know that the firefly and glow-worm pale in comparison, and that probably not even the brilliant display of the tropical elaters can vie with the gorgeous pyrotechnics of certain squids. It is indeed quite possible that the latter exhibit the highest development of the photogenic function known in the entire animal kingdom.

2. Classification of Cephalopods.

Living members of the Molluscan Class Cephalopoda cleave simply and naturally into two well-defined, easily separable groups. The first of these, and that universally regarded as the most primitive, is the Order Tetrabranchiata, comprising only the few species contained in the single genus *Nautilus*. The animals of this group are characterized especially by the possession of a massive, chambered, external shell; of a "funnel" formed by the appression of two lateral folds which remain unfused in the median line below; of a system of suckerless lobes around the mouth, bearing retractile, annulated tentacles; of such traces of metamerism as the presence of two pairs each of ctenidia, "branchial hearts," auricles, renal organs, and osphradia; and of a simple "pin-hole" eye, open to the exterior. Ink-sac and chromatophores are absent.

The second group, Order Dibranchiata, comprising all living cephalopods except *Nautilus*, is characterized by either the complete atrophy of the shell or its reduction in the adult to a concealed loose coil (Spirulidæ), a calcareous plate (Sepiidæ), or a horny pen; by an entire, tubular funnel; by the development of the anterior portion of the primitive foot into a series of eight or ten muscular, sucker-bearing, tentacle-like arms about the mouth; by but a single pair each of ctenidia, branchial hearts, auricles and renal organs; by the presence, at least typically, of highly developed eyes; by the development of complex, specialized pigment cells in the skin; and by the presence of the peculiar "ink-sac." Osphradia are absent.

The Dibranchiata in their turn are sharply divisible into two subgroups, the Decapoda and Octopoda. The former are mainly pelagic; have finned, generally elongate bodies; have not only the eight "primary" arms of the octopods, but a pair of

specially modified "tentacles" as well; have the suckers pedunculate, reinforced with a chitinous ring, and often very curiously modified; and have a so-called buccal membrane surrounding the mouth.

The Octopoda are principally shore or bottom-loving forms; short bodied; lack fins, or have them only secondarily developed; have eight arms only, with their suckers sessile and lacking chitinous rings; and lack the buccal membrane. There are also important internal characters which need not concern us here, but it may be said that few living groups are more sharply delimited. As a whole the group of the Decapoda has seemed to most students more archaic than the Octopoda, at any rate is less uniformly divergent from what must have been the ancestral stock, but it includes many highly specialized types, and probably neither group as we now know it can be taken as especially "primitive."

Of the two groups the Decapoda are much the less uniform, and are therefore still further to be divided. The classical bifurcation, which has found general acceptance until quite recently, is that of d'Orbigny, into Œgopsida, or those forms in which there is a free eyelid, and the Myopsida, in which there is a simple fold-like pseudo-lid, or the skin passes uninterruptedly over the eyeball. There seems to be no doubt but that the Œgopsida, at least, form a monophyletic, natural group, for on this point there is reasonable agreement. This cannot be said of the Myopsida.

The principal alternative system is that of Naef (1916).[1] He sets apart the Sepias and Sepiolids together as one of the two main subgroups, the Sepioidea. The other group, which not altogether satisfactorily he denominates Teuthoidea, is subdivided into Myopsida (here restricted by the elimination of the Sepioidea and hence comprising only the single family Loliginidæ) and Œgopsida, the latter as outlined above. Naef's classification avoids several very serious difficulties involved in the standard arrangement, but encounters, perhaps, certain others, which need not be dwelt upon here.

For convenience the two systems are summarized in the

[1] Naef, A. J., *Pubblicazione Stazione Zoologica Napoli*, V. 1, pp. 14–17, 1916.

accompanying table, that of Naef being slightly modified from his printed synopsis in order better to serve the purposes of the moment.

STANDARD SYNOPSIS OF THE CLASS CEPHALOPODA.

Phylum MOLLUSCA.
Class CEPHALOPODA.
Order I. *Tetrabranchiata.*
Suborder 1. *Nautiloidea.*
Order II. *Dibranchiata.*
Suborder 1. *Decapoda.*
Division A. *Œgopsida.*
Division B. *Myopsida.*
Suborder 2. *Octopoda.*
Division A. *Pteroti.*
Division B. *Apteri.*

SYNOPSIS OF THE CLASS CEPHALOPODA ACCORDING TO NAEF.

Phylum MOLLUSCA.
Class CEPHALOPODA.
Sub-class I. *Tetrabranchiata.*
Order 1. *Nautiloidea.*
Sub-class II. *Dibranchiata.*
Order 1. *Decapoda.*
Suborder A. *Teuthoidea.*
(*a*) *Teuthoidea myopsida.*
(*b*) *Teuthoidea œgopsida.*
Suborder B. *Sepioidea.*
Order 2. *Octopoda.*
Suborder A. *Pteroti.*
Suborder B. *Apteri.*

In number of families and genera now living, the Œgopsida easily preponderate, but the tremendous modern development of the genera *Polypus*, *Sepia* and *Loligo* throws the preponderance in species over to the side of the Myopsids (+ Sepioids) and Octopods. For instance, among recent Cephalopoda are to be recognized some 32 families, and in round figures about 120 genera and 600 species.[1] Of these the Œgopsid Decapods claim 16 families (one half the total), 66 genera (slightly over one half), and around 175 species (nearly one third). The Myopsid (+ Sepioid) Decapods account for but 7 families (nearly one quarter), and 27 genera (nearly one quarter), but

[1] More critical figures compiled from the author's card register will be found in Tables I., II. and III. to follow.

include around 225 species (over one third of the total). The Octopoda, with only 8 families (one quarter) and 25 genera (slightly over one fifth), yet develop nearly 200 species (one third of the total). In comparison to this the single family and genus of Tetrabranchiata (3 generally recognized species) hold a minor place in the fauna.

The extraordinary development of the Œgopsida in families and genera is indicative, as the reader may anticipate, of the more than usual modification to which the different branches of this group have been subject, and we are to see that this is particularly true of the photogenic organs. The number of species on the other hand seems to have been held down by the circumstance that a very considerable proportion of the genera are pelagic types of widespread distribution, and, like so many other animals showing somewhat similar ecologic relations, do not break up well into species with our present degree of refinement in perception.

This brief survey of the classification may seem a digression, but without it as a guide, no consideration of the distribution of the photogenic function within the group as a whole or within its components could be entirely intelligible.

3. Distribution of the Photogenic Function among Cephalopods.

By no means all cephalopods are luminous. Among the entire major division of octopods but two species, *Melanoteuthis lucens* Joubin and *Eledonella alberti* Joubin, have been described as possessing photogenic organs, while even here the fact that the structures so described are actually designed for the production of light still remains to be demonstrated. The various instances where octopi have been observed to emit light are almost always poorly authenticated, though it is not impossible that in some cases, such as the observation by Darwin during the Voyage of the "Beagle," which will be noted later, are explicable on the assumption of infection by photogenic bacteria or protozoa. However that may be, and perhaps the point is not yet definitely settled, in the morphological evidence offered the two species mentioned stand quite alone.

On the other hand among the decapods photogenic properties are so widespread that taking the class as a whole, and even including the Octopoda, I am aware of no other major division of metazoan animals which shows such a proportional development of luminous species as the Cephalopoda.

It is therefore no wonder that the scattered literature, as well as the fragmentary character of the information to be gleaned therefrom, offers almost insurmountable difficulties to the inquiring student, while even reasonably complete information is scarcely to be found in any text-book or work of reference. There seems in fact no more recent effort on the part of teuthologists to meet this need than that of Chun (:10), which seems to have been carried out simply as an incident to the preparation of his monumental work on the "Valdivia" Œgopsids, an expensive and in the United States a relatively inaccessible volume. Furthermore he dealt with but one group of cephalopods, while even for this group there has now accumulated a considerable mass of additional information. In the English language by all odds the best and most trustworthy summary is the brief one of Hoyle (:08).

Probably the best way to convey an accurate idea of the manner of distribution of the photogenic function within the group is to present summarily a systematic survey of the entire class, carried down at least as far as genera, and including at the same time such appropriate supplementary data concerning the possession of this function as in each case is possible. An effort to do this is constituted in the following synopsis. In all cases an attempt is made to state as exactly as one writer can the number of valid or recognized species in each genus, and, in the case of photogenic forms, a full list of the species themselves, as well as an indication of the situation of their photophores. In a catalogue of this kind it must at the outset be admitted that the figures given are only approximate, due to the fact that entire elimination of the personal element is quite impossible, even though the concrete numbers quoted are not mere estimates, but represent in every instance an actual weighing of the validity of each specific name in the light of all available information, information which from the very nature of this paper cannot be

gone into in any detail here. The attempt has been made to be both conservative and judicial, but it of course goes without saying that the result attained must be regarded as far from final. Aside from matters of judgment even, it would be presumptious to claim for this list either completeness or freedom from error, but every effort has been made to reduce unnecessary mistakes to a minimum, and after all it is utility rather than finality which must always be remembered as the end in view. In certain special cases where the number of valid species seems to be more than usually problematic, this circumstance has been so indicated by the use of + or ± signs.

TABLE I.[1]

SYNOPTIC TABLE OF THE CLASS CEPHALOPODA, SHOWING THE OCCURRENCE OF PHOTOGENETIC ORGANS.

Class **CEPHALOPODA.**

Order *TETRABRANCHIATA.*

Suborder NAUTILOIDEA.

Family *Nautilidæ.*

Genus *Nautilus* Linnæus 1758. (No photogenic species known.)

3 or 4 species.

Order *DIBRANCHIATA.*

Suborder DECAPODA.

Division ŒGOPSIDA.

Superfamily Architeuthoidea.

Family *Architeuthidæ.* (No photogenic species known.)

Genus *Architeuthus* Steenstrup, 1857.

14 species.

Superfamily Enoploteuthoidea.

Family *Gonatidæ.* (No photogenic species known.)

Genus *Gonatus* Gray, 1849.

3 species.

Family ONYCHOTEUTHIDÆ.

Genus *Onykia* Lesueur, 1821. (No photogenic species known.)

12 ± species.

Genus ONYCHOTEUTHIS Lichtenstein, 1818. (2 axial photogenic organs in pallial chamber.)

1 established species, *banksii;* several doubtful.

Genus *Tetronychoteuthis* Pfeffer, 1900. (No photogenic species known.)

2 species.

Genus CHAUNOTEUTHIS Appellöf, 1891. (Photogenic organs in ventral integument of mantle—*Chun;* no luminous organs—Pfeffer.)

1 species: *mollis.*

[1] Photogenic families and genera are printed in small CAPITALS.

Genus *Ancistroteuthis* Gray, 1849. (No photogenic species known.)
1 species.

Genus *Moroteuthis* Verrill, 1881. (No photogenic species known.)
3 species.

Family LYCOTEUTHIDÆ.

Genus LYCOTEUTHIS Pfeffer, 1900. (Photogenic organs on eyes, in stalks of tentacles, and in pallial chamber.)
2 species: *diadema, jattai;* possibly identical.

Genus NEMATOLAMPAS Berry, 1913. (Photogenic organs on eyes, in arms, in stalks of tentacles, in pallial chamber, and at posterior tip of body.)
1 species: *regalis.*

Family LAMPADIOTEUTHIDÆ.

Genus LAMPADIOTEUTHIS Berry, 1916. (Photogenic organs on eyes, in stalks of tentacles, and in pallial chamber.)
1 species: *megaleia.*

Family ENOPLOTEUTHIDÆ.

Genus ENOPLOTEUTHIS d'Orbigny, 1844. (Photogenic organs on eyes and in the integument of mantle, funnel, head, and arms, but almost entirely confined to ventral aspect.)
3 species: *leptura, chunii, galaxias.*

Genus ABRALIA Gray, 1849. (Photogenic organs on eyes and in the integument of arms, head, funnel and mantle, but almost entirely confined to ventral aspect.)
7 species: *andamanica, armata, astrolineata, astrosticta, steindachneri, trigonura, veranyi.*

Genus ABRALIOPSIS Joubin, 1896. (Photogenic organs on eyes, at tips of ventral arms, and in the integument of arms, head, funnel, and mantle, but almost entirely confined to ventral aspect.)
5 + species: *affinis, hoylei, lineata, morisii, owenii, pfefferi.*

Genus WATASENIA Ishikawa, 1914. (Photogenic organs as in *Abraliopsis.*)
1 species: *scintillans.*

Genus ENOPLOION Pfeffer, 1912. (Larval form; photogenic organs on tentacle stalks and ventral integument of ventral arms, head, funnel, and mantle.)
1 species: *eustictum.*

Genus ASTHENOTEUTHION Pfeffer, 1912. (Larval form; photogenic organs on eyes.)
1 species: *planctonicum.*

Genus ANCISTROCHEIRUS Gray, 1849. (Photogenic organs in ventral integument of mantle.)
1 species: *lesueurii.*

Genus THELIDIOTEUTHIS Pfeffer, 1900. (Photogenic organs on tentacle stalks and ventral integument of head and mantle.)
1 recognized species: *alessandrinii.*

Genus PTERYGIOTEUTHIS H. Fischer, 1896. (Photogenic organs on eyes, in tentacle stalks, and in pallial chamber.)
4 ± species: *gemmata, giardi, hoylei, microlampas.*

Genus PYROTEUTHIS Hoyle, 1904. (Photogenic organs on eyes, in tentacle stalks, and in pallial chamber.)
3 species: *aurantiaca, margaritifera, oceanica,* + several named larval forms.

Family Octopodoteuthidæ.

Genus Octopodoteuthis Rüppell, 1844. (Photogenic organs on ink sac?)
1 species: *sicula*.

Genus *Octopodoteuthopsis* Pfeffer, 1912. (No photogenic species known.)
1 species.

Genus *Cucioteuthis* Steenstrup, 1882. (No photogenic species known.)
1 species.

Family Histioteuthidæ.

Genus Calliteuthis Verrill, 1880. (Numerous photogenic organs in integument of arms, head, and mantle; best developed ventrally.)
12 ± species: *asteroessa, chuni, dofleini, goodrichi, heteropsis, hoylei, japonica, meleagroteuthis, meneghini, miranda, ocellata, separata, verrilli*.

Genus Histioteuthis d'Orbigny, 1839. (Photogenic organs as in *Calliteuthis*.)
1 species: *bonnellii*.

Genus Histiochromius Pfeffer, 1912. (Larval form; photogenic organs in integument of mantle on ventral aspect?)
1 species: *chuni*.

Family Benthoteuthidæ.

Genus Benthoteuthis Verrill, 1885. (Photogenic organs on arms.)
1 species: *megalops*.

Genus Cienopteryx Appellof, 1889. (Photogenic organs on eyes.)
1 species: *siculus*.

Superfamily Ommastrephoidea.

Family *Brachioteuthidæ*. (No photogenic species known.)

Genus *Brachioteuthis* Verrill, 1881. (+ *Tracheloteuthis* Steenstrup 1881.)
4 species.

Genus *Cirrobrachium* Hoyle, 1904(?)
1 species.

Family Ommastrephidæ.

Genus *Illex* Steenstrup, 1880. (No photogenic species known.)
2 species.

Genus *Todaropsis* Girard, 1889. (No photogenic species known.)
1 species.

Genus *Ommastrephes* d'Orbigny, 1835. (No photogenic species known.)
6 ± species.

Genus *Nototodarus* Pfeffer, 1912. (No photogenic species known.)
2 species.

Genus Hyaloteuthis Gray, 1849. (Photogenic organs in ventral integument of mantle.)
1 species: *pelagicus*.

Genus *Sthenoteuthis* Verrill, 1880. (No photogenic species known.)
4 species.

Genus *Symplectoteuthis* Pfeffer, 1900. (No photogenic species known.)
1 species.

Genus Eucleoteuthis Berry, 1916. (Bands of photogenic tissue on ventral aspect of head and mantle.)
1 species: *luminosa*.

Genus *Dosidicus* Steenstrup, 1857. (No photogenic species known.)
2 species.

Family *Thysanoteuthidæ*. (No photogenic species known.)
Genus *Thysanoteuthis* Troschel, 1857.
2 species.

(Position uncertain.)

Family *Lepidoteuthidæ*. (Naef refers this poorly known group to the Myopsida.)
Genus *Lepidoteuthis* Joubin, 1895. (No photogenic species known.)
1 species.

Superfamily Chiroteuthoidea.

Family *Chiroteuthidae*.
Genus *Doratopsis* de Rochebrune, 1884. (+ *Planctoteuthis* Pfeffer, 1912, and *Leptoteuthis* Verrill, 1884; no photogenic species known.)
7 species.
Genus CHIROTEUTHIS d'Orbigny, 1839. (Photogenic organs on eyes, ventral arms, and in pallial chamber.)
7 species: *imperator, lacertosa, macrosoma, pellucida, picteti, regnardi* (photogenic organs undescribed), *veranyi*.
Genus MASTIGOTEUTHIS Verrill, 1881. (Photogenic organs in integument of the arms, funnel, mantle, or fins, or even absent.)
11 species: *dentata, famelica, levimana, magna*. (Not known to be photogenic.)
cordiformis (small tubercles, possibly photogenic, thickly distributed in dorsal integument of body).
agassizii (photogenic organs numerous in integument of head, arms, tentacle stalks and mantle, both dorsally and ventrally).
grimaldii (photogenic organs on dorsal surface of fins, and ventral surfaces of head, arms, funnel and mantle).
flammea (photogenic organs comparatively few; in integument of dorsal surface of fins, and on ventral surfaces of head, ventral arms, funnel and mantle).
talismani (photogenic organs on ventral aspect of fins).
hjorti (photogenic organs on eyes).
glaukopis (a photogenic organ in ventral border of each eyelid sinus).
Genus *Joubiniteuthis* nov.[1] (No photogenic species known.)
1 species.
Genus *Idioteuthis* Sasaki, 1916. (No photogenic species known.)
1 species.
Family *Grimalditeuthidæ*. (No photogenic species known.)
Genus *Grimalditeuthis* Joubin, 1898.
1 species.

Superfamily Cranchioidea.

Family CRANCHIIDÆ.
Subfamily Cranchiinæ. (Series of small photogenic organs on eyes.)
Genus CRANCHIA Leach, 1817.
3 species or forms: *hispida, scabra, tenuitentaculata*.
Genus LIOCRANCHIA Pfeffer, 1884.
3 species or forms: *globulus, reinhardtii, valdiviæ*.

[1] *Chiroteuthis Portieri* Joubin, 1916, does not seem strictly referable to the genus in which it is placed, as it is said to possess no luminous organs, while the extreme attenuation and length of the three dorsal pairs of arms are at variance with the well known state of affairs in *Chiroteuthis*. Hence I would propose the new group *Joubiniteuthis*, with this species as type.

Genus PYROGOPSIS de Rochebrune, 1884.
4 species: *pacificus, rhyncophorus, schneehageni, zygaena.*
Genus LEACHIA Lesueur, 1821.
3 species: *cyclura, ellipsoptera, eschscholtzii.*
Genus LIGURIELLA Issel, 1908.
1 species: *podophthalma.*
Subfamily Taoniinæ. (A large single or duplex photogenic organ on each eyeball.)
Genus PHASMATOPSIS de Rochebrune, 1884. (Photogenic organs not yet described.)
1 species: *cymoctypus.*
Genus TOXEUMA Chun, 1906.
1 species: *belone.*
Genus TAONIUS Steenstrup, 1861. (Photogenic organs not yet described.)
1 species: *pavo.*
Genus VERRILLITEUTHIS Berry, 1916. (Photogenic organs not yet described.)
1 species: *hyperborea.*
Genus MEGALOCRANCHIA Pfeffer, 1884.
5 species: *abyssicola, fisheri, maxima, pardus, pellucida.*
Genus LEUCOCRANCHIA Joubin, 1912.
1 species: *pfefferi.*
Genus TAONIDIUM Pfeffer, 1900. (Photogenic organs not yet described.)
4 species: *chuni, incertum, pfefferi, suhmi.*
Genus CRYSTALLOTEUTHIS Chun, 1906.
1 species: *glacialis.*
Genus PHASMATOTEUTHIS Pfeffer, 1912.
1 species: *richardi.*
Genus GALITEUTHIS Joubin, 1898.
2 species: *armata, phyllura;* possibly identical.
Genus CORYNOMMA Chun, 1906. (A pair of photogenic organs embedded in the liver in addition to the subocular photophores.)
1 species: *speculator.*
Genus HENSENIOTEUTHIS Pfeffer, 1900 (+ *Sandalops* Chun, 1906, *Helicocranchia* Massy, 1907, and *Teuthowenia* Chun, 1919.)
5 species: *antarctica, joubini, megalops, melancholicus, pfefferi.*
Genus BATHOTHAUMA Chun, 1906.
3 species: *bergeti, bouréei, lyromma.*

Division MYOPSIDA.

Superfamily Loliginoidea.

Family *Loliginidæ.* (No photogenic species known.)
Genus *Acroteuthis* Berry, 1913.
3 species.
Genus *Doryteuthis* Naef, 1912.
5 species.
Genus *Loligo* Schneider, 1784.
32 ± species.
Genus *Lolliguncula* Steenstrup, 1881.
3 or 4 species.

Genus *Loliolus* Steenstrup, 1856.
4 species.
Genus *Sepioteuthis* de Blainville, 1824.
21 ± species.

Superfamily Spiruloidea.

Family SPIRULIDÆ. (A single organ thought to be photogenic at posterior end of body.)
Genus SPIRULA Lamarck, 1799.
11 named forms (number true species uncertain): *atlantica, australis, blakei, fragilis, indopacifica, laevis, peronii, prototypus, reticulata, spirula, vulgaris.*

Superfamily Sepioidea.

Family *Promachoteuthidæ*. (No photogenic species known.)
Genus *Promachoteuthis* Hoyle, 1885.
1 species.
Family *Idiosepiidæ*. (No photogenic species known.)
Genus *Idiosepius* Steenstrup, 1881.
2 species.
Family SEPIOLIDÆ.
Subfamily Rossiinæ. (No photogenic species known with certainty.[1])
Genus ROSSIA Owen, 1834.
14 species.
Genus *Semirossia* Steenstrup, 1887.
2 species.
Subfamily Heteroteuthinæ. (A fused pair of glandular photogenic organs on ink sac in all known cases.)
Genus HETEROTEUTHIS Gray, 1849.
3 species: *dispar, hawaiiensis, weberi.*
Genus STOLOTEUTHIS Verrill, 1881. (Photogenic organs still undescribed.)
1 species: *leucoptera.*
Genus IRIDOTEUTHIS Naef, 1912.
1 species: *iris.*
Genus NECTOTEUTHIS Verrill, 1883. (Photogenic organs still undescribed.)
1 species: *pourtalesii.*
Subfamily Sepiolinæ.
Genus SEPIOLA Schneider, 1784. (Paired glandular photogenic organs on ink sac.)
11 ± species: *affinis, atlantica, aurantiaca, intermedia*(?), *ligulata, pacifica* (?), *penares* (?), *robusta, rossiæformis* (?), *sepiola, steenstrupiana.*
Genus RONDELETIA Naef, 1916. (A fused pair of glandular photogenic organs on ink sac.)
1 species: *minor.*
Genus *Sepietta* Naef, 1912. (No photogenic species.)
4 or 5 species.

[1] This lack is stated by Naef (:12, p. 245) as one of the diagnostic characters of the subfamily, but the possession of photogenic organs by *Rossia macrosoma* is definitely affirmed by Meyer (:06, pp. 390, 392). One is perforce still of unsettled mind in the matter, especially as both observers worked at Naples, and Naef goes into no details beyond the mere negation.

Genus INIOTEUTHIS Verrill, 1881. (Paired glandular photogenic organs on ink sac.)

3 species: *japonica, maculosa, parva.*

Genus *Sepiolina* Naef, 1912. (No photogenic species known.)

1 species.

Genus EUPRYMNA Steenstrup, 1887. (Paired glandular photogenic organs on ink sac.)

8 ± species: *bursa, morsei, pusilla, schneehageni, scolopes, similis, stenodactyla, tasmanica.*

Subfamily Sepiadariinæ. (No photogenic species known.)

Genus *Sepiadarium* Steenstrup, 1881.

2 species.

Genus *Sepioloidea* d'Orbigny, 1855.

1 species.

Family *Sepiidæ*. (No photogenic species known.)

Genus *Sepia* Linnæus, 1758.

80 ± species.

Genus *Metasepia* Hoyle, 1885.

2 species.

Genus *Sepiella* Gray, 1849.

11 ± species.

Genus *Hemisepius* Steenstrup, 1875.

1 species.

Suborder OCTOPODA.

Superfamily Cirroteuthoidea.

Family CIRROTEUTHIDÆ.

Genus *Cirroteuthis* Eschricht, 1836. (No photogenic species known.)

8 species.

Genus *Stauroteuthis* Verrill, 1879. (No photogenic species known.)

3 species.

Genus MELANOTEUTHIS Joubin, 1912. (A pair of supposed photogenic organs on dorsal aspect of mantle.)

1 species: *lucens.*

Genus *Opisthoteuthis* Verrill, 1883. (No photogenic species known.)

6 species.

Genus *Cirrothauma* Chun, 1911. (No photogenic species known.[1])

1 species.

Genus *Froekenia* Hoyle, 1904. (No photogenic species known.)

1 species.

Genus *Vampyroteuthis* Chun, 1903. (No photogenic species known.)

2 species.[3]

Genus *Lætmoteuthis* Berry, 1913. (No photogenic species known.)

1 species.

Superfamily Argonautoidea.

Family *Ocythoidæ*. (No photogenic species known.)

Genus *Ocythoe* Rafinesque, 1814.

1 recognized species.

[1] A possibility seems to exist that this genus is photogenic,—cf. Chun, : 13, p. 23–25, 27.

[2] *Cirroteuthis macrope* Berry, 1911, appears to belong to this genus.

Family *Argonautidæ*. (No photogenic species known.)
Genus *Argonauta* Linnæus, 1758.
12 ± species.
Family *Tremoctopodidæ*. (No photogenic species known.[1])
Genus *Tremoctopus* delle Chiaje, 1829.
Only 1 certainly established species.
Family *Alloposidæ*. (No photogenic species known.)
Genus *Alloposus* Verrill, 1880.
2 species.

Superfamily Amphitretoidea.

Family *Amphitretidæ*. (No photogenic species known.)
Genus *Amphitretus* Hoyle, 1885.
1 species.

Superfamily Polypodoidea.

Family BOLITÆNIDÆ.
Genus *Bolitæna* Steenstrup, 1859. (No photogenic species known.)
1 species.
Genus ELEDONELLA Verrill, 1884.
5 species: (1 species, *alberti*, described as "probablement photogene").
Genus *Vitreledonella* Joubin, 1918. (No photogenic species known.)
1 species.
Family *Polypodidæ*. (No photogenic species known.)
Genus *Polypus* Schneider, 1784.
125 ± species.
Genus *Tritaxeopus* Owen, 1881.
1 species.
Genus *Pinnoctopus* d'Orbigny, 1845.
1 species.
Genus *Scæurgus* Troschel, 1857.
4 species.
Genus *Cistopus* Gray, 1849.
2 species.
Genus *Moschites* Schneider, 1784.
3 species.
Genus *Graneledone* Joubin, 1918.
9 species.
Genus *Eledonenta* de Rochebrune, 1884.
2 species.
Genus *Velodona* Chun, 1915.
1 species.

The increase afforded by the present list over the numbers included in the earlier catalogs of photogenic cephalopods is quite remarkable for the small number of years that has elapsed. Hoyle's list (:08, p. 14) records as photogenic 6 families, 26 genera, and 30 species of Œgopsida, 1 family, 3 genera and 3 species of Myopsida, and none of the other groups, or a total

[1] But cf. Tryon, '79, p. 131.

TABLE II.

RECAPITULATION OF RECENT CEPHALOPODA.

Class CEPHALOPODA.

Order TETRABRANCHIATA.

	Number of Genera.	Number of Species.	Number of Photogenic Species.
Suborder NAUTILOIDEA.			
Family *Nautilidæ*	1	3	0
	1	3	0

Order DIBRANCHIATA.

Suborder DECAPODA.			
Œgopsida.			
Family *Architeuthidæ*	1	14	0
Family *Gonatidæ*	1	2	0
Family ONYCHOTEUTHIDÆ	6	20±	2
Family LYCOTEUTHIDÆ	2	2	2
Family LAMPADIOTEUTHIDÆ	1	1	1
Family ENOPLOTEUTHIDÆ	10	27±	27
Family OCTOPODOTEUTHIDÆ	3	3	1?
Family HISTIOTEUTHIDÆ	3	14±	14
Family BENTHOTEUTHIDÆ	2	2	2
Family *Brachioteuthidæ*	2	5	0
Family OMMASTREPHIDÆ	9	20±	2
Family *Thysanoteuthidæ*	1	2	0
Family *Lepidoteuthidæ*	1	1	0
Family CHIROTEUTHIDÆ	5	28	14
Family *Grimalditeuthidæ*	1	1	0
Family CRANCHIIDÆ	18	41	34+
	66	173±	99+
Myopsida.			
Family *Loliginidæ*	6	72±	0
Family SPIRULIDÆ	1	1+	1+
Family *Promachoteuthidæ*	1	1	0
Family *Idiosepiidæ*	1	2	0
Family SEPIOLIDÆ	12	51±	26+
Family *Sepiadariinæ*	2	3	0
Family *Sepiidæ*	4	94±	0
	27	224±	27+
Suborder OCTOPODA.			
Family CIRROTEUTHIDÆ	8	23	1
Family *Ocythoidæ*	1	1	0
Family *Tremoctopodidæ*	1	1+	0
Family *Argonautidæ*	1	12±	0
Family *Alloposidæ*	1	2	0
Family *Amphitretidæ*	1	1	0
Family BOLITÆNIDÆ	3	7	1
Family *Polypodidæ*	9	148±	0
	25	195±	2

TABLE III.

SUMMARY.

	Familes.			Genera.			Species.		
	Total.	Photogenic.	Per Cent. Photogenic.	Total.	Photogenic.	Per Cent. Photogenic.	Total.	Photogenic.	Per Cent. Photogenic.
Œgopsida	16	10	62.5	66	39+	59.0	173±	99+	57.2
Myopsida	7	2	28.6	27	6+	22.2	224±	27+	11.6
Total Decapoda	23	12	52.2	93	45+	48.4	397±	126+	31.6
Octopoda	8	2	25.0	25	2	8.0	195±	2	1.0
Tetrabranchiata	1	0	0.0	1	0	0.0	3	0	0.0
Total	32	14	43.7	119	47+	39.5	595±	128+	21.3

of 7 families, 29 genera, and 33 species. Chun (:10, p. 39), treating only of the Œgopsida, increases these figures to 8 families, 26 genera and 39 species. The two sets of figures should be compared with those given in the numerical summary in Table III at the top of this page. Here it appears that of the 32 families of recent cephalopods now recognized, 14 (or more than two fifths) contain luminous species; out of 119 genera, 47 (or nearly two fifths) are light producing; and out of 595 species, 128 (or over one fifth) are now held on good ground to be luminous. The rich development in species of the genera *Loligo*, *Sepia* and *Polypus*, which has already been noted, is the circumstance chiefly responsible for the cutting down of the proportion which the luminous *species* bear to the whole to less than one half that which is exhibited by the luminous *families*. Similarly the slight proportional decline in the case of the luminous *genera* is due to the large number of ranking genera in certain mainly non-luminous families such as the Ommastrephidæ, Sepiolidæ, Cirroteuthidæ and Polypodidæ.

The table also indicates very strikingly what is really the outstanding feature of the taxonomic distribution of the photogenic forms, namely, the preponderance both of Œgopsida among the species known to be light producing, and of light producing species among the Œgopsida. In the former instance this preponderance is enormous. 71.4 per cent. of the luminous families, 83.0 per cent. of the luminous genera, 77.3 per cent. of

the luminous species, are Œgopsid. Among the Œgopsida themselves over one half of all the families, genera, and species are described as possessing photogenic organs. Five entire families—the Lycoteuthidæ, Lampadioteuthidæ, Enoploteuthidæ, Histioteuthidæ, and Benthoteuthidæ, all of them of more or less deep sea habit,—have all their species so equipped, and this seems almost certainly true of the very aberrant but numerous Cranchiidæ as well. Among other groups of cephalopods, only the Spirulidæ can aspire to inclusion in the same category, and regarding them our information is still deficient. There may be only one valid species in this family. In addition to those named, one other œgopsid family (Chiroteuthidæ) has more than half its species light producing. On the other hand luminous species for five œgopsid families (the Architeuthidæ, Gonatidæ, Thysanoteuthidæ, Lepidoteuthidæ and Grimalditeuthidæ), five of the seven myopsid families, six of the eight octopod families, and the Nautilidæ, are as yet unknown.

For multiplicity and variety of luminous forms the palm must be awarded to the Enoploteuthidæ and Cranchiidæ, though, as will subsequently appear, the maximum attainment and diversity of structure of the photogenic organs themselves is reached not in either of these families, but in the Lycoteuthidæ.

4. Actual Observance of the Phenomenon.

As compared with other Mollusca, or even with other general groups of Invertebrata, Cephalopoda, and especially those of the decapod section, are extremely difficult either to capture, to maintain alive under artificial conditions, or even to observe with any degree of satisfaction in their free condition. Among the Œgopsida it is probable that a sheer majority of the genera have never been seen at all in the living state, at any rate by any human eyes but those of fishermen. It is therefore not to be wondered at that actual observance of the phenomenon of light production in this group of animals is an extremely rare event, possible only occasionally or under very exceptional conditions. The published records of such observations are consequently so scattered that they have fallen into obscurity, or else, in the case of some of the more spectacular ones become all the more

conspicuous by very reason of their paucity and desultory character.

A brief historical survey of this subject has been given by Hoyle (:08), but the most valuable contributions thereto have been made since that time, while Hoyle himself omitted one or two quite interesting accounts from his summary. It will therefore be well to review briefly the entire field.

I have been no more successful than previous authors in the discovery of any recorded observation of photogenic phenomena in living Cephalopoda prior to that of Vérany in the case of *Histioteuthis bonnellii* (*bonelliana*), ('51, p. 119), a translation of which is quoted in full by Hoyle in the paper cited and is well worthy of repetition here.[1]

"As often as other engagements permitted, I watched the fishing carried on by the dredge on the shingly beaches which extend from the town of Nice to the mouth of the Var. On the afternoon of September 7, 1834, I arrived at the beach when the dredge had just been drawn in, and saw in the hands of a child a cuttle-fish, unfortunately greatly damaged. I was so struck by the singularity of its form and the brilliance of its color that I at once secured it, and, showing it to the fishermen, asked whether they were acquainted with it. Upon their replying in the negative I called their special attention to it, and offered a handsome reward for the next specimen secured, either alive or in good condition, and then passed on to other fishermen and repeated my promise. Shortly afterwards I was summoned and shown a specimen clinging to the net, which I seized and placed in a vessel of water. At that moment I enjoyed the astonishing spectacle of the brilliant spots, which appeared upon the skin of this animal, whose remarkable form had already impressed me: sometimes it was a ray of sapphire blue which blinded me; sometimes of opalescent topaz yellow, which rendered it still more striking; at other times these two rich colors mingled their magnificent rays. During the night these opalescent spots emitted a phosphorescent brilliance which rendered this mollusc

[1] Vérany's monograph is a very rare one in the United States, especially in the West, where I am not aware that any complete copy exists. I am accordingly entirely dependent for the information quoted upon the translation given by Hoyle.

one of the most splendid of Nature's products. Its existence was, however, of short duration, though I had placed it in a large vessel of water. Probably it lives at great depths."

Although not mentioned by Hoyle, the next student whose published observations concern us was none other than Charles Darwin. Among the melange of odd notes in the course of his account of the voyage of the "Beagle" ('60, pp. 7–8) appears the following: "I was much interested, on several occasions, by watching the habits of an Octopus, or cuttle-fish. . . . I observed that one which I kept in the cabin was slightly phosphorescent in the dark." As we have already seen, photogenic organs or tissues are practically unknown among octopods, so that this observation would be quite an anomalous and puzzling one, were it not for the at least plausible explanation that the phenomenon in this instance as so many others in the literature of biophotogenesis was due not to the active functioning of any tissues of the cephalopod itself, but to bacterial infection or even to the presence of effulgent Protozoa in the slime surrounding its skin. Another possibility which occurs to me is that the animal may not have been examined in absolute darkness, but that sufficient light penetrated into the chamber, though imperceptible to the unadjusted human eye, to enable the iridocytes in the skin of the octopus to yield a pseudo-luminous reflection, analogous to that so notorious in the case of the eyes of many mammals. The description by Giglioli of luminescent specimens of a squid which he identified as "*Loligo sagittatus*" and certain Chilean octopods, referred to by Holder in the quotation given in the next paragraph, may be susceptible of similar explanation. To the original of this work with the description of his observations I have unfortunately not been able to gain access. For my own part I have on several occasions attempted to discover similar properties in captive specimens of the common southern California devilfish, *Polypus bimaculatus* (Verrill), but so far with only negative results. Final settlement of the question can only be accomplished by careful experiment.[1]

[1] Since this paragraph was put in type I am reminded that Tryon ('79, p. 131) in his paraphrase of the description of *Tremoctopus gracilis* (Souleyet 1852) says that this species is "phosphorescent and with metallic reflections when living." I have been unable to check this observation by reference to the original work of Souleyet.

In a popular volume by C. F. Holder ('87, p. 46), descriptive of luminous organisms in general, but unfortunately none too carefully compiled, occurs the following paragraph on the Cephalopoda:

"The highest forms of the *Mollusca*, the Cephalopods, cuttlefishes, are probably at times luminous. I have noticed what I presumed was a delicate, sensitive glow about an *Octopus* in a semi-darkened tank, but I am not satisfied to make the statement as a fact. These forms are so remarkable for the waves of color that pass over them, and which seem to make them transparent, that one could readily be deceived.

"The little *Cranchia* (Plate IV., Fig. 2) is a light-giver, its phosphorescence having been distinctly observed. It is an ally of the giant squids, which have been found fifty-five feet in length, and which, if luminous like their pygmy relative, would present a marvelous spectacle, darting veritable living arrows through the depths of the sea.

"Giglioli refers to the phosphorescence of *Loligo sagittatus*, and to that of several small Octopods observed by him at Callao and Valparaiso. Their bodies gave out a pale whitish light, uniformly distributed."

It happens that *Cranchia* is a genus which is now known to possess definite photogenic organs, but these have been found to occur only on the eyeball, whereas the rather poor figure given by Holder represents the animal as brightly and evenly glowing over the entire surface,—body, head, arms, tentacles, and all. As to the supposed photogenic properties of *Polypus* and related octopods,—both Darwin and Giglioli would seem to have been too accurate observers for the explanation advanced by Holder to be entirely satisfactory.

Chun, in his narrative of the cruise of the "Valdivia" (:03, pp. 569–570; also :03*a*, p. 81; :10, p. 50) gives up to this time the fullest account of the actual display of photogenic propensity by a cephalopod we have been able to find, and he followed this in later publications by a very considerable contribution to our morphological knowledge of the organs responsible for the manifestation. The specimen observed proved to belong to a wonderful undescribed species, the *Lycoteuthis diadema* (Chun).

Although it was taken from a considerable depth, he was able to keep it alive in ice water long enough to make a photograph of it by dint of its own light. Again I must quote from a translation by Hoyle: "Among all the marvels of coloration which the animals of the deep sea exhibited to us, nothing can be even distantly compared with the hues of these organs. One would think that the body was adorned with a diadem of brilliant gems. The middle organs of the eyes shone with ultramarine blue, the lateral ones with a pearly sheen. Those towards the front of the lower surface of the body gave out a ruby-red light, while those behind were snow-white or pearly, except the median one, which was sky-blue. It was indeed a glorious spectacle." It is altogether a pity that similar observations have not been possible for the doubtless even more spectacular *Nematolampas regalis*, which, although very nearly related to *Lycoteuthis* is equipped with an entire further battery of photophores.

More detailed from the standpoint of physiology is the account given by Watasé (:05) of a little squid, the "hotaru-ika" of Japanese writers,[1] which is extremely abundant at the proper season and locality on certain of the shores of Japan, and which has since become the best known of all the luminous squids. Watasé's paper is an important one as the first dealing with this species, but, being semi-popular in character and published in Japanese, escaped notice for a considerable time and has only lately received a little of the attention it deserves. Through the kindness of Mr. Sotaro Matsushita, formerly of Redlands, California, I have for some time been in possession of a translation, and a very free transcription of some of its more interesting if quaint passages should be neither inappropriate nor unwelcome here. "Hotaru-ika, when seen externally, does not differ much from other *ika* [squids]. Yet there are many interesting features which we do not see in other *ika*. At each end of the two 'legs' there are three oblong, black spots. These small spots were first discovered by the French scientist Joubin. Yet even he did not know their function. According to the results of my own study of these in living Japanese specimens, the spots were found to produce a considerable light, penetrating to the space of about

[1] The "firefly squid"—*Watasenia scintillans* (Berry).

a foot. . . . While the animal is living these spots are transparent.

"Again there are hundreds of other small spots all over the body. . . . When seen in daylight they appear to be small black spots, but in the night all these spots shine with a brilliant light like that of the stars in heaven. . . . When these spots (while the *hotaru-ika* is alive) are viewed under the microscope, they are very interesting. When the animal is about to produce the light, the membranes [chromatophores] covering the spots will concentrate and remove themselves, thus opening a way for the light. The light is so brilliant that it seems like a sunbeam shot through a tiny hole in a window curtain. Again when the *hotaru-ika* wishes to shut off the light, the membranes will expand and cover the spots. . . ."

In the following year Meyer (:06) described briefly the photogenic activity of the myopsid, *Heteroteuthis dispar* (Rüppell), similar observations having been made some time previously by Lo Bianco, but never published. Meyer found that in the case of the specimen observed by him at the Naples Zoölogical Station he "could in the dark room easily locate the position of the photophore through the transparent mantle, lying on the ventral surface just behind the anus." He further found that when the animal was irritated, "it shot rapidly through the water, and spurted through its funnel a luminous secretion which floated in the water as separate globules, these being drawn out by the currents into shining threads, a pyrotechnic display (*Feuerwerk*) which he was able to repeat many times. The light of the secretion and of the light organ itself had the same pale greenish hue which we observe with our glow-worms." Meyer further reports the discovery by one of his colleagues, Marchand, of somewhat similar photogenic properties in *Sepiola*, except that in this genus "the luminous secretion is not discharged into the water but remains on the surface of the gland. Furthermore, *Sepiola* only shines if it be very powerfully stimulated, as when, for instance, the mantle is cut open." From the foregoing it is at once evident that in both these myopsid genera the mechanism of light production is very different from that of any of the other forms studied, and this conclusion is borne out by the anatomical

features. The possible utility of this peculiar development of the function, so far as *Heteroteuthis* is concerned, is the subject of some interesting speculation in one of Meyer's subsequent papers (:08, pp. 507–508), which will receive more attention later on.

We are now brought to a consideration of some important recent work performed by various Japanese observers in continuation of Watasés pioneer studies on *Watasenia scintillans* (Sasaki, :12, :13, :14; Ishikawa, :13). This constitutes probably the chief work which has been done in this field, and therefore merits consideration in considerable detail.

"In the region where the squids live, that is, in the waters of Namerikawa on the coast of the Japan Sea," writes Ishikawa (:13, pp. 167–169), "this circumstance [the luminosity of the tips of the ventral arms described by Watasé] had already long been known; but none of our zoölogists were aware of it until Watasé by chance made the discovery. At a time when he was engaged in the study of fireflies, he was apprised by a schoolmaster that there occurred a species of squid in the sea at Namerikawa which lighted very strongly. Pursuant to this suggestion he sought the village named, found in due course a species of small squid with powerful light organs, and recognized the same as a species of *Abraliopsis*. As he has orally told me, it was on the 28th of May, 1905, on the memorable day of the battle of Tsusima, hat he saw the light of this squid for the first time. . . . . .

"The large swellings at the tips of the ventral arms, as well as two or three smaller dots, are, as he remarks, luminous organs of the first order. They shine so brilliantly that when one observes the animals in dark water, one sees only two effulgent bodies moving in the dark water, like the glow of an electric contact, and the lively oscillations of the invisible arms produce a very wierd effect. Next to these in the intensity of their light are the eye organs, and then come the remaining organs. The three types of organs do not always shine simultaneously; often only one or the other. But it can also happen that the animal sets all the organs into action at the same time. When the mantle organs light up, the form of the animal springs out spectre-like in the dark water. These organs, arranged in rows, when one

examines them close at hand, shine like an electric illumination. The color of the light is a beautiful clear blue.

"As Watasé writes, the arm organs in dead animals are entirely surrounded by pigment cloaks and only when alive can the animal retract these. The retraction of these cloaks takes place very quickly, and when they are retracted, the organ appears in daylight as a delicate dull-green colored body."

In a paper which comprises a most notable contribution to our knowledge of the ecology and habits of ten-armed cephalopods, Sasaki (:14, pp. 77–80) adds materially to the accounts of his predecessors. Some of his observations are so pertinent to some of the discussion which must follow later that they should be quoted rather fully. Treating the three types of photophore to be seen in *Watasenia* under separate headings, this author writes:

"*Brachial Organ.* This is the largest organ, and when I made observations in the fishing season, it was much more active in phosphorescence than other organs. It is situated at the end of each ventral arm, composed of 3 globules arranged in a series. The globules are ovoid in shape and nearly equal in size, but the middle one in the series is generally a little larger than the others, the dimensions being 1.4 mm. long and about 1 mm. broad. In fresh specimens they show a greenish cobalt colour, and there are 2 or 3 layers of large brownish chromatophores covering a part of the preceding substance. These chromatophores are constantly contracting and expanding. When they were observed at night on the living animals, they were seen to discharge light in all directions much brighter than any of Japanese fireflies. The color of the light is Prussian-blue or tinged a little with purple, and the luminosity is strong enough to outshine the other luminous organs. When the living animal was placed on a glass plate, which was put directly on the case of the dry plate of the photographic camera, and then exposed for four seconds with the Lion's dry plate of the special rapid no. 230, the light of this brachial organ was distinctly taken on the dry plate, although those of other organs made no impression.

"*Minute Organs Scattered on the Ventral Surface of the Whole Body.* There are numerous minute organs distributed on the

ventral surface of the mantle, head and siphon, and they are also on the third and fourth arms. . . .

"Each organ in the fresh specimen has a substance of purplish hue in the centre; this substance seems to be that discharging light when the animal is living. When the organ is exposed in the air, the purplish hue of the substance changes to greenish blue after a while, and finally resolves into a true green. The substance is covered by a pigment layer of darkish brown or deep purple which has a hole resembling the pupil of an eye, through which the substance can easily be seen. The light of the substance at night is whiter and less luminous than that of the brachial organ.

"*Ocular Organ.* When the eyelid of the fresh specimen is removed and the eyeball exposed, there are seen 5 luminous organs arranged in a series along the ventral circumference of the eyeball, the organ on either end of the series being a little larger than the remaining 3. The colour of all these organs is pearly white. When the organ is seen at night in the living animal, the phosphorescence is not distinguishable from that of the minute organ on the body.[1]

"*Difference of Phosphorescence in the Sexes.* On examining the preserved specimens to discover the difference of the external forms as well as the histological structures of their luminous organs as occurring in the male and female, none could be discerned. But in the female specimens there are one hundred or so more of the minute organs of the mantle than in the male. Whether there is any meaning as to sexual selection, it is difficult to say, the data concerned being insufficient at present to announce any opinion.

"Next, as to the difference of phosphorescence between the sexes in their living state, the means of investigation proved to be very difficult. At first I repeatedly undertook to keep the animal in an aquarium, but no success was attained. The reason for the failure is that first of all, the animals are very delicate, and next the aquarium was defective. The animals

[1] A slight discrepancy is noteworthy between the account of Sasaki and that of Ishikawa concerning the character of the light of the subocular organs. According to the latter these photophores are more or less intermediate in brilliancy between the large brachial organs and those of the general integument.

are so weak that in carrying them from the sea to the aquarium they wasted and died. As they wasted, the luminosity in question became very feeble, and naturally with their expiration, the light of the luminous organs gradually vanished altogether. This being so, I then tried to observe the animals directly while they were swimming in the net. But no good means were found easily to distinguish the sexes on such dark nights, even with the feeble light of the moon or of a lantern.

"However in my examinations at night, no special variety of the light could be found, the colour of the light being always the same. And in one case, putting in a vessel and observing about thirty specimens in a fishing boat while they were yet actively on motion, I verified the fact that their luminosity is uniform. In the morning, to my surprise, a male was found dead among those 30 specimens; this proves that it had the same colour of light with the female on that night. The above data seem to prove the fact that the colour of the light of the luminous organs is the same in both sexes.

"Again, in late July of the same year, I made another observation on the phosphorescence under consideration and then it was quite evident to me that the luminosity of the brachial organ was at this season noticeably feebler than in the spring.

"The phosphorescence of the immature animal can never be studied in Namerikawa, young ones thus far not being found there."

In the same paper (pp. 98–99), Sasaki incidentally records the fact that he observed the photogenic property in living specimens of the myopsid, *Inioteuthis japonica* Verrill (= *inioteuthis* (Naef)). These he found to be "discharging a faint cobaltish light from a great luminous organ which is situated in the mantle cavity near the ink-bag." From anatomical observations we know that the luminous organs of this genus are essentially similar to those of the nearly related if not actually congeneric *Sepiola*.

Lastly, Dahlgren (:16, pp. 70–71) describes in a little greater detail than before the photogenic behavior of *Heteroteuthis dispar*, the myopsid species already observed by Meyer. He writes: "When brought into the laboratory in good condition

and allowed to rest quietly it may be taken into the dark-room and gently struck, as it swims in the aquarium, with a glass rod. Fig. 18 is a drawing to illustrate what may and usually does happen under these circumstances. The animal throws out of its siphon several little masses of mucus which show no light at the moment of ejection, but almost instantly, as the oxygen of the water begins to work on them, show a number of rod-shaped particles of a brilliantly luminous matter embedded throughout the very delicate mass. As the mass continues to expand this light continues to glow brightly for as much as three to five minutes, after which it rather suddenly dies out. In color the light is the usual blue-green of luciferine when burning outside the body. The animal can repeat this process for a number of times, when it appears to have exhausted its supply of luciferine, and it is not possible, apparently, to keep it in captivity for a long enough period for the supply to be restored."

From this scanty, but for all practical purposes probably exhaustive summary, we find that except for the doubtful observations by Souleyet, Darwin and Giglioli, the actual process of light production in cephalopods has been observed directly in but seven species, of which three belong to the myopsid family Sepiolidæ and have photogenic organs of a peculiar discharging type, while the other four belong to the Œgopsida. We are fortunate, however, in that each of these latter species is representative of a different family and thus ample support is given to the inferences necessarily drawn from the outward appearance and histological structure of the many types of photophore that they are of a fact photogenic.

(To be Continued)

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BIOLOGICAL BULLETIN

LIGHT PRODUCTION IN CEPHALOPODS, II.

An Introductory Survey.

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Contents.

5. Color and Intensity of Light.

But little is known concerning the color, particularly what may permissibly be termed the *intrinsic* color, of the light produced by cephalopods, in fact next to nothing of any of its fundamental physical qualities. This of course follows as a natural corollary of the scanty nature of the recorded human observations of these animals in the living state. Such as they are the appropriate data gleaned from the preceding section of this paper are briefly tabulated.

Vérany's observations previously quoted are a little ambiguous and it is not just evident whether the "sapphire blue" and "topaz yellow" rays which he describes with such naïve enthusiasm for the photophores of *Histioteuthis* apply to the result of their functional activity at night, or merely to their ordinary brilliant coloration in the daytime. The fact that he was "blinded" would seem to indicate the former.

Table IV.

Color of Light in Cephalopods.

Species.	Date of Published Observation.	Color of Light.
Œgopsida:		
Family Lycoteuthidæ,		
Lycoteuthis diadema (Chun)	Chun, 1902.....	ultramine, sky blue, ruby red, pearly white.
Family Enoploteuthidæ,		
Watasenia scintillans (Berry) ...	Watasé, 1905, 1912.	
	Ishikawa, 1913 ..	clear blue.
	Sasaki, 1914.....	Prussian blue to purplish.
Family Histioteuthidæ,		
Histioteuthis bonellii (Férussac)..	Vérany, 1851.....	?
Family Ommastrephidæ,		
? "*Loligo sagittatus* Lamarck" ..	Giglioli.....	?
Family Cranchiidæ,		
Cranchia sp.....	Holder, 1887.....	?
Myopsida:		
Family Sepiolidæ,		
Sepiola sp. (*rondeletii* Leach ?)..	Marchand, in Meyer, 1906.....	?
Inioteuthis japonica Verrill.....	Sasaki, 1914.....	"cobaltish."
Heteroteuthis dispar (Guppell)...	Meyer, 1906.....	pale greenish.
	Dahlgren, 1916....	blue green.
Octopoda:		
? *Polypus* sp.....	Darwin, 185.....	?
? small octopod.....	Giglioli.....	"pale whitish."
Tremictopus gracilis (Souleyet).	Souleyet.....	?

Of the subsequent observations, only a few trouble to specify the apparent color of the light rays. I use the word apparent advisedly, not alone because of the ever-present subjective considerations by which one and the same ray may yield diverse impressions to different persons at the same time and under the same conditions, or to the same person at different times or under different conditions, but also because there is evidence that the original color values of the light rays may suffer modification, either by reason of the physical features of some of the supervening tissues of the photophore itself, or by the interposition of the chromatophoric color screens to which attention has already been drawn.

The extent to which the brilliantly varied illumination which was described by Chun for *Lycoteuthis diadema* is due to such considerations as these, rather than to differences inherent in the light rays produced by the respective organs is therefore a matter for considerable speculation. In this species Chun (:03, pp. 569–570; :03*a*, p. 81; :10, p. 50) described the light of the central organ in each subocular series as "marvelous ultramarine blue," of the anterior axial organ as "sky blue," of the two anal organs as "ruby red," of the remaining organs as "snow-white" or "pearly." But it should be remembered that no matter what other rays may have suffered absorption to result in the described effect on the human eye, no sort of screen or filter could manufacture those which evidenced themselves and they must therefore have been produced within the photogenic tissues. If, as in all other luminous organisms which have been subjected to examination, this is still a relatively efficient and therefore "cold" light, the question is yet before us whether the "ruby red" rays of *Lycoteuthis* are none the less as "cold" as the ultramarine and blue ones, or as the blue-green luminescence of the firefly. The biochemist and biophysicist have here a tempting field, once the technical biological difficulties of securing and handling the animals can be fairly overcome.

The light of the luminous secretion of *Heteroteuthis dispar* is described by Meyer (:06, p. 389) as "pale greenish," and by Dahlgren (:16, p. 71) as "the usual blue-green of luciferine when burning outside the body."

Sasaki and other observers of *Watasenia scintillans* describe the light of the large organs at the tips of the ventral arms as purplish or Prussian blue, the body organs appearing "whiter and less luminous" than these. In spite of their absolutely different histological structure, the rays emanating from the integumentary and subocular organs do not here appear to be respectively distinguishable and one wonders whether, in this regard, the observations recorded convey the whole truth.

That such elaborate variety in the size, morphological detail, and possession of accessory contrivances as will shortly be described, must find at least partial expression in differences in the physical qualities (intensity and color) of the resulting light rays, seems as inescapable to the present writer as it did to Chun (:03*a*, p. 81). And on the whole the scanty evidence just outlined is in accord, showing that the hues of the light are different, often most strikingly so, not alone as between independent species, but between the organs occupying different situations on the body in one and the same species.

6. Distribution of Photophores on Animal.

The photogenic function in cephalopods is, as has been seen, not a general attribute of the body surface, but is always, so far as is known, localized in the specialized tissue of definitely circumscribed organs disposed in equally definite regions of the body. It therefore becomes appropriate to examine what position or positions on the body these structures have come to occupy. Proceeding accordingly, one is at once struck with the fact that although strong evidence of partiality for certain special situations exists, yet no hard and fast rule may be laid down. The region where the organs occur most commonly seems to be by all means the surface of the ventral hemisphere of the eyeball. Photophores are found in this position in most (probably all) of the Cranchiidæ, in *Enoploteuthis*, *Abralia*, *Abraliopsis*, *Watasenia*, *Asthenoteuthion*, *Pyroteuthis* and *Pterygioteuthis* of the Enoploteuthidæ, in all the Lycoteuthidæ, in *Lampadioteuthis*, in *Ctenopteryx*, and in *Chiroteuthis*,—at least some 25 and more probably around 29 of the entire 44 photogenic genera in the suborder Decapoda. Most of the cranchiid genera, comprising, so far

as known, the entire subfamily Taoniinæ, are peculiar in that the subocular photophores are reduced to one or at most two organs which are frequently so large as to cover nearly the entire lower surface of the eyeball. When two such are present they are semicircular or more or less crescentic in outline, the smaller or anterior organ fitting into the concavity of the larger. The eye-organ in *Ctenopteryx* is a single large falciform structure. In most genera, however, the subocular photophores are smaller and more diffuse in their arrangement, the commonest system being an alignment in a simple, bead-like, longitudinal series on the ventral periphery of the eyeball. Curiously enough, the series usually includes organs belonging to two or more diverse structural types. Such is the arrangement to be found in *Lycoteuthis*, *Nematolampas Abralia*, *Abraliopsis*, *Watasenia* and *Enoploteuthis*, the last-named genus having nine or ten organs on each eye, all the other genera five. *Liocranchia* and *Pyrgopsis* have four organs similarly located, but all of one type. *Chiroteuthis picteti* and *C. imperator* are figured by Chun as having three longitudinal chains of isomorphic organs, 22 to 29 in all, upon each eye. In the latter species he found the number to be somewhat variable, which is an unusual circumstance with the subocular organs. This is a particularly striking fact when the remaining five genera having this type of photophore are considered. In all of these, namely, *Lampadioteuthis*, *Pyroteuthis*, *Pterygioteuthis*, *Cranchia* and *Leachia* the photophores of the eyes, varying in number from four in *Lampadioteuthis* to fifteen in *Pterygioteuthis giardi*, have lost their simple serial arrangement, and the individual organs are scattered to a greater or less degree over the lateral as well as the ventral region of the eyeball. Their distribution thus becomes highly irregular, yet it is almost always absolutely definite and practically invariable within the bounds of each single species. *Chiroteuthis veranyi*, as described by Chun, is unique in having two large bands of photogenic tissue on the ventral convexity of each eye, accompanied by a few small isolated photophores of the more ordinary form, by the coalescence of a number of which they perhaps originated. Since the genera possessing subocular organs are all œgopsid, it follows that

these photophores are covered by the double fold of the integument which forms the eyelid, and consequently in preserved specimens are often invisible without partial dissection. But in the Cranchiidæ the overlying membranes are thin, transparent, and very insufficiently equipped with chromatophores, so that in good specimens the organs may be clearly seen from the exterior. And likewise in certain other groups such as *Enoploteuthis* and the Abralioid genera, we find a delicate, transparent, elongate-oval "window" in the integument, nearly or entirely free of pigmented chromatophores, and overlying that portion of the eyeball where are borne the photogenic organs. There can be little doubt that this functions in aid of the latter by facilitating the passage of their rays.

The next most frequent topographic type of photophore to be met with comprises those occurring in the general integument of the body, primarily on the mantle, head, and arms. A remarkable peculiarity of the integumentary organs is that they, like the subocular photophores, are generally confined to the ventral aspect and this circumstance has given rise to some interesting theories regarding the origin and ecologic significance of the whole phenomenon of light production in this group of animals. Some writers have gone so far as to state that the distribution of these organs is *entirely* ventral, but this is not in strict accord with the facts, there being a few scattered photophores on the dorsal aspect of the mantle in such forms as *Abralia astrolineata* and most of the Histioteuthidæ, while Verrill's figures show them to be quite as strongly developed in this region in his *Mastigoteuthis agassizii*[14] as they are below. Certain other species of *Mastigoteuthis* have them in plenty on the dorsal surfaces of the fins, even if not upon the body proper. Again in Professor Joubin's anomalous *Melanoteuthis* the supposed photophores are entirely dorsal. The possibly photogenic tubercles of *Mastigoteuthis cordiformis* should likewise be recalled in this connection, and finally the presence of photophores on the dorsal arms of *Nematolampas* and *Benthoteuthis*. But even as many exceptions as this serve principally to accentuate the prevalence of the rule. In some genera the integumentary organs are developed on the

[14] *Bull. Mus. Comp. Zoöl.*, V. 8, Pl. I., 1881.

ventral surface of the mantle only (*Ancistrocheirus*, *Hyaloteuthis*, and, according to Chun, though he is controverted by other writers, *Chaunoteuthis*). In others (*Eucleoteuthis*) they occur on the ventral surface of the head as well. In *Calliteuthis*, *Histioteuthis* and some species of *Mastigoteuthis* they are found not only on the mantle and head, but on the aboral surfaces of the ventral and ventro-lateral arms. In *Mastigoteuthis agassizii* they are figured as occurring even on the tentacle stalks, as they do likewise in *Thelidioteuthis*, although this would appear to be an unusual situation for organs of the integumentary type. In the former of these two genera they are numerous in the integument of the head, arms, and mantle as well as the tentacles; in the latter, they are less numerous and although found along the outer side of the tentacles, occur elsewhere only on the ventral aspect of the mantle and head, where they have a very regular and characteristic arrangement. Finally in a number of well-known genera (*Enoploteuthis*, *Abralia*, *Abraliopsis*, *Watasenia*, *Mastigoteuthis*), integumentary organs are plentifully distributed in indefinite number over the entire ventral aspect of mantle, head, arms, and funnel.

On the fins these organs appear less frequently, but they are described as occurring dorsally in several species of *Mastigoteuthis*, and in one (*M. talismani*) on their ventral faces.

In a number of species there is a particular development of the integumentary photophores in the neighborhood of the eyes, usually in the form of a circlet around the margin of the lid opening, and such a circlet may occur, as in *Enoploteuthis*, *Abralia*, *Abraliopsis* and *Watasenia*, in addition to a well developed series of subocular organs. As a general rule, and certainly in the four genera named, the organs comprising this circlet are not to be distinguished from those of the general integumentary surface save by their peculiar arrangement and position. In the Histioteuthidæ, however, comprising the genera *Histioteuthis* and *Calliteuthis*, a most singular modification of this feature is encountered. A peculiar attribute of these genera is that, probably without exception, all the species have the left eye enormously more developed than the right, so much so in fact that a strong lateral torsion or displacement of the entire,

in both genera relatively enormous, head is produced, which would seem to render it a physical impossibility for the animal to propel itself in a straight path without recourse to spiral movement or some violent sort of counter twisting. This asymmetry extends quite inexplicably to the photogenic organs, inasmuch as the "normal" right eye has a well developed circlet of photophores surrounding the lid opening as above described, while the Brobding nagian left eye has the photophores of its circlet not only pulled farther apart by the distention of the lid, but its every component reduced almost to a rudiment, some of them quite atrophied, or they may even be, as Sasaki has stated for *Calliteuthis separata*,[15] absent entirely. It seems as though from the very nature of the case there must be some correlation between such pronounced asymmetry and the habits of the animals, but no reasonable explanation of what might be necessary to bring about or to render advantageous such an anomalous condition seems ever to have been suggested. In *Mastigoteuthis glaukopis* there is no circumocular circlet of photophores, but a single photogenic organ is described as occurring in the ventral edge of each lid sinus.

In a few species the integumentaty photophores are few and consequently definite in number and position (*Ancistrocheirus*, *Thelidioteuthis*, *Hyaloteuthis*, *Eucleoteuthis*). This is probably true also of the very young or larval stages of all the species possessing photogenic organs, but in adults of most species, though still continuing to retain more or less evidence of the primal bilateral symmetry, they are apt to increase to such an extent as to become practically or quite impossible of separate identification and enumeration and thereupon show little constancy in either number or position.

Eucleoteuthis is a genus which deserves discussion by itself. It is unique among known cephalopods in that its photogenic organs instead of forming small rounded or ovoid capsules as in practically all the other genera, are developed as a pair of narrow, more or less interrupted stripes or bands of photogenic tissue extending along the ventral aspect of the mantle for nearly its entire length. A small oval tract of similar tissue flanks the

[15] *Journal College Agriculture Tohoku Imperial University*, V. 6, p. 137, 1915.

outer side of each band at its anterior end, and, in the type species at least, there is a pair of somewhat larger, transversely ovoid photogenic areas on the head at the base of each ventral arms. That these curious tracts should be classified with the remaining organs here collectively referred to as integumentary is by no means certain.

The arms are a favored situation for photogenic organs. The extension along their outer surfaces of the ordinary integumentary photophores in the case of such genera as *Enoploteuthis*, *Abralia*, *Abraliopsis*, *Watasenia*, *Calliteuthis*, *Histioteuthis*, and certain forms of *Mastigoteuthis*, has already been noted. In addition to this certain special types of organs are sometimes developed. One of the generic characters of *Chiroteuthis* is the presence of a series of conspicuous dark photophores along the oral aspect of each of the greatly enlarged ventral arms. *Nematolampas* has a small dark photophore embedded in the extreme tip of each arm of the two dorsal pairs. Not only this but each arm of the third pair bears immersed in its tissues along the outer margin a series of plainly visible photogenic organs which continue as the principal component of a long, chain-like, filamentous extension of the arm which in life must extend like a string of fiery beads far in advance of the animal. There are in excess of thirty individual organs in each chain, but the true number may be much greater as no specimens of the species still retaining these extraordinary structures entirely in their pristine state have yet been captured. In *Abraliopsis* and *Watasenia*, genera so closely allied to one another that one could with about equal ease be regarded as but a subgenus of the other, there are three large, black, bead-like photophores, with perhaps some smaller, more rudimentary ones, in close juxtaposition at the tips of each ventral arm. As previously related, these are known to give forth a brilliant light. Rudiments of similar organs correspondingly situated are known in at least one species of *Abralia*, another nearly related genus. This is *A. astrolineata* Berry of the Kermadec Islands. The curious deep-sea *Benthoteuthis* has a single photophore on the outer periphery of each arm of the three dorsal pairs near the base, and none elsewhere on the body, an arrangement wholly unlike that met with in any other cephalopod.

Until very recently photophores on the tentacles have been supposed to be of rare occurrence, but it has lately been shown that they do actually so exist in quite a number of diverse forms, having tended to escape observation by reason of being embedded so deeply in the fundamental substances of the tentacle stalk as to be quite invisible in preserved material unless thoroughly cleared or otherwise specially treated. In *Pyroteuthis* Vivanti and Mortara have recently established the presence of a series of four such organs in the stalk of each tentacle. I had not only independently made the same discovery in material from both the Atlantic and Pacific, but had likewise found that there is yet a fifth tentacular organ present, and that the same condition obtains as well in the nearly related genus *Pterygioteuthis*. *Lycoteuthis* and *Nematolampas* have two such organs in each tentacle stalk. *Lampadioteuthis* is unique in possessing not only a series of four photophores embedded in the stalk proper, but in addition tucked away at its very base, a single large spherical organ of peculiar structure which is quite invisible without extraction of the entire tentacle from its socket. Conspicuous tentacular photophores are also shown in Verrill's figures of his *Mastigoteuthis agassizii*,[16] but the inference seems to be, as has been indicated above, that these are simply of the ordinary integumentary type, as seems to be true also of the tentacular photophores in the genus *Thelidioteuthis*.

We now come to the class of photogenic organs which is perhaps the most distinctive of the Cephalopoda as compared with other luminous animals, and which, next to the subocular photophores, exhibits the most general distribution within the group. Included here are a large array of very diversely constructed photophores found in quite various situations upon the visceral mass within the pallial chamber. These one and all, however, except in the case of those myopsids which eject their luminous secretion through the funnel, must naturally depend in life upon the more or less complete transparency of the mantle tissues to permit the unobstructed emanation of their beams. In preserved specimens, as would be expected, they can rarely be seen without laying open the pallial chamber, whereupon they are

[16] *Bull. Mus. Comp. Zoöl.*, V. 8, 1881, pl.1.

generally easy to distinguish, many of them being of unusual size and often of conspicuous coloration, while the situations which they occupy are peculiarly limited and, within a given species, constant. By reason of this last fact the intrapallial organs may readily be subclassified into four series, (1) anal, (2) branchial, (3) gastric, and (4) axial. Such a classification, too, in spite of its obviously superficial foundation, is a convenient one. That it is at the same time in all respects a natural or phylogenetic arrangement is probably not true, and it will no doubt be greatly improved upon by the first worker who takes up the relationships of these organs in any sort of adequate detail.

The term anal organs is misleading, but has become so well established in the literature that I use it pending the invention of a more appropriate term. The photophores so classified appear usually as a pair of quite large, often very brightly colored organs of rounded or ovoid outline, lying on the ink sac on either side of the rectum, with which they would otherwise appear to have no particular connection. Being often situated just back of the funnel, or sometimes almost within it, they are therefore sometimes termed the siphonal photophores, a name which in its turn is open to objection as inappropriate to the actual morphological relationships involved. Anal organs occur in a considerable number of little related genera, and the discharging photophores of the luminous Sepiolidæ are noteworthy for occupying an analogous situation.

The branchial organs are always paired, being situated one near the base of each gill. They are confined, so far as known, to the Lycoteuthidæ, Lampadioteuthidæ, and the pterygiomorph section of the Enoploteuthidæ.

The gastric and axial organs are classed together by most writers under the general term abdominal, but I prefer to separate the mesially situated, unpaired organs, which are often extended into a considerable series in the hinder portion of the mantle cavity, from the paired organs which sometimes occur near the middle of the body on either side of and often in close association with the anteriormost of the axial organs. There is evidence that in at least some genera the division here postulated into the

paired gastric and unpaired axial organs is founded upon a good morphological as well as merely topographical basis, but at the same time it is impossible to emphasize too strongly that we are here dealing primarily with the mere somatic distribution of the organs, and not with a true genetic classification based on the embryology or finer anatomy, save where the latter becomes incidentally involved. The need for this qualification has no doubt already been patent to the reader from the foregoing discussion.

Of the dozen genera listed in the synopsis as possessing intrapallial photophores, only *Heteroteuthis*, *Sepiola*, *Euprymna*, *Chiroteuthis* and *Corynomma*[17] are described as having anal organs only, a single pair or organ formed by the fusion of a pair being present in each instance. *Lampadioteuthis* has paired anal and branchial organs (the latter very large) and a single posterior axial organ. *Pterygioteuthis* has paired anal and branchial organs, and four axial organs, the most anterior of which is vastly the largest, the most posterior very minute and pushed far down past the fins into the sharp-pointed tip of the body. *Pyroteuthis* has a quite similar illumination system, but the foremost axial organ is more anterior in position, is only a little larger than the others, and is flanked on each side by a small gastric organ. *Lycoteuthis* and *Nematolampas* have a single pair each of anal, branchial and gastric organs as above, a small anterior axial and a very large posterior axial organ. Branchial, gastric and anterior axial organs are placed at about the same transverse plane so that they form a belt of fiery jewels near the middle of the body. *Onychoteuthis* (*banksii*) is unique in having but two large unpaired photophores, both of which are intrapallial and lie upon the ink sac in the median line, one very large and ensconced in a specially constructed depression on the ink sac proper, the smaller upon the narrow, neck like, anterior portion of the sac.

The minute unpaired organs which have been mentioned as occurring in the spine-like tip of the body in *Pterygioteuthis* and *Pyrotheuthis* are probably correctly interpreted as but the terminal members of an unusually developed axial series. Lo-

[17] Chun rather doubtfully adds *Octopodoteuthis* to this list.

cated in the same general region as these, and, by their appearance, seeming to bear a closer relation to the intrapallial organs than to the other systems outlined, yet scarcely to be regarded as lying actually within the mantle cavity, are the conspicuous paired photophores placed at the extreme posterior tip of the body in *Nematolampas*. *Lycoteuthis* does not possess them. They stand in a class quite by themselves at present, but if the peculiar swellings to be noted in the same situation in certain species of *Abralia* are susceptible of a photogenic interpretation, or if Chun's identification of the posterior disk of *Spirula* as a luminous organ be accepted, a further extension of this division of the classification is afforded.

7. Structure of Photogenic Organs.

Another most remarkable feature of the development of photogenic systems in Cephalopoda is, so far as I am aware, the quite unparalleled variety of structural type manifested by their constituent organs. It is entirely beyond the scope of this paper to enter into any extended account of the histological detail, but it will be useful to call attention to at least a few of the main features. Suffice to say that since the first observations on the finer morphology of cephalopod photogenic organs made by Joubin in 1893, a most bewildering variety of structure within the confines of this single, narrowly limited group of animals has been brought to light, ranging all the way from the simple discharging glands of the luminous myopsids, and the lump of photogenic tissue which forms the proximal photophore in the tentacle of *Lycoteuthis*, through almost innumerable intermediate types, to the astonishingly complex bull's-eye lanterns of *Abraliopsis* and the mirrored searchlights of the Histioteuthidæ. Each species has in fact its own peculiar modifications and sometimes many of them. The histology of all affords a fruitful field of investigation, which, with all due respect to the fine work of Chun, Hoyle and Joubin, we can truly say has been hardly skimmed. This is especially true of the embryology and he who attempts to work out the origin and homologies of even the simplest of these organs will have a virgin field.

Cephalopod photophores appear only rarely to be made up

of masses of photogenic tissue without accessory structures (intrapallial organs of Sepiolidæ; proximal tentacle organs of *Lycoteuthis* and *Nematolampas*; eyelid organs of *Mastigoteuthis glaukopis*). As a general rule they are more or less complicated.

The principal division of the organs on morphological grounds is that already noticed which places the discharging glands of the Sepiolidæ on the one hand,—the enclosed glands of the remaining photogenic genera on the other. The latter it is again possible to roughly separate into three types: the no doubt relatively primitive invaginated epithelial organs of which the subocular photophores of *Cranchia*, *Liocranchia* and *Leachia* are interesting examples, band-like expanses of photogenic tissue as in *Eucleoteuthis*, and the spherical, ovoid or discoid organs, often provided with the most extensive array of accessory mechanisms, which are found in most of the other genera.

The organs of the last mentioned class in their highest development attain to an almost unbelievable degree of complexity To the primary photogenic tissue, with its invariably abundant blood and nerve supply, are here added more or less efficiently developed reflector mechanisms, pigment cups, lenses, diaphragms directive muscles, mirrors, windows, color screens,—even in some cases accessory photophores, giving rise to the puzzling "double organs" which are met with now and then in the most dissimilar situations, so that their purpose and manner of functioning is left even more than it otherwise would be a complete enigma. In some cases only certain ones of these accessory structures are developed, in other cases nearly all, as in the miniature searchlights which yield such beautiful microscopic preparations in the integument of the Abralioid and Histioteuthid forms. Space will not permit a complete description, but the presentation of these various accessories in outline form will give an idea their wonderful variety and serve likewise as a convenient summary. The student desiring more detailed information is referred to the works cited in the bibliography, particularly those of Joubin ('93, '93*a*, '93*b*, '93*c*, '94, '95, :05, :05*a*), Hoyle (:02, :04, :09), Meyer (:06, :08), Vivanti (:14), and the beautiful memoirs of Chun (:03*a*, :10).

TABLE V.

COMPONENT PARTS OF THE CEPHALOPOD PHOTOPHORE.

I. Primary (photogenic tissue).
   1. Photogenic cells.
   2. Veins and arteries.
   3. Nerves.
   4. Connective tissue.

II. Secondary (accessory structures).
   1. Pigment cup (almost always present, but sometimes lacking where photophore is surrounded by other pigmented tissue, as the ink sac or eyeball).
      (*a*) Chromatophores.
      (*b*) Specially modified pigment cells (an adaptation of preceding ?).
   2. Reflector, or Tapetum.
      (*a*) Nucleated cells.
      (*b*) Fibers.
   3. Scale cells, or "Schuppenzellen" of Chun.
      (*a*) as reflector.
      (*b*) as lens or cornea.
      (*c*) in photogenic tissue.
   4. Lens.
      (*a*) Fibrillar.
      (*b*) Cellular.
         (1) Connective tissue.
         (2) Modified mantle musculature.
   5. Diaphragm.
      (*a*) Chromatophores.
      (*b*) Muscles.
   6. Window.
   7. Mirror.
   8. Accessory photophores ("double organs").

The duplex photophores deserve a further word. These comprise two separate masses of photogenic tissue so closely associated together that the conclusion seems unavoidable that in some way they function in common. Organs of this type seem to have been first discovered by Chun, who described them in some detail for a number of species. There is small doubt that histological examination will show the occurrence of similar organs in many other instances also. The double crescentic subocular photophores of certain Cranchiidæ have been briefly described on an earlier page. *Lycoteuthis* (and most probably *Nematolampas* also)[17] possesses a number of duplex organs, the

[17] *Nematolampas* certainly agrees in having the terminal subocular photophores equipped with an accessory photophore. The other organs mentioned have not yet been investigated.

distal organs of the tentacles, the terminal members of the subocular series, and the gastric organs, all being of this category. In the gastric organs, the respective masses of photogenic substance, though entirely distinct from one another, are contained within the same capsule. In the case both of these and the terminal subocular organs, which are separated, the accessory photophore lies beneath the principal one and the rays which emanate from it must accordingly pass through the latter if they are to have egress at all. In *Pterygioteuthis* the branchial organs are duplex, the accessory organ being contained a little to one side of its principal, but still within the same pigment cup.

8. Polymorphic Nature of Photogenic Organs.

The question is now very near, whether so many simple and elaborate morphological types of light-producing organs have any especially closer genetic relationship to one another where they are found within one and the same species or genus. And this leads easily to another, whether the photophores of any given species exhibit such manifold structural diversity as to render improbable their ultimate reduction to a single primordial type. The affirmation of this latter question implies the negation of the former, and I think we may certainly say that this seems most truly to express the facts as we have them. The accompanying table (Table VI.), which it has seemed worth while to elaborate upon the basis of the interesting outline given given by Chun, shows that whereas about a third of the genera cited each possess photophores belonging to a single general type, nearly as many have strongly dimorphic photophores, and an even greater number have trimorphic or polymorphic organs. It is nothing unusual therefore to find organs of extreme simplicity functioning as components of the same photogenic system which contains also organs exhibiting the most varying degrees of complexity in structural plan. While this seems to take place almost in hit or miss fashion, I think it may be taken as a general statement of fact that those species having a relatively abundant development of integumentary photophores distributed over the body generally fail to evolve a great variety of other types, the Abralioid genera providing the nearest to an

exception to this rule (see Table VII.). Those species showing the richest development of structural type in general are the ones which depend upon intrapallial rather than integumentary organs to serve the light producing function. Here there is sufficient divergence among the various organs as to discourage almost at a glance any attempt to homologize them on the basis of reference to a single primal type. Not only their diversity, but their extremely sporadic appearance in connection with organs and tissues of heterogeneous origin, is strongly inhibitive of any such view.

TABLE VI.

POLYMORPHISM IN CEPHALOPOD PHOTOPHORES.

I.	Genera with Isomorphic Photophores.	
	Thelidioteuthis	Integumentary.
	Histioteuthis	"
	Calliteuthis	"
	Benthoteuthis	On arms.
	Mastigoteuthis	Integumentary.
	Cranchia	Subocular.[18]
	Liocranchia	Subocular.
	Pyrgopsis	"
	Henselioteuthis	"
	Bathothauma	"
II.	Genera with Dimorphic Photophores.	
	Enoploteuthis	Integumentary; subocular.
	Leachia	Subocular.
	Megalocranchia	"
	Crystalloteuthis	"
	Toxeuma	"
	Taonidium	"
	Corynomma	Subocular (?); intrapallial.
III.	Genera with Trimorphic Photophores.	
	Abralia (except *A. astrolineata*)	Integumentary; subocular (latter dimorphic).
	Chiroteuthis	On ventral arms; subocular; intrapallial.
IV.	Genera with Polymorphic Photophores.	
	Lycoteuthis	In tentacles; subocular; intrapallial; 10 types (13 if 3 types of accessory organs are counted separately).
	Nematolampas	In arms; in tentacles; subocular; intrapallial; at tip of body; probably 12 or 13 types (15 or 16 if accessory organs are counted separately).
	Lampadioteuthis	In tentacles; subocular; intrapallial;—probably 7 or 8 types.
	Abralia astrolineata, *Abraliopsis*, *Watasenia*	Integumentary; tips of ventral arms; subocular (dimorphic).
	Pterygioteuthis	Tentacular; subocular; intrapallial;—8 types.
	Pyroteuthis	Tentacular; subocular; intrapallial;—probably 8 or more types.

[18] Unequal in size, but showing clear structural evidence of homology.

TABLE VII.

DETAILED OUTLINE OF OCCURRENCE OF POLYMORPHIC PHOTOPHORES IN CEPHALOPOD GENERA.

	Lycoteuthis diadema.	*Nematolampas regalis*	*Lampadioteuthis megaleia.*	*Abralia astrolineata.*	*Abraliopsis* sp.	*Watasenia scintillans*	*Pyroteuthis margaritifera.*	*Pterygioteuthis gemmata.*	*Pterygioteuthis giardi.*
Integumentary:									
Dorsal surface of mantle	–	–	–	+(1)	+(1)	+(1)	–	–	–
Ventral surface of mantle	–	–	–	+	+	+	–	–	–
Ventral surface of funnel	–	–	–	+	+	+	–	–	–
Ventral surface of head	–	–	–	+	+	+	–	–	–
Ventral arms	–	–	–	+	+	+	–	–	–
Circumocular	–	–	–	+	+	+	–	–	–
Subocular:									
Ventral periphery	10(3)	10(3)	6(2?)	10(2)	10(2)	10(2)	} 24(3)	28(4)	30(4)
Lateral	–	–	2	–	–	–			
Arms:									
Tips of dorsals	–	2 } (1?)	–	–	–	–	–	–	–
Tips of dorso-laterals	–	2 }	–	–	–	–	–	–	–
Ventro-laterals	–	62+(1)	–	–	–	–	–	–	–
Tips of ventrals	–	–	–	6(1)	6+(1)	6+(1)	–	–	–
Tentacles:									
At base	–	–	2(1)	–	–	–	–	–	–
Along stalk	4(2)	4(2)	8(1)	–	–	–	10(1)	?	10(1)
Intrapallial:									
Anal	2(1)	2(1)	2(1)	–	–	–	2(1)	2(1)	2(1)
Branchial	2(1)	2(1)	2(1)	–	–	–	2(1)	2(1)	2(1)
Gastric	2(1)	2(1)	–	–	–	–	2(1)	–	–
Axial	2(2)	2(2)	1(1)	–	–	–	4(1)	4(1)	4(1)
Posterior tip of body	–	2(1)	–	?	–	–	–	–	–
Total number photophores	22	90+	23	16+∞	16+∞	16+∞	44	36(+?)	48
Probable number structural types	(10)	(12 or 13)	(7?)	(4)	(4)	(4)	(8)	(8?)	(8)

The photogenic systems of all the species of the eight genera having polymorphic organs are outlined in further detail in Table VII. Those species considered having the mere largest number of photophores are the three Abralioids, occupying the three central columns of the table, but those exhibiting the highest degree of polymorphism are *Lampadioteuthis megaleia*, which has not been investigated histologically but must have not less than seven or eight types of photophores in all, *Lycoteuthis diadema*, with ten types, or thirteen, if the accessory organs are counted in, and *Nematolampas regalis*. *Lycoteuthis diadema*, with the immeasurable advantage of having had its marvelous photogenic properties observed in the living state, is usually cited as the example par excellence of a luminous cephalopod. However, it is evident from sheer morphological grounds that even this wonderful creature must yield the palm to another, if nearly related, genus and species,—the truly amazing *Nematolampas regalis* of the Kermadec Islands. Whether this species will ultimately be found to display all the varied brilliance of the red, white, and blue lights of *Lycoteuthis*, the fact remains that in addition to a complete series of exactly homologous organs, it has an entire battery of pyrotechnic engines of its own, so there is every reason to expect a more rather than a less elaborate illumination. The total number of photophores in this species is in excess of ninety, which are elaborated upon no less than twelve or thirteen different structural principles of uncertain homologies with one another. Counting in the three types of accessory photophores which are to be found in the eight "double" organs (proximal tentacular, terminal subocular, and anal), the total number of types is increased to fifteen or sixteen. Which of the alternative figures quoted is the correct one is still to be established by histological work.

9. Systematic Significance of Photogenic Organs.

It follows almost as a corollary from what has been said in the foregoing sections of this paper that the photogenic system evinces a complex of features of the utmost value to the taxonomist. Of late years ever increasing weight has been given to it, and the presence of constant differences, even though minute, in

its components, is now admitted practically without debate as ample ground for taxonomic discrimination. Where such differences are shown to occur, further differences in the remaining organization seem practically predestined for eventual discovery. Good characters for specific discrimination are to be found, not only in the presence or absence of photogenic organs, but also in their distribution on or within the body, in their number, in their size, and in the veriest details of their intrinsic structure. The taxonomist has in fact few more convenient points of attack in the pursuit of his primary objects of classification and relationship than that afforded by the light organs. And this is exactly what we find, if to somewhat less degree, among the fishes and the few other groups where the photogenic organs have attained some considerable complexity. One can construct a fairly workable taxonomic key based on the photogenic organs alone, for such species as possess them.

10. Probable Polyphyletic Origin of Photogenic Organs.

Before concluding this paper a somewhat general answer may be attempted to a question which has no doubt occurred more than once in the mind of the reader, and which indeed has been touched upon very nearly on more than one occasion—Is photogenesis a primitive function among cephalopods? In other words, are our present day species descended from an ancestral photogenic stem, some branches of which have now yielded up the function? Or has photogenesis arisen several times in this class of animals, possibly to meet altogether diverse conditions or associations in the environment, so that its presence therefore becomes of secondary rather than primary significance?

At first glance the widespread distribution of the function in the great and, comparatively, primitive œgopsid group of cephalopods favors an affirmative answer to our first query. But in reply to this it may be said that the varied pelagic environment of these forms would almost per se favor the development of the light-producing function after a manner which would be hardly likely to hold true among the more littoral Myopsida and Octopoda, the former of which are mainly frequenters of much shallower water than the Œgopsida, the latter hardly ever

pelagic at all, and then generally surface forms or confined to the shallower water like so many of the myopsids.

There are many other arguments which may militate against any theory of monophyletism and as strongly support the contrary view as brought out by the last query above. These, having already been largely elaborated elsewhere or to be dealt with in another connection later on, need be merely summarized here. Such considerations are:

1. The uneven distribution of photogenic organs throughout the entire group, and, as a corollary of this, their appearance in distantly related groups more or less sporadically.

2. The variety and sporadic character of the development of photogenic organs in different regions of the body.

3. The large number of strongly diverse structural types.

4. The evidence from ecological considerations, the distribution upon the body, and similar facts that these organs have arisen in response to very diverse environmental requirements.

How then may one bespeak a photogenic *system*? Exactly as one speaks of a muscular system, or a skeletal system, or a receptor system in almost any animal body. The term is used in the sense not necessarily indicating an aggregation of homologous structures, but an assemblage of organs within a single organic body exhibiting more or less similar or cöordinate physiological reactions, if at times neither in fact phylogenetically nor ontogenetically related.

11. Concluding Note.

This paper is mainly a compilation from the scattered work of other authors. No doubt there are omissions, but the aim has been to present simply a concise summary of the knowledge of this subject which has been gained to the present time. It cannot be too strongly emphasized that not only are many more species of luminous cephalopods likely to be discovered in the future, but some of those now known but not yet recognized as possessing photogenic properties are likely to be revealed as having them. Of the known luminous forms some will no doubt prove to possess luminous organs or properties additional to those described. Bearing all this in mind, if this little paper but fur-

nishes some delving student just a little better base of attack on his problem than might otherwise have been afforded him, its purpose will have been fulfilled.

12. Summary.

1. Light production is an unusually widespread phenomenon in the molluscan Class Cephalopoda.

2. Although unknown in the Order Tetrabranchiata, scarcely developed in the octopod section of the Dibranchiata, and occurring little more than sporadically among the Myopsida, over one half of all described Œgopsida are known to possess photogenic properties.

3. The actual production of light by living cephalopods has been observed only rarely, but in species of sufficiently diverse relationship to confirm the evidence drawn from the morphology and histology of organs found in the remaining species.

4. The light of some species exhibits remarkable brilliance.

5. The color of the light emanating from the respective organs within the same species or in different species may exhibit striking differences in both intensity and quality, but it is not known to what extent this is actually due to inherent diversity in the physical properties of the light rays themselves.

6. Photogenic organs may occur in almost any portion of the body in this group of animals, but the outer integument, eyeball and pallial chamber are the situations most favored. They are often internal and able to function only by reason of the transparency of the body tissues in the living state.

7. The organs are predominantly, but by no means exclusively, ventral in distribution.

8. The organs are strongly polymorphic, even in the same species, varying from comparatively simple bodies of photogenic tissue to the highly complex "searchlight" types.

9. Numerous duplex organs, or organs with accessory photophores, are known to occur.

10. Luminous organs in the Myopsida are usually of the type known as discharging. Those of the other groups are entirely of the enclosed or ductless type.

11. The maximum polymorphism in the photophores of any

single species occurs in *Nematolampas regalis* Berry, from the Kermadec Islands, where the 90 or more organs are elaborated upon 12 or 13 more or less diverse structural principles.

12. The occurrence, distribution, arrangement, and morphological detail of photogenic organs in cephalopods are features of considerable taxonomic importance and yield valuable clues as to the relationship and classification of the genera and species even where still unknown anatomically.

13. The best evidence seems to indicate that the photogenic organs in this group of animals are polyphyletic and more or less sporadic in origin, hence that light production in cephalopods is not an essentially primitive or ancestral function to be regarded as now lost in many members of the group.

13. LITERATURE.[16]

Berry, S. S.

'13 Nematolampas, a Remarkable New Cephalopod from the South Pacific. BIOLOGICAL BULLETIN, v. 25, pp. 208–212, 1 fig., August, 1913.

'13a Teuthological Miscellany No. 1. Zoologischer Anzeiger, Bd. 42, pp. 590–592, October, 1913.

Chun, C.

'00 Aus den Tiefen des Weltmeeres. Jena, 1900.

'03 Aus den Tiefen des Weltmeeres. Zweite Auflage, Jena, 1903.

'03a Über Leuchtorgane und Augen von Tiefsee-Cephalopoden. Verhandl. d. deutschen Zoologischen Gesellschaft, 1903, pp. 67–91, text figs. 1–14, 1903.

'10 Die Cephalopoden. 1. Teil: Oegopsida. Wissenschaftliche Ergebnisse d. deutschen Tiefsee-Expedition Valdivia, Bd. 18, pp. 1–402, 2 pls. and 32 figs. in text, Atlas of 61 pls., 1910.

'13 Cephalopoda. Report Scientific Results "Michael Sars" North Deep Sea Expedition, 1910, V. 3, pt. 1, Zoölogy, pp. 1–28, text figs. 1–11, pl. 1–2, 1013.

Dahlgren, U.

'16 The Production of Light by Animals. Journal Franklin Institute, Feb.-April, 1916, pp. 1–75, text figs. 1–20.

Darwin, C.

'60 Journal of Researches into the Natural History and Geology of the Countries Visited during the Voyage round the World of H. M. S. "Beagle" under the Command of Captain Fitz Roy, R.N. 8vo, pp. 1–551, illustrated, London, 1913 (first edition May, 1860).

Holder, C. F.

'87 Living Lights. A Popular Account of Phosphorescent Animals and Vegetables. 8vo, 127 p., 28 pls., New York, 1887.

[16] A more exhaustive bibliography of the subject is in preparation for a later paper of this series.

Hoyle, W. E.

'02 The Luminous Organs of *Pterygioteuthis margaritifera*, a Mediterranean Cephalopod. Memoirs and Proceedings Manchester Literary and Philosophical Society, V. 46, No. 16, pp. 1–14, text fig. 1–6, June, 1902.

'04 Reports on the dredging operations off the west coast of Central Americ- . . . by the . . . "Albatross," etc. Reports on the Cephalopoda. Bulletin Museum Comparative Zoölogy, V. 43, pp. 51–64, pl. 9–10, March, 1904.

'08 Address to the Zoölogical Section, British Association for the Advancement of Science, Leicester, 1907. Report British Association Advancement Science 1907, pp. 520–539 [1–20], text fig. A–D, 1908.

'09 The Luminous Organs of Some Cephalopoda from the Pacific Ocean. Proceedings 7th International Zoölogical Congress, Boston Meeting (advance print), pp. 1–5, Cambridge, 1909.

Ishikawa, C.

'13 Einige Bemerkungen über den leuchtenden Tintenfisch, Watasea nov. gen. (Abraliopsis der Autoren) scintillans Berry, aus Japan. Zoologischer Anzeiger, Bd. 43, pp. 162–172, text fig. 1–6, December, 1913.

Joubin, L.

'93 Recherches sur l'appareil lumineux d'un céphalopode, *Histioteuthis ruppellii*, Verany. Bulletin Société Scientifique Médicale de l'Ouest, V. 2, pp. 49–80 [1–32], text fig. 1–10, 1893.

'93a Note complémentaire sur l'appareil lumineux d'un céphalopode: Histioteuthis Ruppellii, Verany. Bulletin Société Scientifique Médicale de l'Ouest, V. 2, pp. 161–169 [1–9], 1893.

'93b Note sur une adaptation particulière de certains chromatophores chez un céphalopode (l'oeil thermoscopique de Chiroteuthis-Bomplandi Vérany?). Bulletin Société Zoologique France, V. 18, pp. 146–151 [1–6], 1 text fig., 1893.

'93c Quelques organes colorés de la peau chez deux céphalopodes du genre *Chiroteuthis*. Mémoires Société Zoologique France, V. 6, pp. 331–343 [1–13], text fig. 1–12, 1893.

'94 Nouvelles recherches sur l'appareil lumineux des céphalopodes du genre *Histioteuthis*. Bulletin Société Scientifique Médicale de l'Ouest, V. 3, pp. [1–15], text fig. 1–7, 1894.

'95 Note sur les appareils photogènes cutanés de deux céphalopodes: *Histiopsis atlantica* Hoyle et *Abralia oweni* (Verany) Hoyle. Mémoires Societe Zoologique France, V. 8, pp. 212–228 [1–17], text fig. 1–11, 1895.

'05 Note sur les organes lumineux de deux céphalopodes. Bulletin Société Zoologique France, V. 30, pp. 64–69, text fig. 1–2, 1905.

'05a Note sur les organes photogènes de l'oeil de *Leachia cyclura*. Bulletin Musée Océanographique Monaco, No. 33, pp. 1–13, text fig. 1–7, April, 1905.

'12 Etudes préliminaires sur les céphalopodes recuellis au cours des croisières de S. A. S. le Prince de Monaco. 1re Note: Melanoteuthis lucens nov. gen. et sp. Bulletin Institut Océanographique Monaco, No. 220, pp. 1–14, text fig. 1–12, January, 1912.

'12a Sur les céphalopodes capturés en 1911 par S. A. S. le Prince de Monaco. Comptes Rendus Séances Academie Science, Paris, V. 154, pp. 395–397 [1–3], 1912.

Meyer, W. T.

'06 Über das Leuchtorgan der Sepiolini. Zoologischer Anzeiger, Bd. 30, pp. 388–392, text fig. 1–3, July, 1906.

'08 Über das Leuchtorgan der Sepiolini: II., Das Leuchtorgan von Heteroteuthis. Zoologischer Anzeiger, Bd. 32, pp. 505–508, text fig. 1–4, January, 1908.

Naef, A.

'12 Teuthologische Notizen, 1–2. Zoologischer Anzeiger, Bd. 39, pp. 241–248, March, 1912.

Sasaki, M.

'12 Hotaru-ika. Toyama-ken, Sui-san Kumiai Hokoku, pp. 1–29, 2 pls. and chart, 1912. (In Japanese.)

'13 Hotaru-ika. Zoölogical Magazine, Tokyo, V. 25, pp. 581–590, pl., 1913. (In Japanese.)

'14 Observations on Hotaru-ika Watasenia scintillans. Journal College Agriculture, Tohoku Imperial University, V. 6, pp. 75–107, 1 text pl., pl. 1–3, November, 1913.

Tryon, G. W., Jr.

'79 Cephalopoda. Manual Conchology, V., 1, pp. 1–316, pl. 1–112, Philadelphia, 1879.

Vérany, J. B.

'51 Mollusques méditerranéens, observés, décrits, figurés et chromolithographiés d'apres le vivant, I., Céphalopodes de la Méditerranée. Gênes, 1851.

Vivanti, A.

'14 Contributo alla conoscenza dei Cefalopodi abissali del Mediterraneo. Ricerche sulla Carybditeuthis maculata n. g. n. sp. dello Stretto di Messina. Archivio Zoölogico Italiano, V. 7, pp. 55–79, text fig. 1, pl. 3–5, 1914.

Watasé, S.

'05 Luminous Organs of Abraliopsis, a New Phosphorescent Cephalopod from the Japan Sea. Zoölogical Magazine, Tokyo, V. 17, pp. 119–122, 1 text fig., June, 1905. (In Japanese.)

RAPIDITY OF ACTIVATION IN THE FERTILIZATION OF NEREIS.

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The following experiments were designed as a test of the rapidity of the action of the spermatozoön of *Nereis* in relation to the initiation of the processes of maturation. The almost instantaneous effect of the contact of the spermatozoön in stimulating the formation of the fertilization membrane and, in this case, of the jelly is well known. It is also usually observed that, in the case of those eggs in which maturation follows insemination, that polar-body formation will occur without fail even if later observation shows that no cleavage follows. It might be conceived that the continued action of the spermatozoön were necessary to cause maturation. The results outlined below indicate that maturation in *Nereis* follows almost, if not quite as brief an application of the stimulus as is necessary to initiate the jelly formation.

The experiments of Lillie ('11) on *Nereis limbata* showed clearly that the removal of the spermatozoön later than twenty-one minutes after insemination by a process of centrifuging did not interfere with the maturation of the egg. It was, however, found impossible by this method to remove the sperm earlier than twenty-one minutes after fertilization. Various workers have suggested (I am indebted to Dr. Chambers for first calling this to my attention) that the Barbour apparatus for micro-dissection offered a means of removal during this earlier period. The manipulation of the instrument for this purpose has proved most successful. The spermatozoön may readily be removed shortly after attachment and with more difficulty later because of the increasing strength of adherence to the egg and the viscidity of the head of the spermatozoön. The viscidity is shown in attempts to remove the spermatozoön at about 35 minutes after insemination. The head, remaining attached in the region of the perforatorium may be extended by

the needle some five or ten times its original length. In these later stages the method of removal by centrifuging is more practical.

The experiments were made during the summer of 1919 at the Marine Biological Laboratory at Woods Hole, Mass. Sperm and eggs were obtained from animals caught the evening before and kept in the upper compartment of the refrigerator during the night. Sperm dilutions of about 1/400 to 1/500 were used for most of the experiments. This dilution is not such as will insure that only one spermatozoön will come in contact with each egg (Lillie, '15), but it is sufficiently dilute so that polyspermy rarely results. Polyspermy was noted in a few of the eggs in experiments 6 and 7. The eggs were placed in a dilute suspension of chinese ink, at once fertilized and transferred to the coverslip which forms the roof of the moist chamber. The coverslips had been previously carefully cleaned of all traces of oil or grease in order to allow the drop of water to spread, thus forming a thin film which compresses the eggs slightly as is necessary in order to hold the egg firmly during the operation. The apparatus was always in complete readiness for the operation and it was usually possible to locate promptly an attached spermatozoön and to carefully push or rub it from its point of attachment to the egg. Spermatozoa remained immobile after detachment. Two or three operations were usually carried out on each coverslip. More were not attempted because the delay would allow too great an evaporation of water in the necessarily imperfect moist chamber, thus causing greater compression of the egg and also greater concentration of the sea water. The position of the operated eggs among others was plotted, the coverslip removed and placed under a binocular microscope, the selected eggs were isolated and removed to separate dishes for observation. Frequently the remainder of the eggs on the cover slip was also removed for cleavage counts (Control 2). These were the last to be removed and in some cases had by that time undergone considerable compression (in other cases the cover slip was slightly flooded to prevent this). This may in part account for the low percentage cleavage recorded frequently under Control 2.

The eggs were then kept under observation to note the forma-

tion of the polar bodies and in the second series (Table II.) to note possible cleavage. In all but two of the sixty eggs the polar bodies formed. I am inclined to think that as these two were among the earlier ones observed in this case the negative record may be due to faulty observation. Also in only three cases and one of these from a clearly polyspermic lot of eggs was cleavage observed. The time of removal varied from 1¾ minutes to 13½ minutes after insemination. It is impracticable to remove the sperm in most cases earlier than two minutes after insemination as it is difficult to discriminate between spermatozoa lying against the egg and those that have effected an attachment. The average of elapsed time from insemination to removal was 6.2 minutes and in sixteen cases the spermatozoön was removed in less than 4 minutes after attachment, and in five cases in less than 3 minutes. It seems therefore clear that in so far as maturation is concerned, the full stimulating effect of the spermatozoön is effective within a very few minutes after attachment and quite possibly it is only a matter of seconds.

The results clearly support the concept that the first phase of activation of the egg-membrane formation and maturation is initiated by the spermatozoön which "activates a substance, or ferment-like bodies, contained within the egg" (Lillie, '19, p. 159), rather than by the continuous introduction of some lysin-like substance through the slender perforatorium. For it seems improbable that the spermatozoön could introduce in less than two minutes through the perforatorium which has a cross section area of perhaps one ten-millionth of the surface area of the egg, a sufficient amount of material to take part in reactions throughout the egg. It should, however, be noted that under certain conditions (heating, Just, '15) it is possible to initiate jelly formation without maturation following. This may be taken to indicate that a lesser stimulus gives a lesser result and possibly if it were practicable to remove the sperm more promptly that jelly formation only would result. On the other hand in the cited case we may be dealing with a stimulus differing in kind rather than in degree. It should also be noted that these results are not at variance with the concept that cortical changes form an all important intermediate step in the activation of the egg.

In some cases (Table II.) it was possible to note the time of formation of the first polar body. The average of elapsed time after insemination was 35.8 minutes and no correlation was noted between the duration of attachment of the sperm and the time of formation of the polar bodies.

TABLE I.

Date.	Experiment Number.	Time of Fertilization.	Egg.	Time of Removal of Sperm.	Minutes Elapsed.	Polar Body Formation.	Cleavage.	Controls.	
								No. 1.	No. 2.
July									
17	1	10.48	A	10.56	8	+	−	+	60%
			B	11.02	14	+	−	+	"
	2	2.44	A	2.54	10	+	−[1]	+	90%
			B	2.58	14	+	−[1]	+	"
18	3	5.29	A	5.32½	3½	−	−[1]	+	65%
			B	5.37½	8½	+	−[1]	+	"
19	4	9.59	A	10.03	4	−	−[1]	+	70%
			B	10.08½	9½	+	−[1]	+	"
			C	10.10	11	+	−[1]	+	"
20	5	2.29½	A	2.33½	4	+	−	+	60%
	6	2.40	B	2.51	11	+	−[2]	+	"
21	7	9.40½	A	9.48	7½	+	+[2]	+	90%
	8	10.14½	A	10.18½	4	+	+	+	40%
			B	10.21¾	7¼	+	+	+	"
	9	11.26	A	11.27¾	1¾	+	−[1]	+	50%
			B	11.32	6	+	−[1]	+	"
	10	2.54½	A	3.00	5½	+	−	+	70%
			B	3.03	8½	+	−	+	"
	11	3.13	A	3.16½	3½	+	−[1]	+	
			B	3.21½	8½	+	−[1]	+	

As noted in Table I. some eggs were fixed at about time of the first cleavage with view to study of cytological changes. Too few of these have survived the ordeal of embedding and sectioning to make any detailed study valuable. I noted that in some cases only chromosomal vesicles and in other cases well-formed chromosomes were present at the time of the first cleavage spindle. This may be compared with the observation of Lillie ('15) where chromosomes were formed in eggs from which sperm were removed by centrifuging but not in eggs which were caused to maturate by centrifuging without insemination.

The appended tables outline the experiments. Full data are

[1] Egg fixed at about time of expected first cleavage. No indication of cleavage at that time.

[2] Poly-spermy observed in same lot on cover-slip.

not present in all cases. The experiments were in part preliminary and the desirability of various controls and observation became apparent as they progressed. In as much as the work

TABLE II.

Date.	Experiment Number.	Time of Fertilization.	Egg.	Time of Removal of Sperm.	Minutes Elapsed.	Polar Body Formation.	Time Formed.	Minutes Elapsed.	Cleavage.	Controls. No. 1.	Controls. No. 2.	Controls. No. 3.
July												
23	12	9.47	A	9.50	3	+	10.19	33	—	+	60%	
			B	9.53½	6½	[3]				+		
			C	9.54½	7½	+	10.20	34	—	+	"	
	13	10.20	A	10.23¼	3¼	+	10.52	32	—	+	80%	
			B	10.25⅓	5⅓	+			—	+	"	
	14	11.05½	A	11.08½	3	+			—	+		
	15	11.36½	A	11.38½	2	+			—	+		
			B	11.42½	6	+			—	+		
			C	11.43½	7	+			—	+		
	16	2.40	A	2.48½	8½	+	3.21	31	—	+	90%	
			B	2.53½	13½	+	3.17	37	—	+	"	
	17	3.16½	A	3.24½	8	no. obs.			—[4]	+		
			B	3.24½	9	+	3.49½	33	—[4]	+		
			C	3.26½	10	+			—[4]	+		
			D	3.28	11½	+			—[4]	+		
24	18	10.15	A	10.18½	3½	+	10.46	31	—[4]	+		
			B	10.22	7	+	10.49	34	—[4]	+		
	19	10.51½	A	10.55	3½	+	11.22½	31	—[4]	+		
			B	10.57½	6	+			—[4]	+		
			C	10.58½	7	no obs.			—[4]	+		
	20	11.26	A	11.29	3	+	11.56½	30½	—[4]	+		
			B	11.33½	6½	+	11.57½	31½	—[4]	+		
	21	3.18½	A	3.21¾	3¼	+	3.53	35	—[4]	+		
			B	3.24	5½	+	3.53	35	—[4]	+		
			C	3.25	6½	+	3.52	34	—[4]	+		
25	22	9.33	A	9.36	3	+	10.05½	32½	—	+	60%	
			B	9.38	5	+	10.06	33	—	+	"	
			C	9.39	6	+	10.06	33	—	+	"	
	23	10.16	A	10.18½	2½	+			—	+	58%	
			B	10.21½	5½	+			—	+	"	
	24	10.48½	A	10.51½	3	+			—	+	57%	
			B	10.52	3½	+			—	+	"	
			C	10.55	6½	+			—	+	"	
			D	10.57	8½	+			—	+	"	
	25	11.34	A	11.37½	3½	+	12.15	41	—	+	45%	
			B	11.43½	9½	+			—	+	"	
	26	2.19¾	A	2.42½	4¾	+	2.57	37¼	—	+	25%	
	27	2.47	A	2.49	2	+	3.25	38	—	+	55%	+
	28	3.54½	A	3.57	2½	+	3.37	42½	—	+	80%	+
			B	4.00	5½	+	3.36	41½	—	+	80%	+
26	29	9.52	A	9.55	3	+	10.27	35	—	+	66%	+
	30	10.29	A	10.32¾	3¾	+	11.02	33	—	+	"	+
			B	10.35	6	+	11.03	34	—	+	57%	
	31	11.05½	A	11.07½	2½	+	11.38½	33	—	+	75%	+
			B	11.10¾	5¼	+	11.42½	37	—	+	"	+

[3] Egg disintegrated.

[4] No specific record in original notes of the absence of cleavage.

was stopped by the end of the *Nereis* "Run" and as the observations in regard to maturation were complete it seems to me to be best to present the work in its present form.

TABLES.

The headings are mostly self-explanatory. Under Polar Body Formation and Cleavage a plus sign (+) indicates that the process was observed and a minus (−) the reverse. Control No. 1 are eggs from the same lots as in the experiments fertilized a few minutes before the experiment began from the sperm of the same male used. Control 2 are eggs not operated on from the coverslip and Control 3 are eggs of the same inseminated lot mixed with chinese ink from which those placed on the cover slip were taken. The plus sign (+) under Controls 1 and 3 indicates the practically 100 per cent. cleavage usually realized with *Nereis* eggs.

WESLEYAN UNIVERSITY,
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LITERATURE CITED.

Just, E. E.

'15 Initiation of Development in *Nereis*. BIOL. BULL., Vol. 28.

Lillie, F. R.

'11 Studies of Fertilization in *Nereis*, I and II. Jour. Morph., Vol. 22.

'15 Studies of Fertilization, VII. BIOL. BULL., Vol. 28.

'19 Problems of Fertilization. University of Chicago Science Series.

IMMUNITY AND THE POWER OF DIGESTION.[1]

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The proteins are the fundamental chemical constituents of the living protoplasm in all our tissues and organs. But the cells of the tissues do not take up the proteins as such, they are known to live on the products of protein digestion, on their building stones, the various amino-acids. And by a long process of synthetic character the cells of a multicellular organism build up out of these building stones their own individual proteins of a highly specific structure.

The multicellular organism possesses special organs of digestion which successfully perform the work of splitting the ingested food to the stage of amino-acids, in which stage they become capable of passing through the intestinal wall; they are absorbed here and transported to all our tissues, which in this manner are provided with various substances for their synthetic work in order to replace the wear and tear of the living substance during adult life or to build up organs and tissues during developmental stages.

Now are the cells of all our organs and tissues which, under usual conditions, are supplied for their synthetic work with ready made building stones, without one of the most characteristic and important properties of the living matter, viz., the power of splitting the chemically more complex proteins to the stage of amino-acids? Or are these cells not manifesting a digestive potency inherent in them, because they have no chance of manifesting it, because the ingested food does not reach them primarily and undergoes the hydrolytic changes before it is capable of being absorbed? Is it a lack of power or a lack of opportunity which prevents the cells of our tissues from manifesting a digestive capacity under usual conditions?

[1] Given at the meeting of the Section of Biology, New York Academy of Sciences, October 13, 1919.

A most wonderful property exhibited by the tissues themselves is to inhibit growth of foreign tissue. This resistance of the organism against proliferation of any heterogeneous cellular elements, be it normal tissues, tumor cells or microörganisms, is more generally known under the term of immunity. There is little need to emphasize the importance of any new information concerning this problem. Disregarding, however, the most earnest efforts and attempts to throw upon this property light sufficient to master it, no definitive knowledge has yet been acquired concerning the nature of this property.

In all organisms, plants and animals as well, the power of resistance against proliferation of any foreign tissue does not seem to depend upon the activity of a special organ in the organism. A graft of heterogeneous tissue simply does not take, a tumor graft does not grow, microörganisms do not proliferate, die and disappear in an immune or immunized animal. A living cell endowed with the faculty of proliferation, for example, a tumor cell, well known for its high rate of proliferation and on account of this property disastrous to the organism, on which it has settled, if grafted on an immune organism, will certainly find here all of the elements needed for the building up of its own protoplasm, for we know that the tissues of the organism are abundantly provided with various amino-acids, and these not being specific, can be used equally well by any tissue. And still a tumor cell will not live on an immune organism. It dies and disappears.

If now we inquire into the relation between the digestive power of the cell and resistance in a unicellular organism, we will see that both phenomena in this case are very closely connected. A unicellular organism can ingest another living organism, bacteria as well and the latter, while within a cytoplasmic vacuole of the former, are killed and consecutively digested by the enzymes of the phagocyte. The unicellular organism in this case proves to be immune against a possible intracytoplasmic proliferation of the ingested living individual, primarily and solely because of its digestive capacity.

The question whether in a multicellular organism any relation exists between its resistance against proliferation of a foreign tissue in it and a digestive power of its tissues is obscured by the

fact that the tissues of the organism do not exercise under normal conditions any digestive power, and it is not definitely settled, which of the various tissues, if any, besides those connected with the digestive tract, are endowed with a digestive power. And still there exist sufficient indications that the tissues of a multicellular organism, besides those highly specialized of the digestive tract, are equipped with enzymes which confer on them a digestive power similar to that so characteristic of the special elements in the digestive tract. If the tissues of a multicellular organism be put under the stress of starvation, under which condition no amino-acids will reach them from the absorbing surface of the digestive tract, the tissues will in this case use up tissue proteins, they themselves hydrolyze the proteins or digest them, which work is made possible by the presence of enzymes in these cells. And another example of digestive work performed by tissue cells of a multicellular organism can be found in the growth of tissue on artificial media. Here again the transplanted bits of tissue do not find within the culture medium ready building stones for the building up of their protoplasm in the form of amino-acids, but only proteins of the blood plasma clot. Here again the cells of the tissues themselves have a chance of performing the splitting or digestion of the proteins, and they do it. In both these cases we base our conclusions of the presence of enzymes in the tissue cells on the results of the experiments: on the continuation of the output of nitrogen by the organism during a diet free from nitrogen in the starvation experiment, and on the further growth of the tissue in the culture experiment. The fact only not the mechanism of the digestive activity by tissue cells is determined in both cases.

Some information concerning the mechanism, or rather concerning one of the mechanisms, by which cells of our body may digest, *i.e.*, split and assimilate protein may be gained by microscopical study. And this study informs us that in embryos developing from mesoblastic eggs, *i.e.*, from eggs containing great quantities of yolk, as for example, from birds eggs, all of the tissues contain at an early stage a great quantity of yolk granules in their cytoplasm, which are digested intracellularly and assimilated. Endodermal cells as well as ectoderm and mesoderm cells, even primitive germ cells, all are endowed with

an intracellular digestive capacity. And this capacity is surely not lost during the development of the embryo, at least the mesenchymal cells continue to exercise this faculty, and wherever cells in the developing organism have lost their normal correlation with other tissues, as for example, red blood corpuscles during the rearrangement of vessels, mesenchymal cells are seen to ingest them and digest them within their cytoplasm. The mechanism of this digestive activity is analogous to that observed in unicellular organisms. Enzymes are not given off as in the digestive tract, they work within the cytoplasm, they are endoenzymes. To a digestive activity of the mesenchymal cells is due the disappearance of aseptic emboli, trombi and infarcts; small sequesters may be entirely resorbed and catgut sutures disappear in the organism in a short time.

An intracellular digestive activity is exhibited in an intense way by the tissues of embryos developing from mesoblastic eggs in the early stages of embryonic development and only occasionally during adult condition. This power is dormant in the tissues of mammals during embryonic development, the embryo receiving all the materials needed for its development in the form of amino-acids from the maternal placenta, but is awakened at the first opportunity of being confronted with unsplit protein.

The digestive power of the tissues is well evident in respect to cellular protein in the dead form. Only in a unicellular organism did we see that their digestive activity confers on them a power over living organisms and cells as well and therefore becomes the source and the cause of their immunity against a possible proliferation of the ingested organisms. May the digestive capacity of the tissues of a multicellular organism, of which we have seen a few examples, be also in some way connected with the failure of a heterogeneous graft to take? Is this power not the deleterious factor which cannot be overcome by the grafted heterogeneous tissue and which inhibits its growth and proliferation?

The results of a long series of experiments, which I am going to illustrate, show without any doubt that at least in some cases it is the digestive activity of an adult mesenchymal cell which inhibits the growth of a heterogeneous tumor or rather destroys the actively growing tumor. The experiments were made with

different tumors, but only the Ehrlich sarcoma, and the tumor known at the Crocker Fund Laboratory, under the number 180, gave demonstrable results. It is known that mammalian tumors may be grown easily on chick embryos, but not on adult animals. Also normal adult chick tissue grows well on embryos of the same species. Therefore, embryonic tissues, more particularly the chick allantois, have been used by me as a culture medium, to bring together mammalian tumors and various adult chick tissues. It was expected that the study of the interaction of the tissues of the adult naturally immune fowl and of the mammalian tumor cells grown in a culture medium, for both equally favorable, might show whether the digestive capacity of the tissues more particularly that of the adult splenic mesenchymal cells may in any way be connected with the resistance offered by the adult animal to the grafting of the tumor on it. The experiments have shown that the Ehrlich sarcoma gives invariably a good growth if grafted alone, but if grafted in a mixture with the spleen, disappears even after a short period of proliferation. The short-lived fame of the small lymphocytes thought to be responsible for this disappearance is still in our memory. Only an absolute disregard of microscopical findings can explain how microscopical pictures similar to those shown here (Figs. 1, 2, 3 and 4), have been overlooked and how the small lymphocyte could become the fetish of the immunity.

Microscopical preparations, as seen in retouched photographs, which accompany this paper, illustrate in a striking way the process of disappearance of tumor foci surrounded by the adult splenic mesenchyme in the allantois. Two lines of activity are observed in the mesenchyme, it splits off numerous mobile cells of hemoblastic nature which differentiate further into granular leucocytes. This developmental potency is exhibited in an even more intensive way by the splenic adult mesenchyme, if the splenic tissue is grafted alone on the chick allantois. But the fact of grafting the splenic tissue together with tumor reveals in it a new potency and this is its power of isolating and surrounding tumor cells, of enclosing them in vacuoles and of digesting them within these vacuoles (Figs. 1, 2, 3 and 4). The adult splenic mesenchymal cell is apparently attracted toward the mammalian tumor cell. Contrary to the embryonic mesen-

chyme, the adult mesenchymal cells, once close to the tumor cell, will not indifferently pass by, but together with other mesenchymal cells, will tightly surround it. The tumor cell, in response to the approach of the adult mesenchymal cell, withdraws its cytoplasmic processes, becomes immobile and assumes soon a spherical shape. The Figure 1 illustrates a tumor focus in which the cells still intensely proliferate in the center as may be seen from numerous mitoses present. The tumor focus is however surrounded by a zone of mesenchymal syncytium of splenic origin. The cells of this tissue encircle the tumor cells at first surrounding them very tightly (Figs. 1 and 2, *x*). The adult splenic mesenchymal cells of the fowl treat a heterogeneous mammalian tumor cell, which, if present alone in the embryonic allantois, would live and proliferate in no other manner than if it were a block of dead protein. They gather around the tumor cells and enclose them into a capsule and then secrete a fluid within the little cavity occupied by the tumor cell. The nature of this fluid is such that a disintegration of the tumor cell takes place and a complete splitting of its proteins, it must therefore contain proteolytic enzymes. The tumor cell gradually loses its structure (*Tc'*, Figs. 1, 2, 3 and 4) is transformed into a block of structureless protein (*Tc''*, Figs. 1, 2, 3 and 4) and finally disappears completely. Tumor cell after tumor cell is digested in this manner. In the case of the Ehrlich sarcoma, the rate of digestion of the tumor cells by the splenic mesenchyme is higher than the rate of proliferation of the tumor cells, and the grafted tumor, even after a good start of development, disappears. The same process is observed in relation to the sarcoma 180. Only in this case the rate of proliferation of these tumor cells is higher than the rate of their digestion by the splenic mesenchyme, and the tumor still grows in spite of the existence of a peripheral zone around it, in which the process of digestion of tumor cells by mesenchymal cells is most evident.

The study of microscopical preparations allows us to follow the gradual changes which the tumor cells undergo within the vacuoles surrounded by mesenchymal cells, changes which lead to the full disappearance of the tumor cells. Figures 2, 3 and 4 show very clearly how healthy tumor cells *H Tc* are cut off from the tumor focus, the tumor cells withdrawing their processes

and rounding up, how the tumor cells gradually lose their structure *Tc'*, their cytoplasm becoming vacuolized, their nuclei pycnotic and how finally the tumor cells disappear entirely.

Now in respect to the small lymphocytes, may they not be still connected in some indirect way with the disappearance of the tumor cells? Round-cell infiltration has been described around the disappearing tumor grafts within the allantois. But though the small lymphocyte is a round cell, not every round cell is a small lymphocyte. Round cell infiltration exists indeed around such grafts. This infiltration, however, at a closer study proves to consist not of small lymphocytes, but of round, mobile cells of hemoblastic nature which, as mentioned above, differentiate into granular leucocytes. As has been shown in one of my previous papers, the small lymphocytes are not very viable in the allantois and in most cases quickly disappear in the grafted splenic tissue.

It is a positive tropism between the adult mesenchymal cell and the tumor cell expressed in the phagocytic power of the mesenchymal cell over the tumor cell and the digestive activity of the adult mesenchymal cell which prove to be the direct factors in the disappearance of the tumor cells in a double mixed graft of tumor and spleen. One of the remarkable results of the various experiments undertaken in this direction is the fact, that only the adult splenic mesenchyme, not the embryonic, exhibits the capacity of digesting tumor cells. This observation well corresponds with the fact that the embryo fails to resist growth of heterogeneous tissue.

I have already mentioned that only in respect to the Ehrlich sarcoma and to the sarcoma 180 could I obtain results which clearly demonstrate the phagocytic and digestive activity of the adult splenic mesenchymal cells. The study of the growth of other tumors and of the check of their growth is sufficiently advanced to enable me to conclude that the inhibition of the carcinoma growth is effected by a mechanism not altogether identical to that described in relation to the Ehrlich sarcoma cells. Must this fact astonish us? I do not think so. To expect a literally identical response of the mesenchymal cells to different agents would be just as inconsistent as to expect the digestive activity in all multicellular animals to proceed in a perfectly identical

manner. And we know that this is not the case. We know that in multicellular organisms there exists a digestive cavity, into which free enzymes are poured, we know, however, that in sea-anemones, though a digestive cavity does exist, no ferments had ever been discovered in it and the digestion of the food proceeds through the immediate apposition to it of definite cellular elements.

The fact that at least in some cases the phagocytic and digestive activity of the mesenchymal adult cells is found to be the deleterious factor, which cannot be overcome by some of the tumors and which inhibits the growth of these tumors, or rather destroys an actively growing tumor, throws a new light on the immunity problem in general, since the power of resistance against proliferation of foreign tissue, though manifested in different ways, can certainly not be of a fundamentally different nature.

EXPLANATION OF FIGURES.

The figures are retouched photographs from original preparations. The Figure 1 at about 400, the Figure 3, 700 and the Figures 2 and 4, 1000 diameters.

Abbreviations.

Gr Lc. Granular leucocytes.
H Tc. Healthy tumor cells.
S Ms. Splenic mesenchyme exercising a phagocytic and digestive activity.
Tc'. Tumor cells in various stages of disintegration.
Tc''. Tumor cells transformed into blocks of almost structureless protein.
V. Vessel.
X. Tumor cells tightly surrounded by mesenchymal cells.

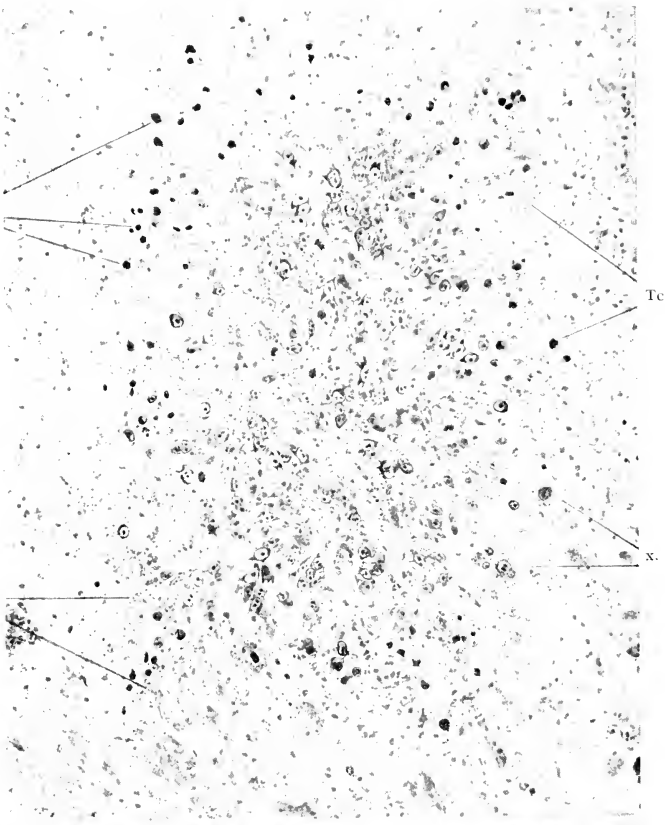
The figures illustrate the result of growth of mixed grafts, consisting of mammalian tumor and adult chick spleen on the allantois.

Plate I.

Fig. 1. Actively growing tumor focus of 4 days growth in the allantois. In the center groups of healthy tumor cells with numerous mitoses. The focus of healthy tumor tissue is surrounded by a zone in which the splenic mesenchymal cells encircle the tumor cells and digest them in closed vacuoles.

Fig. 2. Small area of the peripheral zone around a tumor focus of 4 days growth. Only two tumor cells (*H Tc*) still exhibit a healthy structure. All the others (*Tc'* and *Tc''*) show various changes dependent upon the digestive activity of the splenic mesenchymal syncytium enclosing them in vacuoles.

FIG .1.



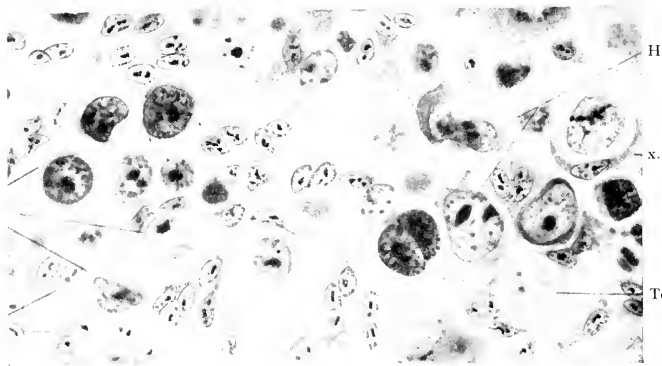


FIG. 2.

PLATE II.

FIG. 3. Small region of the peripheral zone around a tumor focus of 4 days growth, showing a group of healthy tumor cells (*H Tc*) and numerous other tumor cells in various stages of disintegration.

FIG. 4. Small area of the peripheral zone around a tumor focus of 4 days growth, illustrating the disintegration of the tumor cells within the vacuoles, formed by the splenic mesenchymal cells.

FIG. 3.

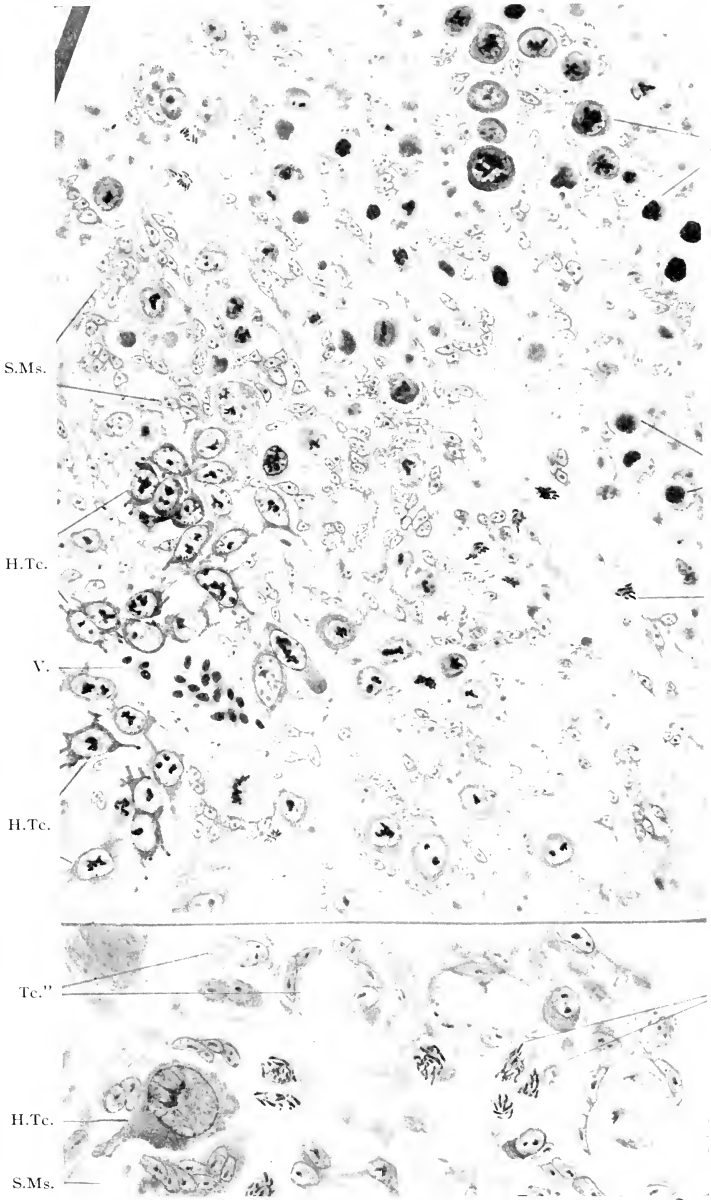


FIG. 4.

CHROMOSOME STUDIES IN TETTIGIDÆ. II. CHROMOSOMES OF PARATETTIX BB AND CC AND THEIR HYBRID BC.[1]

MARY T. HARMAN.

Introduction.

As was stated in a previous paper (Harman '15) the work on the cytological constitution of the germ-cells of *Paratettix* has been undertaken for the purpose of discovering whether or not the microscope will reveal any differences in the germ-cells of very closely related forms which may be correlated with the differences in hereditary characteristics. Three forms have been considered in this paper; two breeding true to type, BB and CC and their hybrid BC (Nabours '14 and '17).

In his breeding work with *Paratettix* Nabours ('17) has considered only the color patterns of the pronota and femora of the jumping legs. He has found fourteen distinct color patterns which behave as a unit and cannot further be broken up. These color patterns he designated as multiple allelomorphs. In most crossings between different pure types the resultant hybrid is readily discernible from either parent. In those hybrids which

[1] Contribution from the Zoölogical Laboratory, Kansas State Agricultural College, No. 27.

are not so readily discerned from the parents, a closer examination shows that the pigment of each parent is distributed in about equal proportions. There is no evidence of dominance nor crossing over, and the proportions of offspring are almost perfectly Mendelian even in small numbers.

It is from these grouse locusts that the material for the present paper was obtained. The author wishes again to thank Dr. R. K. Nabours for this material and express her appreciation to him for furnishing her the material at a time when she could use it and for giving her access to his records from which she has been able to know the pedigrees of her animals.

Observations.

Only the male germ-cells have been studied. Observations have been made upon the spermatogonial divisions, the growth period, synapsis and the maturation divisions. Special consideration has been given to the metaphase plate, polar view, of the spermatogonial divisions, the behavior of the chromatin material during the growth period and the manner of the formation of the first spermatocyte chromosomes.

1. *Spermatogonial Divisions.*

In all three forms the thirteen spermatogonial chromosomes in the metaphase are arranged on the spindle, having the appearance of the spokes on the hub of a wheel (Fig. 1). As may be seen in a metaphase plate, polar view (Figs. 3 to 12), these chromosomes are in pairs and the members of each pair are approximately of equal size and of similar shape. The accessory chromosome is larger than the two smallest pairs and smaller than the other chromosomes. However, many times the difference in size between the chromosomes of the second and third pairs and the X-chromosome is so slight in the spermatogonia that it is difficult to distinguish them. In Fig. 9, the homologous chromosomes of the different pairs are designated by the same number.

In the anaphase the chromosomes take a position more in the same direction of the fibers of the spindle rather than at right angles to it as in the metaphase (Fig. 2). The change from the

perpendicular position in the metaphase to the parallel position in the anaphase would indicate a telomitic fiber attachment. In the late anaphase and early telophase the chromatin material begins to take on a granular appearance before the chromosomes have formed into a spireme (Fig. 13). In fact the cell has completed its division before there has been much reconstruction of the chromatin material.

(*a*) *Chromosomes of CC.*—Figures 3, 4 and 5 are metaphase plates, polar view, of CC spermatogonial cells. Four of the thirteen chromosomes are decidedly larger than the others. The six smallest chromosomes are somewhat narrower at the proximal end than at the distal end. The third pair, marked 3 in these figures, are distinctly pointed at the proximal end, but there is no indication of a bend or hook on this pointed end. The X-chromosome is more ovoid and when lying in certain positions shows a slight constriction in the middle. Figure 3 shows one of the larger chromosomes split at its distal end. This must be a precocious division, as it was not observed in any of the hundreds of other cells examined. Often one of the chromosomes is observed in the center of the spindle (Fig. 4), or the proximal end may be near the center (Fig. 5).

(*b*) *Chromosomes of BB.*—In general the arrangement of the chromosomes of BB are like those of CC (Figs. 9–12). There are the two large pairs, the three smaller pairs and an intermediate pair. The X-chromosome is ovoid with a slight constriction near the middle (Fig. 9). There also occurs the crowding of the chromosomes toward the center of the spindle. The most evident difference between BB and CC is in the third pair, marked 3 in all the figures. Not only are these chromosomes pointed at their proximal ends, but the point is considerably bent to form a definite hook in BB. Sometimes this hook forms an acute angle with the other part of the chromosome (Fig. 12), but more often the bend is rounded (Fig. 10). The hooked chromosome on the left in figure 10 is much smaller than its homologue on the right, but this is due to the fact that a portion of its distal end was cut off as was also the large chromosomes on either side of it. These parts of chromosomes were found in the preceding section.

(*c*) *Chromosomes of BC.*—The hybrid BC (Figs. 6–8) has only one hooked chromosome, while its homologue is merely pointed like in CC. This difference is constant in the many metaphase plates examined. There is some variation in the shape of the chromosomes and the size of the bend of the pointed end. In Fig. 6 the hooked member of the third pair is almost crescent shaped, while in the other cells the hooked chromosome is thick at the distal end, tapers toward the proximal end, and then bends abruptly.

2. *Growth Period.*

Figures 13 to 24 illustrate the changes which take place in the growth period. At the end of the last spermatogonial divisions the chromosomes have a crinkled appearance. Figure 13 shows all thirteen of the chromosomes in this condition. However the X-chromosome is more compact than the others. This crinkled appearance is due to the chromatin becoming less compact and more granular. There is no sign of a nuclear membrane nor any indication of a separation of the nuclear area from the cytoplasm. Soon the chromatin becomes very finely granular and the chromosomes unite end to end (Fig. 14). Shortly after this stage the chromatin has the appearance of a finely granular continuous thread and a light area separates the cytoplasm from the nuclear material (Figs. 15 and 16). The cell begins to increase in size and the chromatin thread becomes contracted into a ball (Fig. 17). There seems to be no definite polarization to the loops of the spireme, but it has more the appearance of a tangled mass. Sometimes it is with difficulty that the individual fibres are distinguished. Now both the nucleus and the entire cell increase greatly in size (Figs. 18–21) and the chromatin material forms into a thicker thread (Fig. 18). Then the chromatin thread becomes looser and the granules are more concentrated, particularly in places throughout the strand (Fig. 19). The portion of chromatin marked X never becomes as granular as the remainder of it. This chromatin is the sex-chromosome and can be identified in all the stages.

Following the opening up of the tangled knot the chromatin thread breaks into pieces (Figs. 20 and 21). Figure 22 shows thirteen pieces, twelve of which are uniting in pairs by an end

to end union. Figures 23 and 24 show successive stages in the formation of the primary spermatocyte chromosomes. As the chromosomes become more compact in appearance the nuclear membrane disappears and there is no definite boundary again between the nucleus and cytoplasm (Fig. 24).

During all these changes there has not been the least appearance of a side by side pairing of the chromosomes, neither has there been any indication of a longitudinal splitting of the chromatin material either as chromosomes or as a chromatin thread. With the breaking up of the chromatin thread always more than the haploid number of chromatin elements may be seen, and in many instances there is evidently the diploid number. This surely justifies the conclusion that at the end of the growth period previous to the formation of the primary spermatocyte chromosomes there appears the diploid number of chromosomes and that the real pairing of the chromosomes does not take place in the spireme stage. It seems, also, very evident that the union of the chromosomes is by telosynapsis. As was previously stated (Harman '15), the dumb-bell shape of the chromosomes is not due to the manner of the division of the chromosomes, but is a result of the way they are formed. This shape is noticed in some of the chromosomes even before the entire chromatin thread is broken up (Fig. 21), and certainly before the first spermatocyte spindle is formed (Fig. 24).

3. *Spermatocyte Divisions.*

There are six dumb-bell shaped bivalent chromosomes and an ovoid univalent chromosome in the primary spermatocyte. These arrange themselves longitudinally on a large spindle (Figs. 25 and 26). The univalent, or X-chromosome, lies near the periphery of the spindle. The other chromosomes are crowded toward the center. Most often they are so closely crowded together that it is difficult to see them all at once in a lateral view of the spindle. Always one of the medium-sized chromosomes lies very near the second-largest chromosome (Figs. 26 and 30).

In the first division all the bivalent chromosomes divide at the constricted part of the dumb-bell (Fig. 27). In other words,

they have divided at the point of union as we have previously seen. As the result of this division the chromosomes of the anaphase are ovoid. The X-chromosome does not divide in this division but passes to one pole much in advance of the others. The first division then is truly a reductional division.

The spindle of the secondary spermatocyte is nearly spherical. Early in the metaphase the chromosomes show a longitudinal split (Figs. 28 and 29). All of the chromosomes divide in this division. It is an equational division in the sense of the dividing of the chromatin material of original chromosomes into halves.

4. *The X-Chromosome.*

The X-chromosome is a persistent portion of chromatin material which can easily be identified throughout the growth period. It never becomes finely granular, nor does it have the woolly appearance of the other chromatin elements. The other chromosomes lose their identity in the coiled thread, but the X-chromosome does not form a part of this coiled knot. With the formation of the primary spermatocyte chromosomes it may be distinguished from the others both by its shape and by its more compact appearance. On the primary spermatocyte spindle it is often found half way to the pole before the other chromosomes have completely divided. It is more difficult to recognize it in the secondary spermatocyte because all the chromosomes here are ovoid in shape, and the differences in size between some of the chromosomes are so much less. It behaves similarly to the other chromosomes here, neither being precocious in its division nor lagging.

Discussion.

Concerning many of the most obvious and essential points of the behavior of the chromatin in the maturation of the sperm, there is no longer any debate. It is agreed that during this period the number of chromosomes is reduced to one half. That this reduction in number is brought about by the union of chromosomes by pairs is also generally accepted. As to how this pairing has taken place has been the subject of much discussion, and upon the answer to this question depends the acceptance or rejection of other theories. It seems to the writer that when we

have accepted the thought that this reduction in number takes place by the union of chromosomes in pairs we have essentially also accepted the theory of the individuality of chromosomes. Then how do these individuals come together and finally separate again?

1. *Synapsis and Heredity.*

One can scarcely follow out the discussion of Janssen on *Batracoseps attenuatus*, and more especially when close attention is given to his drawings, without coming to the conclusion that with this form, at least, the chromosomes unite by parasynapsis and that it is perfectly possible in the twisting of certain chromatin elements to have an exchange of parts of homologous chromosomes, thus forming a convenient mechanism for the "crossing over" of hereditary characteristics if we accept the chromosome hypothesis of heredity. The "chiasmatypie" theory of Janssen ('09) furnishes a very convenient means for explaining some ratios in Mendelian inheritance.

An application of this chiasmatype theory, which should not pass without mention, is the work on *Drosophila*, particularly the work of Morgan and his students ('15). From the behavior of the hereditary characteristics a chromosome map has been constructed in which not only are the determiners of the characteristics located on definite chromosomes, but also the relative distances that these genes are from each other, is given. The position of the genes on the chromosomes is calculated from the percentages of cross-overs. In addition to the vast amount of genetic evidence for this condition in *Drosophila* there is also some cytological evidence.

Metz ('14) in dealing with five different types of chromosome groups of *Drosophila* shows that the chromosomes exhibit a close association in pairs at nearly all times and that before each cell division the pairs become so intimately associated that they may be said actually to conjugate. He further states that the union of the chromosomes is "unquestionably a side-by-side, or *parasynapsis* one." In a later paper (Metz '16) the same author in considering chromosome pairing in about eighty species of Diptera, many of which belong to the genus *Drosophila*, states that the pairing "certainly involves the essential features of a synaptic (parasynaptic) union."

Bridges ('17) confirmed the observations of Metz as to the spermatogonial and oögonial pairing, but deduced the kind of synapsis from his genetic data.

The genetic behavior of *Paratettix* is very different from that of *Drosophila*. In the hundreds of matings of *Paratettix* not a single instance of crossing-over has occurred. In accounting for the hereditary behavior of *Paratettix* without a knowledge of the cytological behavior Morgan has suggested the theory of identical loci. As has been previously shown, at no time during the growth period is there any indication of a double thread, and also there is evidence of an end to end union of the chromosomes to form the tangled thread of the contraction figure. Moreover at the end of the growth period the chromatin material forms the diploid number of chromatin elements. Also these chromatin elements agree in relative sizes to the spermatogonial chromosomes. Undoubtedly these parts unite end to end. In this case there is little chance for crossing-over, as only the ends of homologous chromosomes come in contact with one another. In view then of the cytological behavior, it seems more likely that the series of multiple allelomorphs may be accounted for by the kind of synapsis rather than by the theory of identical loci.

The writer is well aware that there is not perfect agreement as to how synapsis takes place in the Orthoptera, and even within a single family of the Orthoptera. McClung ('14) has recently reviewed the literature on the subject of synapsis in Orthoptera, and Wenrich ('16) has summarized the results. Briefly those who have described or assumed telosynapsis are: Montgomery ('05), *Syrbula;* Stevens ('05), *Stenopalmatus*, ('05) *Blatta*, ('10) *Forficula;* Wassilieff ('07), *Blatta;* Zweiger ('06), *Forficula;* Davis ('08), Acrididæ and Locustidæ; Buchner ('09), *Gryllus*, Œdipoda; Brunelli ('09), *Gryllus*, and ('10) *Tryxalis;* Sutton ('02, '03), *Brachystola;* Baumgartner ('04), *Gryllus*; McClung ('05, '08, '14), various Orthoptera; Nowlin ('08), *Melanoplus;* Pinney ('08), *Phrynotettix;* Robertson ('08), *Syrbula;* Carothers ('13), Acrididæ. Those who have assumed or described parasynapsis are: Gerard ('09), *Stenobothrus;* Morse ('09), Blattidæ; Stevens ('12), *Ceuthophilus;* Robertson ('15), Tettigidæ; Vejodvsky ('11–12), Locustidæ; and Otte ('07), Locusta. In addi-

tion to these Wenrich ('16) gives strong evidence for parasynapsis in *Phrynotettix* and ('17) *Chorthippus* and *Trimerotropis*.

May it not be that within the order Orthoptera, or even within the same family, that the kind of synapsis is different? The behavior in heredity is certainly different. The behavior of *Paratettix* in heredity has already been discussed. Bellamy ('17) has described a similar condition in the genus *Tettigidea*. He found five allelomorphic color patterns without any indication of cross-overs. The cytological constitution of the germ-cells of his material has not been examined. However, Robertson ('17) has described parasynapsis in *Tettigidea parvipennis*. Nabours ('19) has not only found crossing-over in *Apotettix*, but has also found parthenogenesis and crossing-over in those reproducing parthenogenetically. Certainly if we accept the chromosome hypothesis of heredity there must be a difference in the cytological behavior of the germ-cells of these genera. Certainly enough has already been said in this paper to show that the chromosomes pair by telosynapsis in *Paratettix*. With the many cross-overs in *Apotettix*, and the similar behavior in inheritance to *Drosophila*, one would expect the chromosome behavior to be different than in *Paratettix* and more like *Drosophila*. The writer has not yet studied the chromatin behavior in *Apotettix*.

2. *Chromosomes in Hybrids.*

In working with the chromosomes of hybrids Moenkhaus, Morris, Federley, Harrison and Doncaster, Pinney and others have found that despite the fact that the paternal chromosomes are in a foreign cytoplasm they retain their characteristic form, size and number. Moenkhaus ('04) pointed out that when *Fundulus* is crossed with *Menidia* two kinds of chromosomes are present in the fertilized egg and can readily be distinguished in later divisions, furthermore, that these two kinds of chromosomes are like the chromosomes of each parent respectively.

Morris ('14) found two types of chromosomes in the early cleavage stages of the hybrid of *Fundulus heteroclitus* ♀ and *Ctenolabrus adspersus* ♂. These types of chromosomes she identifies as the two types of parental chromosomes.

Pinney ('18) made a number of crosses with teleosts and also

made the reciprocal crosses. She found that some cytoplasm seemed to be more favorable to foreign sperm than others. In some instances a few chromosomes were eliminated, but those that did remain could be identified as to their paternal or maternal origin. Similar results were obtained by Federley ('13) and Harrison and Doncaster ('14) in hybrids of certain moths.

In the above cases cited there is no indication that these hybrids may occur in nature, and these offspring were obtained from animals which are accepted as distinct species. In the case of *Paratettix*, P. BB and P. CC are not yet admitted to be species by taxonomists, and they are known to cross in nature, judging from hybrids obtained in the wild and segregated in the laboratory. The chromosomes of each form are so very similar to those of the other forms in so many ways that it is only with close study that the differences are recognized. Yet, when once recognized it is found to be constant. The chromosomes of the hybrids of *Paratettix* are different from those of the fish mentioned above in that the parental chromosomes are more alike and that the cytoplasm of the egg seems to be perfectly compatible with the chromatin of the foreign sperm. They are different from those of the moths in that there are the same number of chromosomes in both forms and there is a complete synapsis with the homologous pairs of chromosomes.

Conclusions.

1. The third pair of spermatogonial chromosomes of BB are bent at the proximal end so as to form distinct hooks.

2. The third pair of spermatogonial chromosomes of CC are pointed at the proximal end, but there is no bend.

3. The third pair of spermatogonial chromosomes of the hybrid, BC, is composed of one hooked member and one pointed member.

4. During the growth period there is no indication of a parallel condition of the chromatin either in the chromatin thread or in the chromosomes.

5. At the end of the growth period there is evidently the diploid number of chromosomes formed which correspond in relative sizes to the respective spermatogonial chromosomes.

6. Synapsis does not take place in the thread but at the end

of the growth period by an end to end union of the homologous pairs of chromosomes.

7. The chromatin of the sex-chromosomes does not lose its identity during the growth period.

8. The first maturation division is a reductional division, and the second maturation division is equational.

9. The formation of the diploid number of chromosomes at the end of the growth period and the union of their homologous pairs by telosynapsis may explain the absence of any crossing-over in *Paratettix*.

LITERATURE CITED.

Baumgartner, W. J.

'04 Some New Evidence for the Individuality of Chromosomes. BIOL. BULL., Vol. 8.

Bellamy, A. W.

'17 Studies of Inheritance and Evolution in Orthoptera, IV. Multiple Allelomorphism and Inheritance of Color Patterns in Tettigidea. Jour. Gen., Vol. 7.

Bridges, Calvin B.

'17 Non-disjunction as a Proof of the Chromosome Theory of Heredity. Gen., Vol. 1.

Brunelli, G.

'09 La spermatogenesi del Gryllus desertus Pall. (Divisioni spermatogoniali e maturative.) Reale Academia dei Lincei, T. 2, serie 5a.

'10 La spermatogenesi della Tryxalis. Divisioni spermatogoniali. Memarie della Societa Italiana Scienze, T. 16, serie 3a.

Buchner, P.

'09 Das accessorische Chromosom in Spermatogenese und Ovogenese der Orthopteran, Zugleich ein Beitrag zur Kenntnis der Reduktion. Archiv. f. Zellforsh., Bd. 3.

Carothers, E. Eleanor.

'13 The Mendelian Ratio in Relation to Certain Orthopteran Chromosomes. Jour. Morph., Vol. 24.

Davis, H. S.

'08 Spermatogenesis in Acrididæ and Locustidæ. Bull. M. C. Z., Vol. 53.

Federley, H.

'13 Das Verhalten der Chromosomen bei der Spermatogénèse der Schmettalinge, etc. Zeit. Abst. Vererb., Bd. 9.

Gerard, P.

'09 Recherches la spermatogenese Chez Stenobothrus bigutulus (Linn.). Archives de Biologie, T. 24.

Harman, Mary T.

'15 Spermatogenesis in Paratettix. BIOL. BULL., Vol. 29.

Harrison, J. W. H., and Doncaster, L.

'14 On Hybrids between Moths of the Geometrid Subfamily Bistoninæ, with an Account of the Behavior of the Chromosomes in Gametogenesis in *Lycia* (*Biston*) *historia*, *Ithysia* (*Myssia*) *Zonaria* and in their Hybrids. Jour. Gen., Vol. 3.

Janssens, F. A.

'09 La théorie de la chiasmatypie Nouvelle interprétation des cinèses de maturation. La Cellule, T. 25.

McClung, C. E.

'05 The Chromosome Complex of Orthopteran Spermatocytes. BIOL. BULL., Vol. 9.

'08 The Spermatogenesis of *Xiphidium faciatum*. Kansas Univ. Sci. Bull., Vol. 4.

'14 A Comparative Study of Chromosomes in Orthopteran Spermatogenesis. Jour. Morph., Vol. 25.

Metz, Charles W.

'14 Chromosome Studies in Diptera I. A Preliminary Survey of Five Different Types of Chromosome Groups in the Genus *Drosophila*. Jour. Exp. Zool., Vol. 17.

'16 Chromosome Studies on the Diptera II. The Paired Association of Chromosomes in the Diptera and its Significance. Jour. Exp. Zool., Vol. 21.

Moenkhaus, Wm. J.

'04 The Development of the Hybrid between *Fundulus heteroclitus* and *Menidia notata* with Especial Reference to the Behavior of the Maternal and Paternal Chromatin. Am. Jour. Anat., Vol. 3.

Montgomery, T. H., Jr.

'05 The Spermatogenesis of *Syrbula* and *Sycosa*, with General Considerations upon Chromosome Reduction and the Heterochromosomes. Proc. Academy Nat. Sci. Philadelphia, February.

Morgan, T. H. and others.

'15 The Mechanism of Mendelian Heredity. Henry Holt and Co. New York.

Morris, Margaret.

'14 The Behavior of the Chromatin in Hybrids between *Fundulus* and *Ctenolabrus*. Jour. Exp. Zool., Vol. 16.

Morse, Max.

'09 The Nuclear Components of the Sex Cells of Four Species of Cockroaches. Archiv. f. Zellforsch., Bd. 3.

Nabours, Robert K.

'14 Studies of Inheritance and Evolution in Orthoptera I. Jour. Gen., Vol. 3.

'17 Studies of Inheritance and Evolution in Orthoptera II, III. Jour. Gen., Vol. 7.

'19 Parthenogenesis and Crossing-over in the Grouse Locust *Apotettix*. Amer. Nat., Vol. 53.

Otte, H.

'07 Samenreifung und Samenbildung bei Locusta viridissima. Zool. Jahrb. Abt. f. Anat., Bd. 24.

Pinney, Edith.

'08 Organization of the Chromosomes in *Phrynotettix magnus*. Kansas Univ. Sci. Bull., Vol. 4.

'18 A Study of the Relation of the Behavior of the Chromatin to Development and Heredity in Teleost Hybrids. Jour. Morph., Vol. 31.

Robertson, W. Rees Brebner.

'08 The Chromosome Complex of *Syrbula admirabilis*. Kansas Univ. Sci. Bull., Vol. 4.

'15 Inequalities and Deficiencies in Homologous Chromosomes; their Bearing upon Synapsis and the Loss of Unit Characters. Jour. Morph., Vol. 26.

'17 Chromosome Studies IV. A Deficient Supernumerary Accessory Chromosome in a Male of *Tettigidea parvipennis*. Kansas Univ. Sci. Bull., Vol. 10.

Stevens, N. M.

'05 Studies in Spermatogenesis with Especial Reference to the "Accessory Chromosome." Carnegie Inst. Publ. 36.

'10 An Unequal Pair of Heterochromosomes in *Forficula*. Jour. Exp. Zool., Vol. 8.

'12 Supernumerary Chromosomes and Synapsis in *Ceuthophilus*. BIOL. BULL., 22.

Sutton, W. S.

'02 On the Morphology of the Chromosome-group in *Brachystola magna*. BIOL. BULL., Vol. 4.

'08 The Chromosomes in Heredity. BIOL. BULL., Vol. 4.

Vejdovsky, F.

'12 Zum Problem der Vererbungsträger. Böhm. Gesell. wiss., Prag.

Wassilieff, A.

'07 Die Spermatogenese von *Blatta germanica*. Archiv. f. Mikr. Anat., Bd. 70.

Wenrich, D. H.

'16 The Spermatogenesis in *Phyrnotettix magnus*, with Special Reference to Synapsis and the Individuality of the Chromosomes. Bull. Mus. Comp. Zool., Vol. 60.

'17 Synapsis and Chromosome Organization in *Charthippus* (*Stenobothrus*) *curtipennis* and *Trimerotropis suffusa* (Orthoptera). Jour. Morph., Vol. 29.

Zweiger, H.

'06 Die Spermatogenese von *Forficula auricularia* L. Jena. Zeit., Bd. 42.

EXPLANATION OF PLATES.

All figures were made at table level by means of a Zeiss compensating ocular No. 6 and a 1.5 objective with the aid of a camera lucida. Figures 13, 14 and 15 were enlarged three and one half diameters. All others were enlarged two and one half diameters. All were reduced one third.

PLATE I.

FIG. 1. BC, metaphase plate, lateral view, of spermatogonial division.

Fig. 2. BC, late anaphase, spermatogonial division.

FIG. 3. CC, metaphase plate, polar view, spermatogonial division; X is the odd-chromosome, 3, 3 is the third pair of chromosomes, and 2 is one of the chromosomes of the fifth pair showing a precocious split.

FIGS. 4 AND 5. CC, metaphase plates, polar view, spermatogonial divisions; 3, 3 third pair of chromosomes.

FIGS. 6, 7 AND 8. BC, metaphase plates, polar view, spermatogonial divisions; 3, 3^1 third pair of chromosomes, 3^1 is the hooked chromosome of the third pair.

FIG. 9. BB, metaphase plate, polar view, spermatogonial division; the numbers 1, 1–2, 2, etc., to 6, 6 are homologous pairs of chromosomes, X is the odd-chromosome. It will be noted that the members of the third pair are both hooked chromosomes in BB.

FIGS. 10, 11 AND 12. BB, metaphase plates, polar view of spermatogonial divisions, 3, 3 is the third pair of chromosomes.

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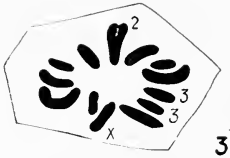

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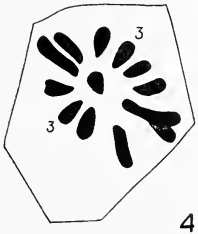

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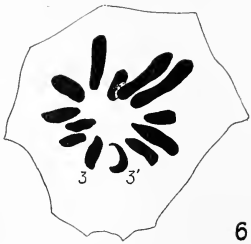

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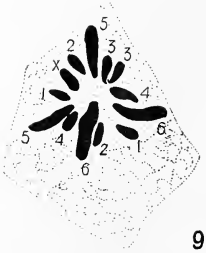

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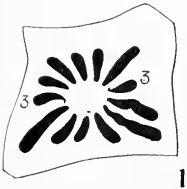

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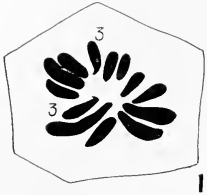

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PLATE II.

FIG. 13. Early telophase of last spermatogonial division. X, the odd-chromosome.

FIG. 14. Beginning of growth period showing the uniting of the chromosomes to form spireme; X is chromatin of the odd-chromosome in all the figures of this plate.

FIG. 15. Early loose chromatin thread.

FIG. 16. Early contraction stage.

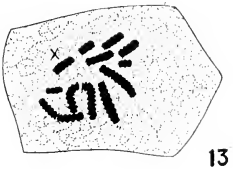
FIG. 17. Later contraction stage and the appearance of the nuclear membrane.

FIG. 18. Large tangled thread.

FIG. 19. Beginning of the concentration of the chromatin in various places throughout the thread.

FIG. 20. Breaking up of chromatin thread into the diploid number of chromatin elements.

FIG. 21. Further breaking of the chromatin thread and the beginning of the formation of the bivalent chromosomes.



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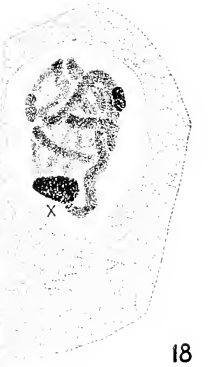

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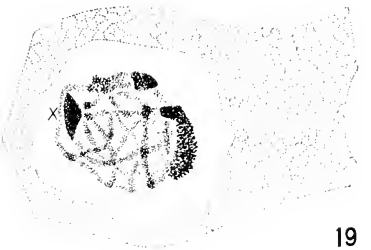

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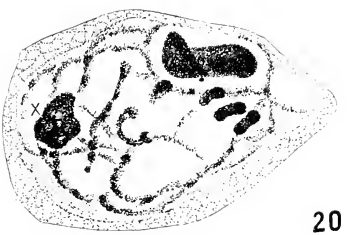

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PLATE III.

FIG. 22. Union of univalent chromatin elements to form bivalent chromosomes. X is the odd-chromosome in all figures of this plate.

FIG. 23. Bivalent chromosomes with the nuclear membrane present.

FIG. 24. Bivalent chromosomes before the formation on the spindle. The two elements of the third chromosome have not yet united.

FIG. 25. First spermatocyte spindle showing the seven chromosomes.

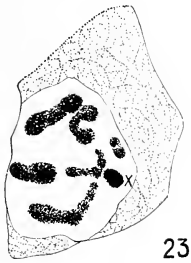
FIG. 26. Metaphase, lateral view of first spermatocyte division.

FIG. 27. Early anaphase of first spermatocyte division. The odd-chromosome approaching one pole in advance of the other chromosomes.

FIG. 28. Metaphase plate, lateral view of second spermatocyte division. Four of the chromosomes show the longitudinal split.

FIG. 29. Metaphase plate, polar view of second spermatocyte division.

FIG. 30. Metaphase plate, polar view of first spermatocyte division.



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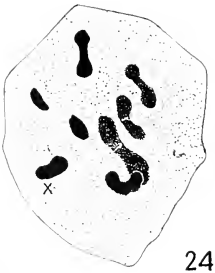

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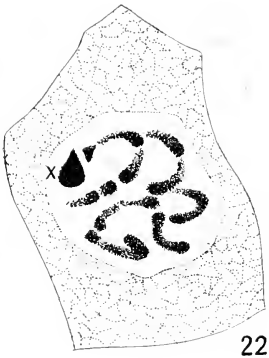

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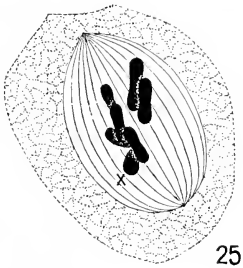

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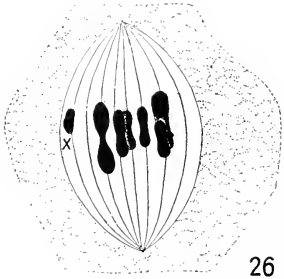

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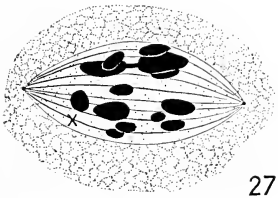

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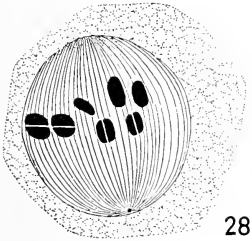

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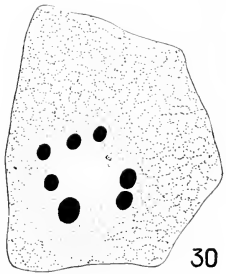

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WHITE-OCELLI—AN EXAMPLE OF A "SLIGHT" MUTANT CHARACTER WITH NORMAL VIABILITY.

CALVIN B. BRIDGES.

Most of the non-lethal mutant characters of *Drosophila melanogaster* may be described as "slight" in the amount of visible change. This is fortunate, for, in general, the mutant genes that cause great changes in an organ, or that cause several distinct changes in different organs, produce races of poorer viability than the wild type of the species. The amount of disturbance to viability is roughly proportional to the extent of the somatic change. In nature these "extreme" mutant types would not be able to survive in competition with the wild type, and in our experimental cultures their presence leads to aberrant ratios that tend to obscure the simple genetic relations. It is possible to reduce the shortage of these relatively inviable mutant characters only by making the conditions of culture exceptionally favorable. Thus, the number of eggs per culture must be restricted by breeding from a single mother; and the amount of food per culture should be carefully regulated. Great progress has been made in improving the quality and methods of using culture media. While it is possible by such improved methods to make the relatively inviable mutants usable in many kinds of experiments, there remain other experiments that require so high a degree of exactness or the simultaneous use of so many mutants that even one poorly viable mutant is inadmissible. As the number of mutant types increases, we are able to drop the use of more and more of the poorly viable mutants and to replace them by new mutants of normal viability. As already implied, these mutants of normal viability are nearly all characterized by somatic changes of slight degree, or by effects, so far as observable, upon a single organ only. Many of these "slight" characters are perfectly definite in demarkation and offer as great precision of classification as do more extreme mutant characters. Another great advantage of slight mutant characters is that they

do not preclude the simultaneous use of other mutants. On the other hand, the use of such an extreme mutant character as white-eyes prevents the effective use in the same experiment of all other eye colors, and likewise the use of vestigial-wing hinders the use of all other wing and venation characters. The most valuable mutants are, then, those of slight but definite somatic change, free from disturbance to viability or masking effects on other mutant characters.

Origin and Description of the White-ocelli Mutant.

One of the best examples of a slight mutant character that fulfills these conditions is that of "white-ocelli," which was found very early in the breeding work with *Drosophila* (June 21, 1912). The ocelli of wild flies are three small simple eyes in a group on the dorsal posterior part of the head. In color they are of a dilute brownish-red, about that of a coffee infusion. Close examination shows that the color of the ocelli themselves is quite light, about that of weak tea, but that there is a crescent-shaped deposit of dense brownish-red pigment about the median side of the two posterior ocelli and against the posterior side of the anterior ocellus. The apparent color of the ocellus is largely due to this outside pigment seen through the transparent lens-like ocellus, and consequently the color changes in intensity with the angle at which the ocellus is viewed.

It was observed that the ocelli of white and also of vermilion-eyed flies were without color or pigment deposit. These ocellar changes are only other effects of the white and vermilion genes. That the color of the ocelli could vary independently of that of the eyes became apparent when it was found that about half of the flies of the stock of the mutant black-body color had white ocelli, while the remainder had the normal coffee-colored ocelli. Some of the white-ocelli flies were bred together and gave a pure-breeding stock of white-ocelli flies (June, 1912). About a year after this, it was found (July, 1913) that the stock of the third-chromosome recessive spineless was also pure for the white-ocellar color. The gene for white-ocelli was thought to be in the third chromosome, since, in crosses in which the spineless was used, the white-ocellar character generally reappeared in association with the spineless, though sometimes not.

The usefulness of the mutant white-ocelli was not appreciated for some years after its discovery. This neglect was due, in large part, to the fact that the regular examination of flies during this period was carried out by aid of a hand lens only, and the separation of the white from the normal ocelli was difficult because of the small size of the region affected. The later work has been done with a binocular microscope, with special attention to proper illumination and magnification; and under these conditions the separation is compiete and entirely accurate, though still somewhat slow.

The Linkage Relations of White-ocelli.

An accurate localization of the gene for white-ocelli was made easy by the use of the two excellent dominant characters dichæte and hairless. The locus of dichæte was near the left end of the map of the third chromosome as then known, being about 13 units to the right of sepia, while the locus of hairless was somewhat to the right of the middle (about 42 units to the right of sepia and 21 to the left of rough). Spineless white-ocelli males were outcrossed to dichæte hairless females; and the F_1 dichæte hairless females were back-crossed by spineless white-ocelli males, with the results shown below:

	0.		1*.		2.		3.		1, 2.		1, 3.		2, 3.		
1918 Sept. 27	D H	ss wo	D ss wo	H	D wo	ss H	D H wo	ss	D ss H	wo	D ss	H wo	D	ss H wo	Total
Total.....	660	528	88	80	116	87	50	53	5	4	2	8	0	1	1,682

*Crossovers in the first region, that between dichæte and spineless, are headed by "1," etc.

The results of this experiment showed that the locus of white-ocelli is to the right of that of hairless by about 6.8 units (a total of 114 crossovers involving region three). The locus of white-ocelli, as thus established, is in what had been the longest unoccupied region of the third chromosome. There had been no workable mutant in the entire distance of about 20 units from ebony (1.5 units to the right of hairless) to rough (21.2 units to

the right of hairless). The ebony rough distance was so great that in constructing a map a correction was required on account of double crossing over. The presence of white-ocelli between ebony and rough gave an opportunity to make a direct test of the amount of double crossing over and consequently of the amount of correction required. The results of the spineless white-ocelli × hairless rough back-cross are given below:

	0.		1.		2.		3.		1, 3.		2, 3.		
1919 Feb. 25	ss wo	H ro	ss H ro	wo ro	ss	H wo ro	ss wo ro	H	ss H	wo ro	ss	H wo ro	Total
Total.....	1,084	1,126	163	195	118	91	226	255	11	9	0	1	3,179

There was 6.6 per cent. of crossing over between hairless and white-ocelli, which agrees with the value 6.8 found in the previous experiment. Likewise, the white-ocelli rough value of 15.8 is in agreement with the expectation from the usual value of 22 for hairless rough. There was only one double crossover in the hairless white-ocelli rough section—a percentage relatively very low. Comparisons show that in the third chromosome (as in the second) the region near the end of the chromosome has a far lower 'coincidence' than has the mid-region. The amount of correction of the observed crossover value for the hairless ebony interval is thus .063 per cent., or somewhat less than one tenth of one unit. Other back-cross tests involving this region have produced a total of 45,971 flies, of which 19.6 per cent. were crossovers. This value is to be corrected to 19.7, which is the map-distance between ebony and rough.

The Viability of the White-ocelli Mutant.

As just seen, the linkage of white-ocelli was worked out through use of the more convenient spineless white-ocelli stock, while the black white-ocelli stock was discarded. The white-ocelli character persisted in the original black stock. No effort was made to eliminate it, nor, on the other hand, to aid in its survival. In May, 1919, a census of the flies of this black stock showed that approximately half were white-ocellars. That is,

the character had persisted in undiminished frequency from June, 1912, to May, 1919, a period that represented fully 175 generations of flies. During this period the black stock had been carried on in mass-cultures. Every two weeks a new culture was started by transferring, without examination or selection, enough flies to insure breeding. In such mass-cultures overcrowding is extreme, and, in spite of the great numbers of parents, not many more offspring succeed in hatching than hatch from successful pair-cultures. The competition grows keener with the age of the culture, since the number of larvæ is continually increasing from eggs laid each day, while the quantity of available food soon begins to diminish and its quality becomes progressively poorer. The mass-culture method of breeding thus exercises a strong and continuous selection against the perpetuation of the weaker or slower hatching individuals or types. In several instances mixed stocks have been started with equal numbers of different mutations, and this stock transferred without selection through several generations. Watch was kept, and in these cases there has been a progressive change in the composition of the stock, rapid at first, until the numbers of one type were quite small, and thereafter slower but in the same direction. Recessive characters of very low viability may persist for many generations as a small proportion of the population. Their existence is maintained by the inter-crossing of the heterozygotes, whereby the mutant gene escapes the adverse selection that the mutant character suffers. Certain of our mutations are so sensitive to larval overcrowding that the ratios in mass-cultures and in pair-cultures seem to belong to different systems of heredity. Thus, the character strap approaches 1 in 4 in pair-cultures, but may approximate 1 in 16 in sister mass-cultures.

The persistence of the white-ocelli character in undiminished proportion through 175 generations of forced competition means that the mutant is under no disadvantage. Such a mutant might easily survive in nature, and one slightly advantageous might ultimately supplant the original type.

The Modification of Eosin Eye Color by White-ocelli.

An examination of the various stocks of eye color mutations showed that there was a strong correlation between the eye color and the ocellar color. The ocelli of white-eyed flies are entirely colorless. The ocelli of vermilion-eyed flies show a slight trace only of color. Indeed, in the case of vermilion, the vermilion gene has a relatively greater effect upon ocellar color than upon eye color. The ocellar color of pink is so faint that pink can not be used in the same experiment with white-ocelli without some confusion in classification. The ocellar color of the dark eye 'sepia' is itself also darker. In the ten multiple allelomorphs of the white series, the ocellar color is proportional to the eye color. This direct effect of eye color genes on ocellar color suggested that the reverse relation might also hold—namely, that the white-ocelli gene might dilute the eye color. A careful examination of the eye color of white-ocelli flies did not show any certain effect. White-ocelli was crossed to vermilion, and the F_2 vermilion white-ocelli flies were not distinguishable from the simple vermilion flies. In the F_2 of the cross between white-ocelli and eosin, a definite modification of the eosin by the white-ocelli gene was observed. In the case of the males, the eye color of the double form was lighter in intensity and less yellow in tone than that of the eosin brothers. In the females, the change was in the same direction but was less marked in degree. Probably 95 per cent. of the diluted males were separable from the simple eosin, while only about 60 per cent. of the females were thus separable. Eosin is known to be especially subject to specific modification,[1] and the effects of the white-ocellar gene give a color intensity and tone and a sexual difference practically identical with those observed in the case of the modifier 'pinkish.' The gene for pinkish was, however, in the second chromosome, and there are other differences between the two cases.

[1] See *Jour. Exp. Zoöl.*, July, 1919.

OBSERVATIONS ON THE SEXUAL CYCLE OF THE GUINEA PIG.

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In the following work, which was undertaken at the suggestion of Professor Leo Loeb, my aim has been to correlate the microscopical and experimental analysis of the sexual cycle which this author had previously made and further studies which he had planned at that time with a careful study of the cyclic changes as far as they are accessible to the naked eye. These observations were made at the breeding establishment of Miss A. E. C. Lathrop, in Granby, Mass., during the years 1913 and 1914.[1]

THE THREE STAGES OF MENSTRUATION AND HEAT.

1. *Præstrum.*—This period continues for one to one and a half days, during which time the external genital organs become congested and swollen and a slight serous secretion is noticeable. This secretion, while in some instances turbid or viscid, is in the majority of cases more or less transparent; while, as before said, this period averages one to one and a half days, in some instances it may continue for two or three days or more. In the last 4 or 5 hours toward the end of this period a maximum in these changes is reached. This is followed by the period of heat. The duration of the proœstrus period is indicated on the following table. Ninety-four (94) animals served for this observation.

	Length of Proœstrus Period.					
	½ Day.	1 Day.	1½ Day	2 Days.	3 Days.	4 Days.
No. of guinea pigs.....	1	39	34	11	8	1

[1] Accidental conditions greatly delayed the publication of this paper. In the meantime there has appeared a study of Stockard and Papanicolaou on the œstrous cycle of the guinea pig. Notwithstanding this long delay in publication, we believe that our observations present new facts of interest and are worth recording.

On the basis of these observations it may be reasonably said that the average length of this period in an animal of normal physical condition is from one to one and a half day, but after puerperium, or in case of weakness or of pathologic conditions, a shortening or prolongation may be brought about.

2. *Œstrus, or Heat.*—The approach of this period is made apparent by excessive menstrual secretion during the last 4 or 5 hours of proœstrum. This continues for 6 or 7 hours of the œstrus period. This is followed by the secretion of a small quantity of moisture which may be just noticeable, or during the last 3 or 4 hours of the œstrus period menstrual secretion may be lacking altogether; still heat is present in this period.

I give the following data as to the length of period of heat:

	Length of Heat Period.				
	5 to 6 Hrs.	7 to 8 Hrs.	9 to 10 Hrs.	11 to 12 Hrs.	13 to 14 Hrs.
No. of guinea pigs.....	6	51	62	30	9

As can be seen from the above figures, from 8 to 11 hours is the average length of the heat period in the healthy animal. I found those guinea pigs in which the periods were longer (12 to 14 hours) or shorter (5 to 6 hours) to have been lacking in vigor or to have shown abnormal physical conditions. In the normal animal I feel justified in claiming, on the average, 9 to 11 hours as the correct length of this period in the normal guinea pig.

3. *Metœstrum.*—This is the period following the œstrus period and might be considered as the period of recovery from the œstrous changes. The length of this period depends almost wholly on the condition of the animal. In the normal animal 2½ to 3 days are required until the normal condition is re-established. From the figures that follow it will be noticed that the length of this period varies from 1½ to 5 days, but my observations have convinced me that where the recovery is shorter or longer than the average period, the animal is not in a normal healthy condition.

	Length of Metœstrus Period.						
	1½ Days.	2 Days.	2½ Days.	3 Days.	3½ Days.	4 Days.	4½ Days.	5 Days.
No. of guinea pigs....	4	9	30	20	15	10	5	4

I may state that at this period a slightly transparent serous or a turbid or viscid secretion can be noticed; but within 2 or 3 days this will disappear, and the swelling of the external organ will cease and normal conditions will again prevail.

Phenomena of First Menstruation or Heat Period in the Guinea Pig.

The first heat or menstruation of the guinea pig differs in some respects from the later periods of heat. The symptoms of proœstrum are of much longer duration. While as stated above, the normal proœstrum lasts about 1 to 1½ days, during the first menstruation a slight secretion and swelling continues for 3 to 8 days; only then appears the period of heat proper. In some instances the secretion and swelling disappear temporarily. Then a few days later it begins again and now develops into the regular heat. As stated, generally the duration of the proœstrum is variable and usually longer in the first menstrual period.

Diœstrus Period of the Guinea Pig.

The diœstrus period is the length of time between two consecutive menstruations or heat periods. Through observation of the condition of heat we determined the length of the sexual cycle in the guinea pig; we used for this purpose a considerably larger material than Loeb[1] and Stockard and Papanicoloau[2] used in their observations. While our average agrees with that of the aforementioned authors, we found a greater range of variation than Stockard and Papanicolaou. From the observations of Loeb it follows that in some exceptional cases the period may be even shorter than that found by us.

	Length of Time Between Heat.							
	14½ Days.	15 Days.	15½ Days.	16 Days.	16½ Days.	17 Days.	17½ Days.	18 Days.
No. of guinea pigs....	1	40	72	62	36	12	8	1

From these figures we may conclude that the average duration of this period was 15 to 16½ days. I found that where the length of time was decidely shorter (14½ days) or longer (17 to

[1] Leo Loeb, Biological Bulletin, 1914, XXVII., 1.

[2] C. R. Stockard and G. Papanicolaou, *Am. Jour. Anat.*, 1917, XXII., 225.

18 days) than the average, the animals were either in a weak or pathological condition, and that menstrual irregularities subsequently followed. My continuous experiments made on this subject covered both winter and summer of 1913, and the winter of 1914 in Granby, Mass., and during all these seasons, through all temperatures, the same condition prevailed.

General Phenomena of Menstruation and Heat and Pregnancy.

Physical Phenomena of Menstrual Period.

One of the foremost indications of the approach of the period of menstruation or heat is the physical change noticed in the animal. The muscles and joints become tender and relaxed, and by this change it can be determined, whether the animal is normal and healthy. During this period nervousness and a certain mental depression are noticeable; and when the period is over the symptoms most apparent indicate fatigue or exhaustion. However, by the end of the second or third day this fatigue has entirely disappeared and within 1½ to 2 days after cessation of heat a normal animal has regained its full vitality.

Relation between Menstruation and Falling Out of Hair.

I have found, particularly in the guinea pig, rat and rabbit an additional visible sign of heat, viz., the falling out of the hair in unusual quantities during the period of menstruation; as a result of this happening, the external appearance of the animal becomes somewhat glossy. We can determine this by comparing the ease with which the hair can be made to fall off by rubbing the skin in the menstruating animal, on the one hand, and in the infantile, pregnant, amœstrous female or male, on the other hand. In exceptional cases a slight menstrual secretion may develop during pregnancy; in this case the falling out of the hair is slight in accordance with the slight degree of menstrual activity.

Specific Odor of Females during the Period of Heat.

Many (but not all) males can evidently clearly distinguish between females which are in the period of heat on the one hand and those in which there is merely menstrual secretion and in

which both are lacking on the other hand. Therefore the male attempts copulation only in the former case. When a number of animals of both sexes are together and among them is a female in the heat period, the males run around and search for that particular female, ultimately finding it.

I am convinced from this searching of the male for the female in heat that he is led to do so by a special odor characteristic of the female, when it reaches the heat period. This peculiarity is common to the mouse, the rat and the rabbit.

Influence of Food upon the Character of Heat and on the Time when Sexual Maturity is Reached.

The vigor of the heat in guinea pigs depends upon the nourishment which the animals have received. Guinea pigs which had been poorly fed develop weak heat. This applies to the duration of the heat as well as to the vigor of the symptoms. The length of the diœstral period does on the other hand not seem to be affected by the state of nourishment.

I made observations on the influence of food on the time when sexual maturity is reached.

I. On especially prepared rich food the first heat was observed 45 to 60 days after birth.

II. On usual food 55 to 70 days after birth.

III. On food of poor quality 75 to 100 days after birth.

The usual food consisted of hay (or grass), carrots (or beets) and oats. In the rich diet corn cake was added, while the poor diet consisted merely of hay (or green grass) and oats. When the quality of the food was above the ordinary, its effect on the size of the guinea pig as well as their physical condition in other respects could be seen. They were of the average condition, when ordinary food was given at regular intervals, and the poorly fed animals were poorly developed physically. The food is therefore of the greatest importance as far as the time of sexual maturity and the character of the heat are concerned.

Influence of Climate on the Character of the Heat and the Length of Sexual Cycle in the Guinea Pig.

The extreme temperature of the summer months (a temperature of over 90° F. in Granby, Mass.) has a weakening effect on

the guinea pigs. And the heat is therefore not so vigorous as it would be under other climatic conditions. The length of the dicestrous period on the other hand seemed to be the same during the warm and cold season of the year.

Does Contact with the Male Influence the Time of Appearance of the First Heat?

If young female guinea pigs are kept in a cage with males and other guinea pigs of the same descent are kept separated from males, the first heat appears in both lots at the same age. This shows that psychical factors such as the stimulation of the male does not influence those conditions which lead to the first heat.

The Copulation-reflex of the Heat Period and the Diagnosis of the Heat Energy.

This reflex can be elicited by the male guinea pig seeking copulation, when it touches or gently massages with his forefeet the lumbar portion of the spinal column of the female which is in heat. The female will then lift up the tail and the vagina will be opened in preparation of the copulation. The same reflex can be called forth if an observer touches with his finger the same part of the body of the female guinea pig. The readiness and vigor with which the reflex occurs can serve as a measure of the energy or vigor of the heat.

Menstruation during Pregnancy.

I very carefully watched twenty guinea pigs during their state of pregnancy for a sign of menstruation and found in ten no evidence of menstrual secretion, but in the remaining ten there was some secretion present; not all of those however showed signs of heat. The menstrual period is not definite during pregnancy. Statistically my results were as follows:

Menstruation was noticed in 3 guinea pigs 27 days after copulation
" " " " 3 " " 30 " " "
" " " " 2 " " 40 " " "
2 " " had menstrual secretion twice, the first time 15 days and the second time 30 days after copulation.

While in these cases the swelling of the external genitalia and the secretions are manifest, they are slight. The secreted material

is mostly transparent, but sometimes turbid. It continued for 3 to 8 days in my observation; then the external genitalia returned to a normal condition.

Some authors suppose that the diagnosis of the menstrual secretion is very difficult in the guinea pig; however careful observation has shown me that the diagnosis can be readily made and hardly misinterpreted.

Diagnosis of Pregnancy about 14 to 15 Days after Copulation.

About 14 or 15 days after copulation we can correctly determine the success or failure of the impregnation. If at that time the external genitalia are in a normal condition we may almost always assume a successful pregnancy. If, on the other hand, there should be within that time a noticeable swelling of the external genitalia, accompanied by secretion, this may in all cases be taken as an indication of failure. As we have stated above, the same phenomenon may be present, perhaps in a milder form, at this period of pregnancy, but this is a very rare occurrence; and as a general rule in the case of failure (early abortion) the secretion and swelling is much more marked.

The Sexual Cycle in Cases of Early Abortion.

In cases of early abortion I found the period of heat in some cases somewhat accelerated and more irregular than in normal animals.

DIOESTRUM.

	Length of Heat Following Copulation.				
	12½ Days.	13½ Days.	14½ Days.	15½ Days.	16 Days.
No. of guinea pigs examined	1	2	2	3	5

As shown in the table, the heat period may appear as early as 12½ or 13½ days after copulation. In control cases the shortest period observed was 14½ days and this occurred only once; and, as I stated above, I believe that in normal cases 15 days represents the minimum and that the shorter periods are usually found in cases of abortion.

The Length of the Period of Gestation in the Guinea Pig.

On the basis of our observations we can give the following statistical data concerning the length of the period of pregnancy in the guinea pig.

	Length of Gestation Period.							
	61 Days.	62 Days.	64 Days.	66 Days.	67½ Days.	68 Days.	69 Days.	71 Days.
No. of pregnant guinea pigs	2	2	4	4	8	15	5	1

The shortest period was 61 days; but those young animals which are born 61, 62 and 64 days following copulation show a certain lack of development; they are really born prematurely and in the majority of cases they soon die, although they may be able to survive. Those born 66 days after copulation usually lived and continued to grow, but those born from 67 to 69 days following copulation show the maximal development after birth; these have the best chance to develop into absolutely healthy, perfect animals. We may therefore conclude that 68 days is the regular period of gestation in the guinea pig.

Separation of the Symphysis Pubis in the Guinea Pig at the End of Pregnancy, at the Time of Birth and During the Period Directly Following Birth.

Through examination with the finger we discern in the guinea pig a gradual separation of the symphysis pubis which begins about 61 to 63 days after copulation and increases as the time of labor approaches to a width of 7 to 8 millimeters or more; but within one or one and a half days after birth the symphysis pubis assumes again its normal condition. The following table shows the time when the separation becomes apparent:

	Beginning Separation of Symphysis Pubis.		
	61 Days, Following Copulation.	62 Days, Following Copulation.	63 Days, Following Copulation.
No. of pregnant guinea pigs.	5	5	4

The length of time from the beginning of the separation of the symphysis to the onset of labor is shown on the following table:

	Duration of Period of Separation of Symphysis Pubis Prior to Birth.		
	4½ Days.	5 Days.	7 Days.
No. of pregnant guinea pigs.....	1	6	7

The following table shows the length of time necessary for the symphysis pubis to return to its normal condition following labor:

	Time when the Symphysis Pubis has again Become Normal Following Labor.			
	½ Day.	1 Day.	1½ Days.	2½ Days.
No. of guinea pigs.....	4	8	5	1

From these data we may conclude that the separation of the symphysis pubis begins from 5 to 7 days previous to birth, or from the 61st to the 63d day after copulation. Under ordinary conditions birth should not take place before the 5th day following this separation. The length of the period of gestation should therefore usually be 68 days.

Preparation for Labor.

In the guinea pig the separation of the symphysis pubis serves as a preparation for parturition, inasmuch as the orifice of the normal pelvis is too narrow to permit birth to take place. Moreover we find at the same time the same remarkable softness and elasticity of the muscles and joints as during the ordinary menstrual period. This same effect (emanating from the ovary or pituitary gland) I have also noticed in the rat and in the rabbit and I presume it is the same in all mammals.

The Time of the First Heat in the Puerperium.

The time of the beginning of heat in the period directly following labor is somewhat variable. In 20 guinea pigs in which parturition set in at the normal time (67 to 69 days after copulation) I found the following figures:

	Beginning Puerperal Heat After Parturition.				
	Soon After	3 Hours After.	5 Hours After.	7 Hours After.	No Heat.
No. of examined guinea pigs	7	7	3	1	2

As our table shows, in 2 out of 20 guinea pigs no heat at all was observed at this period. There was evidence pointing to the conclusion that the heat is less vigorous at this stage than in the normal menstrual period. On the other hand the heat seems perhaps to be of a somewhat longer duration at this period as shown in the following table:

	Duration of Puerperal Heat.		
	7 to 9 Hrs.	10 to 11 Hrs.	12 to 13 Hrs.
No. of guinea pigs	8	7	7

We find therefore that in about one third of the guinea pigs at this period the average duration of heat was 12 to 13 hours, while in normal animals the heat continues for that length of time only in a small minority of cases. Whether or not heat occurs in the period directly following parturition depends upon the duration of the period of gestation as shown in the following table:

	Duration of Pregnancy.							
	61 Days.	62 Days.	64 Days	66 Days.	67½ Days.	68 Days.	69 Days.	71 Days.
No. of observed guinea pigs.	2	2	4	4	8	15	5	1
Puerperal heat	−2	−2	−4	−1/+3	−1/+7	−2/+13	+5	+1

\+ coming in heat; − not in heat.

This table shows that heat does not occur in the period directly following parturition in cases in which the duration of pregnancy was less than 66 or 67 days. In cases in which the duration of pregnancy was less, birth occurred prematurely and the phenomena of the puerperium had not yet fully developed at the time of the completion of labor. If in cases of parturition occurring 61 or 62 days after copulation the first heat following parturition would take place 6 or 8 days later, the first heat would correspond to a typical puerperal heat, provided the duration of pregnancy would have been 68 or 69 instead of 61 days.

On the Duration of the Sexual Cycle following Parturition.

The time of the second menstruation in the period following parturition is rather variable as shown in the following table:

	Development of Second Menstruation Following Parturition.									
	15½ Days.	16 Days.	16½ Days.	17 Days.	17½ Days.	18 Days.	21 Days.	22 Days.	23 Days.	Total.
No. of guinea pigs.....	2	1	4	5	2	3	1	1	1	20

Among 20 guinea pigs in the period following the puerperium I found complete menstruation and heat in 15 cases, while in 5 animals I found only menstrual secretion without heat. The majority of these animals suckled their young and among those the deviation from normal was somewhat less; in these cases the duration was mostly 15½ to 18 days.

Effect of Lactation and Non-Lactation on the Periodicity of the Sexual Cycle after Parturition.

In cases in which the young were suckled the next period of heat took place approximately at the normal time and the conditions continued from then on in a normal manner. It was different in cases in which bodily weakness of the mother prevented the suckling of the young. In the latter cases the return of menstruation was delayed so that it occurred as late as 21 to 23 days after parturition. In the normal suckling animal the sexual periodicity following labor is therefore similar to that of the normal animal, although certain minor variations depending on the vitality of the animal occur even in such cases. The second or third menstruations occur usually after the regular interval of 15 to 16 days even in the weak animal. In the latter, however, we may at these times instead of heat merely find menstrual secretion.

The Effect of Suckling on the Mother and the Young.

Marshall[1] expresses the opinion that in the young separated prematurely from the mother growth will continue. My own observations lead me to a different conclusion. I have found

[1] F. H. A. Marshall, "The Physiology of Reproduction." London, 1910.

that if a mother suckled three young, the growth of the latter was very slow, or death resulted; in addition the weakening effect on the mother was quite noticeable; it sometimes caused her death, and in most cases the death of the young occurred. If the young died, the mother soon regained her health and strength. The result will therefore be disappointing either as far as the condition of the mother or of the young is concerned. It has been my experience that when the young were taken away from the mother within 15 or 20 days after birth and the ordinary vegetable and oat diet was given them, development was very slow and in most cases death occurred. In any case the young to attain healthy growth should not be taken from the mother until more than 25 to 30 days of age. Under natural conditions they cease suckling after 35 to 40 days, when they seek their own food. I am therefore of the opinion that a litter of three very seldom shows normal development when suckled by one mother.

When I combined one mother with two and another with three young, each mother suckled on the average 2½ young. In such cases I obtained good results. While two or three young is the average size of a litter, I have known the number to vary from one to six; the latter number, however, only occurred once in my observation. When but one young is born, it is usually well developed and vigorous; but to be successfully raised it must be suckled for the usual period or at least for a time approaching it. There is another symptom in addition to the bodily weakness, which the mother shows as the result of too intensive suckling: it consists in the changing of the color of the pupil of the eye which becomes pale or white. It may be associated with general exhaustion.

Under what Condition is Copulation followed by Pregnancy?

As we stated above the normal duration of heat is about 10 hours. If copulation took place within 3 or 4 hours after the beginning of heat, pregnancy followed only rarely. If the copulation took place at a later period of the heat, better results were obtained. According to my observations pregnancy occurred in about 73 per cent. to 80 per cent. of the cases. In such cases in which copulation was not followed by pregnancy a second or

third attempt during the next two periods of heat were mostly successful.

Sobotta[1] states that the guinea pig in captivity can become pregnant more frequently in summer than in winter. My observations do not bear out this statement. In my experience the pregnancies resulted almost as often in the cold as in the hot season. The winter climate is better adapted for the guinea pig in captivity than the hot summer weather when the temperature is liable to exceed 90° F. Under those conditions the animal suffers and becomes poorly developed as the result of undernourishment. On the other hand, it is almost always in good condition in winter. Therefore the cold season agrees better with guinea pigs than the hot season under conditions of captivity. In exceptional cases the guinea pig may become in winter time too fat to be favorable for impregnation; and this may even happen in summer time.

Relation between Growth, the First Period of Heat and the Period of Sexual Maturity in the Guinea Pig.

In my observations the weight of the guinea pig is ordinarily three ounces soon after birth. However, the weight of the young is in accordance with the age of the mother and also the number of the litter. As a rule the young born from a young mother are small in size and in litter. On the other hand, when the mother is older than 5 months, the weight of the young is mostly larger. For instance, in one litter the young weighed 6 or 7 ounces soon after birth.

Minot[2] has concluded that the guinea pig is slow in growing and that it attains its full size one year after birth. My observations are as follows: The young guinea pig at the time of the first heat was about two months old; about 7 to 10 days before the beginning of heat, the growth almost stopped or at least progressed very slowly. At the time of the first heat period the average weight was 11 to 13 ounces. After the first heat period had passed it began to grow very rapidly. About two or three

[1] Sobotta, *Anat. Hefte*, 1906, XXXII.

[2] Minot, C. S., "Growth and Senescence," *Journ. Physiol.*, 1891, XII., 97. "Problems of Age, Growth and Death," *Popular Science Monthly*, 1907, LXX., 481, and LXXI., 97 and succeeding numbers.

months later, at the age of 4 to 5 months, it attained its full weight, about 20 to 28 ounces, but one exceptional animal weighed 34 ounces in a non-pregnant condition. However, if we feed a special diet of nutrient material and the animal is otherwise well taken care of, then the growth progresses rapidly and the heat begins earlier than usual. The guinea pig which weighed 34 ounces was fed on the usual farm or laboratory food. Therefore we must remember that the growth of the young depends considerably upon the character of the food.

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SELECTIVE FERTILIZATION IN POLLEN MIXTURES.

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Equality in fertilizing power of gametes dissimilar in the hereditary factors they carry is a corollary of Mendelism. It has been thought that certain exceptions to this general rule may exist. Differential fecundating ability has been suggested from time to time as a possible interpretation of otherwise unaccountable results. The earlier uses of this hypothesis of inequality in sperm efficiency, such as Castle's (1903) theory of sex determination, Cuenot's (1908) conception of the reason for the non-appearance of homozygous yellow mice, have since been found to be unnecessary. The interpretation of linkage phenomena according to the reduplication hypothesis, while essentially different in application, holds somewhat the same implication as selective fertilization, that is, differential operative power of cells of unlike germinal construction.

As the yellow mouse problem was finally resolved to a selective elimination of zygotes so many puzzles have had considerable light thrown on them by a more complete understanding of the factor relations. Many instances of the non-appearance of certain types are known to be the result of the action of lethal factors. Good illustrations of this are found in Drosophila (Muller, 1918). Other cases of elimination immediately after fertilization or early in development are known in maize.

Sometimes abortion of a part of the gametes before fertilization takes place and it is assumed that the elimination is selective. Belling (1914) has shown that in Stizolobium 50 per cent. of the pollen grains and ovules regularly abort in certain types. The aberrant results from the Œnotheras are now generally considered largely, if not wholly, to be due to differential destruction of both

gametes and zygotes. Davis (1915–'17) has called attention to the great amount of pollen and ovule abortion in these plants and to the low germination of the seeds which are produced and to the results which are obtained when a more complete germination is secured.

It is not always easy to distinguish between differential destruction of zygotes immediately after fertilization and selective fertilization. It is still more difficult in the case of abortion of germ cells. In fact it is only a matter of degree between selective elimination of gametes and selective fertilizing ability. From germ cells which are unable to complete development on account of the particular inheritances they carry, to gametes which appear normal but are unable to function perfectly under any circumstances, it is only a step. However, no instance of the latter condition is positively known and the cases of gametic abortion clearly due to the factors which the gametophytes carry are rare.

Although results still remain to be cleared up which indicate a selective action of some kind (Kempton, 1919) the general conclusion holds, which is, that the pollen, although gametophytic itself, has the function of the sporophyte which produces it. This is supported by the well-known cases where pollen color and shape are all of the type of the maternal parent irrespective of the factors for pollen characters which they carry. Many instances are known where gametes, containing lethal factors which stop development immediately after fertilization, are able to function fully as well as others permitting normal growth. East and Park (1917) have shown that compatibility in Nicotiana is alike for all the gametes of any one individual. Different pollen grains may carry factors which will determine sterility or fertility of the future zygotes but all function alike in fertilization according to the sporophyte from which they come. Following up these results East (1919) shows that in the frequency distributions of pollen tube lengths there is no significant difference between pollen from plants greatly unlike in the degree of heterozogosity. In other words gametes carrying markedly diverse germinal complexes are no more variable than those all of like constitution in ability to send pollen tubes down the styles

and to accomplish fertilization. East considers that chance segregation in the germ cells and random mating of these germ cells is a fundamental genetic hypothesis applicable to plants and animals alike. With this conclusion the writer is in accord, with the reservation that the evidence from gametic abortion should not be put aside as belonging to a different category.

The data to be presented here bear upon another phase of the problem. The above conclusion, it should be clearly kept in mind, applies only to gametes produced by one or more individuals of the same type. That is, the gametes may be unlike in the factors they carry, but if they come from the same or similar individuals they are potentially equal in ability to fertilize. But what is the result when germ cells from two individuals of different type are presented at the same time in excess so that not all can fulfill their function? Will fertilization take place at random or not? This is the problem to be considered here. In the one case doses of different kinds of medicine come in the same capsules. In the other the capsules as well as their contents may differ. Anyone accustomed to swallowing a particular kind of capsule made of a familiar substance and all of the same size and shape can take any kind of medicine with equal readiness irrespective of the result which will ensue when the materials within the containers begin to operate. Applying this crude metaphor to plants and animals, in the one case, the germ cells, however they differ in factorial composition, come in the same cytoplasmic envelope; in the other, the cytoplasm as well as the genes may differ.

It has already been shown that, in particular cases, there is a selective action when pollen from different plants is applied to a stigma at the same time. East (1919) has demonstrated that in a mixture of compatible and incompatible pollen placed on the stigmas of self-sterile Nicotianas the compatible pollen alone functions. Two experiments were devised to test this. Small numbers of pollen grains were counted out under a microscope and placed on stigmas known to be receptive to that kind of pollen. The stigmas were then covered with a large amount of incompatible pollen. The former only was able to fertilize as shown by the results from eight such mixed pollinations which

in no case produced a greater number of seeds than the number of compatible pollen grains applied. In the other experiment a constant white-flowered self-sterile plant was pollinated with its own pollen. Several hours later pollen of a red-flowered plant was applied. Abundant seed was secured and when grown red-flowered cross-fertilized plants only were found. In both these cases there was complete selective fertilization in favor of the pollen from dissimilar plants.

According to the results to be reported here a directly reversed effect is obtained from mixed pollinations in maize. In many trials the plant's own pollen has been more efficient in accomplishing fertilization than that from other individuals which differ only in minor features. This same pollen which is less effective when in competition with the plant's own kind of pollen is fully able to function when not applied in mixtures. The results are remarkable in view of the notable advantages which cross-fertilization gives to the immediately resulting seeds and the plants grown from them. The material used consisted largely of self-fertilized strains which had been brought to uniformity and constancy and were considerably reduced in size and vigor. In this material crossing is known to increase the weight of seed within the same inflorescences as much as 50 per cent. in some cases. This greater amount of material is laid down both in the embryo and endosperm, and is apparent in the greater size of the seeds which show a higher specific gravity together with more rapid maturation indicated by their lower water content at the close of the growing period. A large series of mixed pollinations show the ability of the cross-fertilized seeds to germinate better by an average of 16 per cent. The resulting plants start to grow sooner, develop faster, mature in a shorter time and at the end far surpass their self-pollinated sibs. The amount of heterosis shown by maize is possibly greater than that displayed by any other plants in intra-specific crosses. Production of grain has been advanced on an average of 180 per cent., height of plant 27, length of pistillate spike 29, number of rows of spikelets 5 and number of nodes 6 per cent. in a study of a large number of crosses between inbred strains (Jones, 1918).

In spite of these great immediate advantages to be secured the

plants manifest a decided preference for their own kind of pollen. This is a result which would have surprised students of flower pollination in Darwin's time but which, as I shall attempt to point out later, is in agreement with other results from biological investigation.

Methods of Carrying on the Experiments.

In an investigation in which it was desired to compare the chemical composition of seeds of maize having different genetic constitution but produced under as nearly identical conditions as possible advantage was taken of endosperm characters to enable proper classification of seeds produced in the same inflorescences. For example, two kinds of pollen carrying yellow endosperm color and white endosperm color were mixed together and applied to a plant which normally produces uncolored seeds. The resulting yellow and white seeds were distributed at random on the pistillate spikes and were as nearly comparable in respect to external and nutritional factors as it is possible to obtain. It was found that such pollen mixtures could be applied to both strains furnishing the pollen and the two kinds of seeds easily distinguished. On the white-seeded strain the seeds resulting from the plant's own pollen were white and the cross-fertilized seeds yellow. On the yellow-seeded plants the self-pollinated endosperms were dark yellow while the crossed seeds were distinctly lighter in color, in most cases they had a white cap, and were as a rule readily separable.

Attention is directed to the fact that the material used for these mixed pollinations consisted largely of inbred strains which had been reduced to uniformity and constancy so that the genetical differences between the two kinds of seeds sharply differentiated them, much more than in ordinary cross-pollinated varieties of this plant in which the yellow color is usually variable due to more than one factor for this color and various modifying conditions such as the consistency of the endosperm in respect to corneous and floury starch.

A number of pairs of plants were treated in this way by mixing their pollen and applying to both types. After harvesting it was realized that here was an excellent method of determining

whether or not any selective action was shown by the plant's own pollen as compared to that from a plant of somewhat different type. If one member of the pair of plants which furnished the pollen for the mixture is designated A and the other B, the two kinds of seeds grown on A plants are A × A, self-fertilized, and A × B, cross-fertilized; on the B plants B × A cross-fertilized and B × B self-fertilized. Since the same pollen mixture is applied to both, the ratio of the seeds resulting from A pollen to the seeds resulting from B pollen, on A plants, should be the same as the ratio of the seeds resulting from A and from B pollen on B plants. In other words, the numbers form a proportion which, irrespective of the relative amounts of A and B pollen in the mixture, should be a perfect proportion within the limits of random sampling if fertilization takes place at random. The end terms of the proportion comprise the self-pollinated seeds and the middle terms the reciprocally cross-pollinated seeds. If a true proportion is obtained the products of the end terms should naturally equal the products of the middle terms. If they do not the deviation is either in an excess of cross-fertilized or of self-fertilized seeds indicating a selective action in one or the other direction.

The advantages of this method of attacking the problem are readily apparent. It is practically impossible to make up a mixture of large amounts of pollen in which the proportion of each is known. Either measuring or weighing the pollen is out of the question because maize pollen takes up moisture from the air rapidly and when any quantity is brought together it becomes aggregated into a flocculent mass. Moreover this pollen soon loses its viability so that even in case equal numbers of pollen grains could be had there would be no proof that there were equal numbers of functional pollen grains in the mixture.

The method of reciprocal application and arranging the results in the form of a proportion automatically overcomes all these difficulties and the experiment is as simple as could be devised. An attempt was made to have as nearly equal quantities of pollen as possible by measuring out the two kinds roughly. But in many cases the results showed that one kind of pollen was far more effective than the other. This, however, does not destroy

the value of the figures as it is the relative efficiency of each type of pollen, when it is applied to its own and to foreign stigmas, that is being investigated.

In the preliminary experiments there were 18 pairs of plants which produced seed as the result of the application of mixed pollen. When these were counted and the deviations of the proportions found from the closest perfect proportions were obtained there were 12 mixtures which showed a deviation in favor of the self-fertilized seeds and 6 in the opposite direction. The results as a whole showed a tendency to favor the plant's own pollen. This was somewhat unexpected so that it was considered worth while to investigate the matter more fully.

The inbred material was so reduced in growth that the number of seeds produced on one plant was not large enough to give the results much weight. It was therefore decided to pollinate a number of plants of two different self-fertilized strains with the same mixture. Most of the strains used had been self-pollinated for six generations or more, some as many as ten, so that the plants within one strain were practically identical in hereditary constitution. Pollen was collected from about the same number of plants as the pollen was applied to. The two lots of pollen were put together in a paper sack and thoroughly mixed by shaking. This mixture was then applied to plants of the two strains which supplied the pollen. It was desired to have from ten to fifteen plants in each of the paired strains so as to give from 1,000 to 2,000 seeds in each of the two parts of the proportions but the flowers were not always ready at the right time and some pollinations were failures for a variety of reasons so that not as large numbers as desired were secured in every case.

Every effort was made to prevent the entrance of undesired pollen. The technique has been described and the amount of experimental error due to contamination to be expected in artificial pollination of maize has been considered previously (East and Hayes, 1911). In the course of these experiments the effects of extraneous pollen were seen in very small numbers compared to the total number. This source of error could be detected in the seeds when colors or other characters differing from either of the strains used were brought in by the undesired

pollen. In a total of 63,000 seeds produced in these experiments only about 30 illegitimate seeds were observed. A larger number of contaminations, however, would have the same appearance as the legitimate seeds and so could not be detected but giving a reasonable allowance to this source of unreliability the results could not be appreciably altered. Moreover the error of this kind is never all in one direction so that in the main it can be disregarded.

The accuracy with which the seeds have been classified deserves particular attention. Strains were selected to be used which gave sharp differences between self-fertilized and cross-fertilized seeds and in most cases separation was made very satisfactorily. In a few mixtures there was some doubt and in two experiments the seeds on the yellow endosperm plants could not be distinguished. In these two cases the seeds were planted and classification was made with the mature plants. Also in all the other mixtures involving yellow and white endosperms a sample was taken, after all the seeds of one class were mixed together, and grown to determine the per cent. of error in separating the seeds. Since the self-fertilized seeds give small inbred plants, pure for yellow or white color, while the cross-fertilized seeds produce large vigorous hybrids segregating into yellow and white seeds, classification of the mature plants can be made without the least doubt. However, it should be noted that the better germination and greater vigor give the advantage to the cross-fertilized classes in every case if there is any difference. About 120 plants in each lot were grown and the per cent. of error obtained was used to calculate the total amount of error if all the seeds had been grown. Since the numbers of seed ran up into the thousands it was impossible to grow all of them. The figures showing the per cent. of errors found are arranged in Table III. Only in one case is the number of wrongly classified individuals above 3 per cent. In 25 out of 44 lots no faulty separations were discovered. In the remaining cases the misplaced seeds tend to balance each other so that this source of doubt can be largely removed. Calculating the figures without regard to the error of classification gives practically the same results as when this is taken into consideration.

Different characters were used in other mixed pollinations which permitted even more positive classification than the single character difference of yellow and white endosperms. Plants with yellow sweet seeds were paired with white starchy plants. Each strain brought in a dominant character. White starchy plants of the pop or *Zea mays everta* type were used because the clear corneous endosperm differentiates very clearly between yellow and white. In the reciprocal cross the smooth opaque seeds show up plainly contrasted with the wrinkled translucent seeds. Similarly purple sweet and white starchy types were paired. In a few cases the purple crossed seeds were not as distinct as could be desired, but the error is certainly small. These latter mixtures were made the past season and no plants have been grown to test the reliability of their separation but the writer is confident that the number of wrongly classified seeds, if any, is not sufficient to alter the results appreciably.

In many inflorescences a few seeds were found which had failed to reach a stage of development so that they could be classified. Seeds at the tips of the spikes and where the seeds were closely crowded were abortive. This introduces another source of unreliability, that of selective elimination of zygotes. Since crossing gives to seeds of maize an enormous advantage in development, it can confidently be expected that as a rule more of the self-pollinated seeds will be found among the abortions than cross-pollinated. What we are attempting to study is the relative fertilizing efficiency of different types of pollen grains. But one can only arrive at this by counting the zygotes sometime after fertilization has taken place. In the meantime a differential destruction of zygotes may have taken place. This effect must be considered in any organisms employed but because of the short time which elapses between fertilization and the maturation of the seeds, and from the fact that they develop in an exceedingly favorable and uniform environment, maize is the very best material the writer can think of in which this problem can be attacked, especially when the numbers which can be obtained are taken into consideration. All animals and those plants which do not show zenia in the seeds have the objection that a comparatively long time elapses between fertilization and suffi-

cient maturity to permit classification. In plants many cases are known in which there is a selective elimination of certain classes of individuals due to a lesser germination and unequal ability to grow. In Drosophila (Hyde, 1914) crossing does not influence the number of eggs laid but markedly regulates the per cent. that hatch. Therefore the error from this source always tends to show an apparent deviation in favor of cross-fertilization. In maize where the seed progenies can be used this differentiating effect is at a minimum and probably is not sufficient to affect the numbers appreciably but since the tendency is in the opposite direction to the results which have been obtained the data are even more convincing.

TABLE I.

CHARACTERS OF THE SEEDS AND PEDIGREE NUMBERS OF THE INBRED STRAINS AND FIRST GENERATION HYBRIDS USED IN THE MIXED POLLINATIONS.

Pollen Mixture Number.	Characters of the Seeds of		Pedigree Number of	
	A Strain.	B. Strain.	A Strain.	B. Strain.
1.....	Yellow Starchy	White Starchy	1-9-1-2-4-6-7-5-6-2-1	10-4-8-3-5-3-4-8-2-1
2.....	" "	" "	1-9-1-2-4-6-7-5-3-2-1	10-4-8-3-5-3-4-5-2-1
3.....	" "	" "	14-4-6-16-2-12-22	21-13-9-7-57-11
4.....	" "	" "	14-4-6-16-2-12-22	21-13-9-7-57-11
5.....	" "	" "	14-4-6-16-2-12-22	21-13-9-7-57-11
6.....	" "	" "	14-30-4-3-7-11-10	21-13-9-7-57-11
7.....	" "	" "	14-30-4-3-7-11-10	20-4-25-47-4
8.....	" "	" "	14-4-6-16-2-12-22	20-8-5-35-20
9.....	" "	" "	1-9-1-2-4-6-7-5-6-2-1-1	20-4-25-47-4
10.....	" "	" "	(1-6-1-3 × 1-9-1-2) F_1	(20-8 × 21-13) F_1
11.....	" "	" "	(1-6-1-3 × 1-7-1-1) F_1	(20-8 × 21-13) F_1
12.....	" "	" "	(1-7-1-2 × 1-6-1-3) F_1	(21-13 × 20-8) F_1
13.....	Yellow Sweet	White Starchy	146-1-1	65-8-2-2-6-5-3-2-1-1-1
14.....	" "	" "	146-1-1	65-8-2-2-6-5-3-2-1-1-1
15.....	Yellow and White Sweet	" "	(126-1-1-1 × 77) F_1	117-3-1-1
16.....	" " " "	" "	(126-1-1-1 × 77) F_1	117-1-1-1
17.....	Purple Sweet	White Starchy	76-2-2-1-1	117-1-1-1
18.....	" "	" "	76-2-1-2-1	117-1-1-1
19.....	" "	" "	76-2-1-2-1	20-4-25-47-24-1
20.....	" "	" "	76-2-2-1-1	20-4-25-47-24-1
	C Strain		C Strain	
1.....	Yellow Starchy		1-6-1-3-4-4-4-2-4-4-2-5	
19.....	White Sweet		77-2-1-1-1	
20.....	White Starchy		117-1-1-1	

Materials Used and Presentation of the Results.

The figures obtained from the preliminary experiments in which pairs of single plants only were used are not given here because the numbers are too low to give the results much value and for fear of making this report too bulky. It is sufficient to remember that the data taken together indicated a slight selective action favoring the plant's own pollen.

Table II.

Summary of All the Pollen Mixtures giving the Total Number of Seeds, the Numbers in Each Class forming Proportions, the Deviations of which, Expressed as per cent., from the Closest True Proportions, are Almost Wholly in Favor of the Plant's Own Pollen. P is the Probability that these Deviations are Due to the Differences of Random Sampling.

Pollen Mixture Number.	Total Number of Seeds.	A×A.	A×B.	B×A.	B×B.	Deviation from True Proportion, Per Cent.	χ^2.	P.
1	3,430	1,738	46	1,602	44	+ .045	.027	.994
2	5,636	2,133	145	3,080	278	+ .955	7.572	.063
3	1,362	229	14	770	349	+12.715	146.196	.000
4	3,344	710	126	1,856	652	+ 5.465	61.203	.000
5	1,956	589	6	1,290	71	+ 2.105	28.718	.000
6	424	40	71	187	126	−11.850	23.858	.000
7	3,459	23	89	1,507	1,840	−12.245	235.357	.000
8	7,783	2,185	956	2,619	2,023	+ 6.570	144.108	.000
9	8,729	2,550	1,288	2,922	1,969	+ 3.350	42.061	.000
10	5,408	1,084	1,154	997	2,173	+ 8.495	162.687	.000
11	3,314	448	264	1,505	1,097	+ 2.540	8.937	.030
12	3,561	1,724	719	749	369	+ 1.790	5.313	.053
13	736	185	391	95	65	−13.625	55.051	.000
14	792	424	150	156	62	+ 1.155	.533	.894
15	3,168	2,609	47	14	498	+47.750	2,889.561	.000
16	2,224	723	8	74	1,419	+46.975	1,965.981	.000
17	1,410	1,303	3	4	100	+47.960	1,298.993	.000
18	1,599	4	21	1	1,573	+ 7.970	137.532	.000
19	2,606	528	392	343	1,343	+18.525	376.394	.000
20	2,753	897	77	1,174	605	+13.050	283.002	.000
	63,694							

Of those experiments in which pollen was applied to several plants of the same type 20 pollen mixtures in all have been made.

Each mixture is given a number and the seed characters of the materials used and their pedigree numbers are given in Table I. The first number in the pedigree designates the variety from which the inbred strains were derived. The following numbers indicate the progenitors in the successive self-fertilized generations. The total number of units in the pedigree number less one, show the number of generations the material had been self-fertilized at the time the pollinations were made. The inbred strains used in these experiments are as follows:

1. Several distinct strains from a yellow dent variety originally obtained in Illinois and known as Chester's Leaming, self-fertilized ten or more years.

10. A strain with white floury seeds with no traces of corneous starch, self-fertilized nine generations.

14. Two distinct strains from a yellow dent variety from Connecticut known as Stadtmueller's Leaming, selected for high protein content during six generations of self-fertilization.

20. Two distinct strains from a white dent variety originally selected for high protein at the Illinois Exper. Station and further selected during four generations of self-fertilization.

21. One strain from same source as above but selected for low protein in field pollinated cultures and during five generations of self-fertilization.

65. A small, white, round-seeded strain from a variety of popcorn, *Zea mays everta*, self-fertilized ten times and characterized by clear corneous starch.

76. Two similar strains from a sweet variety of latent flint type having purple aleurone and known as Black Mexican, self-fertilized four years.

77. From a sweet variety of latent dent type with deeply wrinkled white seeds known as Evergreen and self-fertilized four generations.

126. From a small, early maturing, yellow, sweet variety of latent flint type with dark yellow kernels, known as Golden Bantam, and self-fertilized three generations.

117. From a variety of popcorn with sharp pointed seeds having clear corneous endosperm, self-fertilized three times.

146. From a yellow, sweet variety, Golden Bantam, of different

source than 126, and somewhat different in type, self-fertilized two years.

Mixtures number 1 to 9 inclusive comprise various inbred strains with yellow starchy and white starchy endosperm. Some of these strains have been described previously (Jones, 1918) and all show marked heterosis in the crossed seeds and in the resulting first generation hybrid plants. A sample of all the different lots of seed secured in these mixtures have been grown to test the accuracy of classification. Pollen mixtures number 10 to 12 are not from inbred strains but from first generation hybrids, one having all yellow seeds the other all white. They were of such a constitution that the second crossing gave still more increase in vigor although not as great as the stimulus following the first cross. The plants being vigorous a large amount of seed was obtained from a few plants. It was desired to know whether the same selective action would be shown by vigorous plants with segregating gametes as contrasted with non-vigorous plants whose gametes were all alike. Classification of the seed was easily carried out and the per cent. of error when tested was found to be quite low. Mixtures 13 and 14 involved yellow sweet in one strain and white starchy endosperm in the other. One dominant factor was carried by each so that differentiation was perfectly distinct in the reciprocal applications. In mixtures 15 and 16 it was intended to make use of the same characters as in the two preceding numbers. The plants which were supposed to be inbred individuals of a yellow sweet strain, and so labeled, when grown in the field were seen to be too vigorous for this material as it had behaved in previous years. The plants were all alike, however, in this respect. It was suspected that this was a lot of first generation hybrids instead of plants from self-fertilized seed. As some crosses had been made with plants of this line the previous season it is now certain that in this instance the seed was not properly labeled at harvest and so was planted for self-fertilized seed when in reality it was all cross-fertilized. As no other plants were available at the time they were needed these were used. Several self-pollinations were made at the same time to show what the seed characters were of this undoubted hybrid. At maturity it definitely showed itself

to be a hybrid as all the ears were segregating for yellow and white sweet seeds. It therefore had certainly been crossed with a white sweet inbred strain the year before as other pollinations of this sort were made at that time. The yellow color of the endosperm was not sufficiently diluted to cause the seed to be suspicioned before planting.

Since the plants were segregating for yellow and white it was not material which would have been used ordinarily for mixing with a white starchy strain. The effects of the starchy-carrying pollen showed up all right among the all sweet seeds but the reciprocal cross-pollination showed only the yellow cross-fertilized seeds. The white cross-fertilized seeds of course could not be distinguished from the self-fertilized seeds. But since half of the pollen grains carried yellow and half of them white the number of yellow seeds can be doubled to give the total number of cross-pollinated seeds and the assumed number of white cross-fertilized seeds subtracted from the white seeds. This increases the error from random sampling somewhat but since the number of yellow seeds is very low in comparison with the white in these mixtures, the data are reliable in view of the great selective action shown in these two mixtures. The fact that the yellow color in this material was a unit factor difference and that there were equal number of pollen grains carrying yellow and white is proven by the self-fertilized ears produced by the hybrid which gave very good 3 to 1 ratios (one ear counted gave 318 yellow and 97 white seeds). Furthermore the starchy crossed seeds produced on the hybrid plants were of two kinds, yellow and white, and were produced in equal numbers (actual numbers: 28 yellow and 27 white. Since the ovules were segregating equally and the self-fertilized seeds gave the mono-hybrid ratio the pollen grains must have carried the two colors in equal amount.

In the last four mixtures the characters, purple sweet and white starchy, were used. In numbers 17 and 18 the plants were not producitve and the numbers of seeds are low. Also the classification of purple starchy cross-fertilized seeds and white starchy self-fertilized was not as sure as in the other mixtures. In the last two mixtures satisfactory numbers were obtained and the differentiation was clear-cut on both sides.

The pollen mixtures 1, 19 and 20 were also applied to a third strain distinct from either of the two used in supplying the pollen. The resulting two lots of seed in each case were both cross-fertilized and probably showed hybrid vigor in the seed in about equal amount. Heterosis is clearly apparent in the plants of similar crosses involving the same or closely related material and is approximately equal.

The data from each pollen mixture are presented in the form of an appendix. Since the arrangement is the same in all the tables these are given in the simplest form possible and a description of one applies to all. In the tables the headings for the four different classes of seed are A × A, A × B, B × A, B × B and in the three mixtures 1, 19 and 20 there are in addition the out-crossed seeds C × A, and C × B. In every case the pistillate parent is given first. These headings when expanded in detail are as follows:

A × A = Self-fertilized seeds from color-carrying pollen.
A × B = Cross " " " non-color " "
B × A = Cross " " " color " "
B × B = Self " " " non-color " "
C × A = Out-crossed " " color " "
C × A = Out " " " non-color " "

On every plant there are two kinds of seeds indicating the relative fertilizing efficiency of the two kinds of pollen in the mixture used. The number of seeds from individual plants are given in the tables under their respective headings, A × A and A × B seed from the same inflorescence and B × A and B × B seeds from another and C × A and C × B from a third. The total number of seeds is summed up below the line. Next to that is given the per cent. of error found upon testing the accuracy of the classification. This figure is used to calculate the amount of error based upon all the seeds obtained. Not more than about 125 seeds were grown in each lot except in two cases as shown in Table III. The calculated amount of wrongly classified seeds is given on the line below the per cent. of error, then follows the corrected numbers after the proper additions and subtractions have been made. After that is placed the observed proportion

of the two kinds of seed in each member of the pair stated as per cent. The closest perfect proportion is calculated from this, based on the results from the A and B plants but not from the C plants, and this subtracted from the actual proportion found gives the deviation in per cent. which appears in the last line. This description applies to mixtures 1 to 12 inclusive. Of the remainder no correction for misplaced seeds is made.

TABLE III.

NUMBER OF PLANTS GROWN AND THE PER CENT. OF WRONGLY CLASSIFIED SEEDS IN THE POLLEN MIXTURES INVOLVING YELLOW AND WHITE, STARCHY ENDOSPERM.

Pollen Number. Mixture	A. × A.		A. × B.		B. × A.		B. × B.	
	Number.	Per Cent.	Number.	Per Cent.	Number.	Per Cent.	Number.	Per Cent.
1.....	414	1.21	26	7.69	107	0	27	22.22
2.....	483	2.69	50	8.00	115	0	128	2.34
3.....	105	0	12	0	119	0	114	1.75
4.....	116	0	101	.99	120	.83	114	1.72
5.....	110	0	6	0	120	0	56	0
6.....	40	—	71	—	112	0	76	2.63
7.....	23	—	89	—	119	0	118	0
8.....	119	0	118	0	120	0	118	1.69
9.....	118	2.54	118	2.54	120	1.67	113	0
10.....	117	1.71	118	0	118	1.69	117	1.71
11.....	114	.88	119	0	123	0	120	0
12.....	116	.86	117	0	116	0	116	0

The total number of seeds, the numbers in each class and the deviations in per cent. from the closest perfect proportion for the 20 pollen mixtures are summarized in Table II. The deviation, if it is an excess of self-fertilized seeds, is given as plus and, if the opposite, as minus. The deviations can range from + 50.0 to − 50.0 per cent. The extremes indicate complete non-functioning of each kind of pollen on one set of plants and exclusive functioning on the other.

Altogether the number of seeds amounts to 63,694. Large numbers are, of course, necessary to be convincing in any experiment on selective fertilization as such investigations are largely studies in sampling. Mixtures number 6, 7, 13, 17 and 18 are less reliable than the others, because they have rather small numbers from either the A or B plants, less than 200 in each case. Like the chain with the weak link the value of each set of figures

is dependent upon the numbers in the less populous half of the proportion.

Some of the mixtures have over one thousand individuals in each of the four classes. Since the experimental error is low and not all in one direction as has been shown and the selective elimination of zygotes tends to obscure the result which has been obtained, such figures as these can not be gainsayed. Of the 20 mixed pollinations 17 show a deviation indicating a selective action in favor of the plants' own pollen. While three of the mixtures show the opposite effect. These three are all low in number of individuals on one or the other side of the proportion. Mixtures 6 and 7 could not be classified by the seeds on the yellow seeded plants so consequently the progenies were grown and classified at maturity. This brings in other sources of error—differential germination and competition between plants which are weak with those that are vigorous—which certainly tend to result to the apparent advantage of cross-fertilization. Two of the deviations showing an excess of self-fertilized individuals are not significant when compared to the allowable differences from random sampling but all the others are. Therefore the conclusion is inevasible that in maize the plant's own pollen is more effective in consummating fertilization than pollen from plants of only slightly different construction. This selective action is shown even though the foreign pollen is perfectly capable of fertilizing the plants when not acting in competition with the plant's own kind of pollen as has been definitely proven. Mixtures number 15, 16 and 17 show 47 out of a possible 50 per cent. deviation, almost complete non-functioning of the unfamiliar pollen. Numbers 15 and 16 include the first generation hybrids in which some calculations had to be made to allow for segregation but these are perfectly justifiable adjustments and the results can be discounted but very little. Mixture 17 is low in numbers having only 104 seeds on the B plants of which 4 are cross-fertilized. But observe the result when this same mixture is applied to the A plants. Here only 3 cross-fertilized seeds are to be found among 1,303 self-fertilized seeds. Surely there is some powerful action working to hold back the unfamiliar pollen. Mixtures number 3, 4, 8, 10, 19 and 20 are by them-

selves convincing as in these the numbers are large, the differentiation of the seeds in both groups is precise and the deviations clearly show the superiority of self-pollination.

The data from pollen mixture number 1 have been published previously (Jones, 1918) although at that time plants had not been grown to test the error in separating the seeds. The greatest number of mistakes of classification of any of the mixtures were made in this lot and the deviation is now well within the limits of random sampling. The data are included here to make this report complete. In the previous publication the probable error used was the familiar formula used for Mendelian ratios. The determinations applied to each half of the proportion alone. Since the ratio on each of the paired plants is dependent upon the ratio on the other, it seems to the writer now that the use of this method of calculating the probable error in connection with this particular problem is wrong.

The method of calculating the significance of the figures as used here is that proposed by Elderton (1901) and is in general use in presenting genetic data. It is obtained in the following manner. The deviations of the terms in the actual proportion found from the closest perfect proportion as calculated are squared and divided by the terms of the perfect proportion. Their sum gives a value χ^2, which by use of convenient tables calculated by Elderton, gives a probability value varying from 0 to 1 proportional to the goodness of fit.

The calculations must be based on the actual numbers of seeds obtained and not on the percentages. To obtain a perfect proportion from which the deviations of the numbers found will be the smallest in the four terms it is necessary to balance the figures so that the same number of individuals are represented on the A and B plants. This is done by reducing the number of seeds of the greater and increasing the lesser keeping the ratios the same, of course. The deviations of the proportions, balanced in this way, from the closest perfect proportions are then used to obtain the probability value in the way described above. The same result is obtained more quickly by calculating the value of χ^2 from the percentages and multiplying this figure by one half the total number of seeds.

With four terms the values of χ^2 greater than 18 have no probability out to three decimal places. Since very much greater numbers were secured in most cases, as shown in Table II., the deviations are clearly not due to the differences of random sampling alone if the application of this method of calculation is justifiable. The writer is not perfectly sure that it is because it should be noted that the theoretical proportion is calculated directly from the results found, that is, there is no possible way of knowing the real amounts of the two kinds of functional pollen contained in the mixtures. Moreover the probable error does not take into consideration the corrections which are made for the mistakes of classification found in a sample drawn from each lot.

There may be a selective action when the pollen is applied to one plant but not to the other or the action may be reversed. All that is measured is the combined effect if both are in the same direction or the excess of one over the other if in opposite directions. It seems reasonable to suppose, however, that the selective fertilization is approximately the same on both members of the pair as a large number of mixed pollinations are avai able made with many different types of plants and the majority give the same result. If this were always true, however, the ratio obtained by out-crossing the mixture onto a distinct strain should not deviate from the ratio of the closest perfect proportion calculated from the figures of the reciprocal crosses beyond allowable limits. That is, the ratio obtained from the out-crossed seeds is supposed to represent very nearly the actual ratio of effective pollen in the mixture since both kinds are more nearly on the same footing. In the three experiments, Nos. 1, 19 and 20 in which such out-crossed seeds were obtained, the deviation is even greater in two cases than that from the reciprocal applications. In pollen mixture number 1 all the deviations are small and probably without significance.

Since the results are convincing when considered without a probable error, it is not necessary to lay much stress on the method of its calculation at this time. Considering the data altogether, magnifying the actual experimental error to its fullest extent, and taking a common-sense view of the allowances to be

made for variations inherent in a problem of this kind the conclusion can be no other than that these plants manifest a definite receptiveness to their own pollen, discriminating against foreign pollen even though it comes from plants only slightly differentiated from them, both of which might easily be descended from the same individual at no very distant period back. This selective action is shown by plants of weak growth or full vigor, whether each strain descended from a line of similar ancestors or whether its immediate parents were diverse and, finally, irrespective of the gametes being alike or unlike in germinal contents. The one significant feature in common in all these experiments is the fact that the cytoplasm which surrounds the male nuclei and which makes up the vehicle that carries them to the egg cells is alike for the gametes of any one type of plant whether this plant is homozygous or heterozygous and in self-fertilization this cytoplasm is the same as the medium in which the pollen fulfills its function. This points very strongly to the probability that the differential effect is due to the rate of pollen-tube growth although it may be determined after the male nuclei are brought to the egg.

The average weight of the seeds in the different classes of all the pollen mixtures is given in Table IV. with the increase in weight of the cross-fertilized over the self-fertilized seeds. Expressed as per cent. these figures permit an estimation of the comparative amount of heterosis shown in the crosses. In fact this is one of the best means of measuring the stimulation of heterozygosis as the environmental differences are reduced to a minimum. It has not been definitely proven that there exists a correlation between the amount of heterosis in the seeds and that shown by the resulting plants grown from those seeds but the indications are that there is a close relation between the two. Since hybrid vigor is roughly proportional to the germinal differences in the two forms united it can be determined whether or not there is a relation between the diversity of the plants used in the several pollen mixtures and the degree of preference shown by those plants to their own kind of pollen. Table V. shows that the coefficient of correlation between the average increase in weight of seeds and the deviation in favor of self-fertilization, both stated

TABLE IV.

THE AMOUNT OF HETEROSIS SHOWN BY EACH CROSS-POLLINATION IN THE INCREASE IN AVERAGE WEIGHT OF SEEDS COMPARED TO THE SELF-POLLINATED SEEDS GROWN IN THE SAME INFLORESCENCES.

Pollen Mixture Number.	Average Weight of Seeds in Centigrams.						Per Cent. Increase.		
	A × A.	A × B.	Increase.	B × A.	B × B.	Increase.	A.	B.	Ave.
1	13.8	18.1	4.3	18.0	15.7	2.3	30.4	14.6	22.5
2	12.2	17.1	4.9	17.6	14.3	3.3	40.0	23.0	31.5
3	29.3	35.5	6.2	39.8	35.7	4.1	21.2	11.5	16.4
4	26.9	31.4	4.5	34.2	29.3	4.9	16.7	16.7	16.7
5	30.0	47.6[1]	17.6	38.3	34.8	3.5	58.7	10.1	34.4
6	—	—	—	36.7	34.7	2.0	—	5.8	5.8
7	—	—	—	19.6	16.3	3.3	—	20.2	20.2
8	25.8	30.6	4.8	23.8	22.5	1.3	18.6	5.8	12.2
9	12.0	15.8	3.8	15.7	13.2	2.5	31.7	18.9	25.3
10	32.2	33.4	1.2	32.5	30.9	1.6	3.7	5.2	4.5
11	27.5	28.6	1.1	34.5	32.8	1.7	4.0	5.2	4.6
12	32.4	32.8	.4	37.4	34.3	3.1	1.2	9.0	5.1
13	25.7	29.3	3.6	16.4	14.8	1.6	14.0	10.8	12.4
14	20.2	22.1	1.9	13.9	12.9	1.0	9.4	7.8	8.6
15	24.2	35.1	10.9	12.9	11.7	1.2	45.0	10.3	27.7
16	32.0	40.0[1]	8.0	11.4	9.6	1.8	25.0	18.8	21.9
17	14.3	20.0	5.7	15.0	10.2	4.8	39.9	47.1	43.5
18	25.0	23.8[1]	−1.2	10.0[1]	8.2	1.8	−4.8	22.0	8.6
19	15.7[1]	22.2	6.5	16.6[1]	15.6	1.0	41.4	6.4	23.9
20	14.3	17.1	2.8	15.9	15.3	.6	19.6	3.9	11.8

in per cent., is $+.496 \pm .093$. Although the numbers are scanty there is a significant relation between the two. This means that the more unlike the plants are the greater the distinction that is made between the two kinds of pollen. *In proportion as the cross-fertilization benefits the immediate progeny in its development the less effective is that pollen in accomplishing the union.*

The same method of experimentation was applied to another plant, the garden tomato, *Lycopersicum esculentum* Mill. Advantage was taken of plant characters such that the seedlings could be distinguished in both reciprocal applications. Pollen from a variety with entire leaves with a tall habit of growth was mixed with pollen from a dwarf variety with normal, serrate leaves. Tall stature and normal leaves are dominant so that the cross-fertilized and self-fertilized seedlings from one variety were

[1] Number of seeds too few to make averages reliable.

visible because of differences in leaf formation and in the other variety by habit of growth. Dwarf plants are characteristically shorter and more compact in stems and leaves which gives them a distinct appearance. The plants used were grown from unpedigreed seed but the tomato is usually self-fertilized and the varieties employed were tested and found to come true to type. Two experiments were made and the results from these are given as pollen mixtures number 21 and 22 in the appendix. The plants from individual fruits are grown separately. The total numbers in the two mixtures are 340 and 272. Differentiation was sharp in the seedling stage in the A lots with either serrate or entire leaves. In the B lots the presence of tall and dwarf seedlings could be easily seen but not all of them could be separated as positively as could be desired. The plants were therefore set in the field and classified after they had grown about two months. They were set out too late to make a satisfactory growth and even at the end of the season classification was not made with certainty in every case.

TABLE V.

CORRELATION BETWEEN THE AMOUNT OF HETEROSIS, SHOWN BY THE PER CENT. INCREASE IN WEIGHT OF CROSSED SEEDS, AND THE SELECTIVE ACTION IN FAVOR OF THE PLANTS' OWN POLLEN. $r = +.496 \pm .093$.

Per Cent. Increase in Weight.

Deviation	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43	
13.5					1				1												2
10.5	1																				1
7.5																					0
4.5																					0
−1.5																					0
+1.5	11		1							1				1		1					6
4.5							1				1										2
7.5	1		1		1																3
10.5																					0
13.5				1			1														2
16.5																					0
19.5										1											1
22.5																					0
25.5																					0
28.5																					0
31.5																					0
34.5																					0
37.5																					0
40.5																					0
43.5																					0
46.5									1			1								1	3
	4	0	2	1	2	0	2	0	2	2	1	1	0	1	0	1	0	0	0	1	20

Pollen Mixture No. 1.

A × A.	A × B.	B × A.	B × B.	C × A.	C × B.
317	1	319	5	361	10
189	2	224	9	429	15
270	7	369	10	421	13
389	8	348	26	445	1
332	8	330	6		
260	1				
1,757	27	1,590	56	1,656	39
1.21	7.69	0	22.22	.39	6.66
21	2	0	12	6	3
1,738	46	1,602	44	1,653	42
97.420	2.580	97.330	2.670	97.520	2.480
97.375	2.625	97.375	2.625	97.375	2.625
+.045	−.045	−.045	+.045	+.145	−.145

Pollen Mixture No. 2.

A × A.	A × B.	B × A.	B × B.
236	11	359	42
206	9	353	20
188	15	380	42
141	3	230	15
194	8	372	39
237	11	214	12
232	11	286	36
167	7	265	19
151	3	273	31
188	8	341	29
245	7		
2,185	93	3,073	285
2.69	8.00	0	2.34
59	7	0	7
2,133	145	3,080	278
93.630	6.370	91.720	8.280
92.675	7.325	92.675	7.325
+.955	−.955	−.955	+.955

The results, taken as they stand, are the same as obtained from maize. There is a deviation favoring the plants' own pollen of 2.06 and 6.84 per cent., in the two cases with the probability values .907 and .082 respectively. In the first instance the difference can easily be due to random sampling, in the other the odds are strongly against such an explanation. In view of the fact that differences in germination of the seeds and viability of plants most certainly tend to decrease the proportion of self-fertilized individuals, the data have some value. They should be corroborated by larger numbers using other characters which can be more surely identified before they are as convincing as the results with maize.

Pollen Mixture No. 3.

A × A.	A × B.	B × A.	B × B.
23	3	90	41
54	7	114	28
27	0	27	26
34	1	15	20
18	0	97	56
43	2	73	19
19	0	76	52
4	0	27	8
7	1	50	3
		41	14
		66	55
		33	5
		22	11
		33	17
229	14	764	355
0	0	0	1.75
0	0	0	6
229	14	770	349
94.240	5.760	68.810	31.190
81.525	18.475	81.525	18.475
+12.715	−12.715	−12.715	+12.715

Pollen Mixture No. 4.

A × A.	A × B.	B × A.	B × B.
189	38	301	67
173	42	208	102
165	11	248	70
64	14	164	167
41	7	194	38
77	15	254	117
		213	38
		278	49
709	127	1,860	648
0	.99	.83	1.72
0	1	15	11
710	126	1,856	652
84.930	15.070	74.000	26.000
79.465	20.535	79.465	20.535
+5.465	−5.465	−5.465	+5.465

Pollen Mixture No. 5.

A × A.	A × B.	B × A.	B × B.
23	2	219	8
123	3	246	14
56	1	313	24
143	0	352	19
244	0	160	6
589	6	1,290	71
98.990	1.010	94.780	5.220
96.885	3.115	96.885	3.115
+2.105	−2.105	−2.105	+2.105

POLLEN MIXTURE NO. 6.

A × A.	A × B.	B × A.	B × B.
40	71	184	129
—	—	0	2.63
—	—	0	3
40	71	187	126
36.04	63.96	59.74	40.26
47.89	52.11	47.89	52.11
−11.85	+11.85	+11.85	−11.85

POLLEN MIXTURE NO. 7.

A × A.	A × B.	B × A.	B × B.
23	89	253	188
		104	189
		185	212
		84	117
		166	174
		115	139
		118	156
		123	190
		116	169
		109	158
		134	148
23	89	1,507	1,840
20.540	79.460	45.030	54.970
32.785	67.215	32.785	67.215
−12.245	+12.245	+12.245	−12.245

POLLEN MIXTURE NO. 8.

A × A.	A × B.	B × A.	B × B.
96	53	173	108
158	112	98	69
154	60	74	83
136	47	102	102
106	38	175	131
103	29	199	147
135	65	109	79
89	53	196	89
130	47	142	153
60	11	203	131
128	49	117	93
63	38	52	52
69	29	166	106
87	34	109	79
186	67	83	72
59	31	138	86
103	54	133	138
77	34	76	75
124	51	45	41
58	18	119	116
64	36	75	108
2,185	956	2,584	2,058
0	0	0	1.69
0	0	0	35
2,185	956	2,619	2,023
69.56	30.44	56.42	43.58
62.99	37.01	62.99	37.01
+6.57	−6.57	−6.57	+6.57

Pollen Mixture No. 9.

A × A.	A × B.	B × A.	B × B.
277	149	209	115
116	69	173	99
168	69	204	156
135	78	188	114
151	52	160	101
78	30	210	142
128	71	172	134
254	102	191	160
286	147	166	112
208	97	195	134
185	88	196	107
113	55	175	108
208	117	207	105
277	130	128	97
		92	73
		123	76
		123	86
2,584	1,254	2,972	1,919
2.54	2.54	1.67	0
66	32	50	0
2,550	1,288	2,922	1,969
66.44	33.56	59.74	40.26
63.09	36.91	63.09	36.91
+3.35	−3.35	−3.35	+3.35

Pollen Mixture No. 10.

A × A.	A × B.	B × A.	B × B.
344	331	189	397
200	272	195	447
284	250	189	522
275	282	233	399
		169	430
1,103	1,135	975	2,195
1.71	0	1.69	1.71
19	0	16	38
1,084	1,154	997	2,173
48.440	51.560	31.450	68.550
39.945	60.055	39.945	60.055
+8.495	−8.495	−8.495	+8.495

Pollen Mixture No. 11.

A × A.	A × B.	B × A.	B × B.
67	57	209	130
385	203	290	199
		279	265
		380	191
		341	312
452	260	1,505	1,097
.88	0	0	0
4	0	0	0
448	264	1,505	1,097
62.92	37.08	57.84	42.16
60.38	39.62	60.38	39.62
+2.54	−2.54	−2.54	+2.54

POLLEN MIXTURE NO. 12.

A × A.	A × B.	B × A.	B × B.
552	196	218	106
86	67	197	92
265	111	163	69
253	114	105	46
182	61	66	56
401	155		
1,739	704	749	369
.86	0	0	0
15	0	0	0
1,724	719	749	369
70.57	29.43	66.99	33.01
68.78	31.22	68.78	31.22
+1.79	−1.79	−1.79	+1.79

POLLEN MIXTURE NO. 13.

A × A.	A × B.	B × A.	B × B.
37	52	14	11
17	87	13	18
11	16	17	11
28	86	6	4
50	91	31	8
42	59	14	13
185	391	95	65
32.120	67.880	59.370	40.630
45.745	54.255	45.745	54.255
−13.625	+13.625	+13.625	−13.625

POLLEN MIXTURE NO. 14.

A × A.	A × B.	B × A.	B × B.
41	13	58	31
91	35	35	15
83	39	46	10
70	27	17	6
139	36		
424	150	156	62
73.870	26.130	71.560	28.440
72.715	27.285	72.715	27.285
+1.155	−1.155	−1.155	+1.155

POLLEN MIXTURE NO. 15.

A × A.	A × B.	B × A.	B × B.
309	11	3	111
415	11	0	53
393	9	1	71
326	7	1	113
276	2	0	67
135	1	2	90
400	4		
355	2		
2,609	47	(7)	(505)
		14	498
98.23	1.77	2.73	97.27
50.48	49.52	50.48	49.52
+47.75	−47.75	−47.75	+47.75

Pollen Mixture No. 16.

A × A.	A × B.	B × A.	B × B.
285	5	12	355
187	1	8	350
251	2	8	408
		4	226
		5	117
723	8	(37)	(1,456)
		74	1,419
98.910	1.090	4.960	95.040
51.935	48.065	51.935	48.065
+46.975	−46.975	−46.975	+46.975

Pollen Mixture No. 17.

A × A.	A × B.	B × A.	B × B.
255	1	4	49
166	0	0	51
185	1		
222	0		
220	1		
255	0		
1,303	3	4	100
99.77	.23	3.85	96.15
51.81	48.19	51.81	48.19
+47.96	−47.96	−47.96	+47.96

Pollen Mixture No. 18.

A × A.	A × B.	B × A.	B × B.
3	5	1	368
1	16	0	432
		0	320
		0	336
		0	39
		0	78
4	21	1	1.573
16.00	84.00	0.06	99.94
8.03	91.97	8.03	91.97
+7.97	−7.97	−7.97	+7.97

Pollen Mixture No. 19.

A × A.	A × B.	B × A.	B × B.	C × A	C × B.
20	16	41	206	75	213
89	53	36	147	71	124
68	86	42	200	61	315
55	47	45	178		
69	39	29	150		
45	20	63	156		
52	37	30	88		
65	8	57	218		
65	86				
528	392	343	1,343	207	652
57.390	42.610	20.340	79.660	24.100	75.900
38.865	61.135	38.865	61.135	38.865	61.135
+18.525	−18.525	−18.525	+18.525	−14.765	+14.765

POLLEN MIXTURE NO. 20.

A × A.	A × B.	B × A.	B × B.	C × A.	C × B.
172	11	193	86	40	52
43	6	104	58	46	56
165	15	117	86	51	55
38	2	80	45	2	5
89	4	47	17		
103	8	128	52		
82	12	81	47		
19	0	94	60		
53	4	92	30		
33	0	99	43		
69	13	75	46		
31	2	64	35		
897	77	1,174	605	139	168
92.09	7.91	65.99	34.01	45.28	54.72
79.04	20.96	79.04	20.96	79.04	20.96
+13.05	−13.05	−13.05	+13.05	−33.76	+33.76

POLLEN MIXTURE NO. 21.

A × A.	A × B.	B × A.	B × B.
39	16	17	25
12	14	1	5
32	25	36	18
14	18		
97	73	54	48
57.06	42.94	52.94	47.06
55.00	45.00	55.00	45.00
+2.06	−2.06	−2.06	+2.06

POLLEN MIXTURE NO. 22.

A × A.	A × B.	B × A.	B × B.
7	22	13	17
10	27	7	27
107	103		
124	152	20	44
44.93	55.07	31.25	68.75
38.09	61.91	38.09	61.91
+6.84	−6.84	−6.84	+6.84

PREVIOUS INVESTIGATIONS ON SELECTIVE FERTILIZATION.

From the work of Kölreuter, Herbert, Gärtner, Darwin, Müller, Knuth and others we are familiar with the phenomenon of self-sterility in plants in which the individual's own pollen is wholly incapable of functioning on the plant by which it is produced although perfectly developed and able to fulfill its duties when brought to other plants of the same species. Numerous investigators have been giving attention to this problem in recent times. East and Park (1917) have made a noteworthy contribu-

tion to its solution and give a complete résumé of the work which has been done on this subject. They have been able to demonstrate that groups exist within which the individuals are all both self-sterile and cross-sterile, but any member of one group is perfectly fertile with any member of any other group. These investigators find that there are about 100 well-endorsed instances of self-sterility in plants scattered over some 35 families. Undoubtedly this discrimination is a means to promote cross-fertilization of approximately the same significance as floral contrivances, dichogamy and diœcism. Even though widespread in its occurrence self-sterility is a special adaptive process fulfilling a particular function. It is directly opposite in its effect to the results found in maize which shows no self-sterility of the type found in Nicotiana and other genera. At least no clear cases are known of maize pollen, which is unable to fertilize the plants which produced it, being able to fertilize other plants.

Darwin has furnished many instances of self-sterility. In addition he reports some experiments which led him to believe that even when a plant was normally self-fertile that pollen from unrelated plants of the same species was prepotent over the plant's own pollen. In discussing means which insure flowers being fertilized with pollen from distinct plants, he says: "We now come to a far more general and therefore more important means by which the mutual fertilization of distinct plants is effected, namely, the fertilizing power of pollen from another variety or individual being greater than that of a plant's own pollen. The simplest and best known case of prepotent action in pollen, though it does not bear directly on our present subject, is that of a plant's own pollen over that from a distinct species. If pollen from a distinct species be placed on the stigma of a castrated flower, and then after the interval of several hours, pollen from the same species be placed on the stigma, the effects of the former are wholly obliterated, excepting in some rare cases. If two varieties are treated in the same manner, the result is analogous, though of directly opposite nature; for pollen from any other variety is often or generally prepotent over that from the same flower" ("Cross- and Self-Fertilization," pp. 391–392).

These statements were based on observations and experiments

with various cultivated plants. Different types of crucifers—kohl-rabi, borccoli, Brussels sprouts, cabbage—were grown near each other and the seed resulting from pollination at will, when grown, showed a large amount of intercrossing. The observation was also made with different varieties of the radish, *Raphanus sativus*. These plants are all partially self-sterile so that cross-fertilization is expected in somewhat greater degree than would result from random pollination. Mixing was also shown by plants which are generally self-fertile such as tulip, hyacinth, anemone, ranunculus, strawberry, orange, rhododendron, rubbarb. The fact that vicinism occurs when varieties of these plants are grown together is established by such observations but this does not prove that one type of pollen is prepotent over the other. Somewhat more significant results were obtained from two other species. *Mimulus luteus* was found to be highly fertile when insects were excluded. Uncastrated flowers of a constant whitish variety were artificially pollinated by a yellowish variety and of the 28 resulting plants all had yellowish flowers so that the "pollen of the yellow variety completely overwhelmed that from the mother plant." A crimson variety of *Iberis umbellata*, which was self-fertile, was crossed with a pink variety, the pollen being applied to uncastrated flowers as before upon the stigmas of which he saw abundant pollen presumably from the same flowers. Out of 30 plants raised 24 showed themselves to be crossed by the altered color of their flowers.

Obviously experiments such as these are not sufficient to establish the prepotency of foreign pollen in self-fertile plants. A number of conclusions might be drawn from such results. The cross-fertilized seeds may have germinated better and the plants grown from them survived in greater numbers. The types may not have been as constant for their flower color as Darwin supposed or the ovules may not have been receptive at the time the plant's own pollen was available but were when the foreign pollen was applied. Taken as they stand the results do indicate a prepotency of pollen from dissimilar plants and it would be desirable to investigate this effect with these species using mixed pollen in reciprocal applications as employed with maize and the tomato.

Darwin knew of many cases of total self-sterility and was so convinced of the necessity for cross-fertilization that he was easily persuaded from these observations that a prepotency of pollen from unrelated plants did exist since he supposed this enabled a plant to choose between its own and unrelated pollen when both were brought at the same time to the stigmas by insects or other agencies. So plausible have been the arguments in favor of such an assumption that the prepotency of germ cells from individuals of somewhat different constitution, even where complete self-fertility exists, has been accepted as an established fact and incorporated in textbooks on biology.

Similarly inconclusive experiments have been performed with animals. Marshall (1910) artificially impregnated a pure bred dog with a mixture of equal quantities of seminal fluids from the same breed and from a mongrel of unknown ancestry. Of the four young which resulted one died early, and three resembled somewhat the mongrel sire. Marshall cites another instance in which a dog copulated with a member of the same breed and two days later with a sire of different type. Out of three puppies one was pure bred and two half-breeds. These cases, according to this writer, indicated a selective action favoring dissimilar rather than related spermatozoa. King (1918) mentions some preliminary experiments with albino and wild gray rats in which advantage was given to the former, yet the results tended to show a prepotency of the latter, so that there was apparently a selective action favoring the out-cross. The details of these experiments are not given.

In attempting to determine whether or not a selective action exists small numbers can never be more than suggestive and unless the mixture is applied at the same time to both types furnishing the sperm cells there is no way of estimating the relative proportions of the two kinds of fertilizing elements present in the mixture which are capable of functioning. Furthermore a constant excess of cross-fertilized individuals over the others may be due to the greater viability of the hybrids and hence there will be a selective elimination of zygotes but not necessarily slective fertilization. Hyde (1914) compared the matings of different types of Drosophila within the strains and in reciprocal

crosses. The dissimilar unions gave greatly increased numbers in both reciprocal combinations. However, the type of mating did not influence the number of eggs laid and there is no proof that cross-fertilization occurred more readily than self-fertilization. The results show that the cross-fertilized eggs hatched better and the offspring survived in greater numbers, a result which is easily understood since there were lethal factors involved in the material worked with.

The only evidence from the animal side of a definite selective action comparable to the many instances of self-sterility in plants is the well-known case of self-impotency in *Ciona intestinalis* (Castle, 1896). Morgan (1905, '07, '10) has experimented with this organism and has found that the self-sterility is not always complete. Material gathered on the Pacific coast showed somewhat greater receptiveness to the individual's own sperm than eggs of the same species at Woods Hole which were almost entirely unresponsive to sperm from the same individual which produced the eggs. In another ascidian, *Cynthia partida*, he found that self-fertilization takes place frequently but the sperm of unrelated individuals is more effective. A third species, *Molgula manhattensis* is self-fertilized as readily as cross-fertilized. From this it seems that incompatibility of uniting gametes as a means of insuring cross-fertilization exists in various grades of effectiveness. Even in extreme cases the degree of self-sterility may be modified by internal and external conditions. In Nicotiana East and Park find that self-fertilization sometimes takes place towards the end of the growing period when the vigor of the plants is reduced.

Discussion.

As far as the writer knows the results obtained from maize stand alone among plants in showing a selective action unfavorable to fertilization by sperm from individuals of different hereditary constitution. The handicap placed upon the foreign pollen is proportional to the germinal unlikeness. If the unequal effect is due to a slower growth of the pollen tube through the tissues of style the selective action may be restricted to plants,[1]

[1] E. C. Miller (Jour. Agric. Research, Vol. 18, pp. 255–266, Dec. 1919), has recently made a detailed study of fertilization in maize and finds that from many

and would also not be surprising to find that the phenomenon is greater in maize than in any other species for the reason that in this plant the pollen tubes have a larger distance to traverse to accomplish fertilization than in any other form known to the writer.

The stigmatic hairs of maize are scattered along a filamentous style which continues to grow until fertilization takes place. The structure withers and dries shortly after pollination takes place. Pollen may adhere at any point along the filament. The total length of style through which the pollen tube grows is normally from 10 to 20 centimeters but in extreme cases may be as great as 50 or more. It will be worth while to see whether or not the selective effect is more pronounced when the styles are long than when short. Such an experiment can be easily carried out and would give some indications as to whether the handicap is placed during pollen tube growth or after the sperm nuclei are brought to the egg.

The lessened ability of moderately different types to fertilize is in line with the impossibility of effecting unions between widely separated forms. In such cases the prevention of fertilization is sometimes due to mechanical difficulties in the way of bringing sperm cells to the eggs but even when this is accomplished there still exists a firm barrier which prevents the passage through the egg membrane. The differential effect demonstrated in maize may be simply a reduced manifestation of this phenomenon.

It is possible that the experiments on anaphyllaxis may throw some light on this problem. It is known that foreign proteins when injected in animal tissues may have a toxic effect and excite an extreme irritability so that in repeated doses they may cause markedly injurious results. By this means it is possible to distinguish between proteins of very slight differences in composition. Since the differences in protoplasmic substances between the types in which a selective action is shown seem to be small there may be some relation between the two phenomena. However, the male gametophyte growing upon the stigmas is, in

pollen tubes which start to grow down the style only one tube in every case in nearly 100 observations was seen to reach the ovary cavity. This indicates that the differential fertilizing power is determined by the rate of pollen tube growth and not after the sperm nuclei have been brought to the egg.

a way, merely a parasitic organism. It would be difficult to find evidence from true parasites that they are restrained by their hosts in proportion as they are genetically dissimilar although in most cases the differences are so great that probably there is no basis for comparison.

The only evidence which has any direct bearing on the problem comes from grafting experiments. It is well known with plants that the affinity of stock and cion is directly proportional to their phylogenetic similarity. In animals the same rule holds, and very fine distinctions are manifested. Morgan (1910) cites the results of Schoene in which the skin of the mouse is readily grafted back upon the same individual or member of the same litter but not upon unrelated mice. Such results as these are quite similar to the greater receptiveness of plants to their own pollen.

For some time there has been current in biological literature the hypothesis that heterogeneity in the structure of organisms favors growth and reproduction and conversely that homogeneity is unfavorable. This is a heritage from Darwinism and has appeared again and again in theories of rejuvenation, vigor derived from crossing, and selective fertilization. It has been stated in many different ways but in general terms it amounts to the supposition that similarity in protoplasmic structure brought about by a line of similar ancestors is not conducive to physiological efficiency and that the differences brought about by the union of unlike elements and the consequent lack of balance stimulates growth. The only basis for this hypothesis is found in the necessity, in most cases, for the union of two differentiated sex cells to start the development in the egg. The attempt to argue by analogy than the union of dissimilar protoplasms is, in itself, an immediate physiological benefit is not supported by the facts and is founded upon fallacious reasoning.

Rejuvenation in vegetatively reproduced organisms by sexual union is no longer looked upon as beneficial in destroying similarity in structure. That the process of forming gametes and their reunion may bring about a reorganization of the protoplasmic substances and an elimination of waste products so as to result in greater growth seems quite plausible. But the

significance of such a process is not to be looked for in the bringing together of differentiated germplasms.

The advantage derived from cross-fertilization is now understood as a phenomenon of inheritance and the older hypothesis of the stimulation of heterozygosis is no longer needed. According to present theory homozygous factor combinations are more efficient than heterozygous combinations of the same factors. In the lowest organisms which are illustrative of a primitive sexuality there is direct proof that the union of different individuals does not result in an increased developmental efficiency. Jennings (1913) finds that in Paramecium, in the generations immediately following conjugation, there is a slowing down in the rate of division. The advantage derived from the pairing of individuals is the greater elasticity in adaptiveness resulting from the mixing of different germ plasms giving to some of the descendants a greater chance for survival. Pearl (1907) has found that in this same organism there is a tendency for like forms to conjugate due to mechanical hindrances to the pairing of individuals dissimilar in form. This has been substantiated by Jennings (1911). Also in gastropods Crozier (1918) has demonstrated that assortative mating takes place between individuals of the same size and this, he considers, results in a greater number of offspring than there would be if random pairing was the rule. It has been proven by Pearson and Lee (1903) that assortative mating occurs in man. This conclusion is reached after extensive investigation in the inheritance of physical characters in which they have found that there are positive correlations between husband and wife with respect to stature, span of arms, and length of forearm. Moreover they have shown that homogamy is a factor favorable to fertility. Parents with like characters are more productive of offspring. This is an important observation and supports the main thesis of this paper.

The occurrence of homogamy in such widely diverse forms of life as the higher plants, protozoa, mollusca and man cannot fail to have significance. The importance of discriminate isolation has long been recognized since it supplies a necessary factor in divergent evolution. The existence of such an assortative action can now be looked for in all forms of life since evidence has been

produced from the corners of the phylogenetical triangle. The evolutionary significance of this phenomenon has been ably reviewed by Peal in the above citation so that it is not useful to go into that phase of the problem in detail here. It is interesting to note that the results from maize fulfill his expectation as in conclusion he says, "the fact that we find such a high degree of homogamy in a protozoan form like Paramecium strongly suggests the possibility that in higher organisms there may be assortative mating of the gametes in the process of fertilization. Should such a homogamy occur it would probably be of far greater importance than any assorted mating of the somas." While the selective agency in plants does not differentiate between gametes produced by one type in the end the result is the same. Individuals with like characters tend to be brought together and virtually to be set apart from the general population.

Biological investigations unite to show that the importance of sex is to make organisms more plastic in adaptability. The advantages have been so great that sexual reproduction is now established as the dominant method for the renewal of organism in both kingdoms at the expense of economy and speed of multiplication. It is not strange then that accessory devices have been developed to insure the fulfillment of the function for which so much has been expended. Self-sterility or self-impotency is one of the means developed to serve this purpose.

The reverse phenomenon, that of self-prepotency, so far is shown among plants only by maize. It would be surprising to find it limited to this one species. Is it not more likely to be a general manifestation? As a fundamental principle it may apply even to those organisms which show self-sterility, this latter being a special adaptation entirely overcoming the handicap placed upon unfamiliar gametes in order to make certain the advantages which exogamy holds out. One cannot insist upon such an assumption with evidence from only one or possibly two species. But the evidence, limited though it may be for the present, is one more indication that homogeneity, similarity, likeness, familiarity, or however it may be described, in protoplasmic structure is consistent with and favorable to the highest physiological efficiency.

In maintaining two opposing tendencies Nature is not necessarily working at cross-purposes. Biparental inheritance with the inclination towards exogamy serves to bring about plasticity. The preferential mating of similar kind operates to make this mixing discriminative. Probably it is not yet time to reconcile completely these two contradictory forces. It may at least be held that assortative mating which favors the pairing of like with like has some importance in evolution since it is an agency in orthogenetic changes. Perceptive reproduction of this kind tends to hold organisms in certain paths once a break from the common type has been made. Having been demonstrated in three of the farthest separated branches of the organic world—angiosperms, protozoa, and man—homogamy may take a somewhat more authoritative part in evolutionary theory.

LITERATURE CITED.

Belling, J.

'14 The Mode of Inheritance of Semi-sterility in the Offspring of Certain Hybrid Plants. Zeitsch. f. ind. Abst. u. Vererb., Vol. 12, pp. 303–342.

Castle, W. E.

'96 The Early Embryology of *Ciona intestinalis* Flemming (L.). Bull. Mus. Comp. Zoöl., Harvard University, Vol. 27, pp. 201–280.

'03 The Heredity of Sex. Bull. Mus. Comp. Zoöl., Harvard University, Vol. 40, pp. 189–218.

Crozier, W. J.

'18 Assortive mating in a Nudibranch, *Chromodoris Zebra* Heilprin. Journ. Exper. Zoöl., Vol. 27, pp. 247–292.

Cuénot, L.

'08 Sur quelques anomalies apparentes des proportions Mendéliennes (6e note). Arch. Zoöl. Exp. et Gén., Vol. 9, pp. 7–15.

Davis, B. M.

'15 The Test of a Pure Species of Œnothera. Proc. Am. Phil. Soc., Vol. 54, pp. 226–245.

'17 A Criticism of the Evidence for the Mutation Theory of DeVries from the Behavior of Species of Œnothera in Crosses and in Selfed Lines. Proc. Nat. Acad. Sciences, Vol. 3, pp. 704–710.

East, E. M.

'19 Studies on Self-Sterility. IV. Selective Fertilization. Genetics, Vol. 4, pp. 346–355.

East, E. M., and Hayes, H. K.

'11 Inheritance in Maize. Connecticut Agr. Exp. Sta. Bull. 167, pp. 1–142.

East, E. M., and Park, J. B.

'17 Studies on Self-sterility. I. The Behavior of Self-sterile Plants. Genetics, Vol. 2, pp. 505–609.

Elderton, W. P.

'01 Tables for Testing the Goodness of Fit of Theory to Observation. Biometrika, Vol. 1, pp. 155–163.

Hyde, R. H.

'14 Fertility and Sterility in *Drosophila ampelophila*. Journ. Exper. Zoöl., Vol. 17, Nos. 1–4.

Jennings, H. S.

'11 Assortive Mating, Variability and Inheritance of Size, in the Conjugation of *Paramecium*. Journ. Exper. Zoöl., Vol. 11, pp. 1–134.

'13 The Effect of Conjugation in *Paramecium*. Journ. Exper. Zoöl., Vol. 14, pp. 279–391.

Jones, D. F.

'18 The Effects of Inbreeding and Crossbreeding upon Development. Connecticut Agr. Exp. Sta. Bull. 207, pp. 1–100, pls. 1–12.

Kempton, J. H.

'19 Inheritance of Waxy Endosperm in Maize. U. S. Dept. of Agr., Bur. P. I. Bull. 754, pp. 1–99.

King, H. D.

'18 Studies in Inbreeding. III. The Effects of Inbreeding, with Selection, on the Sex Ratio of the Albino Rat. Journ. Exper. Zoöl., Vol. 27, pp. 1–35.

Marshall, F. H. A.

'10 The Physiology of Reproduction. London.

Morgan, T. H.

'05 Some Further Experiments in Self-fertilization in *Ciona*. BIOL. BULL., Vol. 8, pp. 313–330.

'07 Experimental Zoölogy. New York.

'10 Cross and Self-fertilization in *Ciona intestinalis*. Arch. f. Entwicklungsmech. d. Org., Vol. 30, pp. 206–234.

Muller, H. J.

'18 Genetic Variability, Twin Hybrids, and Constant Hybrids, in a Case of Balanced Lethal Factors. Genetics, Vol. 3, pp. 422–499.

Pearl, R.

'07 A Biometrical Study of Conjugation in *Paramecium*. Biometrika, Vol. 5, pp. 213–297.

Pearson, K., and Lee, A.

'03 On the Laws of Inheritance in Man. I. The Inheritance of Physical Characters. Biometrika, Vol. 2, pp. 357–462.

'03 Assortative Mating in Man. Biometrika, Vol. 2, pp. 481–498.

STUDIES ON THE CELLS OF CATTLE WITH SPECIAL REFERENCE TO SPERMATOGENESIS, OÖGONIA, AND SEX-DETERMINATION.

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CONTENTS.

I. INTRODUCTION.

Sex-determination with its many attendant problems has always been a subject of great interest to practical animal breeders; and the art of breeding has always been replete with rules by which the sex ratio might be shifted in various ways to the advantage of the breeder. These rules, however, have been founded upon inadequate evidence and unsound reasoning.

The most common beliefs in regard to sex control have from time to time been founded on heat relations, some maintaining that the products of conception in early heat were more often males, others that they were more often females. Pearl and Parshley ('13) have published data on sex-determination in cattle with the following conclusions: (1) That as the time of coitus approaches the end of the œstrous period there is a pro-

[1] This research was supported in part by the Adams Fund of the Idaho Experiment Station.

gressive increase in the proportion of male young born. (2) That in the extreme case this increase in the proportion of male births is probably statistically significant and not to be attributed to errors of random sampling. (3) That these modifications of the sex ratio cannot be attributed to age differences or to any other factor yet suggested.

More recent and extensive data, however, according to Pearl ('17) make the relation of time of service to sex extremely doubtful. He says: "The apparent relation between these two factors, which is believed by many breeders to exist and which our earlier statistics appeared to indicate, seems now to be purely accidental, and to have arisen only because of the comparative meagerness of the statistics on which the matter was discussed." Sex in cattle is a matter of heredity, as is shown by the results of this investigation, and remains a matter beyond the control of the breeder; its ultimate control is problematical. However, the suggestion of experimentally separating the two types of spermatozoa, or destroying the one type without impairing the nature of the other, does not appear to be entirely hopeless. If this can be done, the problem of sex control will be comparatively simple, since artificial insemination can be resorted to.

It appears to be a well-established fact that sex is determined at the time of fertilization. Sex, like other characters of the individual, has a definite factorial basis; and the factorial constitution of the individual with respect to sex as well as to other characters is fixed by the constitution of the two gametes which unite to form the zygote.

The dimorphic condition among the spermatozoa in many of the lower animal forms is well known. Guyer pointed it out in the rat ('10), and man ('10); other investigators have reported it in several other mammals. Among the domestic mammals the same condition has been clearly demonstrated in the pig (Wodsedalek, '13) and in the horse (Wodsedalek, '14). This study on the sex cells of cattle shows that two types of spermatozoa are also produced in the bull; the one type at the time of fertilization determining maleness and the other type determining femaleness. The spermatozoan which determines femaleness is somewhat

larger owing to the presence of a large sex-chromosome or accessory element which is lacking in the type producing maleness. Since it has been found in this study that the oögonia of cattle possess two sex-chromosomes it is safe to conclude, in view of our knowledge of oögenesis in general, that all of the mature ova carry the reduced number or one sex-chromosome. If the ovum is fertilized by a sperm which lacks the sex-chromosome the resulting zygote naturally possess only one sex-chromosome and, therefore, develops into a male. This is evidenced by the fact that the somatic as well as the germ cells of the male possess a single accessory element. On the other hand, if the ovum is fertilized by a sperm possessing the sex-chromosome the resulting zygote possesses two sex-chromosomes and, therefore, develops into a female. The somatic as well as the germ cells of the female possess the two accessory elements which is in exact accord with expectations.

The results presented in this paper were obtained through a most careful and critical study extending over a period of more than four years. The problem was started in the spring of 1915 and every summer since and a great deal of the spare time in the intervening school years, with the exception of the year 1918, has been devoted to it. In the aggregate this means a total of about fifteen months of continuous work. Practically all of the results presented in this paper were at hand at the end of the first two and a half years of study. Over four hundred pencil sketches were made of the various cells, especially those in mitosis, with the aid of the camera lucida and the location of each, as indicated by the mechanical stage, was carefully recorded in a separate booklet. A brief summary of the results of the study was written up and all of the material was put away for a whole year (1918) during which the summer months and other spare time was devoted to the study of the sex cells in another mammal. No effort was made to remember any of the results of the study on the sex cells in cattle.

In the meantime, considerable new cattle material was obtained and hundreds of new slides were made by the departmental technician. At the end of the year the new cattle material was studied and the results were carefully recorded. The original

material was then reinvestigated and new sketches were made of many of the cells, the location of which was recorded before. The results obtained through the study of new slides prepared by the technician, as well as those obtained through the second study of my original material were then compared with the original results. Several hundred different germinal and somatic cells from both sexes were carefully checked up. Especial attention was paid to the number of the ordinary chromosomes and to the sex-chromosomes. Only in a comparatively few cases was there any discrepancy in the interpretation of the nature and number of the ordinary chromosomes. Conditions pertaining to the sex-chromosomes were corroborated in every instance. I was further checked up on my chromosome counts by several of my assistants and senior pre-medical students who had considerable training in microscopic anatomy. In general, these men who knew nothing of my own interpretations, corroborated my counts in a surprisingly large number of instances.

II. Material and Methods.

All of the material used in this investigation with the exception of some ovaries was obtained through the courtesy of the management of the Hagan and Cushing Packing Plant which adjoins the University farm. Some excellent ovarian tissue was given to me by my colleague Dr. A. R. Hahner, formerly professor of veterinary science of the University of Idaho. The ovaries were removed from two five-months-old heifers of the university herd. Testes were obtained from seven mature bulls and one male fœtus of five months and from six smaller fœtuses varying from two to eight and one half inches in length. The ovaries were obtained from four heifers and as many cows, and from five small fœtuses, varying from two and one half to seven inches in length. In addition to this six small embryos were sectioned. Many slides were also made of various somatic structures from the small fœtuses of both sexes.

Several fixing fluids were tried on the testicular material, including Hermann's, Gilson's, Flemming's, and Bouin's. Bouin's fluid used straight or with slight modifications, at 38° C. was the most universally successful fixing agent. When used at some-

what lower temperatures, the material showed little or no modifications. When Bouin's fluid was modified, the alteration took the form of the addition of a small amount of chromic acid, or urea, or the reduction of the percentage of acetic acid. All of these slight modifications gave very good results in the testicular material, and the ovarian, embryological and fœtal tissues appeared to be best when fixed in the fluid modified with chromic acid. The cold method (Hance '17) was also tried in two instances with the testicular tissue but with less success. And while I have not tried this out on the cattle tissue myself, I have every assurance that the laboratory technician carried out the process with great care.

In the study of the male germ cells, smears as well as sections were used. Many stains and counter stains were tried. Iron-hæmatoxylin when used alone was found to be the most satisfactory. All of the figures represented in this paper were made from material stained in this manner.

III. General Arrangement of the Male Germinal Cells.

The structure of the testes of the bull is similar to that of the other well-known mammals and bears a great resemblance to the conditions found in the testes of the horse (Wodsedalek '14). The interstitial cells as in the horse are small and fewer in number in comparison with their large size and great abundance in the testes of the pig. The size of the seminiferous tubules, as well as the general size of the various germinal cells, however, corresponds to the condition found in the pig. The usual types of cells, (1) spermatogonia, (2) primary spermatocytes, (3) secondary spermatocytes, (4) spermatids, and (5) spermatozoa in various stages of development, are present in great abundance.

IV. Spermatogenesis.

In general the spermatogenesis of the bull corresponds to that of the pig and the horse. Since many of the finer cytological points are given in detail in the papers on the pig and the horse (Wodsedalek '13 and '14) they are omitted here to avoid unnecessary duplication. And while all of the finer details involved in a thorough piece of work in spermatogenesis were carefully

studied in this animal, only the phases pretaining to the chromosome numbers and their behavior are emphasized in this paper.

1. *Spermatogonia.*

The spermatogonia usually lie in a single layer next to the wall of the tubule, though occasionally some of the cells are crowded out, thus forming a second layer which is always very irregular. The cells which undergo the last spermatogonial division (Figs. 5–12) are usually beyond the first layer, though occasionally they may be found next to the tubule wall along the entire section of the tubule. At times the cells are far apart, in which case they are flattened out on the tubule wall. The cells also differ considerably in size and appearance, depending on the stage of development they are in.

During the resting stage a large nucleolus is invariably present. As a rule it assumes a somewhat heart-shaped appearance; especially is this true in the larger cells and in those in which the chromosomes begin to form. At the conclusion of the resting stage numerous large chromatin granules appear and arrange themselves along fine threads in an entangled mass. The chromosomes soon become distinct and mitotic figures are fairly numerous. And while, as a rule, there is considerable overlapping and massing of the chromosomes in the early spermatogonial divisions, hundreds of cells were found in which there was little or no overlapping, making accurate counts possible.

Thirty-seven chromosomes appear in the late prophase of the spermatogonial division (Fig. 1). Thirty-six of these are variously shaped, mainly oblong or slightly curved, and differ somewhat in size. One which is much larger is triangular in form or heart-shaped. This is the accessory or sex-chromosome, and is the same thing as the large nucleolus which appears in the resting stages. This point is certain, as the body can be easily traced through the various stages of the cells. A similar condition was reported by Guyer ('10) in man, Wodsedalek in the pig ('13) and in the horse ('14). Several other investigators have reported it in other forms since. During division each chromosome, including the sex-chromosome, divides in two (Figs. 3 and 4).

In the last spermatogonial cells the chromosomes appear in a dense mass. The cells gradually increase in size and great expansion takes place in the nuclei (Figs. 5–9). The chromosomes become decidedly distinct and surprisingly well segregated throughout the spherical nucleus (Figs. 10 and 11). The cells in these stages are numerous and beautiful. The chromosomes appear to be dense in structure and are thicker than those of the early spermatogonial cells. Several of the chromosomes are almost spherical. They are so evenly distributed that hundreds of accurate counts can be made within a short time. This condition prevailed in all of the mature testes studied and the cells were especially numerous in three two-year-old bulls. Even in somewhat stale tissue (Fig. 12) the chromosomes in these cells appear to remain well segregated. Altogether over one thousand accurate counts were made in these cells alone.

2. *Primary Spermatocytes.*

The sex-chromosome can invariably be seen in the spermatocytes where it retains its individuality (Fig. 13). Just how pairing takes place in these cells cannot be stated with certainty. When the chromosomes appear for division they are of the reduced number and bivalent in nature. The thirty-six ordinary chromosomes pair while the sex-chromosome remains unpaired and can easily be distinguished from the others (Figs. 14–18). It occasionally shows its double nature in the late prophase (Fig. 17), and more frequently in the later stages of the primary spermatocyte division (Figs. 22, 24, 25, 27, 28 and 29).

During the primary spermatocyte division the sex-chromosome usually passes to one pole in advance of the other chromosomes (Figs. 19–25). This unequal division of the chromosomes in these cells (Figs. 19–29) gives rise to two different types of secondary spermatocytes. The one type containing the eighteen ordinary chromosomes plus the sex-chromosome. Just before division is complete the chromosomes become loosely paired (Figs. 28 and 29). This peculiar behavior of the chromosomes is apparently quite common in mammalian tissue.

3. *Secondary Spermatocytes.*

No resting stage occurs in the secondary spermatocyte. This condition also occurs in the horse and is frequently found in the pig, according to my former studies. The secondary spermatocytes divide soon after they are formed and not infrequently the spindles are formed in the two cells resulting from the first spermatocyte division while they are still in close contact. Nine chromosomes arrange themselves in the equatorial plate for division in the one type of secondary spermatocyte (Fig. 31), and nine plus the sex-chromosome in the other (Fig. 30). All of the chromosomes, including the sex-element when it is present, divide in these cells (Figs. 32–38).

4. *Spermatids.*

The division of the secondary spermatocyte gives rise in the one case to spermatids containing nine chromosomes (Figs. 36–38), and in the other case nine plus the sex-chromosome or ten (Figs. 33 and 34). All of the chromosomes except the sex-chromosome are bivalent in nature so that in reality we have the equivalent of eighteen chromosomes in the one kind of spermatid and eighteen plus the sex-chromosome in the other. The bivalent chromosomes frequently begin to separate before the division of the cell is complete. Occasionally the eighteen chromosomes can be distinguished as independent elements after the cell divides (Fig. 38), although the chromosomes usually disintegrate before complete separation can be identified. All of the foregoing evidence indicates that eighteen is the reduced number of chromosomes.

V. Dimorphism in the Spermatozoa.

The spermatozoa of the bull vary considerably in size, and careful measurements show that they may be arranged in two separate classes, one type being much larger than the other. Mature specimens, which were free in the lumen of the tubule and parallel to the objective, were selected at random from a single slide and outline sketches of six hundred heads were made with the aid of a camera lucida. The lengths of the sketches were then carefully measured and recorded in quarter millimeters. Figure 1 in the text shows the variation in size of the six hundred

heads measured. It shows a distinct bimodal curve with modes at 12.50 mm., and 14.75 mm. The intermodal depression is

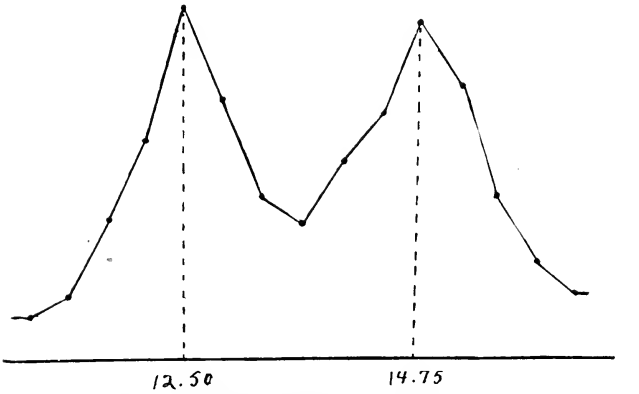


FIG. 1. Diagram showing the variation in size among six hundred mature spermatozoa of the bull.

deep and wide and the two elements of the curve are approximately equal as regards number of individuals. The spermatozoa of the larger type undoubtedly possess the sex-chromosome, while those of the smaller type are without it. Figures 39 and 40 show the comparative size of the two distinct types of mature spermatozoa. They can be distinguished with ease under a high-power microscope.

The general scheme of the development of the spermatozoön from the spermatid of the bull is similar to that in the pig and the horse (Wodsedalek '13 and '14) and, therefore, will not be described here. Bimodal curves were also shown by the writer in connection with the spermatozoa of the pig and the horse, and in a number of other species by Zeleny and Faust ('14 and '15) and by Zeleny and Senay ('15).

VI. Oögonia.

The best ovarian material studied in connection with the number of chromosomes in the oögonia was obtained from some of the small fœtuses. The results obtained from this material

were corroborated in the tissue obtained from cows and heifers, although the adult tissue was not nearly as satisfactory. Most excellent material was found in the ovaries of a four and a half inch fœtus and one six-inch fœtus. These were fixed in the Bouin's plus chromic acid fixing fluid. The oögonia were apparently in extreme activity at the time of fixation. Mitotic figures are abundant and the chromosomes are very distinct, especially in the late prophase and early metaphase stages which are numerous (Figs. 44–46). In general these cells resemble the last spermatogonial cells (compare Figs. 44 and 10), except that the oögonia are somewhat larger.

In well fixed and favorably stained material the oögonia in the resting stage invariably show two large nucleoli each corresponding to the single nucleolus of the resting stage of spermatogonial cells (Fig. 41). These retain their individuality during the spireme stage though at times they are somewhat distended (Fig. 42). When the spireme breaks up the chromosomes are long and narrow and variously curved (Fig. 43). In the late prophase they become shorter and thicker and appear evenly distributed throughout the large nuclei (Figs. 44 and 45). In this stage as well as in the early metaphase stages of division (Fig. 46) hundreds of accurate counts were possible. The two sex-chromosomes can be easily distinguished in all of the prophase stages (Figs. 41–45).

Thirty-eight chromosomes are present in the oögonia (Figs. 43–46). Thirty-six are the ordinary chromosomes corresponding to the thirty-six ordinary chromosomes in the spermatogonia of the male. The two other elements are the sex-chromosomes. When the chromosomes arrange themselves in the equatorial plate for division the sex-chromosomes are always at the periphery (Figs. 46 and 47). During division all of the chromosomes, including the sex-elements, divide in two (Fig. 48). All of the figures of the oögonial cells were made from the same section (Figs. 41–48).

On account of the great significance of the two sex-chromosomes in the femal tissue a tremenduous amount of time was devoted to this particular phase of the problem; this was also true of the studies in connection with the chromosomes of the

somatic tissue in both sexes. The final preparation of the paper for publication was postponed on several occasions, not at all because of any uncertainties, since the results were convincing from the start, but rather because each additional survey of the entire problem from new material proved more fascinating and gratifying than those preceding.

VII. Chromosomes in Somatic Cells.

Numerous slides were made of various somatic structures from fœtuses of both sexes. The organs most frequently used were the brain, lung, liver, Wolffian body, kidney, and intestine. The larger embryos were cut into pieces all of which were then sectioned. Three small embryos, ranging from ten to fourteen millimeter neck-lengths were sectioned in toto. While splendid mitotic figures and late prophase stages were found in many parts of the embryos the very best cells were found in the brain. In the larger specimens the liver and kidneys showed the most favorable cells.

The male somatic cells, like the spermatogonia, contain thirty-seven chromosomes, of which thirty-six are the ordinary chromosomes and one is the accessory element or sex-chromosome (Figs. 51 and 52). The female somatic cells, like the oögonia, contain thirty-eight chromosomes, of which thirty-six are the ordinary chromosomes and the other two are the sex-elements (Figs. 49 and 50). The sex-chromosomes in each case were as distinguishable as they are in the germinal cells. Literally thousands of somatic cells were carefully studied in each sex.

After the chromosomes of the first one hundred of the most favorable male somatic cells were studied and carefully sketched it was found that in ninety-three cases there were thirty-six of the ordinary chromosomes present plus the one accessory. In the other seven cases there were slight discrepancies, usually one or two less. This was in all probability due to unnoticeable overlapping. In two cases there were two extra chromosomes present. This was in all probability due to the fact that two of the chromosomes had divided, since these cells were in the early metaphase stage. This interpretation appears to be correct, since it was found later that occasionally some of the chromo-

somes divide considerably in advance of the others. This point was observed in several of the early metaphase stages; it was fairly common in polar views of late metaphase stages, and in a few instances a chromosome was found almost completely split in two even in the late prophase. For this reason, in selecting cells for accurate chromosome counts great care must be exercised not to select polar views of late metaphase stages. The increase in number may also be due to fragmentation, although one would expect this only in poorly fixed material or general poor technique.

After the counts in the first one hundred camera-lucida sketches showed that over ninety per cent. of the cells contained thirty-seven chromosomes, no more sketches were made. However, hundreds of other counts were made from favorable cells off-hand with about the same results. This shows that in at least ninety per cent. of the male somatic cells thirty-six ordinary chromosomes, and a single sex-chromosome were present. The ten per cent. of discrepancies is undoubtedly due to unnatural states and probable sources of error. The studies of the chromosomes in the somatic cells of the female were conducted in the same manner. Among the first one hundred cells, ninety-one showed thirty-six ordinary chromosomes and two accessories. In several hundred further counts, the percentage of discrepancies was about the same.

In the case of the six small embryos in which sex could not be determined morphologically, or was uncertain, one was poorly fixed, although it was cut into small pieces, and, therefore, could not be used in this study. Of the other five two were unquestionably female and three were male, according to evidences from the cytological standpoint. In the case of the two specimens, cells in various parts of the body repeatedly showed thirty-six ordinary chromosomes and two accessories. In many instances where the ordinary chromosomes could not be counted, the two sex chromosomes were recognizable. In the other three specimens the cells invariably showed only a single sex-chromosome, and in many cases the thirty-six ordinary chromosomes were counted.

These extensive studies indicate quite conclusively that thirty-six is the number of ordinary chromosomes in the somatic cells of both sexes and that the male cells contain one sex-chromosome

while the female cells contain two, making a total of thirty-seven in the male and thirty-eight in the female. These numbers correspond exactly with those of the spermatogonia in the male and the oögonia in the female. This is very significant in relation to our chromosome theory of sex-determination.

VIII. Sex-Chromosomes in Relation to Sex-determination.

It was shown that in the process of spermatogenesis two distinct types of spermatozoa are produced of exactly the same number. The one type contains eighteen ordinary chromosomes plus one sex-chromosome, and the other type contains only the eighteen ordinary chromosomes; this being the result of the unequal primary spermatocyte division, where the eighteen bivalent chromosomes divide and the unpaired sex-chromosome passes over to one pole undivided. In the oögonia there are thirty-six ordinary chromosomes plus two sex-chromosomes. Before the reduction division of the primary oöcytes, in all probability (though this was not actually determined in this animal), all of the chromosomes, including the two sex-chromosomes, pair. This eventually gives rise to ova all of which contain the reduced number of chromosomes or eighteen ordinary chromosomes plus one sex-chromosome.

Since the two types of spermatozoa are produced in equal numbers, fertilization by the one kind or the other is equally possible, and the number of male and female calves born is about equal if a fairly large number of offspring is considered. Sex in the offspring, as determined at the time of fertilization of the ovum by the one or the other type of spermatozoön may be illustrated as follows:

Spermatozoa.	Ova.	Offspring.
(18 + 1)	+ (18 + 1)	= (36 + 2) = female,
(18 + 0)	+ (18 + 1)	= (36 + 1) = male.

The results of the above combinations are in exact accord with the number of chromosomes found in the germinal and somatic cells of the two sexes in cattle; and the relation of the sex-chromosomes to sex-determination can not be doubted. All of the five hundred or more so-called theories or rules for con-

trolling sex, including the one in relation to time of service which is commonly practiced by animal breeders, must be abandoned. There is considerable literature on the metabolic theories of sex-determination which will not be discussed here. However, reference may be made to the brief though able discussion of this subject by Babcock and Clausen ('18).

IX. Sex Ratio in Cattle.

It might be well to quote here Pearl's ('17) more recent results of extensive studies on the control of the sex ratio in cattle. He says: "Some earlier statistics appeared to indicate that there was a possibility of influencing the sex ratio by paying attention to this point. It was believed to be of such extreme importance as to justify the careful study of the matter on the basis of much more extended statistics. These statistics we have now collected and analyzed and shall publish as soon as possible. In the meantime it may be reported that, with the more extended statistics in hand, it appears to be conclusively established that there is no definite or permanent relation between the time in the heat period at which the cow is served and the sex of the offspring. The apparent relation between these two factors, which is believed by many breeders to exist and which our earlier statistics appeared to indicate, seems now to be purely accidental, and to have arisen only because of the comparative meagerness of the statistics on which the matter was discussed.

Table II.

Showing the Sex of the Calves following Service at Different Parts of the Heat Period.

Heat Period.	Lapsed Time in Hours from Appearance of Heat to Service.	Sex of Offspring.		Per Cent. of Males.
		Males.	Females.	
Early.....	Under 3 hours	200	192	51.0
Middle.....	Over 3 and under 8 hours	270	252	51.7
Late.....	Over 8 hours.....	187	212	46.9
Totals.....		657	656	50.0

"The summarized results of 1,313 separate and distinct matings given in Table II. will demonstrate this point. In each one of these 1,313 cases the following facts were accurately known, and

reported in such a way that any bias, conscious, or unconscious, of the observer could not have influenced the result: (*a*) the time in hours from the first appearance of heat (œstrum), as noticed by the breeder, to the time the cow was successfully served; (*b*) the sex of the calf resulting from this service.

"It is evident from this table that there is no significant preponderance of females when service is early in heat. There is not now known any method by which the sex ratio or proportion of the sexes in cattle may be effectively controlled by the breeder. A more detailed account of the results, together with further statistics will be published elsewhere."

X. Sex-limited Inheritance in Cattle.

The discovery of the remarkable behavior of certain characters in heredity which can only be plausibly explained by supposing that they are linked with a sex-chromosome or a sex-determining factor still further strengthens our belief in the existence of such a definite factor. Wentworth ('16) reported a case which seems to fall under this general sex-limited group in the inheritance of black-and-white in Ayrshire cattle. While the general breed color is red-and-white, black-and-white animals have been known for some time, as shown by Kuhlman ('15). It is difficult to state whether the black is due to a true black pigment or whether it is simply a very dense red, since chemical solutions have not yet been attempted.

In summarizing the results of the different crosses, Wentworth says, "If the factor of the black-and-white color is represented by B, the hereditary constitutions are as follows: BB is always black-and-white; bb is always red-and-white; Bb is always black-and-white in the male and red-and-white in the female. All of the nine possible matings were discovered, as shown in Table I.

"The expectations here presented are based on the most probable result of each of the matings, considered on an individual basis with reference to the number of animals produced by each type of mating, but without figuring the proportions of the sexes as equal. From these data it would appear that the black-and-white color of Ayrshire cattle behaves in an ordinary

sex-limited manner similar to the horns in sheep as discussed by Wood ('05) and the rudimentary mammæ in swine as reported by Wentworth ('16)."

TABLE I.

RESULTS OF NINE POSSIBLE MATINGS OF AYRSHIRE CATTLE.

Sires.	Dams.	Male Offspring.		Female Offspring.	
		Black-and-white.	Red-and-white.	Black-and-white.	Red-and-white.
BB.....	BB.....	1	0	3	0
BB.....	Bb.....	0	0	0	1
BB.....	bb.....	10	0	0	10
Bb.....	BB.....	3	0	2	1
Bb.....	Bb.....	1	0	1	0
Bb.....	bb.....	4	5	0	4
bb.....	BB.....	0	0	0	3
bb.....	Bb.....	2	1	0	2
bb.....	bb.....	0	7	0	9
Total.....	.....	21	13	6	30
Expected....	.....	20.75	13.25	5.25	30.75

The simple Mendelian scheme of inheritance is quite common in cattle but, to my knowledge, this is the only case of sex-limited inheritance reported in this animal; nevertheless, it is significant, especially in view of our knowledge of the relation between this scheme of inheritance and the sex-chromosomes in many other species.

XI. THE FREE-MARTIN.

The case of the free-martin, the female of two-sexed twins in cattle, is well known to animal breeders to be perfectly sterile although rarely such females are perfectly normal. I do not wish to enter upon a discussion of this subject here for it is a big problem in itself. However, I wish to call the attention of animal husbandmen, who are not in position to keep in touch with all of the zoölogical literature, to the extensive and most painstaking piece of research on the free-martin by Dr. Frank R. Lillie ('17). In this connection I might also call attention to his article on sex-determination and sex-differentiation in mammals (Lillie, '17).

XII. SUMMARY.

1. Thirty-seven chromosomes occur in the spermatogonia. One, the sex-chromosome, is distinctly larger than the others.

2. Nineteen chromosomes appear in the primary spermatocyte division, of which eighteen are bivalent and the other is the unpaired sex-chromosome.

3. In the primary spermatocyte division the heart-shaped sex-chromosome passes undivided to one pole in advance of the other chromosomes.

4. The primary spermatocyte division is evidently the reduction division, giving rise to two different types of secondary spermatocytes, one with the sex-chromosome and the other lacking it.

5. The one type of secondary spermatocyte, which contains the sex-chromosome, gives rise to two spermatids, each containing the sex-chromosome and eighteen ordinary chromosomes.

6. The other type of secondary spermatocyte, which lacks the sex-chromosome, gives rise to two spermatids, each containing only the eighteen ordinary chromosomes.

7. The mature spermatozoa are of two types, equal in numbers. The one type is larger and contains the sex-chromosome. The smaller type is without the sex-chromosome. The larger type is female producing, while the smaller is male producing.

8. Thirty-eight chromosomes occur in the oögonia; two of these are the sex-chromosomes.

9. The reduced number of chromosomes in the female is, in all probability, eighteen ordinary chromosomes and one sex-chromosome which apparently occurs in all of the mature ova.

10. The somatic cells of the male contain thirty-six ordinary chromosomes and one sex-chromosome.

11. The somatic cells of the female contain thirty-six ordinary chromosomes and two sex-chromosomes.

12. The number of chromosomes in the somatic cells of the two sexes is in exact accord with expectations.

13. There is no relation between the time in the heat period at which the cow is served and the sex of the offspring.

14. Sex in cattle, for the present, remains a matter beyond the control of the breeder. It is determined by the sex-chromosomes; it is a matter of inheritance.

15. Sex-limited inheritance strengthens the belief in the chromosome theory of sex-determination.

LITERATURE CITED.

Allen, Ezra.

'18 Studies on Cell Division in the Albino Rat (*Mus norwegicus albinus*). Jour. Morph., Vol. 31, No. 1.

Babcock and Clausen.

'18 Genetics in Relation to Agriculture. New York.

Guyer, M. F.

'10 Accessory Chromosomes in Man. BIOL. BULL., Vol. XIX., No. 4.

Hance, R. T.

'17 The Diploid Chromosome Complexes of the Pig (*Sus scrofa*) and their Variations. Jour. Morph., Vol. 30, No. 1.

Kingery, H. M.

'17 Oögenesis in the White Mouse. Jour. Morph., Vol. 30, No. 1.

Kuhlman, A. H.

'15 Black and White Ayrshires. Jour. Heredity, Vol. 6, No. 7.

Lillie, F. R.

'17 The Free-Martin; a Study of the Action of Sex Hormones in the Fœtal Life of Cattle. Jour. Exp. Zoöl., Vol. 23, No. 2.

'17 Sex-Determination and Sex-Differentiation in Mammals. Proc. Nat. Acad. Sci., Vol. 3, pp. 464–370.

Pearl, R. and Parshley, H. M.

'13 Data on Sex Determination in Cattle. BIOL. BULL., Vol. 24, No. 4.

Pearl, R.

'17 Maine Agricultural Experiment Station Bulletin, Bulletin No. 261, Part 3, The Control of the Sex Ratio, pp. 130–131.

Pratt, B. H. and Long, J. A.

'17 The Period of Synapsis in the Egg of the White Rat, *Mus Norwegicus Albinus*. Jour. Morph., Vol. 29, No. 2.

Wentworth, E. N.

'16 Rudimentary Mammæ in Swine a Sex-Limited Character. Science, N. S., Vol. XLIII., No. 1114.

'16 A Sex-Limited Color in Ayrshire Cattle. Jour. Agr. Research, Vol. VI., No. 4.

Wodsedalek, J. E

'13 Spermatogenesis of the Pig, with Special Reference to the Accessory Chromosomes. BIOL. BULL., Vol. XXV., No. 1.

'14 Spermatogenesis of the Horse with Special Reference to the Accessory Chromosome and the Chromatoid Body. BIOL. BULL., Vol. XXVII., No. 6.

'16 Causes of Sterility in the Mule. BIOL. BULL., Vol. XXX. No. 1.

Wood, T. B.

'05 Note on the Inheritance of Horns and Face Color in Sheep. Jour. Agr. Sci., V. 1, pt. 3.

Zeleny, C. and Faust, E. C.

'14 Size Differences in Spermatozoa from Single Testes. Science, N. S., Vol. 39, No. 1003.

'15a Size Dimorphism in the Spermatozoa from Single Testes. Jour. Exp. Zoöl., Vol. 18.

'15b Dimorphism in Size of Spermatozoa and its Relation to the Chromosomes Proc. Nat. Acad. Sc., Vol. 1.

Zeleny, C. and Senay, C. T.

'15 Variation in Head Length of Spermatozoa in Seven Additional Species of Insects. Jour. Exp. Zoöl., Vol. 19, No. 4.

EXPLANATION OF PLATES.

Plate 1.

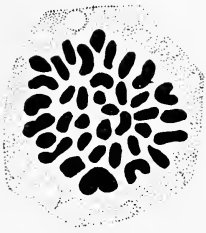
(All of the drawings were made with the aid of a camera lucida, × 2,400, except Figs. 39 and 40 which are × 2,200.)

Fig. 1. Polar view of the metaphase of division in a spermatogonial cell showing thirty-six ordinary chromosomes and the single heart-shaped sex-chromosome.

Fig. 2. Side view of the metaphase of division in a spermatogonial cell showing the thirty-six ordinary chromosomes, and the sex-chromosome at the left.

Figs. 3 and 4. Late anaphases of division of spermatogonial cells showing the division of all of the chromosomes, including the large sex-chromosome.

Figs. 5–9. Late prophase stages of the last spermatogonial cells.



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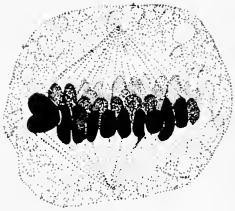

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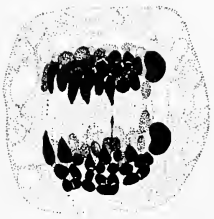

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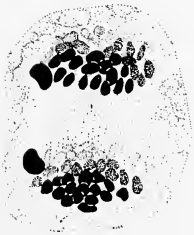

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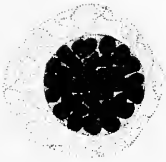

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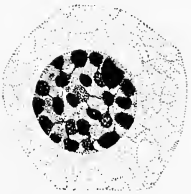

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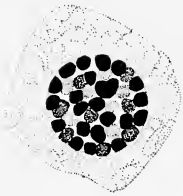

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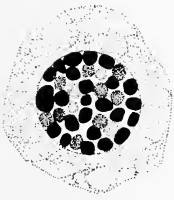

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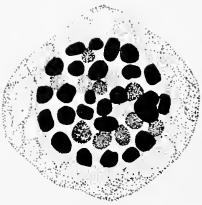

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J. F. WODSEDALEK.

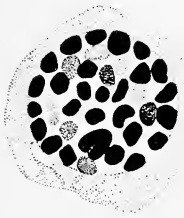
PLATE II.

FIGS. 10 AND 11. Last spermatogonial cells showing thirty-six ordinary chromosomes and the large sex-chromosome.

FIG. 12. Last spermatogonial cell taken from stale tissue showing the persistence of the segregation of the chromosomes in this stage.

FIG. 13. Spireme stage of the primary spermatocyte showing the large sex-chromosome.

FIGS. 14–18. Polar views of metaphase stages of division of the primary spermatocytes showing eighteen bivalent chromosomes and the sex-chromosome which is usually at the periphery of the plate.



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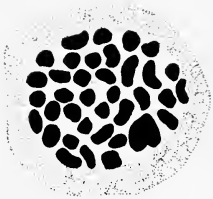

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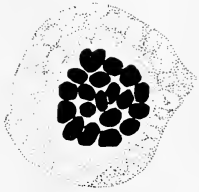

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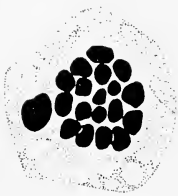

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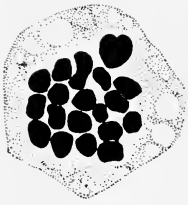

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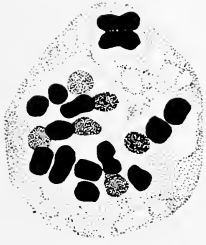

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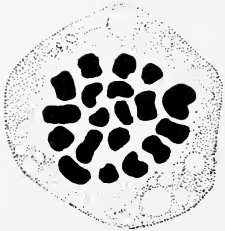

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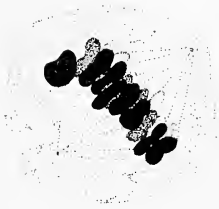
PLATE III.

FIGS. 19–25. Metaphase stages of division of primary spermatocytes showing the passing of the sex-chromosome to one pole in advance of the other chromosomes.

FIGS. 26 AND 27. Late anaphase stages of division of primary spermatocytes showing eighteen chromosomes at one pole and eighteen plus the sex-chromosome at the other.



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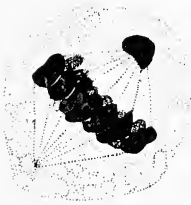

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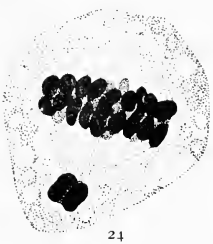

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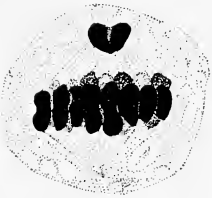

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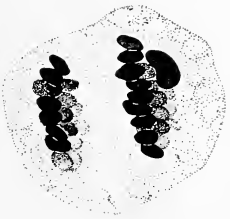

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Plate IV.

Figs. 28 and 29. Cells showing the formation of the secondary spermatocytes from the primary spermatocyte division. The eighteen chromosomes pair loosely to form nine bivalents which are present in the one type of secondary spermatocyte and in the other type are shown the nine bivalents and the sex-chromosome.

Fig. 30. Metaphase stage of division of the one type of secondary spermatocyte showing the nine bivalent chromosomes and the sex-chromosome.

Fig. 31. Metaphase stage of division of the other type of secondary spermatocyte showing only the nine bivalent chromosomes.

Figs. 32–34. Division stages of the secondary spermatocyte with the sex-chromosome showing the division of all of the chromosomes, including the sex-element, giving rise to spermatids both of which contain the sex-chromosome. When the division of the cell is complete (Fig. 34) each of the nine ordinary chromosome splits into two so that in reality there are eighteen ordinary chromosomes plus the sex-chromosome in this type of spermatid.

Figs. 35–37. Division stages of the secondary spermatocyte without the sex-chromosome. Figure 37 shows the splitting up of the nine chromosomes at the poles so that there are in reality eighteen chromosomes passed on to the other type of spermatid.

Fig. 38. A newly formed spermatid showing the eighteen chromosomes.

Fig. 39. A mature spermatozoan of the smaller type undoubtedly without the sex-chromosome.

Fig. 40. A mature spermatozoan of the larger type which undoubtedly contains the sex-chromosome.

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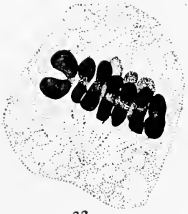

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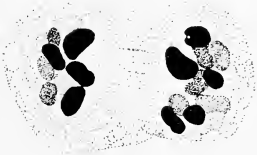

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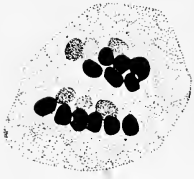

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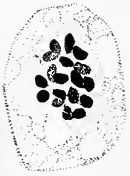

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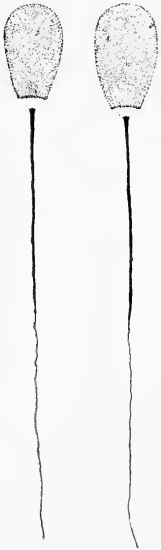

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PLATE V.

FIG. 41. Resting stage of an oögonial cell showing two large nucleoli which are undoubtedly the sex-chromosomes.

FIG. 42. Spireme stage of an oögonial cell showing the two large sex-chromosomes.

FIG. 43. An oögonial cell showing thirty-six newly formed chromosomes and the two large sex-chromosomes.

FIGS. 44 AND 45. Late prophase stages of oögonial cells showing thirty-six ordinary chromosomes and the two sex-chromosomes.

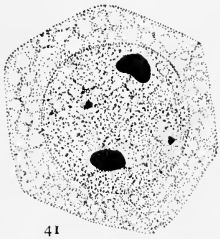
FIGS. 46 AND 47. Metaphase stages of division of the oögonia showing the two sex-chromosomes at the periphery of the plate.

FIG. 49. Liver cell in late prophase stage taken from a female foetus showing thirty-six ordinary chromosomes and the two sex-hromosomes.

FIG. 50. Brain cell in metaphase stage of division taken from a female embryo showing thirty-six ordinary chromosomes and the two sex-chromosomes.

FIG. 51. Brain cell in metaphase stage of division taken from a male embryo showing thirty-six ordinary chromosomes and only one sex-chromosome.

FIG. 52. Liver cell in late prophase stage taken from a male foetus showing thirty-six ordinary chromosomes and only one sex-chromosome.



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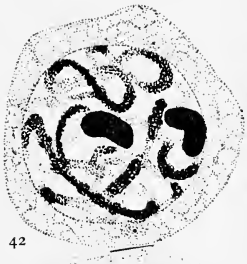

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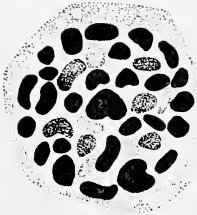

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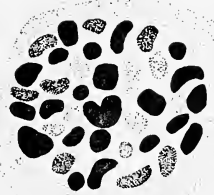

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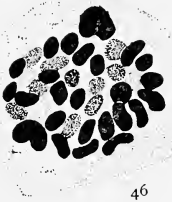

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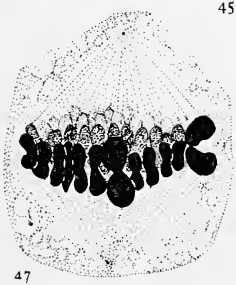

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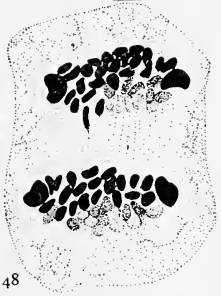

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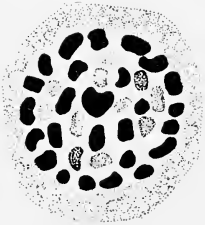

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STUDIES IN ARTIFICIAL PARTHENOGENESIS. III. CORTICAL CHANGE AND THE INITIATION OF MATURATION IN THE EGG OF CUMINGIA.[1]

L. V. HEILBRUNN

This study is a record of experiments performed during the summer of 1916 at the Woods Hole Marine Biological Laboratory.

As Morgan pointed out in 1910, the egg of the lamellibranch *Cumingia* is very suitable for study. Like most other eggs it is still immature when shed into the sea-water. Although the first maturation spindle has formed, no polar bodies are thrown off unless the egg is fertilized or treated with the proper reagents. Doubtless some change is necessary before the egg can throw off polar bodies and begin its development.

An effort has been made to determine the nature of this change. Many diverse reagents cause the egg to mature. Although all of these reagents do not occasion the same morphological transformations nevertheless all of them agree in having one specific physical effect on the egg. All release the egg cytoplasm from the restraint of a rigid enveloping membrane. The immature unfertilized egg is surrounded by a stiff vitelline membrane which presses tightly in on it and effectively prevents the throwing off of polar bodies. It is only when the egg is released from this restraint that maturation can proceed.

Physical Make-up of the Egg.

As in *Arbacia*, the *Cumingia* egg is a mass of fluid protoplasm, surrounded by a rigid membrane. Only a few turns of the centrifuge are sufficient to throw to opposite poles of the egg the substances suspended in the cytoplasm. To one pole pass the presumably lighter oil globules, to the opposite pole the heavier pigment. But these suspended particles can go no farther than the poles, for there they are stopped by the vitelline membrane which surrounds the egg. This is a stiff structure and is easily

[1] Contribution from the Zoölogical Laboratory, University of Michigan, New Series, no. 3.

visible under higher power. It is about one micron in thickness. The vitelline membrane must be thought of as the plasma membrane of the egg. As I have pointed out before, students of cellular mechanics have been blind to the fact that egg cells are provided with visible membranes which are plasma-membranes. They have often insisted that no one has ever seen a plasma-membrane. It is easy to prove that the vitelline mem-membrane of the *Cumingia* egg governs osmotic intercourse and is therefore a plasma-membrane.

When *Cumingia* eggs are put into hypertonic solutions they shrink only very slightly. The stiffness of the vitelline plasma-membrane prevents a marked shrinkage. Moreover, a weakly hypertonic solution produces just as much shrinkage as a strong one provided that it does not alter the membrane. On the other hand if the hypertonic solution makes the membrane less rigid by causing it to swell, then the egg shrinks to a great extent. Facts such as these can be interpreted only on the assumption of a stiff plasma-membrane. That this is the vitelline membrane is certain, for there is no other membrane in the vicinity. Certainly there is none inside the vitelline membrane, for when the egg has been left for some time in a hypertonic solution of considerable strength then the coagulated cytoplasm shrinks away from the vitelline membrane and presents a rough uneven surface. Under these conditions it is obviously not surrounded by any membrane.

As to the chemical nature of the plasma-membrane it is essentially protein. It swells in dilute acids or alkalies, and in sodium chloride or sodium iodide solutions. Moreover it does not contain any large admixture of lipoid as can be shown by testing it with Scharlach R solution.

Like many other marine eggs the Cumingia egg is surrounded by a diffuse jelly of the same refractive index as sea-water and therefore invisible. It can easily be demonstrated by various vital stains (*e.g.*, Nile blue sulphate) or by India ink suspensions. This jelly has no apparent effect on the early developmental phenomena. If the eggs are shaken a few times in a test-tube, they are deprived of their jelly. Such eggs react in the same way as those with jelly intact.

As in *Arbacia* the *Cumingia* vitelline membrane is capable of two kinds of cortical change. Both membrane elevation and membrane swelling can occur. If the egg is placed in solutions of low surface tension the tension of the membrane is lowered and it rapidly lifts away from the egg. No doubt the explanation of this process is the same as that I have offered for the similar process in the sea-urchin egg. But in *Cumingia* membrane elevation is not the normal cortical change. When eggs are inseminated the membrane does not become elevated, it becomes swollen.

Treatment of eggs with a reagent which causes either membrane elevation or membrane swelling will result in a throwing off of polar bodies. Thus just as in the sea-urchin egg either swelling or elevation provides the necessary cortical change, although in this egg it is swelling and not elevation which is normal. Finally there is a third way in which maturation can be initiated. This consists in the removal of the membrane.

I shall now proceed to the experimental data considering first membrane elevation, second membrane swelling, and third the removal of the membrane.

Membrane Elevation.

Any substance which markedly lowers the surface tension of sea-water is effective in producing membrane elevation. But if elevation is to be followed by maturation only certain concentrations and certain lengths of exposure can be used in each case. Too high a concentration or too long an exposure generally leads to coagulation. Frequently an over-exposure produces a rupture of the egg membrane, the protoplasm then flows out to form an exovate. Such eggs soon disintegrate.

Numerous reagents were used to produce a lowering of surface tension. Of course the number of successful reagents could have been increased many fold. Thus many of the higher alcohols no doubt act in the same way as the amyl alcohol which I used.

In the experiments the same general proceedure was always employed. In every case, the eggs of only a single female were used. Owing to the fact that the number of eggs obtainable from a single female is comparatively small, I found it best to

perform all of the experiments in low Stender dishes (containing about 50 cc.) instead of in larger fingerbowls. These dishes were very convenient, for they could without any trouble be put on the stage of the microscope. One Stender dish was made to contain the desired reagent and then the eggs were pipetted into it. After varying lengths of exposure the eggs were transferred from the reagent to fresh sea-water also contained in Stender dishes. Then after twenty minutes or more had elapsed the treated eggs were examined.

As the polar bodies of Cumingia are unusually large it is possible to count the percentage of matured eggs under low power and without removing the eggs to a slide. Counts were usually made in this way. Obviously it is not possible to see the polar bodies if the egg is lying with the animal pole down. Hence the per cent. recorded is always too low. Even if all the eggs had polar bodies not many more than fifty per cent. would show them. If the eggs are turned about this difficulty can of course be obviated, but turning is a tedious process and was only occasionally resorted to. Thus the *counts recorded represent minima* and are really only about half as high as they should be. Counts are given in the form of fractions, in which the denominator represents the total number of eggs counted the numerator the number of eggs with polar bodies. For example the fraction 10/50 would indicate that out of 50 eggs counted 10 showed a polar body.

In making up per cent. solutions of volatile liquids, it was found convenient to use 100 c.c. measuring flasks. Thus if a 2 per cent. ether solution was desired 2 c.c. of ether was placed in a 100 c.c. measuring flask and sea-water was added until the solution reached the 100 c.c. mark. Owing to the diminution in volume on mixing the two liquids such a solution is not exactly a 2 per cent. one. However, after the above method of procedure it is easier to calculate the molecular concentration.

The accompanying table gives the results of experiments with eleven substances which lower surface tension. For each substance used it was necessary first to determine the proper concentration. Sometimes this was a simple matter. Thus for saponin almost any concentration is successful. But usually only a narrow range of concentrations will produce the desired

effect. If the reagent is just a little too strong it can not be effectively employed. In many cases only a certain length of exposure is suitable. Too long exposures generally produce exovates. Formation of exovates is represented by the symbol "e" in the table.

With ether and chloroform it is particularly difficult to obtain maturation. In both instances I at first despaired of success. A 3 per cent. solution of ether in sea-water did not produce maturation. With such a solution exovates generally appeared. On the other hand 2.5 per cent. ether had no observable effect on the eggs. With a concentration intermediate between 2.5 per cent. and 3 per cent. success was attained. In the table are given the figures for a representative experiment with ether.

In the case of chloroform the range of effective solutions is even narrower. In making up chloroform solutions very small quantities of the liquid had to be measured out. This was done by counting the drops from a small pipette which was calibrated for the purpose. About 60 drops from this pipette constituted 1 c.c. By placing 8 such drops into a 100 c.c. measuring flask and diluting to the mark a 0.13 per cent. solution was obtained. This solution produced but little effect upon the eggs. Exposures of 3–5 minutes showed only 1–2 per cent. of polar body formation. Thus a 0.13 per cent. solution was apparently too weak. On the other hand, a 0.17 per cent. solution proved too strong. Such a solution was prepared by diluting 15 drops of chloroform (from the pipette mentioned above) up to 100 c.c. with sea-water. A 0.25 per cent. solution was thus obtained and this was then diluted to 0.17 per cent. by adding 5 c.c. of sea-water to 10 c.c. of the solution. The resultant 0.17 per cent. solution was found to be too strong, for it produced exovates. Moreover viscosity tests with the centrifuge showed that it coagulated the egg cytoplasm. Although neither 0.13 per cent. nor 0.17 per cent. chloroform produced maturation, a concentration slightly under 0.17 per cent. did. This solution was made by diluting 10 drops from the pipette up to 100 c.c. Such a solution would ordinarily give 0.17 per cent., but one particular solution was made on an extremely hot day (when the room temperature was 27°). No doubt the drops from the pipette were smaller, owing to the

lower surface tension. Hence the concentration which resulted was slightly under 0.17 per cent. In the table I have referred to this solution as "0.16 per cent." As the figures show, it proved highly effective.

TABLE I.

POLAR BODY FORMATION AFTER TREATMENT WITH SUBSTANCES WHICH LOWER SURFACE TENSION.

Exposure Minutes.	1 Per Cent. Amyl Alcohol.	2.5 Per Cent. Ethyl Acetate.	0.25 Per Cent. Ethyl Butyrate.	0.5 Per Cent. Ethyl Nitrate.	2.8 Per Cent. Ether.	'0.16 Per Cent.' Chloroform.	5 Per Cent. Acetonitrile.	Saturated Solution Phenyl Urethane.	0.2 Per Cent. Chloretone.	Emulsion[1] Toluol.	0.2 Per Cent. Saponin.
1	0		0	0	0		0		29		12
4	50		50	50	100		100		100		50
1	0	0	0	0	0	13	2	0	18	3	20
2	50	50	50	50	100	50	50	100	100	100	50
1	1	0	0	2	1	29	12	0	0	10	33
	50	50	50	50	100	50	50	100	100	100	50
2	12	4	22	14	6	e	e	0	0	e	
	25	50	100	100	100			100	100		
3	12	10	e	e	15	e	e	0	0	e	33
	25	50			100			100	100		50
4	31	13	e	e	22	e	e	0		e	18
	50	50			100			100			50
5		19^2	e	e	18	e	e	1			20
		50			100			100			50
6	14	12, 23^2	e					2			.
	25	50, 50						100			
7			e	e	15	e	e			e	19
					100						50
8		23						5			
		50						100			
10	3	16	e	e	5	e	e	9			
	25	50			100			100			
15								43			
								200			

All of the substances mentioned in the table produced a similar morphological change in the egg. All of them caused the vitelline membrane to become lifted away from the cytoplasm. The details of this membrane elevation were watched in a great many different experiments. The membrane first detached itself from the egg at a number of points around the periphery. As a result it appeared slightly thicker and for a minute or two it often

[1] This emulsion was made by stirring 1 c.c. of toluol with 3 c.c. or sea water.

[2] Eggs were shaken while counting. As mentioned above this gives a higher count.

showed small outbulgings which gave it a more or less crenate appearance. Very soon, the membrane lifted away with considerable rapidity. If success was to be obtained the egg had to be removed from the solution at just about the time the crenations appeared.

As in *Arbacia* when the vitelline membrane is lifted from the egg surface, a new membrane, no doubt a precipitation membrane, is immediately formed about the egg cytoplasm. Sometimes it is possible to cause this to become elevated also. Saponin produces the best results. When eggs are exposed for 40 minutes to 0.2 per cent. saponin they are found to be surrounded by two membranes. The outer one of these is quite far from the egg and it was thought at first that it might represent the outer edge of the jelly which had become visible. But this was shown not to be true, for two membranes could be produced about eggs from which the jelly had previously been shaken off.

I have used the eggs of *Cumingia* for a number of years and in some years I have found a tendency for a small per cent. of the eggs to mature without apparently any treatment. In the experiments recorded in this paper careful controls were always kept. In every case at least two hundred eggs of the untreated control were examined for polar body formation. Of the experiments cited in the table, in only one instance did the control show any maturation. In the control for the ethyl nitrate experiment one egg showed a polar body out of considerably over two hundred examined.

The table shows that eleven substances can produce polar body formation and this is in every case preceeded by membrane elevation. These eleven substances differ from each other very widely in chemical constitution. It is almost inconceivable that they should have any one chemical effect in common. Their action must be primarily physical. It is believed to involve a lowering of surface tension. The explanation which I have offered for membrane elevation in *Arbacia* applies equally well for *Cumingia*. For details of this explanation the reader is referred to my earlier papers.

Of course by choosing substances similar to those listed in the table numerous other successful reagents could no doubt be

discovered. Thus it is probable that benzol or xylol would behave as toluol, and that many other alcohols and esters could have been added to those given in the table. Such experiments would scarcely add material for the general argument.

On the other hand it might be thought that the eleven substances in the table represent only a few out of the many that I have tried. It perhaps not infrequently happens that experimenters suppress the record of their failures. But in these experiments practically every substance selected because of its effect on surface tension gave the expected result. There were only three exceptions and in two of these cases I performed only a single experiment with a single concentration of the reagent. Moreover in each of these three cases the reagent employed had some secondary effect on the egg. I shall consider each case in detail.

Ethyl Urethane.—3 per cent. ethyl urethane had practically no effect, although in longer exposures (5–7 minutes) a few eggs with polar bodies were observed. The reagent has some action on the jelly that I have not analyzed. Possibly it is a shrinkage effect. Two minutes after the egg entered the solution it was surrounded by queer looking bubbles. Fifteen minutes later the bubbles had disappeared and their place had apparently been taken by a zone of radiating lines.

Nitromethane.—I used a 5 per cent. solution of nitromethane and exposed the eggs to it for intervals of from $\frac{1}{2}$–10 minutes. No polar bodies were produced as a result of the treatment. Exposure for 4 minutes followed by transfer to normal sea-water resulted in rupture of the vitelline membrane and disintegration of the egg. The eggs left in the nitromethane solution showed a queer transformation. They lost their spherical shape and flattened out into discs resembling huge red blood corpuscles. In addition to this queer effect the reagent also appeared to have some action on the jelly, for some morphological changes were visible around the egg. The nature of these changes I did not stop to investigate.

Acetone.—Two concentrations of acetone were tried. 25 per cent. acetone did not produce membrane elevation when eggs were exposed 1–11 minutes. 50 per cent. acetone produced

membrane elevation, but not polar body formation. Of the eggs exposed ½ minute, 72 per cent. had widely elevated membranes. These eggs, however, could not produce polar bodies for they were thoroughly coagulated. This was shown by a viscosity test with the centrifuge.

Membrane Swelling.

If instead of being lifted off, the vitelline membrane is made to swell, much the same effect is produced on the egg. The increased fluidity of the vitelline membrane results in a lower surface tension.[1] Consequently it no longer exerts as great a pressure upon the egg contents. Thus maturation follows membrane swelling just as it follows membrane elevation.

In order to produce a swelling of the membrane the same reagents were used that were previously found to have been effective for the sea-urchin egg. Evidently the vitelline membranes of both *Cumingia* and *Arbacia* are similar, for they swell under the same conditions.

Sodium Iodide.—Eggs were exposed to 0.6 M sodium iodide. After 15 and 22½ minutes they were removed to sea-water in Stender dishes A and B respectively. Of the eggs in A, 14/100 showed polar bodies. The eggs in B formed no polar bodies. The sodium iodide solution caused membrane swelling.

Hydrochloric Acid.—Eggs were placed in 25 c.c. of sea-water plus 0.7 c.c. *n*/10 HCl. After exposures of ½, 1, 2, 3, 4, 5, 7, 10 minutes the eggs were removed from the acid solution and placed in ordinary sea-water in Stender dishes A–H respectively. In the acidified sea-water the egg membrane swelled and the surface of the egg became sticky. Often the eggs adhered to the bottom of the dish. Counts of eggs with polar bodies gave the following results:

A	½ minute exposure	6/50
B	1	5/50
C	1	10/50
D	3	4/50 (This count was made too early)
E	4	13/50
F	5	15/50
G	7	10/50
H	10	8/50

[1] Many biologists apparently do not understand that solids and pseudo-solids (*i.e.*, gels) exhibit surface tension. This tension is greater for a gel than for the corresponding sol. For references to literature on this subject consult Heilbrunn '15, footnote, p. 166.

The eggs which were allowed to remain in the acid sea-water also formed a few polar-bodies.

Potassium Hydroxide.—Eggs were placed in 40 c.c. of sea-water plus 1 c.c. $n/10$ KOH. In this solution membrane swelling occurred. The eggs formed polar bodies while in the alkaline medium. The first polar body observed was noted after 16 minutes exposure. After 33 minutes, a count gave 14/100 with polar bodies.

Potassium Cyanide.—Eggs were placed in a 0.04 per cent. KCN solution, made by diluting 5 c.c. of 2 per cent. KCN up to 250 c.c. with sea-water. During the experiment the cyanide was not allowed to evaporate, for it was kept in a tightly stoppered weighing-tube. After a 36 minute exposure a count showed 28/100 of the eggs with polar bodies. The potassium cyanide caused membrane swelling probably because of its alkaline reaction.

Hypertonic Sodium Chloride Solution.—In the experiments with acids and alkalies and with sodium iodide the solutions used were approximately isotonic with sea-water. In the course of some other work it was noticed that solutions made by adding $2\frac{1}{2}$ M NaCl to sea-water caused membrane swelling. Hence it was expected that these solutions would also cause polar body formation. Eggs were exposed for 7 minutes to a solution made by adding 5 c.c. of $2\frac{1}{2}$ M NaCl to 25 c.c. of sea-water. As a result 21/100 of the eggs formed polar bodies.

To sum up, in all of these experiments the swelling of the vitelline membrane was in every case followed by the throwing-off of polar bodies in a large percentage of the eggs. The controls of untreated eggs did not form polar bodies. In these experiments with reagents which cause membrane swelling there were no failures. No reagent could be discovered which would produce swelling of the vitelline membrane without at the same time causing the eggs to mature.

Rupture or Removal of the Membrane.

There is a third way in which the Cumingia egg may be freed from the binding pressure of its vitelline membrane. If the eggs are shaken vigorously, oftentimes a certain percentage of the

membranes will be shaken off. Apparently the membrane ruptures at the animal pole of the egg and owing to its elasticity it shrinks away toward the vegetal pole. In many cases its wrinkled remains can be found at this pole. No doubt after the membrane is shaken off a new precipitation membrane forms about the cytoplasm but this is very much less rigid than the original vitelline membrane. The results obtained from shaking *Cumingia* eggs are somewhat variable. The eggs must be shaken sufficiently to rupture or remove the membrane, but they must not be shaken too vigorously or too long. Too much shaking interferes with the mitotic processes underlying maturation and a smaller percentage of polar bodies results. This is in accordance with the observation of Wilson ('01) that shaking prevents cell division.

A number of shaking experiments were performed. In one of the best of these, the eggs were placed in a small 10 c.c. test tube and shaken vigorously by swinging the forearm from a vertical to a horizontal position. They were given 40 such swings in 10 seconds. When these shaken eggs were examined an hour and a half later it was found that 27/56 had polar bodies. Thus practically all of them had matured, for as pointed out in the first part of this paper, the counts represent minima. That the shaking process had actually resulted in a removal of the membrane could be demonstrated in three ways. In the first place the remains of the membrane could often be seen at the vegetal pole. Secondly, when the polar bodies formed they did not appear to be within a stiff membrane as in polar body formation after fertilization. Lastly some shaken eggs were placed in a drop of acetone, of these only 3 out of 50 showed membrane elevation. When normal unshaken eggs were similarly treated with acetone all of them showed an elevated membrane.

Finally there is still another method of freeing the egg from the restraint of its membrane. Although this method produces practically the same results as shaking, the procedure is very different. It was found that in diluted sea-water rupture of the vitelline membrane occurred and as was to be expected, maturation followed. Obviously in diluted sea-water the osmotic pres-

sure forces water to enter the eggs and the membrane bursts. In many instances the ruptured membrane could be seen at one side of the egg. Sometimes exovates were produced. Various dilutions were employed, from pure distilled water to a mixture of 1 part of distilled water to 2 parts of sea-water. In one experiment eggs were exposed to distilled water for 40, 60 and 80 seconds and then returned to sea-water. All three exposures were successful. The 40 second exposure was not counted, the 60 second exposure showed 51/100 polar bodies and the 80 second exposure 52/100 polar bodies. The controls were normal and over 200 eggs which were counted showed no signs of maturation. All the exposures showed some signs of cleavage. One egg reached a stage with about 16 cells. In another experiment eggs were placed into 30 c.c. of sea-water plus 15 c.c. of distilled water. Of these eggs 13/50 showed polar bodies.

The Significance of Cortical Change.

It has been shown that three types of cortical change can be produced in the *Cumingia* egg. All of these free the egg from the restraint of a stiff vitelline membrane. This release from restraint may then be thought of as the essential feature of cortical change and as the direct cause of maturation. When it occurs maturation follows; without it no maturation takes place.

It might be argued that all of the types of cortical change have some other common effect besides the one mentioned. Perhaps they all directly produce an increase of oxidations, which then causes maturation to follow. This would be a difficult relation to conceive of chemically. Moreover there is experimental evidence that maturation does not depend on an increase of oxidations.

The supporters of the oxidation theory of initiation of development have always held that dilute cyanide solutions check oxidations. Thus Loeb, '13, states on p. 26: "It has long been known that the oxidations in the cell can be prevented by the addition of a little potassium cyanide, even when oxygen is present. I have found that the addition of 0.5 c.c. of a 1/20 per cent. KCN solution to 50 c.c. of sea-water is sufficient to stop almost immediately the effect of the spermatozoön in the fertilized sea-urchin egg." Such a solution is 0.0005 per cent.

Some *Cumingia* eggs were placed in a 0.04 per cent. solution of potassium cyanide, five minutes after they had been fertilized. The solution was prepared by diluting 5 c.c. of 2 per cent. KCN up to 250 c.c. with sea-water. Solution and eggs were kept in a glass-stoppered weighing-tube to guard against evaporation of the cyanide. Under these conditions practically all of the fertilized eggs formed the first polar body. Actual count without turning over the eggs, showed 24/50.

On the other hand it might be thought that all types of cortical change produce an increase of permeability. In recent years various observers have claimed that the sea-urchin egg undergoes an increase in permeability either after fertilization or after artificial membrane elevation. These observers have endeavored to show: (1) An increased penetration of dyes, (2) a drop in electrical resistance, (3) a more rapid passage of water into or out of the cell.

When fertilized sea-urchin eggs are placed in dilute solutions of methylene blue, they stain more rapidly than do unfertilized eggs, according to Lyon and Shackell, '10. Runnström, '11, obtained similar results with methylene blue although not with neutral red. The experiments of Lyon and Shackell are frequently cited and are always taken to indicate an increased permeability after fertilization.[1] When unfertilized and fertilized *Cumingia* eggs are placed in dilute solutions of methylene blue or neutral red, the unfertilized eggs take up the dye just as rapidly as do the fertilized eggs.

Fertilized and unfertilized eggs were put into Syracuse dishes containing methylene blue solutions of various strengths. From time to time the eggs were examined over a light and over a dark background, and under the microscope. The color of faintly stained eggs can be much better observed with the naked eye than with the microscope. A more accurate method of determination would involve the use of a colorimeter but the method used was sufficient to show that no marked increase of permeability occurred. The dilutions of the dye were made up from a 0.5 per cent. solution of Grübler's "Methylenblau rectif. nach

[1] They might however indicate nothing more than an increased affinity for dyes on the part of the cytoplasm. Such changes in staining properties are common enough, especially after changes in the colloidal state.

Ehrlich." Five minutes after fertilization some fertilized eggs were placed in dishes A2 to E2 containing 0.1 per cent., 0.05 per cent., 0.025 per cent., 0.0125 per cent., 0.00625 per cent. methylene blue respectively. At the same time some unfertilized eggs were placed in dishes A1 to E1 containing similar concentrations of the dye. After ten minutes had elapsed, the eggs in A1 and A2 were navy blue, those in B1 and B2 pale blue, those in C1 and C2 scarcely colored and those in D1 and D2, E1 and E2 not colored at all. Obviously there was no difference between the two sets of eggs. After fifteen minutes eggs in A1 and A2 were navy blue, those in B1 and B2 light navy blue, those in C1 and C2 light blue, the unfertilized eggs in D1 were a very pale blue, the fertilized eggs in D2 uncolored, in E1 and E2 eggs were still uncolored. The eggs were observed at various times, but no marked changes could be observed. After an hour I thought I might be able to detect a slightly deeper color in the fertilized eggs in A2, B2, C2 than in the unfertilized eggs subjected to the same concentrations of dye in A1, B1, C1, but this was probably due to the fact that the fertilized eggs were slightly more numerous. If entrance of stain is a test of permeability, then certainly there is no sharp difference in the permeability of fertilized and unfertilized *Cumingia* eggs. The concentrations of stain used in the above experiment were not injurious. Even in the most concentrated of the solutions used the eggs proceeded in their development and became motile larvæ. A similar experiment was tried with neutral red. A saturated solution of the stain and 1/2, 1/4, 1/8, 1/16 saturated solutions were used. In no case could any difference in permeability between fertilized and unfertilized eggs be noted.

McClendon, '10, and Gray, '16, have maintained that following fertilization there is a drop in the electrical resistance of the sea-urchin egg. They have interpreted this as indicating an increase of permeability, although of course various other explanations might be given. In the sea-urchin egg the normal process of cortical change is membrane elevation, which would interfere with the experiment. McClendon and Gray therefore were obliged either to wait until the eggs lost their power of undergoing membrane elevation or to so treat the eggs that they lost

this power, before they could make their measurements. In the *Cumingia* egg electrical measurements would be easier inasmuch as the normal cortical change is not membrane elevation.

But the following point should be noted. In any measurements of the electrical resistance of masses of egg cells, it is rather doubtful if one is measuring the resistance of the cells at all. If one conceives of a piled-up mass of spheres resting in a liquid, it is obvious after a moment's consideration that the liquid is broadly continuous from one side of the mass to the other. Thus water flows readily through a pile of shot. If now the spheres are poor conductors as compared to the liquid, and an electric current is sent through the mass, it will flow almost exclusively through the liquid. The resistance then depends on the size and shape of the interspaces between the spheres. In the case of egg cells this may vary in several ways. In the sea-urchin egg it is certain that after fertilization the interspaces between individual eggs in a mass are greater than those before fertilization, for it has been shown (cf. Heilbrunn, '15) that after fertilization the eggs offer much more resistance to compression and hence tend to preserve their spherical shape. Thus after fertilization one might expect the resistance of a mass of eggs to be lower even though the electric current did not pass through the eggs at all.

In the last few years R. S. Lillie ('16, '17) has shown that when fertilized or activated eggs are placed in hypotonic solutions, water enters them more rapidly than it does unfertilized eggs. Similarly in hypertonic solutions (R. S. Lillie, '18) water leaves the fertilized eggs more readily. This is due according to Lillie to an increased permeability of the plasma membrane to water. Lillie's reasoning is a bit difficult to follow. Originally he believed in an increased permeability of the membrane to salts and dissolved substances. This would of course decrease the speed of entrance of water from hypotonic solutions, or the speed of exit to hypertonic solutions, for it would decrease the osmotic pressure upon which the exit or entrance of the water depends. Osmotic pressure is, as everyone knows, dependent upon the impermeability of a membrane to dissolved substances. Increase in permeability to salts would therefore produce the opposite

effect from increased permeability to water. Apparently there is a dilemma.

As a matter of fact it appears to be rather far-fetched to assume a change in permeability to water, since we know the plasma membrane to be at all times permeable to it. R. Lillie's results can be much more simply explained on the basis of my conception of the plasma membrane (see Heilbrunn, '15). Normally before fertilization it is a more or less rigid structure and as such resists the entrance or exit of water from the cell. I showed by measurement ('15, pp. 155–158) that when the membrane was made less rigid as a result of membrane swelling then water left the cell more readily. After fertilization the plasma membrane either itself becomes less rigid, as when membrane swelling occurs, or it is replaced by a less rigid membrane as a result of membrane elevation. Hence water enters and leaves the cell more rapidly.

It is easy enough to decide between R. Lillie's interpretation and mine. If the difference is simply one of relative permeability to water, then in hypotonic or hypertonic solutions the water should enter or leave the fertilized eggs more rapidly, but the final equilibrium point should be the same for both fertilized and unfertilized eggs. However on the basis of my view, not only should the water enter and leave the eggs more rapidly, but the actual equilibrium state should be altered. In hypertonic solutions, more water should leave the fertilized eggs and in hypotonic solutions more water should enter them. In the case of hypotonic solutions Lillie's own figures seem to show that at equilibrium more water has entered the fertilized eggs than the unfertilized.[1]

[1] Lillie's measurements were made on the egg of the sea-urchin *Arbacia*. In this egg the presence of the elevated vitelline membrane or fertilization membrane introduces a complication. As is well known this membrane is a stiff structure. When fertilized sea-urchin eggs are subjected to hypotonic solutions they increase in size rapidly until they reach the elevated membrane. Then further increase in diameter is dependent on the power of the eggs to stretch or rupture the membrane. R. S. Lillie does not state which occurs. As a matter of fact these experiments on endosmosis were done in September when the *Arbacia* season is practically over. At this time the normal membrane elevation is difficult to obtain, and usually, unless the sperm concentration falls within certain very narrow limits, the membrane swells at fertilization. For eggs with swollen membranes the experiment is uncomplicated, as in *Cumingia*.

My experiments with *Cumingia* eggs show clearly that after fertilization not only do the eggs swell more rapidly in hypotonic solutions but their total imbitition of water is greater.

Five minutes after fertilization some eggs were placed in 25 c.c. sea-water plus 15 c.c. distilled water. At the same time some unfertilized eggs were placed in a similar solution. Both sets of eggs were measured after about 45 minutes. By this time the eggs had reached an osmotic equilibrium for a second set of measurements 45 minutes later showed no further change. The results are given in Table II.

TABLE II.

DIAMETERS OF FERTILIZED AND UNFERTILIZED EGGS IN 25 C.C. SEA-WATER + 15 C.C. DISTILLED WATER.

Average Diameter of 12 Unfertilized Eggs in Sea-water 62.65 μ.

Fertilized Eggs After 40–45 Min.	Fertilized Eggs After 85–90 Min.	Unfertilized Eggs After 47–55 Min.	Unfertilized Eggs After 92–100 Min.
μ	μ	μ	μ
70.4	69.2	66.9	67.5
69.2	69.8	66.9	67.5
73.9 × 66.9	68.7	66.9	66.9
71.0 × 68.1	69.2	68.1	66.4
69.8 × 66.9	69.2	66.9	68.1
69.8	71.5 × 63.6	68.1	67.5
69.2 × 68.7	71.5	67.5	66.9
68.1	70.4	66.9	67.5
71.0 × 67.5	69.2	66.4	68.7
70.4	70.4	68.1	68.1
72.7	69.8	68.7	67.5
70.4	70.4 × 68.1	68.1	66.9
69.2			
Average, 69.76	69.53	67.46	67.46

Measurements were made with a Spencer movable scale micrometer at a magnification of about 650 diameters. Not all the fertilized eggs remained spherical. For such eggs the longest and shortest diameters are recorded on the table. In computing the averages the mean of these two measurements was taken. It is obvious from the table that a greater amount of water entered the fertilized eggs. This can not be explained by assuming an increased permeability to water.

It should be noted that the vitelline membranes of the eggs in this experiment did not rupture as a result of being placed in the hypotonic solution. Such a rupture sometimes occurs in solutions of this strength.

In hypertonic solutions fertilized *Cumingia* eggs lose more water than do the unfertilized. The water does not merely leave the eggs more rapidly, more of it passes out and the osmotic equilibrium is different in the two cases. This is shown by an experiment in which fertilized and unfertilized eggs were placed in solutions prepared by adding 1 part of 2 *M* $MgSO_4$ to 2 parts of sea-water. Measurements were made as in the previous experiment. The results are given in Table III.

TABLE III.

DIAMETERS OF FERTILIZED AND UNFERTILIZED EGGS IN 30 C.C. 2M $MgSO_4$ + 60 C.C. SEA-WATER.

Average Diameter of 10 Unfertilized Eggs in Sea-water 61.74 μ.

Fertilized Eggs After 13–23 Min.	Fertilized Eggs After 163–172 Min.	Unfertilized Eggs After 27–33 Min.	Unfertilized Eggs After 143–161 Min.
μ	μ	μ	μ
57.7	51.9 × 58.3	59.4	60.6
53.7 × 58.9	57.7	60.0	60.0
56.5	43.3	59.4 (58.3)	61.2 (56.0)
57.7	42.7	61.7	58.9 (53.7)
57.1 × 58.3	57.7	60.0 (53.7)	60.6 (56.1)
57.1 (51.9)	56.5	59.4	58.9
56.5	57.1	59.4	59.4
59.5 × 54.2	53.7	60.6	57.7
56.0	53.1	61.2	58.9
55.4	49.0	59.4	59.4
	54.8	58.9 × 61.2	59.4
Average, 56.78	52.79	60.05	59.55

The above table requires a little explanation. Some of the eggs, usually the unfertilized ones, became slightly flattened at one pole while in the hypertonic solution. These eggs therefore had a slightly smaller volume than their diameter would indicate. In order to show the extent of the flattening a second measurement was taken from the flattened pole to the opposite pole of the egg. This second measurement is in every case shown in parentheses. Another point also needs explanation. The fertilized eggs measured in the second column had lost so much water in the hypertonic solution that their cytoplasm was coagulated and had in most cases begun to shrink away from the vitelline membrane. This shrinkage was most pronounced in the third and fourth eggs measured and this accounts for the very small size of these eggs.

The table shows clearly that more water leaves the fertilized than the unfertilized eggs. This can not be due to an increased permeability to water for an increase of this sort could produce no such effect. It must be due to a loss in the rigidity of the plasma membrane. In order to make this relation clear, I shall quote from p. 154 of the second paper of this series: "The plasma-membrane of the *Arbacia* egg is a protein gel. As such it possesses a certain degree of rigidity. Suppose a hypothetical system completely surrounded by an extremely rigid semi-permeable membrane. If such a system were placed in a concentrated solution no exosmosis could take place, for if the membrane were perfectly rigid, there could be no removal of solvent from the system without the production of a vacuum. But the membrane would be subjected to a considerable pressure which would tend to make it rearrange its particles in such a fashion that the volume enclosed within it might be lessened. Whereas an extremely rigid membrane would resist such forces one with only a certain degree of rigidity would yield (in the case of sufficient pressure) and exosmosis would be possible. Thus osmosis in an enclosed system depends to some extent at least on the rigidity of the confining membrane. These conclusions apply in some measure to the sea-urchin egg, for the vitelline membrane possesses a slight degree of rigidity." They apply even more directly to the Cumingia egg, for its vitelline membrane, which is also its plasma membrane, is stiffer than that of *Arbacia*. Any loss in the rigidity of this membrane favors either endosmosis or exosmosis. That is why the fertilized eggs of *Cumingia* take up more water from hypotonic solutions and lose more water to hypertonic sloutions than do the unfertilized eggs.

Osmotic change in unfertilized eggs is fairly rapid and no one can deny that the plasma membrane is permeable to water. If then R. S. Lillie's measurements are correct and water enters fertilized eggs more rapidly than unfertilized eggs in hypotonic solutions and leaves them more rapidly in hypertonic solutions than it seems certain that the egg plasma membrane has not markedly increased its permeability to dissolved substances as a result of fertilization. For such an increase in permeability,

by diminishing the osmotic pressure, would slow osmotic interchange and if sufficiently great, would prevent it altogether.

All these points show clearly that the permeability theory of fertilization and artificial parthenogenesis rests on rather doubtful evidence. In *Cumingia* certainly, there are no facts which support it.

Discussion.

Bataillon ('12) in discussing the relations between artificial parthenogenesis in amphibia and sea-urchins, states, "Il n'y a pas une parthénogenèse expérimentale des Oursins et une des Amphibiens. Ce sont des matériaux différents chez lesquels le rythme des cinèses est suspendu et peut être rétabli. S'il y a une Biologie générale, les conditions de l'arrêt ont quelque chose de commun, et les conditions de la mise en branle doivent être comparable." Presumably this is true. The essential factors underlying stimulation to development are very probably alike for every sort of artificial parthenogenesis. It is certain, however, that the subsidiary features of the process are different in each case. It is necessary therefore to study each egg individually, to determine exactly its physical make-up, and to attempt to discover what changes are significant in producing an initiation of development. This is what I tried to do in the case of *Arbacia*.

F. R. Lillie ('19) in referring to my explanation of the process of cortical change in *Arbacia* points out that this explanation "can hardly apply to other cases where the cortical changes present a different morphological form." This is true and I never intended that it should. As a matter of fact, I clearly recognized that even in the one egg there were two distinct types of cortical change which might be produced by spermatozoa. The explanation which F. R. Lillie cites was only advanced to cover one of them. At the same time I offered a different explanation for the other.

F. R. Lillie also objects to my considering cortical change as "a mere epiphenomenon . . . the phenomenon of the primary cortical change is too general to be treated in this fashion and its character in different animal groups is too varied for it to be a mere phenomenon of decrease of surface tension." I must point

out that I have always realized the importance of cortical change. On page 183 of my 1915 paper I stated in a section devoted to the significance of cortical change, that "cortical change, whether it be membrane swelling or elevation, always results in the removal of this obstacle [*i.e.*, a stiff membrane]. The vitelline membrane is either rendered soft by swelling or it is lifted away from the egg surface and its place taken by the no doubt less rigid hyaline layer." I showed moreover that "at least two processes which play a part in normal development would be greatly hindered if some kind of cortical change did not occur."

My results with *Cumingia* fully bear out this point of view. Cortical change in *Cumingia* may take the form of a membrane elevation dependent on a sharp decrease in surface tension. It may more simply be just a membrane swelling. In both cases the result is the same. The egg is freed from an obstacle which impedes development. This in *Cumingia* as in *Arbacia* is the restraining influence of a stiff vitelline membrane. In both eggs the same forces are involved in cortical change. The essential features of the process and the effect on further development are as closely alike as they could possibly be in the two cases.

It should be noted that cortical change in *Cumingia* is not ordinarily followed by segmentation. As in *Arbacia* cell-division in *Cumingia* is preceded by a sharp increase in the viscosity of the cytoplasm. This can be demonstrated by tests with the centrifuge. A number of such tests were made and the relation established beyond a doubt.

Summary.

1. The *Cumingia* egg is surrounded by a stiff vitelline membrane which tightly encloses the fluid cytoplasm.

2. A release from the restraint of this membrane is followed by maturation.

3. Such a release from restraint can be accomplished in three ways; by membrane elevation, by membrane swelling, or by the removal or rupture of the membrane.

4. Substances which themselves have low surface tension produce a lowered surface tension of the membrane and this results in its elevation from the egg surface.

5. Acids, alkalies, and certain salt solutions cause the vitelline membrane to swell.

6. The membrane may be removed from the eggs by shaking, or it may be caused to rupture by immersion in dilute sea-water.

7. All of the above mentioned treatments produce polar-body formation. All of them free the egg from restraint.

8. Maturation in *Cumingia* is not dependent on an increase in oxidations.

9. Cortical change in *Cumingia* produces no increase in permeability either to dissolved substances or to water.

10. The essential features of cortical change in *Cumingia* are the same as those previously shown for Arbacia.

BIBLIOGRAPHY.

Bataillon, E.

'12 La parthénogenèse des amphibiens et la "fécondation chimique" de Loeb (étude analytique). Ann. des Sci. Nat. Zool., Ser. 9, T. 16, pp. 249–307.

Gray, J.

'16 The Electrical Conductivity of Echinoderm Eggs, and its Bearing on the Problems of Fertilization and Artificial Parthenogenesis. Phil. Trans. Roy. Soc., Series B, Vol. 207, pp. 481–529.

Heilbrunn, L. V.

'13 Studies in Artificial Parthenogenesis. I. Membrane Elevation in the *Arbacia* Egg. BIOL. BULL., Vol. 24, pp. 343–361.

'15 Studies in Artificial Parthenogenesis. II. Physical Changes in the Egg of *Arbacia*. BIOL. BULL., Vol. 29, pp. 149–203.

'17 Maturation and Initiation of Development in *Cumingia*. Proc. Amer. Assn. Anat. Anat., Rec., Vol. 11, pp. 362–363.

Lillie, F. R.

'19 Problems of Fertilization. Chicago: The University of Chicago Press.

Lillie, R. S.

'16 Increase of Permeability to Water Following Normal and Artificial Activation in Sea-Urchin Eggs. Am. Journ. of Physiol., Vol. 40, pp. 249–66.

'17 The Conditions Determining the Rate of Entrance of Water into Fertilized and Unfertilized Arbacia Eggs, and the General Relation of Permeability to Activation. Ibid., Vol. 43, pp. 43–57.

'18 The Increase of Permeability to Water in Fertilized Sea-Urchin Eggs and the Influence of Cyanide and Anesthetics upon this Change. Ibid., Vol. 45, pp. 406–30.

Loeb, J.

'13 Artificial Parthenogenesis and Fertilization. Chicago: The University of Chicago Press.

Lyon, E. P. and Shackell, L. F.

'10 On the Increased Permeability of Sea-Urchin Eggs Following Fertilization. Science, N. S., Vol. 32, pp. 249–251.

McClendon, J. G.

'10 On the Dynamics of Cell Division. II. Changes in Permeability of Developing Eggs to Electrolytes. Am. Journ. of Physiol., Vol. 27, pp. 240–75.

Morgan, T. H.

'10 Cytological Studies of Centrifuged Eggs. Journ. Exp. Zoöl., Vol. 9, pp. 593–655.

Runnström, J.

'11 Untersuchungen über die Permeabilität des Seeigeleies für Farbstoffe. I. Arkiv för Zoologi, Bd. 7, No. 13, pp. 1–17.

Wilson, E. B.

'01 Experimental Studies in Cytology. II. Some Phenomena of Fertilization and Cell-division in Etherized Eggs. III. The Effect on Cleavage of Artificial Obliteration of the 1st Cleavage Furrow. Arch. f. Entwicklungsmech., Bd. 13, pp. 353–95.

A CONTRIBUTION TO THE LIFE HISTORY OF *AMŒBA PROTEUS* LEIDY.[1]

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It is only within the last few years that a connected life history of *Amœba proteus* has begun to be formulated. This does not seem strange, even in view of its common use in laboratories, since the difficulties attendant upon the study of the life cycle of such an elusive form, are numerous.

The isolated observations upon the various activities of *Amœba*, the accounts of suppositious appendages, and the descriptions of supposedly new species or varieties, are numerous. The fact that so much observational material upon so many apparently nearly related forms has accumulated, and that so many diverse accounts of the habits of one and the same form have been given, has led some investigators to suspect that perhaps much of this material might be combined to throw light upon the life history of possibly some one or two valid species in whose life cycles some of the forms heretofore described might prove to be merely developmental stages. This suspicion has been strengthened recently by the fact that some of the *Rhizopoda* closely allied to the *Amœba proteus*, whose life cycles are now known, show both widely varying forms and habits during different stages in their growth and reproduction.

Investigations of the life history of *Amœba proteus* which have hitherto been made make it possible to generalize, to some extent, upon the course of some of the changes which take place during its development. There seem to be several modes of reproduction in this form, and several intermediate stages between young and adult before the life cycle is completed. In this communication an attempt is made; first, to review briefly what we know of the life history of *Amœba proteus;* second, to

[1] Grateful acknowledgment is here made of the aid of Professor H. D. Reed, of the department of zoölogy, Cornell University.

describe an apparently hitherto unknown method of reproduction; and third to record some observations upon the development and growth of the forms produced by this new method.

The first observer to suggest that another method of reproduction other than simple fission might obtain among the *Amœba* was Varter ('56) who recorded the presence of numerous fine granules which he observed filling the bodies of some *Amœba radiosa*. These granules he looked upon as fragments of the nucleus, and since, when they escaped from the body, they moved with a curious jerky motion, he inferred that they must be provided with flagella, and termed them spermatozoids. The observations extended no farther. Again in 1863 he observed the granulation of the nucleus in *Amœba princeps*, noting that the original nucleus divided several times and gave rise to many smaller nuclei, which he termed, though apparently with meager foundation, reproductive cells.

Wallich ('63) noted that when *Amœba villosa* "died," the nucleus, which had divided several times, escaped from the body surrounded by bits of protoplasm, of globular form. He observes that the fate of these globules is unknown to him, but believing that they might function in some reproductive process, termed them scaroblasts. Referring to the many different species of *Amœba* which appeared in his cultures, he writes (with reference particularly to *Amœba princeps*, *diffluens*, and *radiosa*): "It will, I think, eventually be found that these are mere transitory phases of one and the same species." Later, he observes with greater conviction: ". . . though not prepared to affirm that the whole of the varieties of *Amœbæ* are reducible to a single, primary, specific type, I candidly confess that the balance of the evidence appears to me to point to such a conclusion, and to indicate that the divergence in the form and outward characters may be wholly dependent on the local and even temporary conditions of the medium in which the young animal happens to make its appearance in the world."

It is to Scheel ('99) that we owe our first complete knowledge of a type of reproduction different from the familiar method of binary fission. In 1899 he described a process of reproduction in *Amœba proteus* which he termed *schizogony*, after a somewhat

similar mode of multiplication occurring among the *Sporozoa*. As it occurs in *Amœba proteus* its course is as follows: The individual encysts; the nucleus divides into several smaller nuclei; these migrate to the periphery of the cell; the protoplasm of the cell divides into as many equal portions as there are nuclei; the cyst wall ruptures; and the nucleus-containing bits of protoplasm emerge, each a complete *Amœba* and an epitome of its parent. In analogy with the spores of the *Sporozoa* these were given the name, pseudopodiospores. More recently Calkins ('05) has noted a method of reproduction in which the nucleus produced by repeated division, gametic nuclei, whose fusion resulted in other nuclei which became the nuclei of the smaller individuals after the manner of the pseudopodiospores just mentioned. The young *Amœbæ* soon assumed the form hitherto called *Amœba radiosa;* passed through this stage and became the common *Amœba proteus*.

Another apparent mode of reproduction was observed by Metcalf ('10). In this method the observer is of the opinion that the sequence of events is: Mature individuals produce globular masses of protoplasm, which are termed gemmules. These develop flagella and assume a cercomonadoid form; later they fuse by two and two, lose the flagella, and develop into adult *Amœbæ*. It is stated that possibly the life cycle of *Amœba proteus* may require a year or more for its completion, and may exhibit during its course three, or even more, modes of reproduction.

Schepotieff ('08, '09) states: "All of these examples suggest that in the case of the *Amœba* [*proteus*] the developmental cycle may be completed in very different ways."

It has been shown, as we have said, that *Amœba radiosa*, formerly regarded as a distinct species, is merely a stage in the development of *Amœba proteus*. It may be that this also is true of such recognized species of *Amœba* as *villosa*, *princeps*, *diffluens*, etc. It is suggested elsewhere in this paper that the species known as *Amœba guttula* is but a developmental stage in the cycle of *proteus*. Vahlkampf ('05) has shown that *Amœba limax*, at least, is to be regarded as a distinct species.

Preparation of Cultures.

The material from which the *Amœbæ* herein described were reared was obtained from a pool in a cattail marsh, in about two feet of water among decaying lily pads and *ceratophyllum*. The thin glutinous deposit upon the bottom and especially on the lily pads which had fallen to the bottom was found to be rich in *Amœba proteus*. In the laboratory the material was distributed into several battery jar aquaria after having been filtered through cheese cloth to remove the larger creatures.

An immediate examination of the material showed that beside the active *proteus*, *radiosa*, *guttula*, and *limax*, there were many encysted protozoa. One encysted form which appeared in large numbers, and which confusingly resembles some of the smaller encysted *Amœbæ* was *Vorticella microstoma* (Fig. 4). Under ordinary magnification its distinguishing feature, the crescentric nucleus, is not visible. With the 1.8 mm. objective it becomes faintly discernible, but it is best seen, and the cyst is indubitably identified apparently only when stained. Methyl green and iodine gave the best results.[1]

After some weeks had elapsed *proteus* and *radiosa* appeared in large numbers. The material was now transferred to a dozen small petri dishes and kept in a constant temperature of about 75 degrees Fahr. After a space of a fortnight there was begun the transfer of inoculation of *Amœba proteus* to 4 cm. stender dishes, furnished with straw infusion or oak leaf infusion, and free from all protozoan forms. The infusions were prepared by boiling the straw or leaves for several hours, and decanting off the dark brown liquor, to be diluted to the optimum strength. A slimy scum formed upon the surface of the infusions after a few days time, which when stirred up and caused to sink to the bottom furnished a nutritive substance upon which the *Amœbæ* throve.

From the stender dishes individual *Amœbæ* were removed from time to time and kept in shallow cells sunk in slides of unusual thickness. The slides employed were furnished with a device

[1] Methyl green stain: Saturated alcoholic solution methyl green, 3 parts; 2 per cent. aqueous solution acetic acid, 1 part; water 3 parts. Iodine stain: saturated alcoholic solution iodine, 1 part; water, 2 parts. For various protozoan stains, see Hausman, "Fresh Water and Marine Gymnostominan Infusoria" (in press).

for supplying water to take the place of that carried off by evaporation (Fig. 3).

The method of removing individual *Amœbæ* from stender dishes was as follows, and can be used with success for the isolation of any of the larger protozoan forms: A drop of water containing the *Amœbæ* was placed upon an ordinary slide, and, uncovered, searched with the 16 mm. objective and 8 × eyepiece. When *Amœbæ* were located they were removed by means of what is termed an isolation pipette (Fig. 2). A long rubber tube attached at one end to a glass tube drawn out to a very fine tip, and at the other to a small compression bulb, enables one to select and withdraw very minute objects with considerable precision. Both the stender dishes and growing slides were kept at a temperature of 80 degrees Fahr. in a large aquarium jar heated by a carbon filament lamp placed in the bottom, the current being controlled by a rheostat. By means of this simple device unvarying temperatures could be maintained for any desired length of time. The advantage of such a culture oven is that light is admitted freely on all sides (Fig. 1).

Formation of Apseudopodiospores.

One of the stender dishes which had been inoculated with adult *Amœba proteus* proved very productive, the individuals increasing rapidly, apparently by means of binary fission, since many were observed in process of division. The bodies of the largest individuals became filled with minute bodies, which upon staining seemed to be nuclei. The individuals bearing these were extremely sluggish. The pseudopodia were short; exhibited very little movement, changed their shape but slowly, and upon the functional posterior of the body absent altogether (Fig. 5).

After an interval of four days had elapsed a reexamination of some of the sediment from the same stender dish was made and it was found that the numbers of the large *proteus* had appreciably decreased and that their places were taken by a multitude of very small amœboid forms, of an average diameter of 4 or 5 microns. The majority of these exhibited feeble movements of an amœboid kind. Some were globular, some possessed an irregular body outline, though definite pseudopodia were lacking

(Fig. 10). These, I was later led to believe, originated from the bodies of the larger multinucleated *proteus*. Several of these latter individuals while under observation were observed to give rise to smaller individuals of the form already described. The process was as follows: the animal, which had been moving slowly and apparently without much vigor, gradually came to rest with the pseudopodia upon the then functional anterior portion of the body lobate with slightly pointed tips (Fig. 6). The posterior portion became semi-globular and towards this the greater number of the minute suppositive nuclei within the body plasm migrated. After an interval of about seven to ten minutes the ectoplasm surrounding this globular posterior extremity appeared to disintegrate and from the interior there floated forth several hyaline globules about 4 or 5 microns in diameter. These were followed, after a few minutes by several others, and then a constant outflow began that continued until upwards of thirty of the hyaline spheroids had been extruded (Fig. 7). These were apparently identical with the forms which had made their appearance in the culture. Since these are judged not to be fundamentally different from the pseudopodiospores, of Scheel, but since they exhibited no lobose pseudopodia, they are here called for convenience, *apseudopodiospores*. Some of these, immediately after extrusion began to move slowly, bulging the very thin ectoplasm at several points, yet without forming any definite pseudopodia. Others, synchronous with their emergence, disintegrated. Still others floated away without exhibiting any signs of motion (Fig. 8). It may be that these were gametic forms, and fused before further development.

Towards the time of the completion of apseudopodiospore ejection, the parent *Amœbæ* usually gave signs of renewed activity, elongated perceptibly, and then began apparently to make efforts to move away (Fig. 9). However, after an interval of from twenty minutes to half an hour, they disintegrated. Some disintegrated at once, leaving the apseudopodiospores behind, but usually they were extruded from the globular posterior portion of the animal, the anterior part retaining its integrity. It appeared as though not all of the minute nuclei were used up at the time of the production of one "litter" of apseudopodio-

spores, and that some of them (those in the anterior portion) were lost.

M. Popoff ('11) has recorded a type of reproduction in *Amœba minuta*, a marine species, similar to the one described above, with the difference, however, that the resulting spores were gametes, which later fused. Schmidt ('13) likewise, described this same sort of reproductive activity as occurring in another marine species, *Amœba aquitalis*.

The apseudopodiospores however, at least the majority whose development was watched, were not gametes.

The smallest of the young *Amœbæ* (as we shall call them) those which have just been separated from the parent body are about 3 to 5 microns in diameter and are extremely sluggish. The body is sub-globular and changes its outline but little during the very slow movement. No pseudopodia are developed. The protoplasm is clear, and contains a few small, angular, transparent granules. No contractile vacuole could be seen, nor where the creatures observed to feed (Fig. 10).

With growth comes an increase in activity and a progressively greater irregularity of the body outline (Fig. 11), until at length true lobate pseudopodia make their appearance (Fig. 12). The number of granules within the body increases, food is taken by engulfing, and the protoplasm assumes a grayish hue. This color may be due both to the number of particles within the endoplasm and to the augmentation of its volume.

At the time of the appearance of the true pseudopodia the body is unsymmetrical, but as growth proceeds are more or less radiate arrangement of the pseudopodia takes place, at first not well defined, but becoming more and more pronounced with the increase in size (Fig. 13).

The pseudopodia now become more extended, and tend to develop more acuminate tips. With increasing length and sharpness the pseudopods seem to become more rigid, and spine like, and the granules migrate from them into the more globular central mass of the body, leaving them clear (Fig. 14).

During the time when the young *Amœbæ* are passing from the apseudopodia stage to the *radiosa* stage they confusingly resemble, if indeed they are not exactly similar to the species

known as *Amœba guttula* (Fig. 11), and it is suggested that possibly this creature hitherto accorded specific rank, may be merely a developmental stage in the cycle of *proteus*. That *Amœba radiosa* was named from the *radiosa* stage of *Amœba proteus* has been indicated. The *radiosa* stage which we have observed in this developmental series may be similar.

A new type of modification now takes place, as has been said, when the pseudopodia become longer and more spinous. During this stage, in respect to size, configuration, and characteristic spineous, immobile, hyaline, ray-like pseudopodia, the creatures are apparently indistinguishable from the species known as *Dactylosphærium radiosum*. Hence we shall term this stage the *Dactylosphærium* stage (Fig. 15).

The genus *Dactylosphærium* was established by Hertwig, and Lesser ('74) to receive the organism which they described as *Dactylosphærium vitreum*. The species now known as *Dactylosphærium radiosum*, however, was not referred originally to that genus, but to the genus *Amœba*, as *Amœba radiosa*, by Ehrenberg ('30). It was transferred to the genus *Dactylosphærium* by Bütschli ('80), who however erroneously called it *Dactylosphæria*. Cash ('05) says of it: "The body consists of granular protoplasm and when all the pseudopodia are withdrawn it may become spherical or bluntly lobed; or it may assume an active amœboid phase, when it is hardly, *if at all*,[1] to be distinguished from the smaller forms of *Amœba proteus*." It occurs in marshes and pools, "less common than *Amœba proteus*, *with which it is usually associated*." [2]

It was observed that not all of the young *Amœbæ* acquired this *Dactylosphærium*-like form. Some became small *radiosa*, passed on to large *radiosa*, and thence on to the *proteus* form. It seemed to be the usual thing, however, for the majority to assume the form of *Dactylosphærium*, and the suggestion is made that perhaps *Dactylosphærium radiosum*, like other former species, may be forced to relinquish its specific distinction.

From the bodies of those individuals which had assumed the *Dactylosphærium* form, lobate pseudopodia were occasionally protruded, and at length the creature became almost globular, and

[2] The italics are the author's.

then proceeded to take on a form similar to the adult *proteus*. Such individuals were isolated, and soon grew in size to adult form, and were, apparently, *Amœba proteus*. Fission was observed to take place among some of these, but no further reproductive activities were noted. The entire sequence of events which have been enumerated took place within three months, the cultures being kept in the glass oven already described, at an average temperature of 80 degrees Fahr.

Some of the individuals of nearly adult proportions developed long, whip-like, and flexible pseudopodia, often more like long threads (Fig. 16). These were clear, even the stouter ones being devoid of granules. Gruber ('11) reported a similar type of pseudopodium (there was but one in this case) which was sent out from the body and moved about as if an organ of exploration.

Some of the most bizarre forms occur during the transition period between the smallest form and the *Dactylosphærium* stage. Fig. 14 represents forms, all but one of which were taken from the same slide.

In Fig. 18 an attempt has been made to group the various forms observed in a tentative cycle of development. The nine arbitrary stages appear to occur in the following order:

1. Adult stage.
2. Division of the nucleus, and migration of the nuclei to the posterior extremity of the body.
3. Escape of apseudopodiospores.
4. *Amœba guttula* stage.
5. Small *Amœba proteus* stage.
6. *Amœba radiosa* stage.
7. *Dactylosphærium radiosum* stage.
8. Resumption of amœboid form.
9. Growth to adult size.

BIBLIOGRAPHY.

Bütschli, O.

'80 *Protozoa*, in Bronn, H. G. Klassen und Ordnungen des Thier Reichs, parts 8 and 9.

Calkins, G. N.

'05 Evidences of a Sexual Cycle in the Life History of *Amœba proteus*. Archiv für Protistenkunde, Vol. 5, p. 1.

'07 Fertilization of *Amœba proteus*. BIOL. BULL., Woods Hole, Mass., Vol. 13, p. 219.

Carter, H. J.

'56 Notes on the Freshwater *Infusoria* of the Island of Bombay. Ann. & Mag. of Nat. Hist., Vol. 18, p. 115.

'63 On *Amœba princeps* and Its Reproductive Cells. Ann. & Mag. of Nat Hist., Vol. 12, p. 30.

Cash, J.

'05 British *Rhizopoda* and *Heliozoa*. London.

Ehrenberg, H.

'30 Beiträge zur Kenntniss der Organization der Infusorien, etc. Abhl. der Konigl. Akad. der Wiss. Berlin, p. 30.

Grüber.

'11 Ueber Einartige Körperformen von *Amœba proteus*. Archiv. für Protistenkunde, Vol. 23, p. 253.

Hertwig & Lesser.

'74 Ueber Rhizopoden Denselben Nahstehende Organismen. Archiv. für Mikr. Anat., Vol. 10, suppl., p. 35.

Metcalf, M.

'10 Studies upon *Amœba*. Jour. Ex. Zoöl., Vol. 9, p. 311.

Penard.

'02 Faune Rhizopodique du Bassin du Leman, Geneva.

Popoff, M.

'11 Ueber der Entwicklungscyclus von *Amœba minuta*. Archiv für Protistenkunde, Vol. 22, p. 179.

Schaudinn, F.

'03 Untersuchungen ueber die Fortpflanzen einiger Rhizopoden. Arbeit aus dem Kaiserl. Gesundheitsamte, Vol. 19, p. 547.

Scheel, C.

'99 Beiträge zur Fortpflanzung der Amœben. Festschrift zur Siebensigsten Geburtstag von Carl von Kupffer, Jena, p. 569.

Schepotieff, A.

'09–'10 Amœbenstudien. Zool. Jährbucher, Abt. für Anat., Vol. 29, p. 485.

Schmidt, H.

'13 Faunistische und Entwicklungsgeschichtliche Studien an Sarcodinen der Umgegend von Bonn. Archiv für Protistenkunde, Vol. 29, p. 203.

Wallich, C. G.

'63 (*a*) On an Undescribed Indigenous Form of *Amœba*. Ann. & Mag. of Nat. Hist., Vol. 11, p. 287.

(*b*) Further Observations on *A. villosa* and other Indigenous Rhizopods. Ann. & Mag. of Nat. Hist., Vol. 11, p. 434.

Vahlkampf, E.

'05 Beiträge zur biologie und Entwicklungsgeschichte von *Amœba limax*. Archiv für Protistenkunde, Vol. 5, p. 167.

Vejdovsky, F.

'80 Ueber die Rhizopoden der Brunnewässer Prags. Sitsber. der böhm. Gesell. der Wiss. Prag., p. 136.

PLATE I.

FIG. 1. Aquarium jar culture oven. *a*, thermometer; *b*, cardboard cover; *c*, aquarium jar; *d*, cultures; *e*, iron tripod; *f*, lamp; *g*, copper wire rack for lamp.

FIG. 2. Isolation pipette.

FIG. 3. Growing slide. *a*, sponge, for absorbing excess water; *b*, cover glass; *c*, glass tube carrying thread, as siphon; *d*, lower half of glass vial, cemented to the slide with balsam, as reservoir.

FIG. 4. *Vorticella microstoma*, encysted.

FIG. 5. Unusually large *Amœba proteus*, with body filled with minute nuclei.

FIG. 6. *Proteus*, resting before extrusion of the apseudopodiospores.

FIG. 7. Extrusion of the apseudopodiospores.

FIG. 8. Immobile, globular apseudopodiospores.

FIG. 9. *Proteus* after extrusion of the apseudopodiospores.

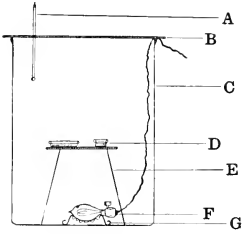
FIG. 10. Active apseudopodiospores.

FIG. 11. Apseudopodiospores during the stage when they resemble *Amœba guttula*.

FIG. 12. Small *proteus* stage.

FIG. 13. *Amœba radiosa* stage.

FIG. 14. Apparent transitional forms between the *radiosa* and the *Dactylosphærium* stages.



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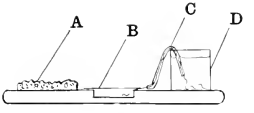

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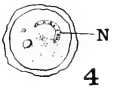

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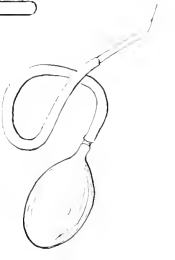

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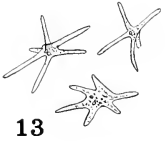

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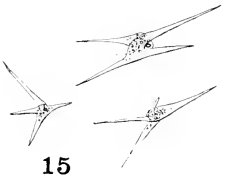
PLATE II.

FIG. 15. *Dactylosphærium radiosum* stage.

FIG. 16. *Amœba proteus* in the adult stage, exhibiting long attenuated pseudopodia.

FIG. 17. Adult normal *Amœba proteus* (stained).

FIG. 18. Tentative Cycle of Development. 1. Adult stage. 2. Division of nucleus; migration of nuclei to posterior. 3. Extrusion of the apseudopodiospores. 4. *Amœba guttula* stage. 5. Small *Amœba proteus* stage. 6. *Amœba radiosa* stage. 7. *Dactylosphærium radiosum* stage. 8. Resumption of amœboid form; retraction of pseudopodia. 9. Growth to adult.



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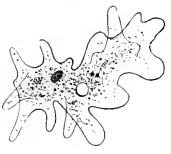

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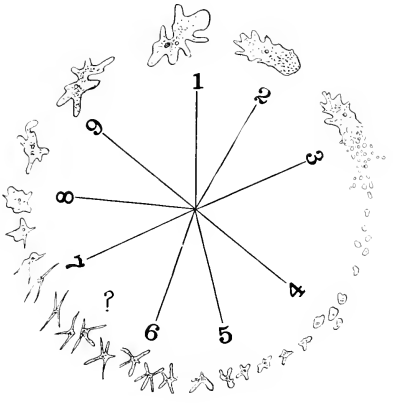

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BIOLOGICAL BULLETIN

THE AXIAL GRADIENTS IN HYDROZOA. III. EXPERIMENTS ON THE GRADIENT OF TUBULARIA.

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A. INTRODUCTION.

In a recent paper Banus ('18) states that there is no difference between the time of regeneration of oral hydranths on apical and basal pieces of the stem of *Tubularia*. The data presented by Banus apparently support this conclusion. Such data are, however, of no significance unless complete information is given as to the manner in which they are obtained. Perusal of Banus's paper reveals the fact that absolutely no information is imparted concerning the conditions under which the experiments were performed or the manner of handling the material. Those who have worked on the physiology of the lower forms are well aware that experimental results can be readily controlled and modified by conditions. It is, therefore, impossible for the impartial mind to accept the validity of Banus's conclusions, until further information concerning his experiments shall be forthcoming. Grave doubt is cast upon the correctness of Banus's statements by the fact, completely ignored by him, that experiments of this kind had already been performed several times, with results contrary to his. In addition to these omissions, Banus has made a number of exaggerated and misleading statements.

Banus begins by saying that Child "assumes the existence of metabolic gradients in a great number of species of animals and plants and on this assumption he builds a theory of individuality." In view of the great mass of data which has been presented concerning metabolic gradients and the extent to which these

data have been checked by several different methods it seems hardly scientific to dismiss the matter under the word "assumes." Gradients are not assumptions; they are facts. It is legitimate, of course, for any one to question and criticize the interpretation of these facts, and desirable that other possible explanations of them should be suggested; but to ignore such facts by designating them as assumptions is not the way to arrive at scientific truth. The nature of the axial gradients has been so thoroughly and frequently discussed in numerous papers from this laboratory that presentation of the subject here seems to me superfluous.

Banus next remarks that the hydroid *Tubularia* is extensively used to support Child's conceptions. It is scarcely necessary to point out to the zoölogical world the exaggeration conveyed by this statement, as it is well known that other cœlenterates have been used as extensively and other lower forms much more extensively in accumulating the experimental evidence upon which those conceptions rest.

We are next informed that Child "has made no measurements of the rate of metabolism of different regions of the stem of *Tubularia*." We would be pleased to carry out such experiments if Banus would kindly suggest a suitable method. The matter would be relatively simple were it not for the fact that the relative proportions of perisarc and cœnosarc vary in different regions of the stem of *Tubularia*. It is therefore difficult or impossible to determine the amount of living material in portions of the stem and impossible to establish any basis for comparison of the metabolism of different regions. Naked hydroids, such as *Corymorpha*, would be required for experiments of this kind. The metabolic rate of different regions of the first zoöid of *Planaria dorotocephala* has been determined and has been found to accord with the metabolic gradient conception.

Banus then proceeds to discuss the regional differences in rate of regeneration of *Tubularia*. He says that Child "assumes" the existence of such differences. In his summary he states that "the rate of regeneration of the oral hydranth of an apical piece is on the average identical with the rate of regeneration of the oral hydranth of the basal piece"; and further that "there is no evidence of the existence of level or regional

differences in the stem of *Tubularia*." Such statements as these display an unpardonable ignorance of the literature dealing with the regeneration of *Tubularia*. This form has been investigated by a number of well-known zoölogists, and twenty years ago the regional differences which Banus denies were demonstrated to exist.

The first experiments on the regeneration of *Tubularia* were those of Loeb ('91 and '92), performed at Naples. In the first of these publications (on p. 15) he states that he took very long stems, cut off the roots and polyps, and then divided the remaining portion in half. No difference was observed in the rate of regeneration of the oral hydranths on the apical and basal pieces.

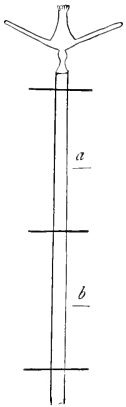


FIG. 1. Diagram of *Tubularia* to illustrate method of cutting apical and basa pieces of the stem of equal length; employed in experiments recorded in Tables III., IV., V., VII., IX., and X. *a*, apical; *b*, basal.

The following year he repeated the experiment and this time he observed that in one experiment (he does not state how many experiments were performed) the oral hydranths emerged about twenty-four hours earlier on the apical than on the basal pieces. Owing to the meager details which are furnished regarding these experiments, it is impossible to determine why the same investigator working on the same material should at one time obtain

one result and on another occasion the contrary result. Loeb has chosen to disregard the one contrary experiment since later ("Organism as a Whole," p. 171), he again asserts, in spite of all the evidence at that time available to the contrary, that apical and basal pieces of the stem of *Tubularia* regenerate hydranths simultaneously. This statement has never been confirmed, except by Banus; every other zoölogist who has worked upon the matter has found the contrary to be true. In 1899, Driesch working also at Naples expressed himself as fully convinced that Loeb was mistaken. He found many evidences of regional differences in *Tubularia*. He observed that the length of the primordium is greater the nearer the piece is to the original distal end of the stem; that in very small pieces, the more apical pieces produce larger hydranths and tend to give rise to distal structures only, while the basal pieces produce smaller hydranths and proximal structures; and that when long pieces of the stem are cut in half, the apical halves give rise to oral hydranths earlier than the basal halves. Driesch, therefore, as he emphatically stated in this paper, disagreed with Loeb on this point. In Table X, p. 131, Driesch gives a record of thirty pieces in which the apical halves regenerated oral hydranths one to twenty-three hours earlier than the basal halves in twenty-five cases and simultaneously with them in but five cases. Table XI, p. 132, presents similar data. These statements of Driesch were verified by Morgan ('01, '05, '06a, '08), and by Morgan and Stevens ('04). Thus Morgan says ('05, p. 496),—"the rate of both oral and aboral development is determined by the level at which the end lies." Again in 1906, p. 497, he states: "It has been shown in *Tubularia* that the time required for the formation of a new hydranth depends on the distance of the cut surface from the old hydranth. The nearer the cut surface to the oral end the quicker the regeneration. The same law also holds for the development of the aboral hydranth from the aboral end of a piece." These statements were reiterated in 1908, p. 157—physiologically polarity is "shown in the more rapid regeneration of the cut surfaces the nearer they are to the distal end."

Child ('07) again performed the experiment in question and

agreed with the results obtained by Driesch, Morgan, and Stevens. These experiments of Child's are the only ones considered by Banus and the false impression is thereby conveyed that no one but Child had ever experimented upon the matter. Banus has criticized these experiments of Child's on the grounds that they are not extensive and that the differences recorded are in some cases so slight that they may be due to experimental error. It

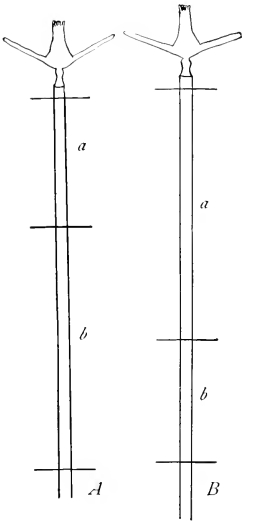


FIG. 2. Diagram to show method of cutting unequal pieces for the experiments given in Table VI. 2A, apical pieces half as long as basal pieces; 2B, apical pieces twice as long as basal pieces.

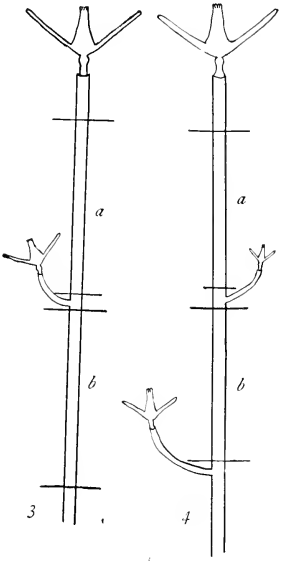
is true that no very extensive series of experiments were carried out because Child felt that the point had already been settled by the work of Driesch and Morgan and that any further experimentation was superfluous. Such observations as he made were therefore incidental to other matters. It is also true, as Banus says, that the differences obtained were not very large, but since they always vary in the same direction, the results cannot be due, as claimed by Banus, to experimental variation. In Table

I., p. 2, of Child's paper we find that of 24 pairs of pieces, the distal oral hydranths emerged first in 14 cases, at the same time as the proximal oral hydranths in 5 cases, and later than the latter in 5 cases. A result which is due to experimental error should vary equally in both directions. It should also be stated that part of the pieces given in this table were regenerating in modified sea-water and not under normal conditions. In regard to Tables II. and III., p. 5, Banus has misrepresented the facts. In these tables the differences between the times of emergence of distal and proximal oral hydranths are less than in the preceding table, although they still vary in the same direction; but it is distinctly stated by Child in the text that the difference is decreased owing to the smaller size of the pieces, and he further shows that with still greater reduction in the length of the pieces, the proximal oral hydranths will emerge first. In short, these experiments were directed towards demonstrating the effect of reduced length on the time of emergence of the oral hydranths, a fact which Banus in quoting them omits to mention.

After Banus's paper appeared the experiment was repeated at Woods Hole in the summer of 1918 by Dr. W. C. Allee. Dr. Allee was entirely unable to agree with Banus's statements, but found, on the contrary, that the oral hydranths arise earlier on apical than on basal pieces of the stem of *Tubularia*. He communicated this result to Professor Child and other members of this laboratory and also showed his experiments to a number of people at Woods Hole. In the summer of 1919, Dr. Allee assigned the experiment to his class in Invertebrate Zoölogy at Woods Hole. Twenty-seven sets of experiments were performed by the students, each set consisting of from two to eight pairs of pieces. Twenty-four hours after cutting, the apical halves (as indicated by the red color of the regenerating ends) were in advance in twenty-five of the sets, the basal halves in advance in one set, and in the other, there was no difference. Of the 112 pairs of pieces cut, 95 of the apical pieces survived, and 71 of the basal pieces. After forty-eight hours, 52 or 55 per cent. of the apical pieces had produced hydranths, while this had occurred in only 15, or 20 per cent. of the basal pieces. It should be stated that the material was not in first-class condition at the

time, and, as I shall show, the difference between the time of regeneration of apical and basal pieces is reduced under such circumstances. Professor Child and I are greatly indebted to Dr. Allee for his interest in the matter, and for his kindness in putting through the experiments.

In view, therefore, of the overwhelming preponderance of the evidence already at hand in support of the existence of the



FIGS. 3 AND 4. Diagrams to show method of cutting pieces for experiments given in Table VIII. Figure 3, method for all experiments in Table VIII., except number 36; figure 4, method used for experiment 36.

regional differences along the axis of *Tubularia* which are denied by Banus, further experimentation seems superfluous. Under the circumstances, however, a repetition of the experiment has been deemed necessary by various members of this laboratory as an answer to Banus's paper. I therefore undertook to repeat his work and for this purpose made trips to Woods Hole in June, and in December, 1919. The results were identical at both

seasons of the year and were completely at variance with Banus's statements. I was unable to verify any of his results. In numerous experiments conducted for this purpose, the apical halves of *Tubularia* stems regenerated markedly faster than the basal halves of the same stems. I further believe that I discovered the cause of Banus's peculiar results. My results are presented in detail in the present paper.

Not only are the researches just enumerated opposed to Banus's statements but a large number of other facts concerning the regeneration of *Tubularia* clearly point to the existence of axial differences in metabolic rate in this form. Thus all of the facts collected by Driesch, Morgan, and Child concerning the phenomena of "polarity" in *Tubularia* are entirely irreconcilable with the view point of Banus and Loeb. If there is no axial difference along the stem of *Tubularia*, why should the apical end of a piece produce a hydranth and the basal end a stolon, or if a hydranth, only later than the apical end? Why do heteromorphic hydranths arise simultaneously on the two ends of very short pieces while on long pieces the aboral hydranth is delayed? This question has received no adequate answer except that based on the axial gradient conception; in short pieces, there is practically no gradient, and hence each end of the piece begins to produce a hydranth at the same or nearly the same time; while, in long pieces, the apical end by virtue of its higher metabolic rate gets the start in hydranth formation and hence gains control of the stem for a certain distance, thus inhibiting the formation of the aboral hydranth. Why, as shown independently by Driesch, Morgan, and Child, in an axial series of very short pieces, do the apical pieces produce larger distal structures with much reduced or absent proximal structures while the more basal pieces give rise to smaller distal structures and larger proximal parts? Longer pieces from the distal region thus resemble shorter pieces from the proximal region in the structures which they produce, owing to the pronounced tendency of the distal pieces to use up their substance in the formation of distal structures only. Why is the primordium of the oral hydranth larger in apical than in basal pieces and the emerged hydranth likewise larger? These and numerous similar facts

have been repeatedly ascertained by several investigators and are totally inexplicable on the point of view maintained by Loeb and Banus that there are no regional differences in the stem of *Tubularia*.

There is one further statement made by Banus to which we must take exception. This is the assertion on p. 266 and again on p. 273 that regional differences in rate of regeneration constitute the "actual basis" for the axial gradient conception as

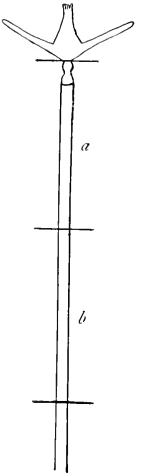


FIG. 5. Method of cutting pieces usually employed by Banus and used for the experiments recorded in Table XI.

applied to *Tubularia*. They are part of the basis, but not the entire basis, as Banus implies. Axial differences in metabolic rate along the stems of *Tubularia* had been demonstrated at the time when Banus published his paper by two other methods—the differences in susceptibility to potassium cyanide and other substances, and the differences in electric potential. The results yielded by both of these methods are again considered in this paper.

In view of the fact that Banus's results are in total disagreement with a considerable number of researches, a critical examina-

tion of his method of procedure is necessitated. But, as has already been pointed out, this is impossible because no description is given by Banus concerning his material, his methods, or his experimental conditions. We need to know the season of the year, the temperature, the vegetative condition of the material, the length of time material was kept in the laboratory before being used, the presence or absence of lateral buds on the stems employed (since buds mark the limits of the individual), and particularly the level from which the pieces were taken, with reference to the original hydranth. We have repeatedly pointed out that metabolic gradients are not fixed and static things, but markedly dynamic and labile, and particularly in the lower forms, they may result from external conditions and may be readily modified and altered by conditions. The failure of Banus to describe or consider the various factors mentioned, any one of which might alter the experimental result, is evidence that he really does not understand the metabolic gradient conception and has not interested himself in understanding it. This is further shown by certain remarks made in his paper such as for example the naïve statement on p. 268 that the pieces were "long enough to show a marked difference according to Child's opinion," whereas in fact "according to Child's opinion," and as even a hasty perusal of the work put out from this laboratory would show, the axial differences are most clearly marked in most respects in relatively short pieces.

In order, therefore, to obtain any information concerning the experiments of Banus, it has been necessary for me to communicate with him. Mr. Banus replied to the first letter which I wrote to him, but did not reply to two others requesting further details. It is therefore not possible for me to furnish all of the details necessary for a correct evaluation of these experiments but the information I was able to obtain is sufficiently astonishing. Banus states that his experiments were performed in New York City in November and in Woods Hole in December. Since most of the other researches on *Tubularia* were carried out in the summer season, it was at first thought that seasonal differences in the vegetative condition of *Tubularia* might account for Banus's results. I found, however, that *Tubularia* is in prac-

tically the same condition at Woods Hole in June and in December and yields identical results at these two seasons of the year. Banus further states that the "temperature during regeneration was in New York 22° C., and in Woods Hole it was about 15° C." Upon examination of Banus's tables, one finds that the time interval between section and emergence of hydranths is much too great for these temperatures. At a temperature of 22° C., apical pieces of *Tubularia* produce hydranths as early as 36 hours after section and the great majority of such pieces will have completed regeneration in 48–60 hours, yet in no case do any of the pieces recorded in Banus's tables regenerate in less than 53 hours and the majority of them require more than 60 hours. Even at a temperature of 15° C., the times given by Banus are surprisingly long, since I found that at 12° C., the majority of the apical pieces will regenerate within 70–80 hours (see Table IV.). It is therefore evident that in Banus's experiments some factor is acting to delay the time of regeneration of the apical pieces, whose regeneration precedes by an average of 10 to 12 hours, according to my findings, the regeneration of the basal pieces.

The cause of the delayed regeneration in Banus's experiments lies in all probability in his method of cutting the apical pieces. In reply to my inquiry concerning the level of the stem from which he cut the pieces, Banus made the following statement: "the most distal cut was usually made as near as possible to the hydranth without including any part of it." Such a method of procedure explains the aberrant results obtained by Banus. It has long been known that the short stalk below the hydranth of *Tubularia* is incapable of regeneration. When the apical pieces are cut in the manner described by Banus, this stalk forms the distal end of such pieces. It dies and disintegrates, thereby markedly delaying the regeneration and time of emergence of oral hydranths on these pieces. I shall present evidence in this paper (see Table XI.) that when the apical pieces are cut in such a way that their distal ends are just below the base of the original hydranth, the time of emergence of the oral hydranth is greatly delayed and falls behind that of the basal pieces. Hence when such a procedure is followed, all

sorts of irregular results are obtained and it is not surprising that under such circumstances, the experimenter would be lead to question the existence of axial differences along the stem of *Tubularia*. When the correct method of cutting the apical pieces is observed, namely, when the hydranth, its stalk, and the first millimeter or two of the stem are discarded, then there is not the slightest question that the apical halves of stems prepared in this manner give rise to oral hydranths very much in advance of the basal halves.

The experiments presented in this paper were performed at Woods Hole in June and in December, 1919. I am greatly indebted to Professor F. R. Lillie for a research room at the Marine Biological Laboratory on both of these occasions and for a grant from the departmental funds covering the expenses of the December trip. I am also indebted to Professor C. M. Child for advice and suggestions throughout the course of the work.

B. Susceptibility Gradients in Tubularia.

The death gradients in lethal concentrations of various substances have already been described by Child ('19*b*) for a number of hydroids including *Tubularia*. I have repeated and confirmed these observations for a number of forms. In *Tubularia*, the disintegration (1/100 to 1/400 mol.KNC) begins at the tips of the proximal tentacles and proceeds down these tentacles to their bases; soon after the proximal tentacles have begun to disintegrate, the process is initiated in the distal tentacles and proceeds to their bases. At about the same time as the tips of distal tentacles begin to die, the mouth region disintegrates and the disintegration extends along the body of the hydranth to its base. In many cases, it was observed that the outer surface of the proximal tentacles (*i.e.*, the surface that contains the most nematocysts) preceded in disintegration the inner surface. In the stalk of the hydranth there is a specialized region bearing a ring of nematocysts; in many cases it was noted that this region disintegrated early and without any definite relation to the progress of disintegration in other parts of the hydranths, as was also noted by Child. Such specialized regions, owing to functional activity, are commonly highly susceptible to toxic agents,

a fact that has been noticed by us on many different forms. It is to be understood that the disintegration of the ectoderm precedes that of the entoderm by a considerable time interval except in the case of the rim of the mouth where both germ layers seem to disintegrate almost simultaneously forming a great bulging mass of disintegrated particles.

The remarks in the foregoing paragraph refer to fully developed hydranths. In young hydranths, it was commonly observed that the distal tentacles disintegrated first and the disintegration then extended basipetally along the body of the hydranth, the proximal tentacles disintegrating later than the body of the hydranth. In medusa-buds, the disintegration proceeds from the free to the attached end. Young hydranths are more susceptible than fully developed ones only after they have reached a certain stage.

The hydranths are much more susceptible than the stems. The disintegration of the stems is obscured by the presence of the perisarc. Nevertheless the progress of disintegration was observed in many cases in the stems but was never followed for more than a short distance. The disintegration proceeds from the base of the hydranth down the stem. It might be said in criticism that the killing agent can gain access to the stem only from the open top of the perisarc and that the death gradient in the stem may therefore be simply a consequence of the diffusion path followed by the agent. To answer this objection short apical pieces of stems bearing hydranths were cut off and their disintegration followed in cyanide. In such cases the open cut surface at the proximal end of the stem affords a readier point of entrance for the reagent than does the top of the perisarc; nevertheless except for a small area of disintegration around the cut surface, the disintegration proceeds in the same manner as before, from the distal end of the stem proximally.

Direct observation of the course of disintegration in the stems is not, however, entirely satisfying, as the process is admittedly obscured by the presence of the perisarc and could be observed for only the most distal region of the stems. Another method was therefore employed, namely, the differential survival of distal and proximal pieces of the stem in nearly lethal concentrations of toxic substances. The substance employed for this purpose

was ether. It was found that distal pieces are more susceptible to ether than proximal pieces, that more of them die when both kinds of pieces are exposed for a certain length of time to a given concentration, and that of those that survive, the regeneration is more delayed in the apical than in the ~~distal~~ basal halves. An experiment of this kind is recorded in Table I. In this experiment the

TABLE I.

DIFFERENTIAL SUSCEPTIBILITY OF APICAL AND BASAL PIECES OF EQUAL LENGTH (10–12 MM.) TO 2 PER CENT. ETHER.

Exposed to ether for twenty hours. Table records condition of the pieces seven days after cutting. Temp. 12 ± 2° C.

Condition.	Apical.	Basal.
No. of hydranths emerged	5	14
No. of pieces with primordia of hydranth[1]	5	7
No. of pieces living but without primordia[1]	2	15
No. of pieces dead[1]	38	14

hydranths and first millimeter or two of the stems were discarded, the stems were then cut into two equal pieces, each 10–12 mm. long, and all of the distal pieces placed in one finger bowl and the proximal in another. A solution of 2 per cent. ether in sea-water was then poured on both sets of pieces as soon as possible after they were prepared and they were left in this for twenty hours. They were then thoroughly washed and left in normal sea-water. The temperature throughout was 12° C. ± 2. The condition of the fifty pairs of pieces seven days after cutting is shown in the table. The greater susceptibility of the apical pieces is perfectly evident. More of them have died than in the case of the basal pieces and of those that survived, the basal pieces are much in advance in the process of regeneration.

C. GRADIENTS IN REDUCTION OF POTASSIUM PERMANGANATE.

Child (19*a*) has called attention to a new method of demonstrating the metabolic gradients. This method consists in exposing the organisms to appropriate concentrations of a readily reducible substance like potassium permanganate. This substance is reduced by protoplasm, a brown precipitate of manga-

[1] Pieces were examined with the compound microscope. Pieces showing smooth rounded ends and circulation were counted as living, while stems containing only masses of granules were regarded as dead.

nese dioxide being formed. It was found by Child that this capacity of organisms to reduce permanganate exhibits the same kind of a gradation in relation to the body axes as does their time of death in toxic solutions. The apical ends reduce the most permanganate and take on a very deep brown or almost black color and the depth of this color decreases basipetally.

I have observed the staining of *Tubularia* by potassium permanganate. The picture thus presented is identical with the course of disintegration already described. The tips of the proximal tentacles stain first, the tips of the distal tentacles next; the stain progresses rapidly down the tentacles (on the outer surfaces of the proximal tentacles before their inner surfaces) to their bases. Meantime the stain appears on the distal end of the body of the hydranth and progresses basipetally along its ectoderm. After the staining is completed, it is found that the tips of both sets of tentacles and the mouth region of the hydranth are very deeply stained and the stain shades off to the bases of tentacles and hydranth. This was best observed by turning the hydranth with a needle so that it faced upward.

The staining of the stem was naturally difficult to observe. The short stalk below the hydranth became stained soon after the hydranth but the staining of the stem proper was very slow. As far as could be observed the stain proceeded from the distal end of the stem proximally. The observations were, however, unsatisfactory.

The younger hydranths stain much more rapidly than the larger ones, but exhibit less distinct graded differences between different regions of the hydranth.

D. Electrical Gradients in Tubularia.

Differences in electrical potential along the axes of organisms form still another manifestation of the metabolic gradients and are at present being investigated by members of this laboratory for a number of the lower forms. These electrical gradients correspond in all respects to the death and staining gradients. The regions of highest susceptibility and reducing power are electronegative (galvanometrically) to regions of lower susceptibility and reducing power. This matter has been discussed

elsewhere (Hyman, '18) and further papers upon the subject will appear shortly. Briefly it is believed that chemical differences are as a rule responsible for these permanent differences in potential and that such chemical differences arise in the final analysis through differences in metabolic rate at different levels.

The existence of such a difference in electrical potential along the axis of *Tubularia* was discovered by Mathews ('03). Mathews found that the hydranth is negative to any region of the stem and that distal levels of the stem are negative to proximal levels. He correctly attributed these potential differences to differences in metabolic activity.

I have repeated these experiments on *Tubularia* and verified all of Mathews' statements. A galvanometer constructed on the principle of the D'Arsonval galvanometer and put out by the Leeds and Northrup Company was used. Although not as sensitive as some other types of galvanometers in use, the instrument was found adequate for the purpose. Non-polarizable electrodes were employed, made in the usual way of the glass tubes from medicine droppers, packed at the small end with kaolin paste made with sea-water, and filled at the other end with saturated zinc sulphate solution. Zinc rods, amalgamated with mercury, dipped into the zinc sulphate solution, and were connected by copper wires with the binding posts of the galvanometer. Small rolls of hard filter paper were thrust into the ends of the electrodes and the stems to be tested placed on these. These filter paper rolls were kept soaked with sea-water. Although such electrodes are made as nearly alike as possible, there is almost always some difference of potential between them. Such difference increased with use so that it was necessary to renew parts of the electrodes or to make wholly new ones at frequent intervals. The existence of a potential difference between the two electrodes of course makes it impossible to obtain an absolute value of the amount of current originating from the organism; but absolute values were not desired in the present experiments. It was my purpose merely to discover which regions of the organism were electronegative as compared with other regions.

In performing the experiments isolated stems of *Tubularia* in

perfect condition only were employed. They were placed across the filter paper ends of the electrodes and the reading of the galvanometer recorded. The stem was then reversed on the electrodes and the reading again taken. The difference in the two readings, particularly the difference in the direction of swing of the indicator on the scale gives the desired information about the electrical condition of the stems. Each reading was repeated once, sometimes twice. When dead organisms are tested in this way, the galvanometer gives the same reading regardless of the position of the material on the electrodes.

The galvanometric readings obtained on *Tubularia* are recorded in Table II. In connection with this table it is necessary to explain that the scale of the galvanometer used is printed in red on the right side of the zero point and in black on the left side. When the right electrode, which is connected with the right hand binding post of the galvanometer, is negative, the indicator swings to the right of the zero point and hence reads on the red half of the scale; when the left electrode is negative, the indicator reads on the left hand or black side of the scale. In some cases, both readings may be on the same side of the zero point but one farther to the right or left than the other. Left and right refer, of course, to the hands of the observer as he sits facing the instrument.

In the table, each number refers to one individual and all of the data given under that number were obtained on one individual. In Table II., *a*, are recorded the readings of the galvanometer when the *hydranth* is compared with nearby portions of the stem or with distant portions or with regions where branches are present. Table II., *b*, gives the readings when distal portions of the *stem* are compared with more proximal regions or with far proximal regions or with proximal regions bearing branches. In each table, the first column gives the number of the individual, the second describes the material, the third gives the reading in one position between regions not very far separated, the fourth column the readings for the same regions in the reversed position on the electrodes, the fifth and sixth are the same as the third and fourth except that they compare regions more widely separated or distal regions with levels bearing branches.

TABLE II., *a*.

ELECTRICAL GRADIENTS OF *Tubularia;* HYDRANTH COMPARED WITH NEARBY PORTIONS OF THE STEM AND WITH FAR PROXIMAL PORTIONS OR REGIONS BEARING BRANCHES.

Each number refers to one individual. Scale of the galvanometer reads with red figures to the right of the zero point, with black figures to the left of zero, hence *r* means red, and *b* means black, and numbers accompanying *r* and *b* are divisions on the scale. The right electrode is negative when the reading is on red or farther to the right than before; and the left electrode is negative when the reading is on black or farther to the left than before. Hydr., hydranth, br., region of branches, prox., proximal, dist., distal.

No.	Material.	Readings with Reference to Position of Material on Electrodes as Designated in These Columns.			
		Hydr. Right, Stem Left.	Hydr. Left, Stem Right.	Hydr. Right, Far Prox. or Br. Zone Left.	Hydr. Left, Far Prox. or Br. Zone Right.
1.	Hydr. and stem	5 *r*	7 *b*		
		3 *r*	7 *b*		
2.	Small hydr. and stem	2 *b*	4 *b*		
		2 *b*	2 *b*		
3.	Hydr., stem, and br.	0	15 *b*	0	8 *b*
		2 *r*	10 *b*	3 *r*	5 *b*
5.	Ditto	2 *r*	5 *b*	0	4 *b*
		0	5 *b*	0	4 *b*
6.	Large hydr., stem and br.	4 *r*	8 *b*	2 *r*	6 *b*
		5 *r*	8 *b*	2 *r*	6 *b*
8.	Small hydr. and stem	0	3 *b*	0	2 *b*
		0	5 *b*	0	2 *b*
10.	Large hydr., stem and br.	2 *r*	6 *b*	0	5 *b*
		2 *r*	9 *b*	0	5 *b*
11.	Large hydr. and stem	0	5 *b*		
		2 *b*	6 *b*		
12.	Medium hydr. and stem	3 *r*	6 *b*		
		0	5 *b*		
13.	Ditto	0	4 *b*	2 *b*	0
		0	4 *b*	2 *b*	0
14.	Ditto	0	6 *b*		
		1 *r*	4 *b*		
15.	Ditto, and br.			0	5 *b*
				1 *r*	5 *b*
16.	Ditto			1 *b*	3 *b*
				0	3 *b*
17.	Small hydr. and stem	2 *b*	4 *b*		
		0	2 *b*		
18.	Medium hydr. stem and br.	5 *r*	2 *b*	2 *r*	5 *b*
		0	6 *b*	2 *r*	2 *b*
19.	Small hydr. and stem	0	3 *b*		
		1 *r*	2 *b*		
21.	Very large hydr. and stem	13 *r*	18 *b*		
23.	Medium hydr. and stem	3 *r*	4 *b*		
		5 *r*	4 *b*		
35.	Ditto	0	14 *b*		
		6 *r*	15 *b*		

TABLE II., *a*, *Continued.*

No.	Material.	Readings with Reference to Position of Material on Electrodes as Designated in these Columns.			
		Hydr. Right, Stem Left.	Hydr. Left, Stem Right.	Hydr. Right, Far Prox. or Br. Zone Left.	Hydr. Left, Far Prox. or Br. Zone Right.
37.	Ditto, and br.	7 *r*	19 *b*	2 *r*	6 *b*
		4 *r*	10 *b*		
38.	Medium hydr. and stem.	13 *r*	1 *r*		
		5 *r*	0		
39.	Ditto, and br.	7 *r*	0	2 *b*	2 *b*
				0	1 *b*
40.	Medium hydr. and stem.	8 *r*	18 *b*	9 *r*	6 *b*

The tables give all of the readings which were made. There have been no omissions or selection of data. Forty-two individuals in all were tested. The first forty of these came from the same lot of *Tubularia*, collected on December 6, and tested on December 7 and 8. The last two individuals came from another lot of material collected on December 8 and tested on the same day. Material was kept at a temperature of 10° C. A number of different readings were commonly made on each stem, various levels of the stem being tested in order to obtain a picture of the potential differences along the whole organism.

The following conclusions may be drawn from the data presented in Tables II., *a*, and *b*:

1. The hydranth is always electronegative to nearby regions of the stem. This is shown without exception in the twenty-three cases given in Table II., *a*. The galvanometer invariably reads to the right when the hydranth is on the right electrode, and to the left when the position is reversed.

2. The difference between hydranth and distal regions of the stem is greater in the case of larger hydranths and much less in the case of small hydranths. Thus in nos. 2, 8, 17, and 19, where small hydranths were used, the potential difference between hydranths and stem is 2 to 5 points of the scale; while when medium or large hydranths are used, the differences are much greater. These conditions are probably associated with age.

3. Hydranths are usually more negative to distal portions of the stem than to more proximal regions or regions where lateral

TABLE II., *b*.

ELECTRICAL GRADIENTS OF *Tubularia*.

Distal regions of stem compared with proximal or with far proximal or region bearing branches. Otherwise as in table II., *a*.

No.	Material.	Readings with Reference to Position of Material on Electrodes as Designated in These Columns.			
		Dist. Stem Right, Prox. Left.	Dist. Stem Left, Prox. Right.	Dist. Stem Right, Far Prox. or Br. Left.	Dist. Stem Left, Far Prox. or Br. Right.
1.	Hydr., stem and br.			2 *b*	4 *b*
				2 *b*	4 *b*
4.	Stem without hydr., hydr. regenerating	1 *b*	5 *b*	0	0
		1 *b*	5 *b*	1 *b*	1 *b*
		1 *b*	5 *b*		
7.	Stem without hydr., like 4	0	3 *b*		
		0	3 *b*		
9.	Stem without hydr., like 4	1 *r*	5 *b*	2 *b*	0
		0	3.5 *b*	2 *b*	0
13.	Medium hydr. and stem	4 *b*	2 *r*		
		5 *b*	0		
20.	Ditto	3 *b*	5 *b*		
		2 *b*	10 *b*		
		2 *b*	8 *b*		
22.	Ditto	4 *b*	5 *b*		
		3 *b*	4 *b*		
24.	Ditto	3 *b*	5 *b*		
		2 *b*	5 *b*		
25.	Ditto and br.			2 *b*	1 *b*
				2 *b*	1 *b*
26.	Medium hydr. and stem	1 *b*	3 *b*		
		1 *b*	3 *b*		
27.	Ditto	2 *r*	4 *b*		
28.	Ditto	No potential difference			
29.	Ditto	2 *r*	1 *b*		
		2 *b*	4 *b*		
30.	Ditto	1 *b*	2 *b*		
		0	3 *b*		
		0	2 *b*		
31.	Ditto	0	2 *b*		
		0	3 *b*		
32.	Ditto	1 *r*	2 *b*		
		1 *r*	4 *b*		
33.	Ditto, and br.			1 *b*	1 *b*
				1 *b*	1 *b*
34.	Small hydr. and stem	No potential difference			
36.	Medium hydr. stem and br.,			0	2 *r*
				0	2 *r*
41.	Hydr., very long stem	12 *r*	0		
		12 *r*	5 *b*		
42.	Like 41	7 *r*	9 *b*		
		8 *r*	0 *b*		

branches are present. This is shown in numbers 3, 5, 6, 8, 10, 15, 16, 18, 37, and 40, Table II., *a*. Thus for example, in no. 3, the difference between the hydranth and the distal stem is 15 divisions of the scale in the first trial, 12 in the second, while the difference between the same hydranth and a more proximal region of the stem where a lateral branch was present was 8 divisions in both trials. In one case, no. 13, the region of branching was negative to the hydranth; in another case, no. 39, there was practically no potential difference between the hydranth and the branching region. We therefore see that proximal regions of the stem are more negative than distal, especially when they bear branches. This is due to the fact that the hydranth dominates only a certain length of stem, and beyond that length physiological isolation has occurred with the formation of a new individual, expressed by the development of lateral branches. Such new individuals like the original one are electrically negative apically.

4. Distal regions of the stem are nearly always electronegative to nearby proximal regions. This was the case in 14 of the 17 cases tested in Table II., *b*. In two cases, nos. 28 and 34, there was no potential difference between two such regions of the stem; in one case, no. 13, the gradient was reversed, the distal region being positive to the proximal region. Such cases as these three account for the fact that occasionally, distal and proximal pieces of the stem regenerate simultaneously, or that the proximal piece may precede.

5. The potential difference between distal and proximal regions of the stem is always very much less than that between hydranth and distal regions of the stem.

6. The potential difference is usually slight or absent or may be reversed between distal regions of the stem and far proximal regions, or regions bearing branches. Of six cases tested, two showed no potential difference (nos. 4 and 33, Table II., *b*); in one case, the distal region was negative (no. 1); and in the other three cases, the far proximal region or branching region was negative to the distal region (nos. 9, 25, 36). This verifies what was said in paragraph 3. These far proximal regions are really beginnings of new individuals and hence are more electronegative than the regions immediately distal to them.

I therefore find, as Mathews did, that within the limits of a single *Tubularia* individual, any distal region is electronegative (galvanometrically) to any proximal region. Since electronegativity is usually associated with a higher rate of oxidative metabolism in organisms, these experimental data constitute strong evidence that there is a metabolic gradient along the axis of *Tubularia*, that the apical end of this axis has the highest rate of activity, and that this rate diminishes proximally.

E. Differences in Rate of Regeneration of Distal and Proximal Pieces of Equal Length.

A large number of experiments were performed with reference to this point with the result that the apical pieces were found to regenerate markedly faster than the basal pieces in practically all cases. A few cases were observed in which the proximal piece regenerated first.

1. *Method of Procedure.*—The method of cutting the pieces was invariably as follows unless specifically stated otherwise. Stems free from branches and filled with cœnosarc throughout their length were removed from the colony and placed on a glass plate. The hydranth and the first millimeter or two of the stem were then removed by a cut and discarded and the basal end injured by removal from the colony also cut off and discarded. The piece of stem was then cut into two equal halves, a distal or apical half and a proximal or basal half. In most cases, unless otherwise stated, such halves were 8–12 mm. long. Figure 1 illustrates the method of cutting the pieces.

After cutting the pieces were handled in two different ways. In the majority of the experiments all of the apical halves were placed in one finger bowl and all of the basal halves in another finger bowl. Such experiments are designated throughout this paper as *mass* experiments. The number of oral hydranths emerged in each finger bowl at a given time was then recorded. In other experiments, which are designated as *individual* experiments, each half was placed in a separate stender dish and the time of emergence of the oral hydranth on each half stem recorded as accurately as possible. In all cases the record was taken only when the hydranth had emerged completely from the perisarc.

Observations were made and the hydranths emerged recorded every two to four hours during the daytime. No observations were made during the night and hence there are in all of the experiments gaps of from six to ten hours for each night period. The first morning observation after such a gap is indicated in all of the tables by an asterisk.

There has been no selection of experiments for presentation in this paper. Practically all of the experiments performed are presented.

2. *Mass Experiments in June.*—These experiments were performed between June 16 and July 9, 1919. The material was in excellent condition up to July 1, when the pieces for the last experiment were cut. There was a great abundance of material, growing rapidly and containing hydranths of all sizes. Material was always cut on the same day as collected since, as is well known, *Tubularia* will not keep in good condition in the laboratory in the summer. The hydranths fall off within twenty-four fours, new ones being subsequently regenerated; further the cœnosarc either dies away in the basal regions or else retreats to other parts of the colonies. The regenerating pieces were kept in the laboratory during the earlier experiments; the temperature was naturally variable and as recorded in the daytime ranged from 15° to 24° C., with probably lower temperatures at night. The later experiments were placed in the refrigerator at a constant temperature of 13° C.

The mass experiments performed in June are recorded in Table III. As already stated, all of the apical halves were placed in one finger bowl and all of the basal halves in another; the two finger bowls were kept under the same conditions. At frequent intervals the regenerating pieces were examined and the number of oral hydranths emerged recorded. The record was taken only when the hydranth had completely emerged from the top of the cœnosarc and had spread its tentacles.

Details of these experiments not given in the table are as follows. Experiment 1 was performed on slender stems; experiments 2 on stout stems; the other experiments were with medium sized stems although there was some variation in the diameter of the stems. Experiments 1, 2, 5, and 10 regenerated in the

TABLE III.

MASS EXPERIMENTS PERFORMED IN JUNE SHOWING RATE OF REGENERATION OF APICAL AND BASAL HALVES OF STEMS OF *Tubularia*.

Pieces 8–10 mm. long. Hrs. means numbers of hours elapsed since cutting; *a*, apical half; *b*, basal half; columns under *a* and *b* give number of hydranths emerged at time indicated. Asterisk indicates first morning observation.

1			2.			5.			6.			10.			11.			15.			16.			21.		
Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*b.*	*b.*	Hrs.	*a.*	*b.*
*37.5	13	0	*38.5	3	0	*36.5	2	0	47	1	0	38	2	0	60	1	0	63	1	0	63	1	0	*65	9	0
39	15	3	40	6	0	38.5	2	0	50	3	0	40	3	0	64	2	0	66	5	0	67	6	0	68	13	0
40.5	17	3	43	7	2	41.5	4	0	52	5	0	42	7	1	66	5	1	72	15	0	70	8	0	70.5	18	0
42		4	44.5	9	3	44	9	0	*60	12	4	44	9	1	68	8	3	*82	24	8	73	13	1	73.5	20	2
43.5		5	46	10	3	47	11	0	63	15	6	46	14	2	72	19	5	86	25	10	*83	19	10	77.5	24	5
45		8	48	13	5	50	15	2	66	18	6	48	16	6	74	25	7	90		16	86	22	17	79.5	24	7
47		12	50	15	7	52	15	3	69	19	10	50	18	11	*81.5	33	11	93		17	89	22	19	*89	28	15
51		15	52	18	8	*60	20	15	72	21	12	52	22	12	84	35	13	96		20	93	25	23	93	31	17
*61		17	*62	21	18	63	22	18	75	22	12	*58	29	15	86	36	14	*105		21	97		25	95	31	19
			70		18	66	24	19	*83	24	17	60.5	30	15	88	38	16	111		22				98	33	19
			74		20	69	25	23	85	25	18	62.5	35	17	90	38	16	119		24				101	34	21
			76		21	75		23	88		20	64.5	36	19	92	40	19	*130		25				*114	41	26
						*83		23	90		21	66.5	37	25	94	42	20							116	42	27
						90		24	95		22	68.5	38	27	102	46	23							118	43	28
						107		25	97		23	72.5	41	29	107	47	25							120	43	31
												74.5	41	29	110		31							127	43	32
												*82	43	30	112		33							*137	43	35
												84.5	45	32	117		34							143	44	36
												86.5	48	32	*126		35							*162		38
												88.5	50	32	140		37							165		39
												90.5		33	151		38							*188		41
												95.5		34										198		43
												97.5		34												
												*106		36												
												108.5		37												
												117.5		39												

laboratory at variable and generally moderately warm temperatures, from 15° to 24° C. (day records). In experiment 6, the pieces were placed in the refrigerator (temp. 13° C.) for the first twelve hours and in experiment 11 for the first twenty hours after section, and were then removed to laboratory temperature. In experiments 15, 16, and 21 the pieces were kept in the refrigerator (temp. 13° C.) for the entire period of regeneration as the weather had become unfavorably warm by this time. There was some mortality, particularly among the basal pieces, owing probably to the warm weather. In experiment 6, two basal pieces were living but had not regenerated when the experiment was concluded. In experiment 10, where fifty pairs of pieces were cut, seven basal pieces died and four had failed to regenerate when the experiment was discontinued. In experiment 11, three apical halves and twelve basal halves had died or failed to regenerate when the experiment was discontinued. One basal piece died in experiment 21.

The length of the pieces in all of the experiments recorded in Table III. was 8–12 mm. It was not possible to find stems free from branches long enough to give longer pieces in the summer material, owing to the fact that the colonies are growing rapidly and branching extensively at this season. The number of pieces cut in each experiment depended on the number of healthy stems of sufficient length available in the day's collection. Although material was very abundant and large quantities of it were brought in whenever desired, most of the colonies consisted of stems so short as to be useless for the experiments.

3. *Mass Experiments in December.*—According to the statements of Mr. Gray, head of the supply department at Woods Hole, *Tubularia* is most abundant and in excellent condition in the early summer reaching a climax in June. After that, as the weather becomes warm, the colonies die away, the protoplasm withdrawing into the perisarc and apparently passing into a dormant state. In the fall, as the water becomes colder, the colonies begin to grow again, reaching their height in November and December, and then with still colder weather, once more passing into the quiescent state, emerging in the spring. In November, 1919, no *Tubularia* could be found at Woods Hole,

in spite of diligent search by the collectors. It was, however, obtainable early in December in fair abundance, and experiments were performed upon it from December 6 to 16. The colonies at this time were in excellent condition, branching freely and growing rapidly. The general appearance of the material was much the same as in June except that the hydranths attained a larger size than in June and a few lots of material consisted of very long stems, much longer than any observed in June. The majority of the December material, however, was branching so freely that most of the stems were relatively short and in some cases it was necessary to cut pieces less than 8 mm. long. The temperature of the running water in the laboratory in December was 8° C. and it was therefore possible to keep the material for two or three days in excellent condition. Two collections of material were used in the December experiments; one collected on December 6 was cut for experiments on December 6, 7, and 8; the other, collected on December 8, was used on December 8 to 11. All of the pieces were kept on the water tables in slowly running water at a temperature of approximately 12° C., varying, however, for slight periods from 10° to 14°.

The results of the mass experiments performed in December are given in Table IV. In experiment 26, the pieces were about 5 mm. long; in experiment 27, 5–8 mm. long; and in experiments 35, 44, and 47, 10–12 mm. long. The pieces were cut as in Fig. 1, except in the case of experiment 44, in which the basal pieces were cut at the proximal end of long stems so that some 10–15 mm. of stem was removed between the levels of the apical and basal pieces in this experiment. There was no mortality among the pieces. The temperature throughout was 12° C. ± 2.

4. *Conclusions from Mass Experiments.*—The data given in Tables III. and IV. permit us to draw the following conclusions concerning the rate of regeneration of oral hydranths on apical and basal pieces of the stem of *Tubularia* of equal length:

(*a*) Hydranths invariably emerge first on the apical pieces and a considerable number of such pieces will have regenerated before any of the basal pieces have produced a hydranth.

(*b*) At any given time there are in nearly all cases a greater

number of apical pieces with oral hydranths than basal pieces. The difference is always more marked in the early part of the regeneration period; later the basal pieces may catch up with the more tardy of the apical pieces with the result that the number of regenerated basal pieces may in a few cases equal the

TABLE IV.

MASS EXPERIMENTS PERFORMED IN DECEMBER SHOWING RATE OF REGENERATION OF APICAL AND BASAL HALVES OF STEMS OF *Tubularia*.

Hrs. means number of hours elapsed since cutting; *a*, apical half; *b*, basal half; figures under *a* and *b* give number of hydranths emerged at time indicated; asterisk indicates first morning observation.

26.			27.			35.			44.			47.		
Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.
*61	18	7	45	1	0	*60	16	0	52	2	0	61	1	0
63	19	9	51	2	0	62	19	0	58	4	0	65	2	0
65	21	11	53	8	2	64	22	4	61	8	0	67	4	0
67	21	14	55	15	3	66	23	9	*69	16	1	69	5	0
69	22	15	57	25	8	68	29	18	71	18	8	73	12	3
71		17	59	27	19	70	31	25	73	19	10	75	14	3
73		18	*68	40	40	72	35	29	75	22	16	*85	26	14
76		18	70	42	42	74	37	29	77	25	19	87	29	18
*84		20	72	48	44	77	41	30	79	26	20	89	32	20
90		21	74	49	45	*84	44	36	81	27	23	91	33	26
*112		22	76	51	46	87	45	42	83	28	25	95	33	30
			80	53	50	89	46	45	*93	30	27	97	34	31
			83	54	51	91	47	47	105		28	99	35	33
			*92	55	55	93	49	47	*117		29	101	36	33
			94	57	56	95	49	49	119		30	*111	37	36
			96	57	57	97	51	49				119		37
			98	58	57	99		51						
			100	60	57									
			104		58									
			*117		60									

number of regenerated apical pieces (exps. 27 and 35, Table IV.); but in no case are the basal pieces in advance.

(*c*) In all cases the apical pieces complete their regeneration first.

(*d*) When other factors are equal the rate of regeneration is a function of temperature.

(*e*) When other factors are equal, the rate of regeneration is a function of the diameter of the stem. More slender stems regenerate more rapidly than stouter stems. Thus in experiment 1, Table III., the pieces were cut from slender stems bearing small hydranths; those in experiment 2, same table, from stouter

stems bearing larger hydranths, cut at the same time and from the same lot of material. It is perfectly apparent that the more slender pieces regenerate more rapidly, and this was also evidenced throughout all of my experiments. It is probable that this relation of the rate of regeneration to the diameter of the stem is connected with the age of the stem, but since one does not certainly know that slender stems are younger than stouter ones, the matter must be left open at present. Morgan ('06 *b*) found that young stems regenerate more rapidly than old ones and that when the hydranths are removed from the top and lateral branches of a stem, the lateral branches regenerate first. At any rate, these facts dispose of the suggestion which has been made that apical pieces regenerate more rapidly than basal pieces because they are of larger diameter and hence contain more protoplasm. As a matter of fact it is the pieces of smaller diameter which regenerate the more rapidly. Further in slender stems there is no difference in diameter along the stem, and yet the apical halves of such stems regenerate hydranths earlier than the basal halves.

5. *Individual Experiments.*—These experiments were identical with the mass experiments except that each piece was placed in a separate dish and the number of hours required for it to produce an oral hydranth recorded as accurately as possible. The records of the four experiments of this kind which were performed are given in Table V. Experiments 9 and 17 were performed in June at room temperatures; experiments 29 and 45 in December at a temperature of 12° C. ± 2. Pieces were 8–12 mm. long except in experiment 29, where they were 5–8 mm. long. There was some mortality in the June experiments but none in December.

6. *Conclusions from Individual Experiments.*—The results of these experiments lead to the same conclusions as previously stated from mass experiments. Of 122 pairs of pieces in which both pieces regenerated, the apical halves regenerated hydranths first in 111 cases, or 91 per cent.; the basal halves first in 10 cases, or 8 per cent.; and the time of emergence of the hydranth was practically the same in both pieces in one case. Cases where the basal piece preceded in regeneration are indicated by

TABLE V.

RECORDS OF THE TIME OF EMERGENCE OF INDIVIDUAL APICAL AND BASAL HALVES OF THE SAME STEM.

Dagger calls attention to cases where the basal half emerged first; other abbreviations and symbols as before.

No.	Hours Since Cutting. Exp. 9.		No.	Hours Since Cutting. Exp. 17.		No.	Hours Since Cutting. Exp. 29.		No.	Hours Since Cutting. Exp. 45.	
	a.	*b.*		*a.*	*b.*		*a.*	*b.*		*a.*	*b.*
1	43	dead	1	40	*64	1	45	71	1	73	*91
2	*34	*58	2	77	86	2	103	*114	2	*67	77
3	42	75	3	*39	*64	3	65	79	3	60	75
4	44	50	4	*39	53	4	*88	79†	4	73	77
5	41	dead	5	43	77	5	57	73	5	60	73
6	42	dead	6	*39	45	6	56	69	6	*91	73†
7	38	48	7	*39	76	7	54	71	7	79	*67†
8	42	61	8	45	67	8	75	*88	8	59	71
9	38	44	9	68	67†	9	*64	66	9	56	*67
10	40	50	10	*64	74	10	*64	72	10	59	71
11	38	50	11	*64	dead	11	56	74	11	*115	93†
12	42	48	12	70	dead	12	56	76	12	*67	95
13	46	50	13	71	*87	13	53	*64	13	72	74
14	42	48	14	45	*64	14	*64	*87	14	72	73
15	36	50	15	41	53	15	66	*87	15	*66	*114
16	42	50	16	41	dead	16	*64	68	16	*90	78†
17	36	46	17	dead	116	17	68	90	17	*66	74
18	53	dead	18	75	112	18	92	97	18	72	78
19	42	67	Aver.	53	73	19	66	*87	19	68	76
20	36	46				20	*64	70	20	*66	76
21	42	63				21	76	*87	21	72	76
22	36	46				22	70	96	22	68	78
23	42	46				23	70	96	23	68	80
24	38	40				24	68	*87	24	*66	76
26	42	44				25	*64	76	25	68	76
27	42	42				26	*64	72	26	68	*90
28	40	*58				27	68	*112	27	68	72
29	42	63				28	90	95	28	78	74†
30	50	*58				29	73	104	29	68	78
31	48	63				30	66	76	30	58	70
32	65	67				31	72	*112	31	*66	72
33	61	78				32	68	78	32	*66	76
34	63	62.5†				33	114	99†	33	68	76
35	40	46				34	69	*86	34	68	78
Aver.	42	52				35	*86	91	35	72	76
						36	69	75	36	*66	92
						37	75	*86	Aver.	69	77
						38	75	*86			
						39	67	*86			
						40	*86	101			
						41	66	97			
						42	118	103†			
						Aver.	71	83			

Total number of regenerated pairs 122
Number of cases where *a* preceded . . . . . 111 or 91%
Number of cases where *b* preceded . . . . . 10 or 8%
Number of cases where *a* and *b* equal 1

a dagger in Table V. The average difference between the number of hours required for the emergence of the hydranths on apical and basal halves was 10 hours in exp. 9; 20 hours in exp. 17; 12 hours in exp. 29; and 8 hours in exp. 45. The individual differences range from half an hour to more than forty hours.

It may be inquired why in a small percentage of cases the basal piece precedes the apical piece in regeneration, and why there is such a great variation in the difference between the time of regeneration of the two pieces. It is highly probable that these variations are related to the degree of physiological isolation existent in the basal pieces before they were cut from the stems. It has already been pointed out that a hydranth controls only a certain length of the stem proximal to it and beyond that limit a new individual arises which eventually expresses its presence by the formation of a lateral bud. Now it is evident that such new individuals must exist physiologically before they give morphological expression of their existence by bud formation. It has already been stated that in these experiments the longest obtainable stems free from buds were used. Such stems are the exception rather than the rule since the majority of the material obtainable, particularly in the summer, will furnish only a small proportion of long stems free from branches. It is therefore obvious that the basal regions of such long stems must be in various stages of the process of physiological isolation and branch formation. The nearer such basal regions are to branch formation the more rapidly will they regenerate when isolated and those that are on the very verge of branch formation may conceivably regenerate as rapidly as or even more rapidly than more apical pieces. This matter is referred to again in connection with experiments on the rate of regeneration of basal pieces cut below branches.

7. *Remarks on the Temperature Coefficient.*—It has generally been accepted that when the rate of a biological process increases two to three times with each ten degrees rise in temperature that such a process is chemical in nature. It may be doubted that this line of reasoning is strictly correct. The use of the temperature coefficient to analyze the nature of a biological process involves the unwarranted assumption that such processes

may be purely chemical; but it is very doubtful that they ever are solely chemical in nature, and, of course, equally doubtful that they are ever the consequence of purely physical changes. In all probability biological processes are neither complexes of purely chemical reactions nor purely the resultants of physical changes but they involve both types of changes occurring simultaneously and mutually interacting. On *a priori* grounds, however, it may be accepted that the chemical processes are of paramount importance in living things, since, while substances having physical properties similar to or identical with those of protoplasm exist which are not alive, in no case do non-living materials carry on the chemical reactions characteristic of protoplasm; further, the "signs of life" are chemical or of chemical origin, and protoplasm in which the chemical reactions have fallen to a low level is to all intents dead. Granting, therefore, that chemical reactions play the most important rôles in life processes and that in many cases physical changes are insignificant, it may be valid to draw conclusions from the value of the temperature coefficient. But it must always be borne in mind that the chemical reactions which occur in living things are subject to processes of regulation in the organism. The relation of chemical changes to temperature is therefore in the organism a variable quantity. Thus Behre ('18) found that the rate of respiratory metabolism of *Planaria* is lowered when the animals are maintained at a high temperature and raised when they are maintained at low temperatures. The temperature coefficient for the rate of respiration of *Planaria* is therefore not a fixed value for a certain range of temperature but depends to some extent upon the temperature at which the animals had been living previous to the experimental test. Since such modifications or regulations are known for emulsoid colloids, their behavior at any given time depending upon the conditions to which they had previously been exposed (phenomenon of hysteresis), it is possible that this ability of organisms to modify the rate of processes presumably chiefly chemical with reference to temperature is due to the colloidal substratum in which the chemical reactions take place.

In *Tubularia* similar regulations to temperature are observable.

The rate of regeneration of pieces of the stem of *Tubularia* is, as has long been known, dependent in large part upon the temperature and the temperature coefficient of this process is described as corresponding to that of chemical reasions (Moore, '10). The rate of regeneration is not, however, wholly dependent upon the temperature at which regeneration occurs but is to some degree affected by the temperature at which the particular stems used had been living previous to their utilization. Thus in the experiments recorded in Tables III. and IV., it can be noted that summer material regenerates more slowly at 13° C. than does winter material at 12° C. While other possible explanations of this fact could be suggested it seems reasonable in the light of other results along this line to suppose that this is another case of acclimation to temperature; material living for some time at low temperature has elevated the rate of its chemical processes above that which would result if the material were suddenly lowered to the same temperature from a higher temperature—a procedure usually practised in experiments on the temperature coefficient.

F. Rate of Regeneration of Distal and Proximal Pieces of Unequal Length.

Banus refers to Child's experiments on pieces of unequal length in which Child found that longer pieces will regenerate slightly faster than shorter ones provided the factor of level is eliminated by always making the apical pieces the shorter pieces. Since apical pieces regenerate faster than basal pieces no conclusions could be drawn regarding the effect of length on the time of regeneration unless the apical piece were the shorter. Banus has "repeated" this experiment and claims that the longer piece always regenerates first regardless of level. Here Banus has again misrepresented Child's statements and he has not in reality repeated Child's experiment. Child distinctly states that changes in the length of the piece "produce only very slight or no appreciable differences in time of emergence of oral hydranths provided the length of the piece is above a certain minimum. But with reduction in length below the minimum the appearance of the hydranth is delayed and this retardation increases with

further reduction in length." The apical pieces will therefore regenerate later than the basal pieces only when they are reduced below a certain minimum length. This minimum length is very much less than any used in Banus's experiments. In order to get the result mentioned by Child it is necessary to cut the apical pieces as small as 2 mm. Yet Banus in "repeating" Child's experiments has used no pieces less than 10 mm. in length. In pieces as long as this, length makes very little difference; according to Child's results and my own, the apical pieces will regenerate oral hydranths earlier than the basal pieces, just as when both pieces are of equal size. In his tables 3, 4, and 5, Banus presents data on apical and basal pieces in which the lengths of the pieces were: 10 and 20 mm., 10 and 30 mm., and 10 and 40 mm. Banus found that the longer pieces in all of these cases, regardless of whether they are apical or basal, regenerate oral hydranths slightly in advance of the shorter pieces.

With these results and statements of Banus I am quite unable to agree. There is some truth in the statement that a longer piece will regenerate slightly faster than a shorter piece with apical end at the same level. Yet in the case of apical pieces, the difference between pieces 10 and 20 mm. long is very slight indeed, in fact, practically nil; but it is plainly marked in shorter pieces, say 5 and 10 mm. long. In the case of the basal pieces the difference in time of regeneration between 10 and 20 mm. pieces is somewhat greater but here it must be remembered that the apical end of a basal piece 20 mm. long is in these experiments at a level 10 mm. more distal than that of a basal piece 10 mm. long, and the factor of level again comes into play. In all cases in pieces exceeding 5 mm. in length, the apical pieces will in general regenerate more quickly than the basal pieces, regardless of their relative lengths; and a basal piece twice as long as an apical piece will still regenerate more slowly than the apical piece, notwithstanding the effect of length.

I have repeated Banus's experiment on relatively long pieces of unequal length and the results are given in Table VI. Three pairs of experiments were performed. In one experiment of each pair the apical piece was *half* as long as the basal; in the other

experiment, the apical piece was *twice* as long as the basal. Pieces for the two experiments of each pair were cut from the same lot of stems at the same time and kept under the same conditions. All of the apical pieces were kept in one finger bowl and the basal in another. The method of cutting the pieces for such experiments is given in text-figure 2. All of these experiments were performed in December at a temperature of 12° ± 2. The length of the pieces is stated in the table. No experiments were attempted with pieces in which the ratio of length was 1 : 3 or 1 : 4, as it is difficult if not impossible to obtain a sufficient number of unbranched stems of the requisite length.

The data given in Table VI. show quite clearly that an

TABLE VI.

RECORDS OF MASS EXPERIMENTS WITH APICAL AND BASAL PIECES OF UNEQUAL LENGTH.

Columns under *a* and *b* record number of hydranths emerged at time indicated.

Exp. 32. *a* = 4 — 6 Mm., *b* = 8 — 12 Mm			Exp. 33. *a* = 8 — 12 Mm., *b* = 4 — 6 Mm.			Exp. 37. *a* = 8 — 10 Mm., *b* = 15 — 20 Mm.			Exp. 38. *a* = 15 — 20 Mm., *b* = 8 — 10 Mm.			Exp. 42. *a* = 10 — 11 Mm., *b* = 20 — 22 Mm.			Exp. 43. *a* = 20 — 22 Mm., *b* = 10 — 11 Mm.		
Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.
56	1	0	58	1	0	*59	18	0	*59	7	0	53	3	0	53	7	0
58	2	0	*67	15	2	61	24	1	61	11	0	55	7	0	55	9	0
*67	11	9	69	16	4	63	35	3	63	17	0	57	11	0	57	10	0
69	13	11	71	18	5	65	41	7	65	22	0	59	18	0	59	12	1
71	15	16	73	21	7	67	47	13	67	24	3	62	20	0	62	17	2
73	17	17	75	22	14	69	49	18	69	28	10	*69	25	25	*69	26	18
75	20	19	77		17	71	49	25	71	28	14	72	26	26	72	28	24
77	21	20	79		19	73	50	27	73	30	18	74	26	27	74	28	26
79	22	22	81		20	76		30	76	32	21	76	26	28	76	29	27
84	23	22	84		21	*83		35	*83	35	34	78	26	29	78	29	29
*91	23	23	*91		22	86		38	86	37	38	82	27	30	82	30	29
94	24	23				88		40	88	38	45	84	29		84		29
104		24				90		41	90	43	46	*96	30		*96		30
						94		43	94	43	47						
						96		44	96	44	49						
						98		46	*108	49	50						
						*108		47	114	50							
						110		48									
						120		49									
						*132		50									

G. ALTERATION OF THE REGIONAL DIFFERENCES IN RATE OF REGENERATION.

apical piece will regenerate faster than a basal piece of twice its length, in pieces at least as long as 5 mm. Although the factor of length is of some consequence, the factor of level is of vastly

greater importance. In experiments 32 and 33, the longer pieces in each case regenerate faster, but the effect of length does not overcome the effect of level, the apical pieces in both experiments regenerating first on the whole. The influence of length is most marked in experiments 32 and 33, where short pieces were employed. It is very little evident in experiments 37 and 38, and 42 and 43, where pieces 10 mm. in length were employed. In fact, in experiment 37, the apical pieces 10 mm. long regenerate slightly faster than the apical pieces 20 mm. long; and in experiments 42 and 43 there is practically no difference. We may therefore say that length is of little consequence in pieces exceeding 10 mm. in length, in agreement with Child's previous statement and in contradiction to the claims of Banus. In all experiments the longer basal pieces regenerate faster than the shorter basal pieces, but it is probable that this effect is one of level rather than of length, because the longer basal pieces have their apical ends at a higher level than the shorter basal pieces.

It may therefore be concluded that in the case of apical and basal pieces of unequal length, the apical pieces will still regenerate more rapidly on the whole regardless of whether they constitute the shorter or the longer pieces, always provided that their minimum length is 5 mm. The level at which the pieces are cut is still the dominant factor in such experiments. In pieces below 10 mm. in size, a longer piece will regenerate slightly faster than a shorter one; but in pieces above 10 mm. length, length is of practically no consequence. Long basal pieces in such experiments regenerate faster than shorter basal ones mainly because their apical ends are at a higher level than the apical ends of the shorter pieces. These results are the contrary of those of Banus whose experiments are invalidated owing to his erroneous method of cutting the apical pieces as discussed at greater length below.

The data already presented incontestably demonstrate that a regional difference in rate of regeneration exists along the axis of *Tubularia*, such that regeneration is the more rapid the nearer the piece lies to the apical end. It may next be inquired whether this regional difference is modifiable under either certain normal conditions or under experimental conditions. To this inquiry

an affirmative answer may be returned. It is possible to modify or eliminate the regional differences in question. The various methods by means of which this was attempted or accomplished are discussed in this section.

1. *The Effect of Cold.*—A number of experiments were performed in which both apical and basal pieces were exposed to a lowered temperature for a number of hours after cutting or during the entire period of regeneration. The pieces were removed from room temperatures (approximately 20° C.) to the temperature of the refrigerator (13° C.) for various periods of time. Although such a proceeding invariably retards the rate of regeneration, the differences between the regeneration of apical and basal pieces were unaltered by such exposure to low temperature. These experiments are therefore included in Table III. (exps. 6, 11, 15, 16, and 21), as showing the typical difference between the rate of regeneration of apical and basal halves. It is highly probable, however, that with very low temperatures, in the neighborhood of zero, the typical difference between pieces of different level would be reduced or eliminated.

2. *Effect of Using Material Kept in the Laboratory.*—Two experiments were performed in June upon material which had been kept in the laboratory aquaria for a week preceding the cutting of the pieces. As is well known under such circumstances, the hydranths of *Tubularia* fall from the stems and new hydranths are subsequently regenerated. Such new hydranths are smaller than and have a lower rate of activity than the original hydranths; it is therefore to be expected that the regional differences along the axis will be reduced in such cases. As already stated only two experiments were performed as the weather had become warm and little material was available. The material for these experiments was collected on June 30 and cut on July 6. The pieces were kept in the refrigerator (13° C.) throughout the regeneration period. The results are given in Table VII. Experiment 21, Table III., furnishes a control for these experiments. It will readily be seen that the differences between the rate of regeneration of apical and basal pieces are plainly reduced as a consequence of the depressing effect of laboratory conditions upon the physiological axis of

Tubularia. This experiment shows that the metabolic gradient of *Tubularia* is not a fixed and permanent gradient in the stem but is readily variable under the conditions of the animal's environment. Experiments such as those of Banus in which no account is taken nor any description given of the conditions of the material or the environment do not therefore merit serious consideration.

TABLE VII.

RECORD OF MASS EXPERIMENTS WITH APICAL AND BASAL PIECES OF EQUAL LENGTH, THE PIECES BEING TAKEN FROM MATERIAL KEPT ONE WEEK IN LABORATORY CONDITIONS BEFORE CUTTING.

Temp. 13° C. Control, exp. 21, Table III., in which the pieces were cut on the same day as the material was collected.

Exp. 24.			Exp. 25.		
Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.
72	1	0	72	4	0
*84	11	5	*84	9	3
87	12	9	87	11	6
94	22	16	89	13	9
96	22	18	94	22	18
*107	33	28	96	23	23
110	33	30	*107	27	26
114	35	33	110	28	28
117	36	33	114	29	28
119	36	33	117	29	30
123	37	35	119	30	
*146		36			
158		37			

3. *The Effect of the Presence of Branches*.—In the consideration of the data on the electrical gradient in *Tubularia* it was pointed out that the control of a *Tubularia* hydranth extends only for a limited distance down the stem and that the stem beyond this limit is more or less differentiated as another individual. This differentiation, at first purely physiological, is later morphologically apparent by the formation of a bud at the level of the apical end of the new individual. The appearance of the bud not only indicates the formation of a new individual but also is an expression of a loss of control of the basal portions of the stem by the original hydranth. It is therefore to be expected that pieces taken above such lateral branches will have a lower metabolic rate than pieces of the same level from unbranched stems; and further that pieces taken below the branch, since

they are near the apical ends of new individuals (the real apical end being the hydranth of the branch) will have a higher metabolic rate than ordinary basal pieces. Owing to the operation of both of these factors it may be expected that the difference between apical and basal pieces will be reduced when they are cut above and below, respectively, the level of a branch. This was found to be the case. Banus in his paper does not state whether or not he used stems free from branches and did not reply to inquiries on this point.

In preparing pieces for this kind of experiment, the following procedure was usually adopted. Stems having one branch at about the middle of the stem were selected, the terminal hydranth, upper millimeter or two, and basal end cut off and discarded as usual. An apical piece was then cut anterior to the branch, and a basal piece of equal length posterior to the branch; the small piece bearing the branch was discarded. As found by Morgan and verified in my experiments, the stumps of lateral branches left on pieces will regenerate hydranths more rapidly than the distal end of such pieces, and these lateral hydranths will then inhibit the formation of the terminal hydranth; hence in experiments of this kind it is necessary to avoid using pieces bearing stumps of branches. The method of cutting the pieces in most of the experiments is illustrated in Fig. 3. In one experiment, stems having two branches were selected, the apical piece cut in front of the first branch, and the basal piece between the two branches as illustrated in Fig. 4.

The results are presented in Table VIII. All experiments of this kind were mass experiments. Experiments 12*a* and 12*b* were performed in June at room temperature, the remaining experiments in December at 12° C. ± 2. The controls for these experiments are indicated at the top of the table; such controls were cut at the same time and from the same lot of material, with the exception that they came from stems without branches, and were kept under the same conditions. The pieces in experiments 28, 30, and 34 were short pieces, 5–10 mm. long; those in experiments 12 and 36, approximately 10 mm. long. The pieces for all experiments except number 36 were prepared according to Fig. 3; those for experiment 36 as in Fig. 4.

The effect on the relative times of regeneration of apical and basal pieces by cutting them above and below a lateral branch is of course slight but nevertheless it is evident in most cases. If the experiments given in Table VIII. are compared with those

TABLE VIII.

RECORDS OF MASS EXPERIMENTS WITH APICAL AND BASAL PIECES OF EQUAL LENGTH, THE APICAL PIECES BEING TAKEN IN FRONT OF THE FIRST BRANCH, THE BASAL PIECES BELOW THE BRANCH.

Columns under *a* and *b* give number of hydranths emerged at time indicated. Controls in Table III. and IV., exp. 10 for exp. 12; exps. 26 and 27 for exp. 28, 30 and 34; exp. 35 for exp. 36.

Exp. 12*a*.			Exp. 12*b*.			Exp. 28.			Exp. 30.			Exp. 34.			Exp. 36.		
Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.
37	1	0	33	1	0	53	1	0	54	1	0	56	1	0	*59	2	0
43	2	0	37	2	0	55	2	0	*62	4	0	58	3	0	63	4	0
45	3	0	*43	6	2	57	3	0	66	5	2	*67	17	2	65	5	0
49	4	1	47	10	3	60	11	0	68	7	4	69	17	3	67	8	2
51	4	3	49	13	6	*68	13	5	70	9	8	71	17	8	69	11	4
*57	6	4	51	16	9	70	14	6	72	9	9	73	22	9	71	14	10
60	8	4	53	17	13	72	15	7	79	11	10	75	25	14	73	15	11
66	9	5	55	18	15	74	16	9	82	11	13	77	32	20	76	18	12
68	10	6	59	25	19	76	17	11	*90	12	13	79	35	26	*83	28	20
72	11	7	61	28	20	78	18	12	93	13	14	81	38	27	86	35	23
74	11	8	*68	29	22	80	19	13	95	15	15	84	40	30	88	36	27
*81	11	9	71	30	24	83	20	15	99	15	16	*91	55	41	90	43	31
88	12	10	75	31	24	*91	22	18	*116	16		94	57	41	92	47	36
95	13	10	77		25	94		19				96	59	42	94	48	40
105	14	10	85		25	117		20				98		44	96	48	41
108		11	*92		26	119		22				100		47	98	48	43
110		12	97		27							106		51	*108	51	46
			100		28							*116		58	110		48
			107		29							122		59	114		49
															120		51

in Tables III. and IV., it will be found that in general more basal pieces have regenerated in the experimental series when the same number of apical pieces have regenerated in both control and experimental series. This appears chiefly in the early part of the regeneration period. A few such comparisons may be pointed out; in making them it is necessary to select experiments in which the total number of regenerating pieces is similar in experiment and control, since the number of regenerated apical pieces in the early stages of an experiment is greater relative to the basal pieces, the greater the total number of pieces. In experiment 12*b*, Table VIII., 2, 9, and 19 basal pieces have

formed hydranths as compared with 1, 6, and less than 15 basal pieces in the control experiment, number 10, Table III., when 6, 16, and 25 apical pieces have regenerated in both cases. Similarly, in experiment 30, for which experiment 26 is a control, the regeneration of the apical and basal pieces is practically simultaneous, a result which is never obtained when stems free from branches are employed. Comparison of experiment 36, Table VIII., with its control, experiment 35, Table IV., shows the same effect; in the former case 12 basal pieces, in the latter case no basal pieces have regenerated at the time when 18 apical pieces have regenerated in both experiments. In other cases, as in experiment 34, little difference from the control could be observed. The decrease in the time difference between the apical and basal pieces in these experiments is apparently largely due to a delay in the regeneration of the apical pieces. This is to be expected, since as already explained all basal pieces are probably more or less isolated as new individuals, and hence are slightly accelerated in both experimental and control series. The "apical" pieces, on the other hand, in the present experiments, since they are taken in front of the level of a branch, really represent the basal end of the first zoöid of the stem, and hence are delayed in regeneration as compared with pieces similar in position from stems where branches have not yet arisen and where the hydranth still controls most of the length of the stem. In regard to the basal pieces, it should further be pointed out that the really high metabolic point of the new individuals formed at the base of *Tubularia* stems is in the hydranth of the branch, and the basal piece itself below the level of the branch retains only part of the increased metabolic rate after the bud has formed.

Banus has presented one table in which he has compared the rate of regeneration of three equal pieces, each 10 mm. in length, from different levels of the same stem. I have not repeated these experiments as they seem to be lacking in point. The reason why the apical pieces in these experiments of Banus regenerate more slowly than the middle pieces is doubtless, as in the case of the other experiments reported in his paper, the consequence of an erroneous method of cutting the apical pieces. That some

basal pieces may precede the middle pieces is to be expected on the basis of what has already been said. It should be obvious that in stems as long as 30 mm., the length required for these experiments, the basal regions must already be more or less physiologically isolated as new individuals whether branches are present or not. Therefore it may be expected that at least some of these basal pieces will regenerate more rapidly than the middle pieces. It is furthermore to be remarked that the metabolic gradient is steepest near the hydranth and gradually diminishes in slope down the stem; and it has never been claimed by us that any marked axial difference exists along the basal parts of the stems of hydroids. Indeed, we believe that in many cases the gradient has disappeared in these basal regions, as shown by the tendency for such levels of the stem to produce numerous adventitious buds, irregularly arranged, while in the more distal levels of the stem, bud formation proceeds in a very definite and orderly manner.

4. *The Effect of Depressing Agents.*—It has been pointed out by us on numerous previous occasions that a certain relation exists between metabolic rate and depressing agents, such that regions of higher metabolic rate are more affected by depressing agents than regions of lower metabolic rate. If this general statement is correct, and various lines of evidence establish its accuracy, then it should be possible to reduce, eliminate, or reverse the differences in rate of regeneration that normally exist between apical and basal pieces. This is the case. Only two depressing agents were employed, ethyl ether and potassium cyanide. Apical and basal pieces of equal length were cut in the usual way and both exposed to the same concentration of these substances, made up in sea-water, for a certain length of time. The pieces were then thoroughly washed in several changes of sea-water and completed their regeneration in normal sea-water.

These experiments are presented in Tables IX. and X. In Table IX. are given the results of all the mass experiments performed with cyanide. They were performed in June, at room temperature, except number 22, which was placed in the refrigerator later. The concentration of cyanide used and the number of hours during which the pieces were exposed to it are

given in the table. In experiment 7, the concentration employed, 1/20000 mol., was too weak to produce any effect, but the effects of 1/10000 and 1/5000 mol. solutions are very striking. The rate of regeneration of both apical and basal pieces is retarded, but that of the apical pieces, in accordance with the hypothesis, is more retarded so that the basal pieces regenerate on the whole the more rapidly.

TABLE IX.

RECORD OF MASS EXPERIMENTS ON THE RATE OF REGENERATION OF APICAL AND BASAL HALVES WHEN BOTH ARE EXPOSED FOR A NUMBER OF HOURS AFTER CUTTING TO THE SAME CONCENTRATION OF POTASSIUM CYANIDE.

Columns give numbers of hydranths emerged at hours indicated. Exp. 10 control for exp. 13; exp. 21 for exp. 22. See Table III.

Exp. 7. KNC 1/20000 Mol. for 20 Hrs. After Cutting.			Exp. 13. KNC 1/10000 Mol. for 12 Hrs. After Cutting.			Exp. 22. KNC 1/5000 Mol. for 9 Hrs. After Cutting.		
Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*
36	1	0	60	1	0	84	1	0
40	2	0	62	2	2	93	2	0
46	5	2	*69	3	7	put into refrigerator		
48	11	3	72	8	9			
50	13	8	74	12	14	178	2	1
*58	18	16	76	13	16	205	7	3
61	22	18	78	15	20	210	8	7
64	23	20	80	17	20	216	8	9
67		21	83	20	23	*227	11	13
73		23	85	24	24	229	11	14
			*93	34	36	231	12	16
			101	35	40	237	13	20
			106	35	41	*249	16	22
			108	36	42	256	17	22
			111	37		264	19	22
			*120	38		*273	23	23
						276	24	23
						280	24	26
						283	24	29
						287	26	29
						*297	28	30
						300	29	
						305	30	

The results of the experiments with ether are given in Table X. Experiments 14 and 20 are mass experiments; 18 and 46 give records of the number of hours required for the regeneration of each piece. Experiments 14, 20, and 18 were performed in June at room temperatures, except that exp. 20 was kept in the refrigerator for part of the time after regeneration had begun. Experiment 46 was performed in December at a temperature of

12° C. ± 2. It will be observed that in the case of the mass experiments, the basal pieces on the whole regenerate more rapidly than the apical pieces, although both are retarded as compared with the controls. The individual experiments bring out the same point. The number of deaths was considerable but of 39 pairs in which both pieces regenerated, the basal pieces preceded the apical pieces in 14 cases, or 38 per cent. as compared with the result under normal conditions as given in Table V., where but 8 per cent. of the basal pieces precede the apical pieces.

A number of interesting points are brought out by these experiments with ether and cyanide. In the first place the rate of regeneration is greatly retarded. In the case of cyanide, where different concentrations were employed, the retardation is proportional to the concentration used. This retardation is evidenced by both kinds of pieces, the apical pieces being, however, more retarded than the basal pieces, with the consequence that the usual relation between the time of regeneration of apical and basal pieces is reversed. Now there can be little doubt that the rate of regeneration of pieces of *Tubularia* primarily depends upon the rate of chemical processes in those pieces. The effect of temperature upon the rate of regeneration is sufficient proof of this. Therefore, since depressing agents retard the rate of regeneration, it is impossible to doubt that they bring about this effect by lowering the rate of chemical reactions in the pieces. This is further evidenced by the fact that concentrations of these reagents which are effective at room temperatures are entirely without effect at temperatures of 12° and 13° C. Thus 1 per cent. ether is very effective at 20° C. but has no effect at 12° C. In order to alter the relations of apical and basal pieces at 12° C., it is necessary to use 2 per cent. ether. The same is true of cyanide. When, therefore, the metabolic rate is already lowered by low temperature, the action of depressing agents is diminished. This further supports the statement made at the beginning of this section that the action of depressing agents is related to the rate of chemical activity of the protoplasm which is exposed to them, and that such effects are greater the higher the metabolic rate of the living material. The differential effect, therefore, of ether and cyanide on the rate of regeneration of apical and basal

TABLE X.

RATE OF REGENERATION OF APICAL AND BASAL HALVES WHEN BOTH ARE EXPOSED FOR A NUMBER OF HOURS AFTER CUTTING TO THE SAME CONCENTRATION OF ETHER.

Experiments 14 and 20, mass experiments; experiments 18 and 46, individual experiments. Exp. 10, control for exp. 14; exp. 17 for 18 and 20; exp. 45 for 46; in Tables III. and V.

Mass Experiments.						Individual Experiments.					
Exp. 14, 1 % Ether for 12 Hrs. After Cutting.			Exp. 20, 1 % Ether for 12 Hrs. After Cutting.			Exp. 18, 1 % Ether for 15 Hrs. After Cutting.			Exp. 46, 2 % Ether for 17 Hrs. After Cutting.		
Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	No.	*a.*	*b.*	No.	*a.*	*b.*
53	0	1	60	3	3	1	70	74	1	dead	dead
55	2	1	65	8	7	2	*87	115	2	dead	*137
59	5	4	67	8	12	3	*87	90	3	dead	dead
61	7	6	69	10	14	4	115	64†	4	dead	dead
*68	10	12	73	13	19	5	68	dead	5	91	160
71	10	14	75	14	19	6	74	94	6	100	91†
73	14	16	*83	16	25	7	92	dead	7	94.5	95
75	16	19	88	17		8	87	115	8	137	125†
77	20	23	90	19		9	99	dead	9	73	88
79	22	24	92	20		10	dead	99	10	89	79†
81	25	28				11	99	96†	11	113	127
83	26	29				12	74	70†	12	dead	125
*91	30	32				13	dead	dead	13	127	77†
94	31	34				14	dead	87	14	dead	dead
99	33	35				15	87	dead	15	71	91
101	36	36				16	dead	76	16	100	71†
106	36	36				17	94	89†	17	95	*137
*115	37	36				18	96	dead	18	74	*89
129	37	37				19	90	*107	19	*88	90
*140	37	38				20	63	63	20	68	dead
									21	*88	112
									22	90	100
									23	*88	*88†
									24	100	94†
									25	*88	90
									26	78	*116
									27	dead	115
									28	75	89
									29	89	77†
									30	dead	77
									31	69	dead
									32	77	91
									33	dead	77
									34	69	*111
									35	*111	87†
									36	91	101
									37	89	98
									38	*111	91†
									39	71	87
									40	dead	dead

Total number of regenerated pairs..... 39
Number where *a* preceded..... 24 or 61%
Number where *b* preceded..... 14 or 38%
Number where *a* and *b* equal..... 1

pieces indicates very clearly that the apical pieces have a higher rate of chemical activity. They are more affected by the depressing agents and more retarded.

5. *The Effect of Cutting the Distal End of the Apical Piece at the Base of the Hydranth.*—When questioned regarding his manner of cutting the pieces for his experiments, Banus replied as follows (I quote verbatim from his letter): "The most distal cut was usually made as near as possible to the hydranth without including any part of it. Other times more basal parts were used. No difference in the results was found." To two subsequent letters requesting more specific statements concerning this matter and asking for a diagram showing the exact relation of the most distal cut to the base of the hydranth, Banus returned no replies. The first sentence quoted leaves little doubt that Banus made his most distal cut just below the base of the hydranth, therefore including in the apical pieces, the little neck or stalk region of the hydranths. The rest of Banus's statement is too vague to merit any attention. What is meant by "more basal parts"? How is one to know in the experiments reported by Banus in which cases the distal cut was made at the base of the hydranth, and in which cases more basally? Certain it is that in some of Banus's pairs of pieces the apical piece emerges first, and in others the basal piece. This indicates some great irregularity in his method of procedure. Probably those cases where the apical pieces emerged first are the ones in which "more basal parts were used." In the absence of more definite information, speculation is idle. We are here concerned with the fact that *usually the distal cut was made at the base of the hydranth.*

I have performed three experiments in which the apical pieces were cut in the manner usually employed by Banus and as represented in text-figure 5. Such apical pieces include the stalk of the hydranth. This stalk is incapable of regeneration. It together with that portion of the cœnosarc which occupies the distal end of the perisarc dies away and disintegrates. This process of death and disintegration of the apical end of apical pieces cut in this manner naturally delays the regeneration of the apical pieces, because regeneration does not begin until the end of the piece has rounded off and become covered with a layer of

cells. But this is not the only retarding factor in such pieces. The cœnosarc after the death of the apical end withdraws into the perisarc leaving a short apical region of empty perisarc. This empty perisarc crumples to a greater or less extent. Therefore when the hydranth does regenerate it has to push out through this empty region before it can unfold, and this of itself would further delay the time of emergence of the oral hydranth; but to make matters worse, the crumpling of the empty perisarc renders it very difficult for the hydranth to push its way to the surface. On account of all of these factors, the regeneration of the apical pieces is very greatly delayed when they are cut in the manner employed by Banus. In fact, in many cases, the oral hydranth is so greatly retarded that the aboral hydranth emerges first, and in a few cases, the oral hydranth never emerged on such pieces, a complete reversal of polarity with disappearance of the primordium of the oral hydranth having been observed. Presumably Banus failed to notice whether oral or aboral hydranths had emerged, but the two ends of such pieces are easily distinguished by the bit of empty perisarc so that there is no doubt of the correctness of my statements. Not only are the oral hydranths of these pieces delayed but they are often abnormal in appearance; they are enlarged and distended, owing probably to the pressure to which they are subjected in being forced out through the crumpled perisarc, and their tentacles are short and stumpy. They regulate to normal within a few hours after they have emerged. In two or three cases, partially doubled hydranths were produced.

The three experiments performed with pieces cut in the way employed by Banus and as represented in Fig. 5 are presented in Table XI. They were performed in December and regenerated at a temperature of 12° C. ± 2. Experiment 31 consisted of pieces 5–8 mm. long, the other experiments of pieces 10–12 mm. long. In connection with experiments 39 and 49, the number of both oral and aboral hydranths emerged on the apical pieces at each observation is given. These records include of course only those cases in which the aboral hydranths emerged first. In some of these cases the oral hydranths subsequently emerged, and this is indicated by the number in parenthesis which follows

the number of pieces having aboral hydranths. In experiment 49, six such pieces had failed to give rise to oral hydranths when the experiment was concluded and in three of these cases, no primordia of the oral hydranths were present, the polarity having been completely reversed.

The data given in Table XI. show in a very striking manner that the regeneration of apical pieces cut so that their distal ends

TABLE XI.

RECORD OF MASS EXPERIMENTS ON THE RATE OF REGENERATION OF APICAL AND BASAL PIECES OF EQUAL LENGTH WHEN THE DISTAL END OF THE APICAL PIECES IS TAKEN AT THE BASE OF THE ORIGINAL HYDRANTHS.

Exp. 31.			Exp. 39.				Exp. 49.			
Hrs.	*a*.	*b*.	Hrs.	*a* Oral.	*a* Aboral.	*b*.	Hrs.	*a* Oral.	*a* Aboral.	*b*.
60	1	2	62	5		0	61	0		1
*69	8	19	64	12		2	65	3		2
71	12	23	66	16		23	67	7		8
73	13	28	68	25		38	69	8		15
76	16	33	70	29		43	71	13		19
78	21	39	72	31		44	73	22	1	28
80	28	47	75	34		45	75	25	2(1)	36
82	33	49	*82	42		47	*85	31	4(1)	46
84	38	49	85	43	2	48	87	32	7(1)	47
87	44	49	87	45	3	49	89	34	8(1)	49
*94	47	52	89	48	3	50	91	35	10(2)	49
101	48		91	48	3	51	93	38	10(2)	49
103	49		95	49	3	51	96	40	10(2)	49
105	50		*107	50	3	52	98	40	10(2)	50
107	52		109	51	3(1)		*110	44	10(4)	
			111	52	3(3)					

are just at the base of the original hydranths is markedly delayed and that in the majority of cases, the basal pieces regenerate first. It should be remarked that the delay is chiefly in the time of emergence of the oral hydranths and not in its formation; for the primordia of the hydranths in these apical pieces form in advance as a rule of those of the corresponding basal pieces; but these hydranths can not emerge as rapidly owing to the fact that they must be pushed through the piece of empty crumpled perisarc left by the death of stalk region.

The data in Table XI. furnish the explanation of Banus's results. It is obvious that anyone who practices the method of cutting the apical pieces described in connection with this table and who mixes up such a method with procedures where "more

basal parts are used" can expect nothing but irregular and inexplicable results. By using such methods and failing to describe them it is possible to accumulate data which appear to contradict everything that previous workers have obtained. As long as no one takes the trouble to inquire by what procedures such data were obtained and as long as the author refuses to furnish any information about his methods, such data might stand on record indenfiitely in the scientific journals to puzzle future investigators. I believe that I have conclusively shown that Banus's data are completely invalidated by his experimental method. This work and that of previous investigators—Driesch, Morgan, Child, Stevens, and Allee—demonstrate incontestably that in *Tubularia* when other factors are equal the rate of regeneration of pieces of equal size depends upon the level which they occupied in the intact stem; it is more rapid the nearer the pieces lie to the original distal end of the stem. A metabolic gradient exists in the stem of *Tubularia* which is the primary cause of these regional differences in rate of regeneration.

II. Summary.

1. This experimental work was undertaken as a reply to a paper published by Banus ('18).

2. The existence of a metabolic gradient in the stem of *Tubularia* is demonstrated in this paper in four different ways.

(*a*) Differential susceptibility of apical and basal regions of the stem to ether and cyanide. Apical regions are more susceptible.

(*b*) Differential capacity of apical and basal regions to reduce potassium permanganate. The apical end of the organism has the greatest reducing power.

(*c*) Difference in electrical potential along the stem. Apical regions are electronegative (galvanometrically) to basal levels within the limits of the individual. (At a certain distance from the original hydranth of *Tubularia* a new individual is arising and the apical end of this is likewise electronegative to regions anterior to its level.) Since in general electronegativity is associated in protoplasm with increased oxidative metabolism, this difference in electrical potential along the stem of *Tubularia* is

evidence that distal levels have a higher metabolic rate than proximal levels.

(*d*) Difference in the rate of regeneration of apical and basal pieces. Work upon this point constitutes the bulk of the paper and is summarized under the subsequent heads.

3. Apical halves of the stem of *Tubularia* regenerate oral hydranths markedly faster than basal halves. In cutting such pieces it is essential to discard the original hydranth and the first millimeter or two of the stem; the remaining stem is then cut into two equal halves. The difference between such halves has been demonstrated by:

(*a*) Mass experiments in which all of the apical halves have been placed in one dish, the basal in another. In such cases, the number of apical pieces which have regenerated oral hydranths is nearly always in excess, rarely equal to, and never less than the number of basal pieces which have regenerated.

(*b*) Individual experiments, in which the number of hours required for the emergence of the oral hydranth on each piece was recorded. The apical pieces regenerated oral hydranths first in 91 per cent. of the cases (122 pairs of pieces observed).

4. Apical pieces regenerate on the whole more rapidly than basal pieces, even when the latter are twice as long as the apical pieces. Such apical pieces must not however be less than 5 mm. in length. In pieces over 10 mm. in length, length has very little effect upon the time of regeneration; in pieces less than 10 mm. in length, the longer pieces regenerate faster than shorter ones having their distal ends at the same anterior level but this effect of length is not sufficient to overcome the influence of level except in very short pieces (under 5 mm.).

5. The difference in rate of regeneration of apical and basal pieces which exists under normal condition can be somewhat reduced by using stems bearing branches and cutting the apical piece above the branch and the basal piece below the branch. Since the first branch marks the limit of the *Tubularia* individual, the apical pieces above such branches are really the basal regions of the principal *Tubularia* individual, and the basal pieces below the branch are near the apical end of the second individual. In consequence of these relations, the difference between the

time of regeneration of such apical and basal pieces is less than is the case when pieces are cut from corresponding levels of stems without branches.

6. The difference in rate of regeneration of apical and basal pieces of the stem of *Tubularia* can be reversed by putting both sets of pieces for a certain time after cutting into appropriate concentrations of depressing agents like cyanide and ether. Under such circumstances the basal pieces regenerate in advance of the apical ones on the whole. This is due to the fact that depressing agents affect most strongly those regions having a higher rate of chemical activity. Since apical pieces have a higher metabolic rate than basal pieces, they are more affected by the same concentration of depressing agent and hence their regeneration is more retarded. In such cases the basal pieces regenerate the more rapidly. That this explanation is correct is further evidenced by the fact that the action of depressing agents is greatly influenced by temperature. At lowered temperatures a higher concentration of the agent must be employed to obtain the same effect produced at higher temperatures by lower concentrations.

7. These results are in accord with those obtained by a number of previous investigators and are directly opposed to the results presented by Banus. Banus claims that there is no difference on the average between the time of regeneration of oral hydranths on apical and basal pieces of the stem of *Tubularia*. Personal communication with Banus has elicited the fact that his usual method of cutting the apical pieces was erroneous. He cut them in such a way that the distal end of the apical piece was taken just below the base of the hydranth. In such cases, as shown in this paper, the distal ends of the apical pieces die away and the regeneration and time of emergence of the oral hydranth on these pieces is greatly delayed. It is believed that Banus's results are invalidated by such a method of procedure.

8. The results presented in this paper together with those of others quoted in the paper show that the rate of regeneration of pieces of *Tubularia* depends, when other factors are equal, upon the level which those pieces occupied in the intact stem; it is more rapid the nearer the pieces lie to the original distal end of

the stem. A metabolic gradient exists in the stem of *Tubularie* which is the primary cause of these regional differences in rata of regeneration.

LITERATURE.

Banus, M. Garcia.

'18 Is the Theory of Axial Gradient in the Regeneration of *Tubularia* supported by facts? Jr. Exp. Zoöl., Vol. 26, pp. 265–275.

Behre, Ellinor H.

'18 An Experimental Study of Acclimation to Temperature in *Planaria dorotocephala*. Biol. Bull., Vol. 35, pp. 277–317.

Child, C. M.

'07 An Analysis of Form Regulation in *Tubularia*. IV. Regional and Polar Differences in the Time of Hydranth-Formation as a Special Case of Regulation in a Complex System. Arch. f. Entw'lungsmech., Vol. 24, pp. 1–28.

'19a Demonstration of the Axial Gradient by Means of Potassium Permanganate. Biol. Bull., Vol. 37, pp. 133–147.

'19b The Axial Gradients in Hydrozoa. II. Susceptibility in Relation of Physiological Axes, Regions of Colony, and Stages of Development of Certain Hydroids. Biol. Bull., Vol. 37, pp. 101–125.

Driesch, H.

'99 Studien über das Regulationsvermögen der Organismen. II. Quantitative Regulationen bei der Reparation der *Tubularia*. Arch. f. Entw'lungsmech., Vol. 9, pp. 103–136.

Hyman, L. H.

'18 Suggestions Regarding the Causes of Bioelectric Phenomena. Sci., N.S., Vol. 48, pp. 518–524.

Loeb, J.

'91 Untersuchungen zur Physiologischen Morphologie der Thiere. I. Ueber Hetermorphose.

'92 Idem. II. Organbildung und Wachstum.

Mathews, A. P.

'03 Electrical Polarity in the Hydroids. Am. Jr. of Physiol., Vol. 8, pp. 294–300.

Moore, A. R.

'10 The Temperature Coefficient for the Process of Regeneration in *Tubularia crocea*. Arch. f. Entw'lungsmech., Vol. 29, pp. 145–148.

Morgan, T. H.

'01 Regeneration in *Tubularia*. Arch. f. Entw'lungsmech., Vol. 11, pp. 346–381.

'05 Polarity Considered as a Phenomenon of Gradation of Materials. Jr. Exp. Zoöl., Vol. 2, pp. 495–506.

'06a The Physiology of Regeneration. Jr. Exp. Zoöl., Vol. 3, pp. 457–500.

'06b Hydranth Formation and Polarity in *Tubularia*. Jr. Exp. Zoöl., Vol. 3, pp. 501–515.

'08 Some Further Records Concerning the Physiology of Regeneration in *Tubularia*. Biol. Bull., Vol. 14, pp. 134–149.

Morgan, T. H., and Stevens, N. M.

'04 Experiments on Polarity in *Tubularia*. Jr. Exp. Zoöl., Vol. 1, pp. 559–587.

THE GERM-CELLS OF CICADA (TIBICEN) SEPTEMDECIM (HOMOPTERA).[1]

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CONTENTS.

[1] A thesis presented to the Faculty of Princeton University, in candidacy for the degree of Doctor of Philosophy.

A. Introduction.

The study presented here was done at Princeton University during the last year. The material was collected during the appearance of the 17-year locust (*Cicada septemdecim*) in the vicinity of Princeton, N. J., during the spring of 1919 at the suggestion of Professor E. G. Conklin. With such an interesting life cycle as these insects have, it was thought that a cytological study of their germ cells might reveal some important facts in the history of the mitochondria and the chromosomes. Both of these are constant and important structures of all cells, and it is the opinion of the writer that no cytological study can be complete which neglects either one or the other. It is only by a correlated study of cytoplasmic and nuclear structures that we can ever hope to solve the many perplexing questions in cell economy. We use the almost mystic phrase: "interaction between nucleus and cytoplasm" in many cases to cover our ignorance concerning certain cell activities, but we are far from knowing any of the specific actions and reactions between the nucleus and cytoplasm. Within recent years the study of mitochondria of animal and plant cells has attracted many workers, with the result that many cytologists have come to regard these structures as of vital importance in cell activities. Attention, which for many years has been centered on the nuclear activities, has been drawn to a more intensive study of the cytoplasm and its structures. All these studies have emphasized the importance of such structures as mitochondria in relation to cell metabolism. Moreover, although the chromosome hypothesis of heredity seems to be firmly supported by a vast amount of evidence, yet one group of cytologists (Meves, Benda, Duesberg, etc.) maintain that the mitochondria also have a rôle as the bearers of hereditary characters. While the evidence which these workers have gathered is not of a convincing nature, nevertheless the facts are worthy of careful consideration. There are some reasons for believing that inheritance in some cases is through the cytoplasm, and we must not loose sight of the fact that "cytoplasm as well as nucleus is concerned in heredity and differentiation" (Conklin, '16). However, whether or not such cytoplasmic structures as

mitochondria constitute the idioplasm is an entirely different problem. Modern researches in genetics have shown that, despite mutations, hereditary characters are relatively stable and that the hereditary constitutions of organisms are definitely organized; hence the idioplasm which is causal in the development of the hereditary characters must similarly be stable and highly organized.

Keeping in mind the "cell as a whole," I have studied the chromosomes and mitochondria in the oögenesis and spermatogenesis of *Cicada*, and my observations give added evidence to the chromosomes as the idioplasmic substance, while there is no evidence from an unbiased standpoint that the mitochondria behave as idioplasmic substances. There is evidence, however, that the mitochondria are intimately concerned in cell metabolism.

Throughout this work I have had the constant advice and encouragement of Prof. E. G. Conklin, and it is with great pleasure that I here express my indebtedness.

B. Materials and Methods.

The youngest specimens of *Cicada* obtained were those of the second pupal stage about three weeks prior to their emergence from the ground and their final moult into the imago. These specimens were collected about the middle of April by digging under trees in the vicinity of Princeton. The pupæ were found lying about a foot from the surface of the ground and were most abundant several feet away from the tree trunks. In the testes of such pupæ, one finds most of the cells in the maturation stages besides an abundance of spermatids, spermatozoa, and a few spermatogonia. In the adult or imago, the testes are almost completely filled with sperm except for a small number of spermatogonia. After copulation, the testes are reduced to about one tenth their former size and contain only a small residue of spermatozoa, some degenerated cells and a few spermatogonia which also show signs of degeneration.

There are two testes, each consisting of a great many radiating ellipsoidal follicles which give the testes a berry-like appearance. In the female there are two typical ovaries, each consisting of a

great number of ovarian tubules containing a great many oöcytes. The oldest oöcyte of a second stage pupa is about one seventieth the linear size of the oldest oöcyte of the adult, which shows the tremendous growth that takes place in a few weeks. It has been estimated (Marlatt, 1898) that the female *Cicada* lays between 400 and 600 eggs.

The male and female gonads were dissected out in Ringer's solution and fixed in either Flemming's (strong), Bouin's, Benda's, or Regaud's fixing fluids. Mitochondria were well preserved by the Flemming, Benda and Regaud fluids, but were either partially or wholly destroyed in Bouin's fluid depending on the length of time the gonads remained in the fixing fluid. Material fixed in Flemming's fluid (10 to 12 hours) was usually the best for studying both chromosomes and mitochondria. Sections were cut 8 to 10 micra in thickness and the stains used were iron-hæmatoxylin (with and without counterstain), Benda's crystal-violet and alizarin, and Altmann's fuchsin-methylene green.

The developing eggs were also collected from time to time for a study of the chromosomes of the embryonic cells. These were fixed in either Bouin's or Carnoy's fluids and were imbedded by the celloidin-paraffine method.

C. Observations.

1. *Diploid Chromosome Groups.*

(*a*) *Spermatogonia.*—The spermatogonia are found in the proximal end of the ellipsoidal follicles of the testes. They form a cap of cells at this end of the follicle containing the primary and secondary spermatogonia. From the proximal end of the follicle there proceeds a short narrow filament which contains the spermatogonia of the multiplication stages. Mitotic figures are quite abundant among the spermatogonia of the multiplication stages, but the metaphase plates are usually so crowded that it is impossible to make accurate counts of the chromosomes. On the other hand, the primary and secondary spermatogonia rarely show cell divisions, but the metaphase plates when they appear are very clear.

The male chromosome number is 19, which indicates that there

is present an unpaired sex element (Figs. 1 and 2). One pair of the complex is strikingly larger than the rest, being in the form of somewhat curved rods (Text-fig. 1). This pair corresponds to the "macrochromosome" pair described by Kornhauser ('14) in *Enchenopa*.

Two other chromosome pairs (*BB*, *CC*, Text-fig. 1) can also be distinguished from the other chromosomes by their size, being approximately half the size of the macro-chromosomes (*AA*). The other 13 chromosomes show no size differences which would enable us to arrange them in pairs or distinguish

TEXT-FIG. 1. Spermatogonial chromosomes, showing the relative sizes of the chromosomes; the macrochromosome pair, *AA*, the *BB* and *CC* pairs, and 13 other chromosomes which show no size differences.

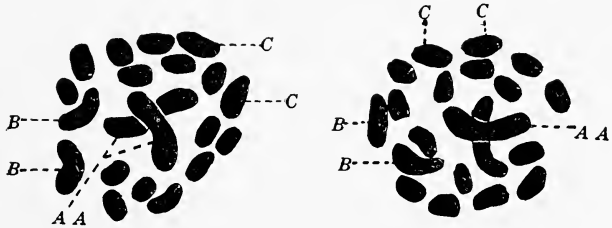
them from each other. However, as will be later shown, the *AA*, *BB*, and *CC* chromosome pairs are so characteristic in their form and size that they can be recognized in all the diploid groups. The size relations of the chromosomes of *Cicada* correspond to those described by Kornhauser ('14) in *Enchenopa*. Also in the *Cercopidæ* (Homoptera), Boring ('13) has described three pairs of chromosomes (*A*, *B*, *C*) which bear similar size relations to the three pairs here described in *Cicada*.

The sex-chromosome cannot be identified in the spermatogonial groups either by its size nor by any peculiarities in its staining reactions. In the resting spermatogonia there is, however, always present a single chromatin nucleolus (Fig. 13) which probably is the persisting sex chromosome.

(*b*) *Ovarian Follicle Cells.*—Among the follicle-cells of the ovary, mitotic figures are very abundant. I have found a great many mitoses not only among the follicle-cells surrounding the young growing oöcytes, but even among the follicle-cells surrounding the almost mature oöcytes. I have searched for evidences of amitotic division among these cells, which has often

been described, especially in the follicles surrounding mature oöcytes; but I have failed to find any strong evidence for the occurrence of this method of nuclear division. The only indication that amitotic division may take place is found in the follicles surrounding the old oöcytes. Here the cells are usually binucleate and appear to be the end stages of amitotic divisions. However, I have found many karyokinetic figures among such cells and it seems reasonable to suppose that the binucleate follicle-cells arise through the failure of the division of the cell body following mitotic division of the nucleus.

All metaphase plates of dividing follicle-cells show 20 chromosomes (Figs. 3, 4, 5, 6, Text-fig. 2), and it is possible in all of these to recognize the chromosome pairs *AA*, *BB*, and *CC* as in the spermatogonial plates, by their size relations. Figure 12 is that of the late telophase of a dividing follicle cell in which



TEXT-FIG. 2. Two metaphase plates from ovarian follicle-cells showing the *AA*, *BB*, *CC* pairs of chromosomes; note that the *AA* pair lies in the center of the group.

one of the macrochromosomes (*A*) can be recognized in the daughter cells. It will also be seen that there is present a precocious longitudinal split of the chromosomes in preparation for the next cell-division.

In the follicle-cells surrounding the older oöcytes, the chromosomes do not have the thick compact appearance found in the younger follicle-cells. The chromosomes are usually thinner, poor in chromatin content, and the respective chromosomes appear somewhat longer, often showing an equational split (Figs. 10, 11). I am unable to account for this difference in appearance of the chromosomes (compare Figs. 7, 10, 11). In

the resting nuclei of the old follicle-cells, it is very noticeable that they are very poor in chromatin content, all the basichromatin being accumulated in one or two small masses (nucleoli). When such cells prepare for mitosis, the chromosomes which are reconstituted are correspondingly poor in chromatin. However, the linin basis of the chromosomes is still present and consequently the number and size relations of the chromosomes is maintained. This, I believe, gives added evidence to the view previously expressed (Shaffer, '20) that the linin is the morphologically stable substance which maintains the chromosomal organization and structure.

(*c*) *Embryonic Cells.*—Although a number of the developing eggs of *Cicada* were collected, I was unable to obtain good material for a study of the chromosomes due to the difficulties in sectioning. The eggs were so full of yolk that it was possible to cut them only by imbedding by the celloidin-paraffine method.

Figure 9 is that of a metaphase plate of a cell from the blastoderm showing 20 chromosomes, and hence of the female type. The chromosome pairs *AA*, *BB*, and *CC* can be distinguished as in the other diploid nuclei. While I have been unable to make an exhaustive study of the chromosomes of the embryonic cells, yet I have found no variations in the chromosome numbers or in their size relations.

(*d*) *Somatic Cells.*—On dissecting the female locusts to remove the ovaries, a number of round, brown-pigmented bodies resembling eggs were found in the abdomen. On sectioning these it was found that they were of a glandular nature and are perhaps concerned in the secretion of adhesive materials for the eggs. In a small cap of cells which lies at one end of these glandular bodies, mitotic figures were found in abundance. There are always 20 chromosomes which show similar size relations to the diploid chromosome groups previously described. After mitotic division of the nucleus the cell-body fails to divide, resulting in the formation of binucleate and multinucleate cells. At the time of division of such multinucleate cells, typical triasters are formed (Fig. 52). In the telophase of such a division, the tripartite daughter nuclei reconstruct to form six separate nuclei, the cell-body again failing to divide. At this time the

cytoplasm becomes active in the secretion of large globules and although the nuclei increase in number, I have been unable to find mitotic figures and it seems possible that the increase in their numbers is brought about by amitosis.

2. *Spermatocytes.*

(*a*) *Growth Stages.*—Unfortunately the material which was collected showed very few of the early growth stages of the spermatocytes and consequently I was unable to make a detailed study of the process of synapsis. Apparently the early growth stages must take place some time before the month of April. In the youngest pupæ which I have collected, the only growth stages of the spermatocytes which I have been able to find are those of the pachytene-bouquet stage (Fig. 17) in which the thick synaptic threads are polarized at one side of the nucleus. Sections across the bouquet usually show 18 chromatic blocks representing the end view of the threads. Since each loop has been cut twice, this would indicate that there are 9 pachytene loops. At the base of the polarized bouquet is usually found the compact, deeply-staining nucleolus (Fig. 17, *X*) which is the persisting sex-chromosome. This is undoubtedly the same compact chromatic nucleolus found in the resting stages of the spermatogonia (Fig. 13). As is usual, the bouquet is polarized toward the pole of the cell containing the idiozome (Fig. 17, id.). It has often been stated that the idiozome exerts some attraction on the synaptic threads influencing their polarization. There is no evidence to support this view and I am inclined to believe that the same factors which determine at which point in the cell the idiozome should lie also determines the polarization of the synaptic threads.

Occasionally the pacyhtene threads show a longitudinal split, and in such cases it is noticed that the chromatic granules (chromomeres) of the two halves of the thread do not correspond either in size or location. Consequently the longitudinal split cannot be interpreted as an equational split, but is rather the primary split or point of synaptic union. Usually there is present a single loop of the bouquet which is much larger than the other loops (Fig. 17, *AA*) and this undoubtedly represents

the synaptic condition of the macrochromosome pair of the spermatogonial chromosomes.

(*b*) *Tetrads and Maturation Divisions.*—Stages of the early prophases of the spermatocytes were quite abundant and it was possible to follow the formation of the first maturation tetrads. In the early prophases, the homologous threads show a great variety of twisting about each other (Fig. 20). One pair (*AA*, Fig. 20) is easily distinguishable from the others by its large size and is derived from the spermatogonial macrochromosomes. Figure 18 represents the stages in the formation of the definitive tetrad from this pair (Figs. 61, 62, 63, 64). In the early prophases the homologous threads of the *AA* tetrad are very long and twisted about each other. However, they retain their connections at the ends, thus making the tetrad a large ring if its twists were straightened out. The space enclosed by this ring is the interchromosomal space which marked the point of synaptic union of the threads. In the later prophase stages, the large ring condenses, the threads become thicker retaining their point of union at the ends and the interchromosomal space becomes reduced in size until in the definitive maturation tetrad it is reduced to a small oval slit between the two halves of the ring (Figs. 18, 21, 62). In the first maturation metaphase, the macrochromosome tetrad no longer appears in the form of a ring, but rather in the form of a ring flattened at the poles, or as two slightly bent rods whose concavities oppose each other. In a similar way, the tetrad derived from the *BB* pair of the spermatogonia goes through the formation of a ring tetrad (Fig. 19), resulting in a tetrad similar to the macrochromosome tetrad (*AA*), but approximately half its size. The other tetrads show no ring formation; usually the homologous threads become free at one of the synaptic ends, retaining their connection at the other end, thus giving the appearance of two chromosomes joined end-to-end (Fig. 20). The condensation of such tetrads produces the typical dumb-bell form tetrad, with the narrow portion of the dumb-bell marking the retained point of synaptic union. Thus, if the point of synaptic union is retained at both ends, rings like the *AA* and *BB* tetrads are produced; if the synaptic union is retained at one end only, the dumb-bell type

tetrad is produced. Payne ('14) has described a different method of ring-formation in *Forficula*. The two univalent chromosomes are first joined end-to-end; while retaining this point of union, the free ends come together by a bending process with the resulting formation of a ring each half of which represents a univalent chromosome. A similar method of ring-formation has been described by Sutton ('02) in *Brachystola* and by Davis ('08) in several Orthopterans. In *Forficula* besides the "bending process" of ring-formation, Payne describes ring-formation of the type here described in *Cicada*.

(*c*) *Maturation Divisions.*—In the metaphase plates of the first maturation division, the chromosomes are always grouped in a characteristic manner (Figs. 23, 24, 53 to 57). There are 10 chromosomes in the metaphase plate, 8 of which are arranged in a circle surrounding the macrochromosome tetrad (*AA*, Fig. 23) which always lies in the center of the group. The sex chromosome (Fig. 23, *X*) always lies outside this circle of chromosomes and often does not lie in the same plane. Boring ('07) has figured the chromosomes of a number of species of Homopterans in which the sex-chromosome lies outside the group of autosome tetrads. The constant position of the *AA* tetrad in the center of the spermatocyte complex can be traced back to the diploid chromosome groups in which the macrochromosome pair shows a marked tendency to lie in the middle of the metaphase plate with the other chromosomes grouped about them (text-fig. 2). In a previous paper (Shaffer, '20) it was pointed out that the characteristic grouping of the chromosomes in the metaphase had its explanation in the persistence of the interchromosomal linin fibres (Fig. 24) which undoubtedly persist as a part of the chromosomal architecture and maintain definite spatial relations between the chromosomes. The evidence in *Cicada* seems to support this view.

In the side-view of the metaphase, the *AA* and *BB* tetrads are arranged on the spindle in the direction of the spindle axis. Hence in polar views only a half of each tetrad can be seen (Fig. 23). In both the *AA* and *BB* tetrads the spindle fiber attachments are median (Figs. 18, 19) or atelomitic (Carothers, '14), and since the tetrads lie in the direction of the spindle axis, they

will separate in the anaphase at the point of synaptic union and the division is reductional. The other tetrads have terminal spindle fiber attachments, but I am unable to say whether they divide reductionally or equationally. In the case of the *CC* tetrad, there is evidence that the narrow portion of the dumb-bell actually marks the point of synaptic union, and since separation of the dyads in the anaphase occur at this point, the division is also reductional.

The sex-chromosome (Fig. 25, *X*) usually lies on the outer surface of the spindle. In the anaphase it usually lags behind the other dividing chromosomes, sometimes appearing bipartite, and passes undivided to one of the daughter cells (Figs. 27, 70). As the dyads come into the late anaphase of the division, a secondary (equational) split can often be seen (Fig. 27, *AA*).

Following the first maturation division, there is no interkinesis or construction of a telophase nucleus. The dyads again become arranged in the metaphase, each showing the secondary split. Figure 28 (also Fig. 58) is that of second spermatocytes (daughter plates), one having 9 dyads the other having 9 dayds plus the X-chromosome. It will be noted that the grouping of the dyads is exactly similar to the grouping of the tetrads in the first maturation division metaphase, namely 8 dyads arranged in a circle around the macrochromosome dyad. In *Notonecta*, Browne ('16) found that the chromosomes always assumed a definite grouping in the metaphase, but the grouping was different in the two maturation divisions. In the anaphase of the second maturation division all the dyads divide and there are no lagging chromosomes.

(*d*) *Giant Spermatocytes.*—It is quite common to find spermatocytes with double or more the number of chromosomes. Figure 59 is a photograph of such a spermatocyte in the metaphase which has over twice the normal number of tetrads. These giant spermatocytes develop normally and give rise to giant spermatids and spermatozoa (Figs. 31, 33*c*). The origin of these giant cells may be traced back to the spermatogonia in which there has been a failure of division of the cell-body resulting in cells with double the diploid number of chromosomes. Such cells are quite common among the spermatogonia of the multi-

plication stages. Wilcox ('95) has described giant spermatids and spermatozoa in *Cicada tibicen;* he also finds that they are derived from abnormal spermatogonia in which there has been a failure of the division of the cell-body, and that their development insofar as the spermatozoan is concerned follows a normal course with the production of typical spermatozoa which merely differ from the normal ones by their large size. It seems difficult to ascertain the significance of these giant cells or whether there is any possibility that they play a part in the fertilization of the egg.

3. *Growth Stages and Synapsis in Oöcytes.*

While it has not been possible to study the process of synapsis in the spermatocytes due to the absence of the proper stages in my material, in the oöcytes I have been able to follow the various growth stages in some detail.

The young oöcytes in varying stages of growth are found at the base of the nurse chamber or "Keimlager" (Fig. 34). They are always distinguishable from the nurse-cells by their definite cell outline, by the thread-like appearance of the chromatin as compared to the granular nuclei of the nurse cells, and by the presence of a definite mitochondrial zone. Only occasionally could oögonial divisions be found in the ovaries of the youngest pupæ, and hence I have been unable to trace the chromosomes from the last oögonial division into the early growth stages. The very early growth stages (leptotene, etc.) are not found in the material collected after the latter part of April but in the material collected earlier, there is an abundance of oöcytes in the presynaptic stages.

Figure 35 is that of the nucleus of a young oöcyte corresponding to von Winiwarter's "protobroque" nucleus. The chromatin is in the form of a network with the nodal points of the net staining somewhat more deeply than the rest of the reticulum. On closer analysis of this nucleus it is seen that the net-like appearance of the chromatin is due simply to the optical effect of numerous delicate chromatic threads crossing each other in all directions. In the later stages the individual threads become more evident and this stage (Figs. 36, 74) no doubt corresponds to von Winiwarter's "deutobroque" nucleus. Gradually these threads as-

sume more and more a definite individuality, and the nucleus then becomes filled with a number of very delicate leptotene threads (Figs. 37, 75). These often show a distinct polarization, usually being attached at one side of the nuclear membrane with the free ends suspended in the nuclear sap. During this stage there is also a marked tendency for the leptotene threads to become associated in pairs and this pairing becomes more marked in the later stages (Fig. 38). The threads are now markedly polarized and it becomes quite clear that they are actually pairing side-to-side (zygotene stage). This paired appearance of the threads is not an accidental one, for I have observed it in a great many cells with great clearness. That it is also not due to a longitudinal split of a single thread is evidenced by the fact that the chromatin granules in homologous threads do not lie at the same level. After this pairing has taken place and the threads have become well polarized, they gradually become shorter and thicker (Fig. 39), forming the typical pachytene stage, the threads sometimes showing the primary split or point of synaptic union. A typical pachytene bouquet stage follows, in which the loops have both their free ends attached at one pole of the spindle (Figs. 40, 76). Usually there is one especially large loop (*AA*, Fig. 40) similar to the large loop found in the bouquet stage in the spermatocytes, which undoubtedly represents the macrochromosome pair. Cross-sections of cells in the bouquet stage show the threads on end view (Figs. 41, 77), and it is possible to count these. Usually there are 20 such cut ends of the pachytene threads and since each thread has been cut twice, we can deduce that there are ten pachytene loops, and it is at once seen that this number corresponds to the reduced number of chromosomes.

The release from the bouquet stage sets free the thick woolly-looking pachytene threads (Fig. 42) and the primary split comes clearly into evidence, and often the homologous elements become separated along the synaptic line. As this separation continues, the homologous threads become twisted about each other assuming the typical strepsistene condition (Fig. 43). The separation may begin at either or both ends of the threads or they may retain their union at the ends and separate along their

middle points. As the strepsistene stage advances, the threads lengthen considerably, become more lightly staining, and as they separate more widely their individuality becomes less and less distinct. In the older oöcytes, the chromatin of the germinal vesicles appears more or less granular, much of it still retaining its affinity for the basic stains. On close analysis of such nuclei (Figs. 46, 47) much of the chromatin shows evidences of still retaining, in part, the arrangement in threads, and I interpret these as being the original conjugating threads of the early growth stages which have been greatly expanded.

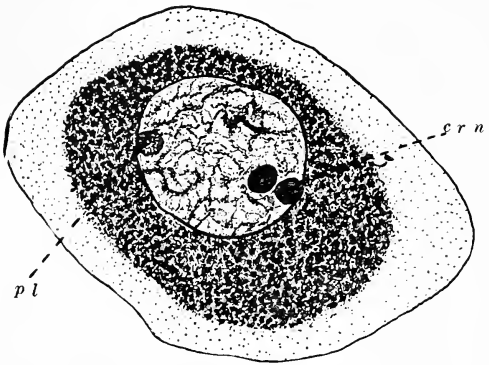
4. *Chromatin Nucleoli.*

I shall not attempt to review the literature concerning nucleoli since there is considerable confusion of interpretation in this field. It is evident that many of the different opinions expressed in the literature have arisen from the fact that these structures are not always homologous. Recently Nakahara ('18) has advanced the view that in the oöcytes of *Perla*, the nucleoli are derived from the yolk-nucleus which consists of a dense granular mass in the cytoplasm closely applied to the nucleus. As will be shown later, Nakahara's "yolk-nucleus" is really an accumulation of mitochondria, and it is difficult to see how these could give rise to nucleoli.

In the oöcytes of *Cicada* true chromatin nucleoli are found, and whenever they are present take the basic stains. The interest connected with chromatin nucleoli in the oöcyte is bound up with the possible homology of such structures with the chromosome nucleoli or persisting sex-chromosomes found in the growth stages of the spermatocyte. Since one sex-chromosome of the female is derived from the sperm, there is no à priori reason why we should not expect to find such a body persisting in the stages in the oöcyte homologous to those stages in the spermatocyte where it is found to persist as a nucleolus.

In the very young oöcyte of *Cicada* (Figs. 35, 36, 73, 74), there are usually present two chromatin nucleoli. In the leptotene nuclei these are no longer found, nor can they be found in the succeeding pachytene stages. However, in the strepsistene nuclei, two deeply staining bodies again appear (Figs. 43, 78)

which are similar to those found in the protobroque and deutobroque (preleptotene) nuclei. In the later stages these bodies become closely associated (Text-fig. 3, Figs. 81, 84) and persist as definite chromatin nucleoli. A true plasmosome is also present in these stages which becomes vacuolated and grows in size with the age of the oöcyte (pl., Figs. 44, 46). In the older germinal



TEXT-FIG. 3. Oöcyte of post-synaptic period, showing mitochondria arranged in perinuclear zone, and two chromatin nucleoli (*cr.n.*) and a plasmosome (*pl.*).

vesicles, the two chromatin nucleoli may persist (Fig. 87), but more commonly a single large chromatin nucleolus is found together with six to eight smaller nucleoli (Fig. 46).

It is difficult to say whether the two chromatin nucleoli really represent persisting sex-chromosomes and are therefore homologous to the chromosome nucleoli of the spermatocytes. There is some evidence that this is the case. The disappearance of the definite nucleoli during the synaptic stages (leptotene and pachytene) leads me to believe that they are resolved into chromatic threads which go through a synaptic process similar to the autosome threads. The fact that there are ten pachytene threads in the bouquet stage indicates that all the chromosomes are in synapsis. The reappearance of the two chromatin nucleoli in the strepsistene stages are perhaps brought about by a re-condensation of the synaptic threads representing the sex pair resulting in the formation of the two compact chromatin nucleoli.

This view is supported by the fact that in those stages where the nucleoli re-appear, they usually lie close to each other (Figs. 43, 78), and in the strepsistene stages they are more loosely granular in appearance than in the later stages. The later increase in number of the nucleoli in the germinal vesicle stages may be an expression of the increased metabolic activity of the nucleus during this period.

Wilson ('06) was unable to find chromosome-nucleoli in the oöcytes of *Anas*, *Euschistus* and other forms during the "contraction figure of the synaptic period" and he is inclined to doubt the persistence of nucleoli in the oöcytes homologous to those found in the spermatocytes. As has been shown in *Cicada*, it is quite probable that the sex-chromosomes go through synaptic stages just like the autosomes and hence do not persist as compact bodies in these stages as does the odd chromosome of the spermatocytes. However, they may be present in the form of nucleoli before and after the synaptic period. Foot and Strobell ('11) have described a chromatin nucleolus in the oöcytes of *Protenor* which gives rise to two large idiochromosomes at the time of cell-division. In *Gelastocoris*, Payne ('12) has described a chromatin nucleolus which appears after the last oögonial division and persists until shortly after synapsis. It later becomes reduced in size and disappears, and Payne interprets it as having been derived from the sex-chromosomes. The reason for the persistence of the sex-chromosome in the spermatocyte and its non-persistence in the oöcytes during synapsis may lie in the fact that in the former the odd sex element has no homologue with which to pair, while in the latter the two sex-chromosomes are homologous and synapsis becomes possible. However, with the wide variations which these nucleolar structures exhibit in the oöcytes, it is not possible to make any generalization as to their homology with the nucleoli of the spermatocytes.

5. *Discussion of the Chromosomes in Homopterans.*

(*a*) *Review.*—The earliest work on the chromosomes of the Hemiptera homoptera is that of Wilcox ('95) on *Cicada tibicen* in which he states that there are 12 chromosomes in the spermatogonia and 24 "elements" in the spermatocytes. The work of

Stevens ('05*a*, '05*b*) and of Morgan ('08, '09) on the *Aphids* and *Phylloxerans* has dealt mainly with the problem of sex determination. It was found that only spermatozoa bearing the sex-chromosome are functional and consequently only females are produced from fertilized eggs. Males are produced parthenogenetically from eggs which during the maturation processes reduce the chromosome number to one half the somatic number; hence there is only one sex-chromosome remaining in the male. Stevens ('06) and Boring ('07, '13) have studied the chromosomes of over 20 species of Homopterans mainly in relation to sex determination. Kornhauser ('14) has made a comparative study of the chromosomes of two species of *Enchenopa* in which he describes parasynapsis and pre-reduction.

In all the Homoptera studies thus far with one exception, the sex-chromosomes consist of but a single (X) element which persists as a chromosome nucleolus through the growth stages of the spermatocyte and passes undivided to one pole of the first maturation spindle. The only exception to this is that described by Kornhauser ('14) in *Enchenopa binotata* in which there are two sex-chromosomes (XY). These behave very much like the autosomes in the growth stages of the spermatocyte, becoming resolved into synaptic threads and pairing in synapsis much as the autosomes do. In the preleptotene stages the sex pair is found persisting as nucleoli. In the strepsistene stages, when the chromosomes become more diffuse and lightly staining, the chromatic nucleoli again appear by a condensation of the threads representing the sex pair. It will at once be noted that this behavior of the sex pair in *Enchenopa* resembles the behavior of the two chromatic nucleoli which I have described in the oöcytes of *Cicada* and I am, therefore, led to believe that the chromatic nucleoli of the oöcytes are homologous to the chromosome-nucleolus of the spermatocytes.

A common characteristic of the diploid chromosome complexes of many Homopterans is the usual presence of a pair of large rod-shaped chromosomes (*AA* pair of *Cicada*). Stevens ('06) has shown these in *Aphrophora*, and Boring ('07) has figured these in many of the Homoptera that she has studied. Kornhauser ('14) had described them in two species of *Enchenopa* that he has

studied and applies the name "macro-chromosome" to them. Besides the macrochromosome pair, there are usually present one or two chromosome pairs (the *BB* and *CC* pairs of *Cicada*) which are somewhat smaller than the macrochromosome pair, but which can easily be distinguished by their size from the other chromosomes. The presence of a pair of macrochromosomes and one or two pairs of somewhat smaller chromosomes therefore seems to be characteristic of the diploid chromosome groups of the Homoptera.

(*b*) *Ring Tetrads.*—McClung ('14) has described two types of ring tetrads in the Orthoptera which he terms (1) the *Hippiscus* type, and (2) the *Stenobothrus* type. Both types of ring tetrads are essentially the same as far as the formation and relation of their elements (chromatids) are concerned. Half of each ring represents a whole chromosome and each univalent chromosome is secondarily split, so that each ring tetrad really consists of two rings superimposed one upon the other. The difference between the two types lies in their relation to the spindle-fiber attachments and their position in the metaphase plate. In the *Hippiscus* ring type, the spindle-fiber attachments are at the synaptic ends (terminal) and the rings lie flat in the metaphase plate. Consequently polar views of the metaphase plate show the entire ring, while side views show only half of the ring. In the anaphase the two superimposed rings separate and consequently the division is post-reductional. On the other hand, the rings of the *Stenobothrus* type usually have median spindle-fiber attachments and the ring lies in the metaphase in the direction of the spindle axis. Only lateral views of the spindle would therefore show the complete ring, while polar views show only half of the ring. In the anaphase the two halves of the ring (which are univalent chromosomes) separate as simple V's and the first maturation division is pre-reductional.

In *Cicada*, the large ring of the early prophases is derived from the macrochromosome pair, *AA*, of the spermatogonia. In the spermatogonial divisions this pair has median spindle-fibre attachments and the spindle-fiber attachments of the ring tetrad derived from this pair are also median (Fig. 18). In the metaphase the ring is arranged on the spindle in the direction of the

spindle axis with the sister chromatids directed toward the same pole. Polar views of the metaphase (Fig. 55) show only half the tetrad, but oblique views show the relation clearly (Fig. 57). Thus the rings of *Cicada* are of the Stenobothrus type and they divide reductionally in the first maturation division.

(*c*) *Synapsis.*—Although I have been unable to study the process of synapsis in the male germ cells, my observations on the oöcytes indicates that the chromosomes conjugate parasynaptically, and there is no reason for supposing that it might be different in the spermatocytes. From Fig. 38 it is quite evident that the leptotene threads pair side-to-side, but the essential point is whether each leptotene thread actually represents a single univalent oögonial chromosome. Wenrich ('16) has shown in *Phrynotettix* that the leptotene threads are derived from the telophase chromosomes of the last spermatogonial division by a process of unravelling of the chromatic blocks contained within separate chromosomal vesicles. In *Cicada* I have been unable to trace the oögonial telophase chromosomes into the early growth stages of the oöcyte, but there is some indirect evidence that each leptotene thread is derived from a single oögonial chromosome. In the late leptotene stages, the threads are well polarized and all extend in one direction through the nucleus. I have studied a great many such nuclei in cross-section and it is possible to count the number of leptotene threads cut on end view. Such counts usually approximate the diploid chromosome number (20) and points to the fact that each leptotene thread is derived from a single chromosome.

As has been noted before (page 417), a casual study of the germinal vesicles of the oöcytes does not indicate any persisting individuality of the chromosomes; however, careful and minute analysis of such nuclei reveals the fact that the synaptic threads are still present but in a much-expanded and diffuse condition. Similarly in the male germ cells following the strepsistene stages, the synaptic threads appear to loose their individuality, becoming diffuse and widely expanded ("confused stage" of Wilson). This diffuse stage in the spermatocytes, although of relatively short duration, is no doubt homologous to the "germinal vesicle" stage of the oöcyte, since both occur at homologous periods in

the male and female germ-cells, namely, after synapsis and before the appearance of the definitive maturation tetrads.

D. Observations on Mitochondria.[1]

1. *Mitochondria in Spermatogenesis.*

(*a*) *Spermatogonia.*—Mitochondria are present in the spermatogonia of all stages in the form of granules usually localized at one end of the cell, usually the end directed toward the cyst cavity (Fig. 13). This is quite the usual location of mitochondria in spermatogonia, but as I have shown in *Passalus* (Shaffer, '17), the mitochondria may be diffusely spread in the cytoplasm. There is no indication that the mitochondria of the spermatogonia ever assume the form of filaments. During mitosis the mitochondria are spread along the outer spindle-fibers and by the time of the telophase and the cell-constriction, the mitochondria become grouped as granular masses lying above the daughter nuclei.

The amount of cell-degeneration taking place in the testes of *Cicada* was so striking as to call for more than mere casual observation. Sometimes an entire half of a follicle would be filled with cells in various stages of degeneration. This degeneration is usually found only among the spermatogonia prior to the commencement of the growth stages of the spermatocyte, which seems to be a critical time for the cells. Once they begin the synaptic processes, they apparently go through to maturation normally. It is quite noticeable that cell-degeneration is more abundant in the adult testes than in the testes of pupæ.

[1] I shall continue to use the term "mitochondria" to denote those cytoplasmic structures, regardless of their form, which are preserved by special reagents (destroyed by acetic acid, etc.) and which take specific stains. I see no advantage in the employment of the term "chondriosome" as recently urged by Duesberg ('19). The term "mitochondria" is not only more commonly used in this country, but also conveys the idea of their nature quite as well as "chondriosome." It can be equally well applied to those structures when filar in form as when granular and it seems to be etymologically as proper as the term "chondriosome." The various names which have been proposed to designate these structures (chondrioconts, plastosomes, plastidules, karyochondria, etc.) are based either upon their supposed origin or else upon their morphology. Since there is still much confusion as to their origin and since their morphology may vary at different times in the cell-cycle, it seems best not to commit ourselves to the use of new terms but to employ the original name, "mitochondria," of Benda.

In studying the process of degeneration it is at once evident that the mitochondria are concerned in the process. The first noticeable change in the degenerating cells is that the mitochondria become larger and more deeply staining and the nucleus often becomes polymorphic (Fig. 14). While the mitochondria have increased in size, they have decreased in numbers and it is quite evident that the large mitochondria have grown in size by an agglutination of the smaller normal ones. I have described a similar process in the degenerating spermatogonia of *Passalus* (Shaffer, '17), but was unable to follow in detail the succeeding stages of degeneration. In *Cicada*, the further processes in the degeneration involve a continued agglutination or coalescence of the mitochondria which results in the formation of large bodies of globular form staining intensely (Fig. 15). This process is continued (Fig. 16) until finally the degenerated cell becomes a deeply staining mass with the nucleus barely visible. Often the large globules show vacuoles which I interpret as being due to a partial dissolution of their substance by the reagents used.

Scott ('16) found that in experimental phosphorus poisoning of white mice the mitochondria are the first elements of the acinus cells of the pancreas to show any pathological changes. They begin to loose their characteristic filamentous form and finally agglutinate in large compact masses in the cell. "The mitochondria in these agglutinated masses fuse to form droplets possessing the characteristic properties of lipoids" (Scott,'16, p. 251), hence developing a fatty degeneration of the pancreas. The significance of these observations of Scott is at once apparent in connection with the degeneration of cells above described in *Cicada*. The behavior of the mitochondria in the degenerating spermatogonia of *Cicada* is essentially similar to their behavior in the fatty degeneration of the pancreatic cells and I am consequently of the opinion that the degeneration of the spermatogonia is a fatty degeneration.

Recently, Athias ('19) has studied the mitochondria in the interstitial cells of the ovary of the bat, *Vespertilion idæ*. The mitochondria are present here in the form of granules and filaments, which gradually become transformed into fat globules.

These represent "sans doute le produit de sécrétion des cellules interstitielles." Athias is of the opinion that the mitochondria are transformed into lipoid globules by a change in their chemical constitution. "On observe tres souvent des images qui montrent que le produit graisseux doit resulter d'un changement chimique de la substance mitochondriale; on peut suivre, en effet, les differentes phases de la transformation des plastosomes en gouttelettes de graisse" (page 195).

What the significance of such cellular degeneration, as found in the testes of *Cicada*, can be is difficult to say, since it occurrs regularly and is moreover found in almost all insect testes. It may possibly be that these degenerated cells in some way supply nutriment to the spermatozoa, as suggested by Wieman ('10) in the case of degenerated cells in the testes of *Leptinotarsa*, and are hence homologous to the nurse cells of the ovaries. I have often noticed deeply staining bodies, somewhat resembling the degenerated cells, lying among the spermatozoa; but other than this there is no evidence that the degenerated cells are a source of nutriment for the spermatozoa.

(*b*) *Spermatocytes.*—Since the spermatocytes of the early growth period are not present in my material, it has been impossible to follow the mitochondria from the last spermatogonial division into the spermatocytes. However, in the pachytene stages (Fig. 17) mitochondria are present in the form of filaments which sometimes appear granular. As will be noticed they are most abundant at the pole of the cell where the idiozome is located, which is the usual localization of the mitochondria in the spermatocytes, but which is by no means general. In *Passalus* (Shaffer, '17) I have found that the mitochondria of the early spermatocytes are in the form of diffusely spread granules and are often most abundant in a zone immediately surrounding the nucleus.[1] Undoubtedly, the filar mitochondria

[1] Duesberg ('18) has questioned these observations stating (foot-note, p. 138): "it is characteristic, even if not quite general, that the male auxocytes have their chondriosomes accumulated at one pole of the nucleus around the idiozome." Duesberg's ('10) own figures (Figs. 51, 52, 53) of the spermatocytes of the guinea-pig seem to contradict this, for he describes the mitochondria as being spread diffusely in the cytoplasm. "Ils (mitochondria) cessent d'etre groupes exclusivement autour de l'idiozome, pour se repandre dans toute le cytoplasme" (p. 65).

of the spermatocytes are genetically related to the granular ones of the spermatogonia. There is no evidence that the mitochondria go through a process of pairing during the growth stages; on the contrary they increase in numbers. This increase in the numbers of the mitochondria is a most important point in an understanding of their nature and significance. Such an increase in numbers of mitochondria during the growth period has been explained by some workers (Goldschmidt, Buchner, etc.) as being due to an elimination of material from the nucleus into the cytoplasm (chromidial theory). On the other hand, another group of workers insist that mitochondria are always derived from pre-existing mitochondria by a process of autonomous division. Wilke ('13) has described such a process in *Hydrometra*. As will be shown later, it is quite possible that the mitochondria may arise in another way.

As the first maturation spindle is forming (Fig. 21), the mitocondria begin to migrate from one pole of the spindle towards the opposite pole and finally surround the entire spindle by the time of the metaphase (Fig. 22). As the chromosomes begin to divide in the anaphase, there is no indication of any division of the mitochondria (Fig. 25). In the late anaphase, when the cell-constriction begins to appear (Fig. 27), the mitochondria begin to divide, so that when the cell-constriction is complete, the daughter cells (second spermatocytes) each contain approximately equal amounts of mitochondria (Fig. 28). The view of autonomous division is not supported here and it is quite evident that their division is due to their separation by the cell-constriction.

In the second maturation division, the behavior of the mitochondria is the same as in the first maturation division; they surround the spindle peripherally and become divided by the cell constriction (Fig. 29), so that the daughter cells (spermatids) receive equal amounts of mitochondria. As is usual in the insects, the mitochondria of the spermatid become resolved into a compact body, the Nebenkern (Fig. 30, *N*), which usually shows a lighter peripheral area. It is interesting to note that

There are many other cases in which the mitochondria are not localized at one pole of the nucleus in the spermatocyte, and while such localization is common, it is not general as Duesberg maintains.

in the giant spermatocytes (Fig. 59) the mitochondrial content is much greater than in the normal cells and the giant spermatids derived from such giant spermatocytes have Nebenkerns which are correspondingly larger than the normal ones (compare Figs. 30 and 31).

(*c*) *Transformation of the Spermatid.*—Besides the deeply staining Nebenkern, there are also present in the cytoplasm of the spermatids, an acrosome-sphere, a centrosome and often a chromatoid body (Fig. 30). The latter is found in some of the spermatogonia and some of the spermatocytes, passing undivided to one of the daughter cells at the time of mitosis. During the transformation of the spermatid, the chromatoid body is cast off in the elongating tail, as has been described by Wilson ('13) in *Pentatoma*.

The centrosome of the spermatid is derived from the centrosome of the second maturation division. In the late anaphase (Fig. 29), the centrosomes lie close to the chromatin masses at both poles and in the telophase, when the spermatid nucleus is reconstructed, it can still be seen closely adhering to the surface of the nuclear membrane (Fig. 30). The axial filament begins to grow from the centrosome and pierces the Nebenkern (Fig. 32). The division of the Nebenkern into two halves lying on each side of the axial filament is not quite so clear as in the case of other insects. In the further elongation of the spermatid (Fig. 33), the Nebenkern becomes drawn out, as the axial filament grows, into two narrow filaments forming a sheath around the axial filament.

What I have termed the "acrosome-sphere" is a derivative of the spindle; but whether it represents the "sphere" material or whether it is a portion of the mitosome could not be determined. It is a rather large compact body and stains intensely with hæmatoxylin. (Figs. 30, 32). As the spermatid goes through the stages of transformation, the acrosome-sphere becomes somewhat compressed and forms a typical acrosome at the head of the spermatozoön.

(*d*) *Discussion.*—Leaving aside for the present the question of the origin of the mitochondria, let us turn to consider some of the facts concerning the behavior of the mitochondria in spermato-

genesis. Not only do they have a characteristic behavior which seems to be quite general in the insects, but they are present in all generations of the male germ cells. There are many discrepancies in the literature on this point, some workers maintaining that the mitochondria disappear at various times. Buchner ('09) has described a disappearance of the mitochondria during mitotic division in the spermatogonia of *Gryllotalpa*, but on the contrary Duesberg ('10), working on the same form, has shown that the mitochondria do not disappear at that time. Wilke ('13) states that in *Hydrometra* the mitochondria are absent from some of the spermatogonia and in some cases from the spermatocytes. I am inclined to believe that the disappearances of mitochondrial structures is in all cases due to improperly fixed material. In fact, there is some evidence from Wilke's figures that this is the case. In the spermatocytes of *Hydrometra*, Wilke describes a deeply staining perinuclear zone of the cytoplasm in which are located "yolk-spherules" (Dotterkugeln). These spherules at first homogeneous, begin to show the appearance of threads within them and finally definite mitochondria are set free in the cytoplasm, being formed out of the substance of the "Dotterkugeln." His results, however, do not indicate that a reversal of this process might not be taking place. I have often seen in material which has been improperly fixed bodies resembling Wilke's "Dotterkugeln," which are produced by an artificial agglutination of the mitochondria. As I shall later attempt to show, certain methods of fixation may produce a variety of changes in mitochondrial structures ranging from a slight distortion of their form to their complete disappearance. I merely wish to urge the fact here that mitochondria are constant cytoplasmic structures of the cell and when proper technical methods are employed, they can be demonstrated at all periods in the cell-cycle.

The rôle of the mitochondria in spermatogenesis is difficult to interpret on the basis of their morphological behavior. The formation of the compact Nebenkern from the filar mitochondria may be an indication of some chemical change in the mitochondria or it may be merely an expression of the compactness which the other cell elements, notably the nucleus, show in the transforma-

tion of the spermatid into the spermatozoön. O. Vander Stricht calls the Nebenkern of the spermatid a "vitelline body." Wildman ('14) ascribes a nutritive function to the mitochondria (karyochondria) of the spermatid of *Ascaris*. The view has often been expressed that the mitochondria of the spermatozoön are concerned in locomotion, since they usually form a part of the tail. On the basis of the behavior of the mitochondria in spermatogenesis, we can only say that, just like the acrosome or the axial filament, they form a definite structural element of the spermatozoön, and it may be that their only significance is bound up with their function as an organelle of the spermatozoön. This view is contested by Meves, Duesberg and others on the basis of their observations on fertilization in certain forms where the mitochondria of the sperm apparently enter the egg and can be traced into the cleavage cells. If this behavior of the mitochondria can be found to be of general occurrence in fertilization, their significance from the point of view of heredity and development becomes of utmost importance. It may be said that the evidence for this is still in a very unsatisfactory state. I shall reserve for later discussion the questions bearing on this problem, until added observations on the mitochondria during oögenesis may be presented.

2. *Nutrition of the Egg.*

Without entering into a detailed description of the structure of the ovary of *Cicada*, I wish merely to mention some facts in regard to the nutrition of the egg during the growth period. The study of insect ovaries dates back over a century, many of the works being concerned with the question of the origin of the various cell-elements, viz., germ-cells, nurse-cells, epithelial cells and follicle-cells. Much of this work is of little value for the solution of these problems, since the study has been usually confined to that of the adult ovaries, and it is evident that only an embryological as well as a histological study of the ovary can enable us to come to any definite decisions. As pointed out by Hegner ('14), the origin of the nurse-cells and germ-cells may be different in different forms. In the case of *Miastor*, Hegner states that the nurse-cells are mesodermal in origin, while in the Chrysomelid beetles "the nurse-cells in the ovaries seem to be

of germ-cell origin" (Hegner, '14, page 119). In *Dytiscus*, *Giardina* ('01) has shown that germ-cells and nurse-cells arise by differential divisions of a stem-cell. In *Cicada*, I am unable to ascertain the origin of the cell-elements of the ovary from a study of the pupal and adult ovaries. The differentiation of the cells must take place at a comparatively early time in the long life cycle of the insect.

There are two ovaries, each consisting of a great many ovarian tubules, the ovaries of the adult being much larger than those of the pupæ, due to the presence of a large number of mature eggs. Figure 34 is that of a longitudinal section through an ovarian tubule of an adult recently emerged from the pupal case and it will at once be seen that it is a typical Hemipteran ovarian tubule. At the proximal end of the tubule is the narrow end-filament which is undoubtedly of a ligamentous nature helping to support the ovaries in the abdomen. The tubule may be divided into three zones depending on the character of the cells present. At the proximal end is the nurse chamber, containing all the nurse-cells. These stain very deeply and it is impossible to make out their cell walls. The chromatin of their nuclei is in the form of diffusely spread granules, and usually there is present a chromatic nucleolus and a true plasmosome (Fig. 45, *n.c.*). At the base of the nurse chamber (Keimlager) are found numerous young oöcytes in various stages of synapsis. Proceeding distally from the nurse chamber are the older oöcytes of the post-synaptic stages, those farthest away from the nurse chamber being the oldest and largest (Fig. 34, *oöct. 2*). In this region are also found the follicle-cells (*f.c.*) which begin to form definite follicles around the oöcytes. As the young oöcytes begin to migrate distally from the base of the nurse chamber, they still retain protoplasmic connections with the cytoplasm of the nurse-cells, resulting in the formation of pseudopod-like projections from the oöcytes, the egg-strings (*e.s.*), by means of which nutriment is passed from the nurse chamber to the oöcytes. Even old oöcytes in which yolk is beginning to form still retain connection with the nurse chamber by means of the egg-string (Fig. 47). In the ovaries of young pupæ the egg-strings appear simply as cytoplasmic protrusions of the oöcytes, but in the

adult ovaries, the egg-strings loose their cytoplasmic appearance and seem to be of a fibrous structure (Figs. 34, 45, 47, *e.s.*). This fibrous appearance of the egg-strings has often been figured in the ovaries of other insects, but I am inclined to doubt its normality; it seems rather that the fibrous appearance is due to fixation and really represents a compression of the cytoplasmic processes of the oöcytes.

In the nurse chamber, the nurse-cells are arranged in what appears to be a syncytium, and only occasionally can cell-walls be distinguished. In the young pupæ, the egg-strings of the oöcytes pass through the region of the follicle-cells into the nurse chamber and seem to fuse and become continuous with the cytoplasm of the nurse-cells. In the adult ovaries when all the cells are at the height of their functional activity, the egg-strings become much enlarged assuming the fibrous appearance above described. In the nurse chamber, the egg-strings end in a central fibrous mass (Figs. 34, 71, 72, *i.n.c.*), the substance of which is continuous with the egg-strings and from which they lead to the oöcytes. The nurse-cells immediately in the region of the central plasmatic mass stain deeply and show evidences of degeneration. Within the plasmatic mass can be seen many nurse-cell nuclei in various stages of disintegration (Figs. 72, 34, *i.n.c.*). The nuclei become smaller, staining intensely, and finally become broken down and the products of their disintegration can be seen passing down the egg-string into the oöcyte (Figs. 45, 71). The nurse-cells are thus ingested by the protoplasmic process of the oöcytes which are probably furnished with some substances (enzymes) enabling them to digest the nurse-cells. In the adult ovaries, at the height of the breeding season, the ingestion of the nurse-cells has taken place to such an extent that almost half of the nurse-cells have disappeared and the central plasmatic mass has grown in size containing a great many ingested nurse-cells. The growth of the central plasmatic mass is undoubtedly correlated with the disappearance of the nurse-cells in this region of the ovary.

It is a noteworthy fact that the ingestion of the nurse-cells takes place at a time when there is a rapid growth of the oöcytes. The oöcytes at the beginning of the synaptic processes have large

nuclei surrounded by a small amount of cytoplasm containing mitochondria. As the synaptic period progresses the oöcyte grows somewhat in size, chiefly by an increase in the size of the nucleus and a slight increase in the cytoplasmic volume. After the synaptic processes have been completed, the cytoplasmic volume increase rapidly, so that the oöcyte becomes over four times its linear size before yolk begins to form. After the cytoplasmic volume has reached its maximum, the yolk-building process commences and at the end of this period the almost mature oöcyte is about three times its linear size at the beginning of the process. This great increase in size is not due to any increase in cytoplasmic volume, but due to the great enlargement of the yolk spherules. It is doubtful whether the cytoplasmic volume increases at all after the beginning of the yolk formation.

The ingestion of the nurse-cells by the oöcytes in *Cicada* is perhaps homologous to the ingestion of the cells in the ovaries of other forms, the classical example of which is found in *Hydra*. As is well known, in *Hydra* one cell in the ovary grows large by ingesting the other cells in the ovary and becomes the functional oöcyte. In *Dinophilus*, Conklin ('06) has described the fusion of from 25 to 30 oögonia to form a single large (female-producing) egg. In *Ciona* and other Ascidians, the test-cells are ingested by the oöcytes and remain in the egg, perhaps aiding in the elaboration of nutrient materials, but are cast out of the egg prior to fertilization. In the ovaries of the certain insects (*e.g.*, *Dytiscus*) of the type in which the oöcytes are supplied with a separate group of nurse-cells, the process of absorption of the nurse-cells as the oöcyte grows has been often described. Korschelt ('86) has described in the ovaries of *Notonecta* and *Reduvius* a disintegration of nurse-cell nuclei in the nurse chamber through the action of the oöcytes, with the production of a central space, "Plasmatische Raum," in the nurse chamber, which is free from nuclei. Foot and Strobell ('11) have also described a similar plasmatic area free from nuclei in the ovaries of *Protenor*, but are not inclined strongly toward Korschelt's view that in this region the nurse cells disintegrate and furnish nutriment for the oöcytes. In *Cicada* a study of Figs. 34, 45, 71, 72 will show that there is no doubt that Korschelt's view is correct, for, not only

can nurse cells be found in various stages of disintegration, but the products of such disintegration can actually be seen to pass down the egg-strings to the oöcytes. From Fig. 45, the impression may be gathered that the material derived from the disintegrated nurse cells passing down the egg-string is accumulated in a zone about the nucleus. Such, however, is not the case and as will be shown later, the deeply staining perinuclear zone consists of mitochondria and in material fixed in Bouin's fluid, the mitochondria disappear while the granules in the egg-string are preserved.

3. *Mitochondria in Oögenesis.*

(*a*) *Mitochondria in Growth Stages.*—The young oöcytes of *Cicada* lying at the base of the nurse-chamber are at all times distinguishable from the nurse-cells not only by their characteristic nuclei, but also by the presence of definite aggregations of granular mitochondria which are characteristically localized in the cells.

During the entire synaptic period of the oöcyte, the mitochondria are found lying in the cytoplasm as a cap of deeply staining granules closely applied to the nuclear membrane at one pole (Plate V.). As will be seen from Figs. 38, 39, 40, the pole of the nucleus at which the mitochondria are found always corresponds to the pole of the nucleus towards which the synaptic threads are polarized. It will at once be seen by comparing Figs. 17 and 40, that the mitochondria of the oöcyte and spermatocyte are localized in the cytoplasm at homologous positions, namely at the side of the nucleus where the idiozome, the sphere or centrosome is probably located. In the oöcytes, however, no centrosome or sphere can be distinguished and similarly Montgomery ('11) states that in *Euschistus* "it is not clear whether this body (idiozome or sphere) has any homologue in the oöcytes," etc.

The mitochondria of the oöcytes of *Cicada* are always in the form of granules, never assuming the filar form as found in the spermatocytes. During the later synaptic stages (pachytene stage, Fig. 40) there is an increase in the amount of mitochondria, but their localization at one pole remains constant. In the post-synaptic stages we find that, although the mass of mito-

chondria is still found at one pole of the nucleus, they gradually completely encircle the nucleus. After this period, when the synaptic threads have become diffuse and the "germinal vesicle" is established, the mitochondria increase greatly in numbers and are found localized in a zone immediately surrounding the nucleus (text-fig. 3, and Figs. 83, 84).

This perinuclear zone of mitochondria is sharply delimited from the rest of the cytoplasm. Fauré-Frèmiet ('08) has described the mitochondria of the oöcytes of *Julus* as being similarly arranged in a perinuclear zone whose cytoplasmic limit is definitely marked by the presence of a membrane formation. I can find no evidence for the presence of such a limiting membrane in *Cicada*. The mitochondria continue to increase greatly in numbers in the perinuclear region up until the stage in which the cytoplasmic volume of the oöcyte is greatest (Fig. 85). Throughout the period in which mitochondria are forming in the perinuclear zone, the nuclear membrane remains intact at all times as is evidenced in material improperly fixed in which the nuclear membrane has shrunken away from the cytoplasm as shown in Fig. 85. Neither is there any evidence that any nuclear materials are discharged or extruded into the cytoplasm at any time. There are many descriptions in the literature of bodies in the cytoplasm of oöcytes which have been derived from the discharge of nuclear materials. Goldschmidt and his pupils have maintained that the mitochondria are derived from the passage of nuclear materials into the cytoplasm, but there is no evidence that this is the case in *Cicada*. Vejdovský ('12) has described in the oöcytes of *Aphrophora* large deeply staining globules and vacuoles (compare my Fig. 42). He does not relate these structures to the mitochondria in any way, but indicates that these nucleolar-like bodies in the cytoplasm are derived by a "nucleolization" of the chromosomes and a casting-out of the resulting nucleoli into the cytoplasm. He is of the opinion that the vacuoles represent the escaped nuclear sap, there being no nuclear membrane present at this time. In the first place, it seems very doubtful that the nuclear membrane does disappear at any time in the resting nucleus. Secondly, if there is a nuclear membrane present it is difficult to see how such solid bodies as nucleoli

could pass through the membrane unless they be in a fluid, diffusible state, and if so they would become diffused when they entered the cytoplasm. Cases of "extruded nucleoli" have often been mentioned in the literature, but it must be said that many of the interpretations are exceedingly doubtful. In *Cicada*, when the ovaries have been fixed for a considerable length of time (24 hours) in Bouin's fluid, the mitochondria entirely disappear, leaving occasionally traces of their dissolution in the form of cytoplasmic vacuoles in the region of the nucleus (Fig. 82). By fixing the ovaries in Bouin's fluid for varying lengths of time (5 to 12 hours) a variety of peculiar structures may be found in the cytoplasm which are all referrable to the various stages and degrees of dissolution of the perinuclear zone of mitochondria (Figs. 39, 41, 42, 80, 81, 82). When the ovaries are fixed for ten hours in Bouin's fluid, a zone of the cytoplasm is found around the nuclei of the oöcytes which takes the plasma stain intensely (Fig. 42). Within it may be found vacuoles and deeply staining bodies resembling those structures which Vejdovskŷ ('12) figures in *Aphrophora*. In studying the effect of acetic acid of fixing fluids upon the mitochondria, I am led to believe that many of the peculiar cytoplasmic bodies which have been described and figured in germ-cells under various names such as extruded nucleoli or plasmosomes, vacuoles, idiozomes, spheres, yolk-spherules (Dotterkugeln), etc., are the result of imperfect fixation of mitochondrial substances. O. Vander Stricht ('04) has shown a similar effect of reagents in the distortion and dissolution of mitochondria in the "couche vitellogène" of the oöcyte of the bat.

(*b*) *Yolk-formation.*—Throughout the growth period of the oöcyte, the mitochondria increase greatly in numbers and continue to be located in the well delimited perinuclear zone. At the time when the cytoplasmic volume of the oöcyte is at its maximum (Fig. 85) the zone of the mitochondria occupies approximately a third of the cytoplasmic volume. After this stage in the growth of the oöcyte, the perinuclear mitochondrial mass begins to loose its well-defined zonal limits and the granules become dispersed in the cytoplasm towards the periphery of the oöcyte (Fig. 86). The migration of the mitochondria from the

perinuclear zone to the periphery of the cell continues, until in the older oöcytes all the mitochondria have been localized in the cortical region of the oöcyte (Fig. 47). A similar centrifugal migration of the mitochondria from the perinuclear zone toward the cell periphery has been described by Payne ('16) in the oöcytes of *Gryllotalpa*, by Fauré-Frèmiet ('08) in the oöcytes of *Julus*, by Govaerts ('13) in the oöcytes of the beetles *Trichiosoma* and *Cicindella*, and by many other workers who have studied the mitochondria in the oöcytes.

At this time the cytoplasm of the oöcyte is of a hyaline, homogeneous appearance, except for the mitochondrial granules located in the peripheral zone. In somewhat older oöcytes (in which yolk-spherules have not as yet formed) numerous vacuoles appear in the cortical zone of the cytoplasm where the mitochondria are located. These vacuoles are at first very small in size and within them can be seen the mitochondrial granules. That the granules in the vacuoles are actually mitochondria is proved by the fact that they respond to all the specific stains and are only found in material which has been fixed according to the mitochondrial technique. The further history of the mitochondria and the vacuoles of the cortical layer is concerned with the process of formation of the yolk-spherules. The mitochondria within the vacuoles begin to disintegrate, often showing small vacuoles within themselves. At this time the vacuoles containing the disintegrated mitochondria appear very much like numerous small nuclei. These structures are no doubt similar to the "pseudo-nuclei" of Blochmann. Korschelt ('89) has described similar "pseudo-nuclei" in the oöcytes of several insects, and he considers them as follicle-cell nuclei which have migrated into the oöcyte much as the test-cells of the Ascidians migrate into the oöcyte to aid in the elaboration of nutrient materials. Hegner ('15) has studied similar "secondary nuclei" in the oöcytes of *Camponotus* (Hymenoptera) and has also given a satisfactory review of the literature dealing with these structures. However, he is of the opinion that the "secondary nuclei" arise as buddings from the oöcyte nucleus and that this process is, hence, comparable to chromatin diminution processes in other forms (*e.g.*, *Miastor*, *Ascaris*). Hegner finds that the "secondary

nuclei" surround the oöcyte nucleus increasing in numbers presumably at the expense of the oöcyte nucleus. Later they become scattered toward the periphery of the oöcyte, increasing in numbers and finally disappearing. According to Blochmann the "pseudo-nuclei" may become incorporated in the yolk-spherules. From this general behavior of the "pseudo-nuclei" and the "secondary nuclei" of Hegner I am convinced that in all cases they represent stages in the transformation of mitochondria into yolk. Loyez ('08) has shown that these structures are not nuclei in any sense of the word and similar to Govaerts ('13), she has shown their relation to mitochondria and yolk-formation.

Figure 48 shows the various stages in the development of the yolk-spherules from the substance of the mitochondrial granules contained in the vacuoles. The substance of the vacuoles takes the plasma stain lightly at first, and as the vacuole grows in size the mitochondrial granules disappear and the substance of the vacuole begins to take the plasma stain more deeply. These plasma staining bodies grow considerably in size and often a small area of their substance begins to show a marked affinity for the basic stains. This basic staining area grows in extent and finally the entire body, which is none other than a yolk-spherule, takes the basic stain intensely. The relative size of the yolk-spherules is enormous as compared to the size of the mitochondrial granules from which they have been derived, and this indicates, together with the changes in the staining reactions, that a series of chemical reactions take place during this transformation. The mature egg is filled with these large deeply staining yolk-spherules, between which lie small granules which give the characteristic mitochondrial staining reactions similar to the mitochondria described by Duesberg ('08) in the egg of the bee. Apparently not all of the mitochondria are transformed into yolk; or perhaps new ones are being formed which have as yet not been transformed into yolk.

The transformation of mitochondria into yolk has been described by Loyez, Russo, Fauré-Frèmiet, Govaerts, Hegner, Lams, Vanderstricht, and others. L. and R. Zoja have described a transformation of the "plastidules fuchsinophiles" into yolk

in the egg of *Helix*. R. Vander Stricht ('11) has described in the oöcyte of the cat a "couche vitellogène" or perinuclear ring of mitochondria which grows in size during the early growth stages of the oöcyte and which later becomes dispersed toward the periphery of the cytoplasm and there gives rise to yolk. O. Vander Stricht ('94) has also described a "couche vitellogène" (of mitochondrial nature) in the oöcytes of the bat, which in the young oöcytes is arranged in the perinuclear zone, but in the older oöcytes it becomes diffused in the cytoplasm. This diffused substance forms the "pseudochromosomes" of the oöcytes of the adult ovaries and from these are derived the yolk bodies. Wildman ('13) has described two kinds of cytoplasmic inclusions in the spermatocytes of *Ascaris*, the "karyochondria" and the "plastochondria," both of which are derived from nuclear material. The "karyochondria" become transformed into yolk granules which later fuse to form the "refractive" body of the spermatid. The "plastochondria" have a negative behavior and take no part in spermiogenesis. Recently Gajewska ('19) has described a perinuclear ring of mitochondria in the oöcytes of *Triton* and indicates the relation of this to yolk formation.

I have cited only a few of the cases in which the mitochondria appear to have a genetic relationship to yolk formation, and as will be shown later, this relation is more general than is usually supposed. The chemical processes involved in the transformation of mitochondria into yolk cannot involve complicated chemical changes since the mitochondria themselves are of a phospholipoid nature and closely allied chemically to yolk.

(*c*) *Discussion*.—In almost every work dealing with oögenesis and the study of oöcytes, mention is made of certain cytoplasmic inclusions which are either of a granular, globular or filar nature, or else assume larger proportions and appear as single compact bodies. Various names have been applied to these structures, such as "Dotterkern" or yolk-nucleus, yolk-matrix, "couche vitellogène," "corps de Balbiani," pseudochromosomes, extruded nucleoli, "zona plasmatica perinucleare," etc. In nearly all these cases it will be noted that these special portions of the cytoplasm are involved in the process of yolk elaboration, and I shall attempt to point out some of the homologies existing be-

tween the above-named structures and the perinuclear zone of mitochondria as found in the oöcytes of *Cicada*. First, however, it may be said that the occurrence of a special zone of mitochondria immediately surrounding the nucleus is by no means uncommon. Fauré-Frèmiet ('08) has described a perinuclear ring of mitochondria in the oöcytes of *Julus*, as does Payne ('77) in the oöcytes of *Gryllotalpa*, Vejdovskỳ ('12) in the spermatocytes of *Diestramena*, Schaefer ('07) in the spermatocytes of *Dytiscus*, Shaffer ('17) in the spermatocytes of *Passalus*, etc. In fact in almost every case where the mitochondria have been studied in the early growth period of the germ-cells, they have been found in close spatial relations to the nucleus.

In the early literature dealing with insect oögenesis, there is almost always figured granules or deeply staining areas of the cytoplasm whose homology with the perinuclear ring of mitochondria as described here in *Cicada* becomes at once evident. Korschelt ('89) describes in the oöcytes of *Dytiscus* deeply staining portions of the cytoplasm in the region of the nucleus which he interprets as representing nutrient materials derived from the nurse-cells, and into which the nucleus of the oöcyte sends amœboid processes. Marshall ('07*a*) describes small nuclear-like bodies around the nuclei of the oöcytes of *Polistes*. These increase in number and later migrate to the periphery of the oöcyte, but Marshall offers no explanation as to their significance. The same author (Marshall, '07*b*) figures a deeply staining granular zone around the nucleus of the oöcyte of *Platyphlax* (Hymenoptera). Often this granular mass is cone-shaped extending toward the egg-string (compare my Fig. 45). In the older oöcytes, he describes the appearance of deeply staining bodies which lie scattered in the cytoplasm (when fixed in Flemming's fluid); but their further history is not traced. McGill ('06) describes a deeply staining perinuclear zone of granules in the oöcytes of *Plathemis* and *Anax*, which she considers as the "yolk-nucleus." It later breaks away from the nuclear wall and becomes scattered in the cytoplasm. Hegner ('15) describes a perinuclear zone of granules in the oöcytes of *Camponotus* which he states "resembles chromatin in some respects and may represent chromatin which has passed through the nuclear membrane

into the cytoplasm" (page 508). More recently, Nakahara ('18) has described in the oöcytes of *Perla* a deeply staining mass of granules in the cytoplasm closely surrounding the nuclear membrane, which he calls the "yolk-nucleus." This later breaks away from the nuclear membrane, becomes insignificant and finally disappears. Nakahara is of the opinion that the nucleoli of the oöcyte nucleus are derived from the passage of material from the "yolk-nucleus" into the oöcyte nucleus, and hence nucleoli are extra-nuclear in origin. These interpretations of Nakahara are hardly excusable since at this late date he has had the advantage of a large mass of data bearing on mitochondria, and it is evident that he has not made a proper study of the literature. Many similar cases of almost riotous interpretations of mitochondrial structures might be mentioned, many of them inexcusable in light of the recent studies on the mitochondria. As has been before stated, many of these erroneous interpretations are based upon material prepared without regard for the special technical processes involved for the demonstration of mitochondrial structures. Perhaps the best example of this is the work of Giardina ('04). This writer has studied the oöcytes of a number of insects (*Mantis, Periplaneta, Stenobothrus, Gryllus*) and the gastropod, *Helix arvensis*. In all of these forms, Giardina has described a special zone of the cytoplasm immediately surrounding the nucleus to which he assigns the name "zona plasmatica perinucleare." At times this zone may appear granular, striated, vacuolated, homogeneous, etc., and is always sharply delimited from the rest of the cytoplasm by a membrane. After considerable discussion as to its physical state, Giardina comes to the conclusion that it is a formation *in situ* of the cytoplasm under the action of substances from the nucleus, and that it acts as an intermediary between the nucleus and the cytoplasm in the nutrition of the egg. From a study of Giardina's figures and a comparison with the oöcytes of *Cicada* fixed in Bouin's fluid for varying lengths of time (Figs. 39, 41, 42), it becomes at once evident that the "zona plasmatica perinucleare" of Giardina is nothing more than poorly-fixed mitochondria arranged in a perinuclear zone. Duesberg ('12) is of this opinion as is also Fauré-Frèmiet ('10).

It thus seems that perhaps it is quite general in the insects that the mitochondria of the oöcyte are first arranged in a definite zone around the nucleus during the period in which they increase in numbers, and in the older oöcytes the perinuclear zone of mitochondria becomes dispersed towards the periphery of the oöcyte and becomes concerned in the process of yolk-formation.

4. *Significance of the Perinuclear Zone of Mitochondria.*

(*a*) *Relation to Yolk-nuclei.*—As I have before indicated, the occurrence of a perinuclear zone of mitochondria is quite commonly met with especially in the female germ-cells during the growth period. I shall not attempt a lengthy review of the literature in this regard since we already have the excellent review of Duesberg ('12), but I wish to point out some of the significant facts which have a bearing on the problems presented here. I wish merely to discuss the origin and homologies of the yolk-nucleus or "corps de Balbiani," mention of which has been made in almost every work in oögenesis which has appeared in the past two decades. As has been shown in Duesberg's ('12) review, much of the recent work in this connection has demonstrated that these bodies (yolk-nuclei) are in many cases partly constituted of mitochondrial substance. O. Vander Stricht ('04) has shown that the "couche vitellogène" surrounding the nucleus of the oöcyte of the bat is of a mitochondrial nature, and von Winiwarter and Sainmont ('09) have described a zone of granular mitochondria around the nucleus of the oöcyte in the kitten, which they homologize with the yolk-nucleus and "couche vitellogène" of Vander Stricht. Recently, Gajewska ('19) has shown that in *Triton* the yolk-nucleus is made up of three substances: "Zuerst eine Anhäufung von Ergastoplasmatischer Substanz (Ergastoplasma-Dotterkern), dann ein Mitochondrienkonglomerat (Dotterkern als Körnerkonglomerat) und endlich ein Haufen von Fettkugeln und Eiweissplättchen. Die Muttersubstanz fur den Dotterkern ist der perinukleare Ring (couche vitellogène)" (p. 116).

The fact which I wish to emphasize here is that in all cases the yolk-nucleus has its beginning in the young oöcyte in the form

of granular masses closely applied to the nuclear membrane, and these masses grow in amount during the period in which they are close to the nucleus. This is clearly shown in the early works on the yolk-nucleus such as Munson ('99) in *Limulus*, Crampton ('99) in *Molgula*, Calkins ('95) in *Lumbricus*, Van Bambeke ('95) in *Scorpæna scrofa*, Vander Stricht ('98) in the human oöcytes and in *Tegnaria*, and by others of the early workers on these structures. In almost all these cases, the close association of these granules with the nucleus and the similarities in the staining reactions with that of the chromatin have given the earlier workers the opinion that the granular masses represent extruded nuclear material. In many of these cases, the granular masses surround the centrosome forming a compact body with sharp outlines.

The formation of the typical yolk-nucleus (corps de Balbiani) as found in the Arachnids has been described by Vander Stricht ('98, '04) and Faurè-Frèmiet ('10). Here, as in other cases, the granules (mitochondria) forming the yolk-nucleus are first found in a perinuclear zone. Within this granular mass is found a deeply-staining vesicle, and later the mitochondria gather about it in concentric layers forming the typical compact Arachnid yolk-nucleus, which usually shows a ray-like structure. The relation between the yolk-nucleus and the attraction-sphere has often been noted. I have previously called attention to the fact that the mitochondria are usually (although not always) localized at the pole of the cell at which the centrosome, sphere or idiozome lies. In the formation of the compact Arachnid yolk-nucleus, the mitochondria gather about the centrosome-sphere as a center, the relation between the two being only spatial. Duesberg ('12) has noted this relation and applies the term "idiozom" or "centrotheka" to the central vesicle of the yolk-nucleus and points to the fact that this conception corresponds to Henneguy's definition of the "corps de Balbiani": "Un corpuscle centrale entouré d'un zone d'aspect homogène ou finement granuleux."

It thus seems that in oöcytes the perinuclear ring of mitochondria may behave in two ways; it may become dispersed in the cytoplasm and give rise to yolk in the periphery of the cell (as seems to be the case in the insects), or the mitochondria of the

perinuclear zone may become massed about the centrosome, forming the compact "corps de Balbiani" which later disintegrates in the formation of yolk (as in the Arachnids, etc.).

(*b*) *Origin of the Mitochondria.*—There are two facts of importance to be noted in the foregoing discussion: (1) the quite usual presence of mitochondria in a zone of the cytoplasm immediately surrounding the nucleus; (2) the presence of this perinuclear zone at a time when the mitochondria are increasing greatly in number. One of the most important questions bearing on the nature and rôle of the mitochondria is bound up with the mode of their origin and increase in number, and there has been considerable controversy regarding these questions. According to the view of Meves, Bouin, Duesberg and others, the mitochondria have no "de novo" origin, but are always derived from pre-existing mitochondria by a process of division. The chief evidences to support this view are: (1) the constant presence of mitochondria in all cells at all times; (2) the mitochondria within the cell are actually distributed to the daughter cells at the time of mitosis; (3) in some few cases (Meves in *Ascaris*, Duesberg in *Ciona* and *Apis*) the mitochondria of the fertilized egg have been traced into the embryonic cells. In the first place, it may be said that the omnipresence of mitochondria in all living cells may be interpreted upon an entirely different basis as I shall attempt to show later. There is, of course, a genetic continuity of mitochondria to a certain degree; the mitochondria of a cell are certainly carried into the daughter cells at the time of mitosis, but the view that individual mitochondria give rise to "homologous" mitochondria of succeeding cell-generations is entirely without evidence. Duesberg ('18) found that the yellow oöplasm of the Ascidian egg was rich in mitochondria, and, using the results of Conklin ('05) who traced yellow oöplasm of the egg into the embryo, consequently maintained that the mitochondria were genetically continuous from the fertilized egg to the embryonic cells. While there is no question that the yellow oöplasm is continuous, yet this is far from establishing that the individual mitochondria are continuous.

Opposed to the "genetic continuity" hypothesis of the mitochondria, we have the "chromidial" hypothesis developed by

Goldschmidt ('09) and his students. As has been before mentioned, there have been many descriptions of cytoplasmic bodies whose origin has been attributed to material extruded from the nucleus. According to Goldschmidt and others (Buchner, Jorgensen, Schaxal, Wasilieff, Popoff, etc.), mitochondria are derived from the chromidia and ultimately from the chromatin of the nucleus, and are hence similar to Hertwig's "chromidia." The fact that the mitochondria lie at the pole of the nucleus where the idiozome lies and toward which the synaptic threads are polarized, has been taken by Buchner and Wasilieff to be the place where mitochondria arise by the emigration of chromatic materials from the nucleus into the cytoplasm. Buchner ('09) derives the mitochondria of the spermatocytes of *Gryllus* from the material of the sex-chromosome, which in the bouquet stage becomes vacuolated and shows evidences of disintegration. While chromatin from the nucleus may at times come to lie in the cytoplasm (*e.g.*, chromatin diminution processes in *Ascaris* and *Miastor*, etc.), yet there is no strong evidence that it may give rise to the mitochondria. It is difficult to see how escape of chromatin from the nucleus could account for the tremendous increase in the mitochondria as found during the growth period of the oöcytes of *Cicada*. Furthermore, the difference of behavior of chromatin and mitochondria towards fixing fluids and specific stains indicates that they are of a totally different chemical nature (see Cowdry '16, p. 426).

According to the view of Vejovskỳ ('07, '12) the mitochondria are cytoplasmic structures having their origin in the "regressive modification" (Duesberg, '12) of the sphere material. Montgomery ('11) expresses the opinion that "it is probable that they (mitochondria) are produced by either idiozome or nucleus or by a joint action of both" (p. 787). Although it is quite usual that the mitochondria are found lying close to the idiozome or sphere, there is no conclusive evidence that they have their origin in or from the sphere material.

We have now discussed the three views prevalent regarding the origin of the mitochondria. From a study of the mitochondria of *Cicada* and from a review of much of the literature bearing on the subject, still another view presents itself which

has hitherto been only occasionally expressed in the literature. This is what might be called the "interaction theory," and according to it the mitochondria are neither self-perpetuating structures, nor are they derived from the chromatin of the nucleus, nor by a disintegration of the sphere material. According to this view the mitochondria are cytoplasmic differentiations which arise through specific chemical actions of the nucleus upon materials in the cytoplasm. I have before emphasized the almost universal presence of a particular zone of mitochondria immediately surrounding the nucleus at certain times in the cell-cycle, and I believe this perinuclear zone to be one of the best morphological demonstrations of an "interaction" between nucleus and cytoplasm. The presence of this perinuclear zone of mitochondria during the period in the oöcyte when the mitochondria are increasing in number as the cytoplasmic volume is also growing by assimilation of products from the nurse-cells, supports the view that the mitochondria are being built up by nuclear action on substances in the cytoplasm. After the maximum amount of mitochondria has been elaborated, the perinuclear zone is dissipated and the mitochondria become diffusely spread in the cytoplasm followed by their transformation into yolk.

Montgomery ('11) and Browne ('13) have also expressed views that the mitochondria may arise as a result of the interaction between nucleus and cytoplasm.

The view of mitochondrial continuity has no more than weak circumstantial evidence to support. Granting that the mitochondria of the spermatozoa are brought into the egg and that the cells of the embryo and adult all possess mitochondria, it is far from establishing the fact that the mitochondria of these cells are derivable from those of previous cell-generations which go back to the fertilized egg. Mitochondria do arise, as such, *de novo* in the cells by the chemical actions of the nucleus on the cytoplasm immediately surrounding it, although there may be a limited amount of mitochondria carried over from the previous cell-divisions.

Just what portion of the cytoplasm is acted upon by the nucleus is another matter. In the oöcytes of *Cicada*, nutriment is

brought in from the nurse chamber through the egg-string and assimilated by the cytoplasm; the nucleus exerts a chemical influence (enzyme?) on these products whereby the mitochondria are differentiated in the cytoplasm immediately surrounding the nucleus. According to this view it is possible to explain the presence of mitochondria in all cells (animal and plant) at all times, for all cells are constantly receiving nutriment which is taken into the cytoplasm (assimilated) and then acted upon by the nucleus resulting, among other things, in the elaboration of mitochondria. It thus seems that the *mitochondria arise as differentiated parts of the cytoplasm through specific chemical (enzyme) reactions of the nucleus upon the products of assimilation of the cell.* What the significance of such cell structures in the cell economy may be is quite another problem.

E. General Considerations.

1. *Chromosomes.*

My study of the chromosomes of *Cicada*, I believe, presents added evidence to the already large body of facts bearing on the individuality of the chromosomes and my observations indicate a persisting chromosomal organization which is constant throughout the cell-cycle. I have studied the metaphase plates of hundreds of cells, germinal and somatic, and have found no variations in either chromosome number, the relative sizes of the chromosomes or their characteristic grouping. The only exceptions to this is found in the giant spermatocytes and the multinuclear cells in the adhesive gland of the female, where the increase in chromosome number is due to the suppression of the division of the cell-body at mitosis resulting in the formation of polyvalent chromosome groups. In such polyvalent cells, we can still recognize double, triple, quadruple, etc., sets of each of the chromosome pairs.

There have appeared at various times in the cytological literature discrepancies of chromosome numbers in certain species, purporting to show that chromosomes vary in number and cannot be regarded as persistent structures of the cell. McClung ('17) has dealt ably and at length with such criticisms and has particularly concerned himself with the work of Delle Valle. Ac-

cording to Delle Valle, chromosomes are not constant structures of the cell and their number varies as ordinary fluctuating variations, since the number in a particular cell depends upon the mean size of the chromosomes, the amount of chromatin in the nucleus being constant (McClung, '17, p. 548). I have already (page 409) called attention to the remarkable difference between the metaphase chromosomes of the young follicle-cells and those of old follicle-cells (compare Figs. 7, 10, 11). In the old follicle-cells, the chromosomes are somewhat longer, thinner and poor in chromatin constitution. The nuclei from which such chromosomes are derived are much poorer in basichromatin than the nuclei of the young follicle-cells, usually having only a single small mass of basichromatin. Nevertheless, as will be seen from Figs. 10 and 11, the chromosome number remains constant and their relative sizes are similar to the chromosomes of other diploid groups; their only difference seems to be that they possess less chromatin. It is at once evident that this condition cannot be interpreted on the basis of Delle Valle's hypothesis. It also brings to light the fact that the material concerned in maintaining the chromosome number and size is not the chromatin of the nucleus, which is apparently more or less variable in amount, but the underlying structural basis of the chromosomes, namely the linin. In a previous paper (Shaffer, '20), I have emphasized the importance of the linin as being responsible for the architecture and organization of the chromosomes and for the maintenance of the stability of the nuclear elements. The chromomeres of the chromosomes are linearly arranged in a definite order (Wenrich, '16) which is maintained constant through the agency of the linin, the structural basis. Besides this, the linin is also concerned in the movements and localization of the chromatic elements of the nucleus. Some of the most fundamental problems of the cell are bound up with such phenomena, namely, what determines how the chromomeres shall be arranged in the chromosome, or by what agency are homologous chromosomes brought together in synapsis. "As long as 'conjugation' of the chromosomes is dealt with as though they were entities with independent power of movement, instead of the processes back of it, the super-

structure of theory must remain as unwieldy as at present" (Kingsbury, '12, p. 48). As I have before pointed out, it is possible that we may find an explanation for such movements of the chromatic elements in the linin ground-work of the nucleus. Homologous chromosomes have linin connectives running between them and it is possible that their union in synapsis is brought about by a contractility of these interchromosomal linin fibers, very much as the spongioplasm acts in localizing substances in the egg (Conklin, '17). The linin is, therefore, the persistent material of the nucleus, while the chromatin may be variable in amount in certain phases of the cell-cycle.

(*b*) *Mitochondria.*—The function or the rôle of the mitochondria in the cells of animals and plants still remains one of the unanswered cytological problems of to-day. A few cytologists have insisted that the mitochondria are idioplasmic materials which have a rôle in the transmission of hereditary characters similar to the chromosomes. According to this view, the chromosomes bear the determiners for the generic or racial characters of the organism, while the mitochondria bear the determiners for the specific or individual hereditary characters. From what little we do know of "cytoplasmic inheritance" it seems that the reverse is true (Conklin, '17) and that the larger orientations of development are fixed by the cytoplasm, particularly that of the egg. The idioplasmic view of the mitochondria was developed from the fact that they were found in all cells at all times and that they behaved characteristically during mitosis, becoming equally distributed to the daughter cells. These facts seemed to indicate a persistence and continuity of the mitochondrial substance through the cell-cycle which would fit in with the view of their idioplasmic nature. As I have before indicated (p. 445) there is no basis for maintaining their genetic continuity, but rather that "new" mitochondria may be formed in the cell without any relation to previously existing mitochondria.

According to another view the mitochondria may become transformed into certain histological elements of the cell, such as muscle and nerve fibrillæ, collagenic fibrils, and certain of the glandular secretions (pancreas, thyroid, etc.). Without

entering into a detailed discussion of the "histogenetic" view of the mitochondria, it may be said that the evidence is far from being convincing. From what we know of the chemical nature of the mitochondria, it becomes difficult to understand how they may become transformed into structures so different chemically. According to Cowdry ('16, p. 435), "it is apparent that the doctrine of an actual chemical transformation of mitochondria into substances of diverse constitution is weak."

As to the chemical nature of the mitochondria, practically all workers agree that they are combinations of lipins with varying amounts of albumin (phospholipins). The transformation of the mitochondria into the yolk-spherules of the egg at once indicates their lecithin nature. N. H. Cowdry ('17) has given a summary of the more important data bearing upon the chemical nature of the mitochondria. Löwschin ('13) has been able to make mitochondria artificially in lecithin and albumin solutions. These mitochondria behave in every way (form, solubility, fixation and staining) like true mitochondria of organic cells. Russo ('12) has described an increase in the number of mitochondria of the oöcytes of the fowl following injections of solutions of lecithin.

While we are beginning to know something about the chemical nature of the mitochondria, we are far from knowing their rôle in the physiology of the cell. That they bear an important relation to metabolism is conceded by many workers, and the class of chemical compounds to which the mitochondria are allied chemically (the lipins) have recently been emphasized in biochemical works as being intimately concerned in metabolic processes. In fact, Mathews ('15) believes that the phospholipins are the most important substances in organic matter.

In the oöcytes of *Cicada* it is quite clear that the mitochondria are related to the nutritive metabolic processes and that they are actually transformed portions of the products of assimilation. According to Cowdry ('17), in plants the "mitochondria are concerned in the formation of chlorophyll, and thus the very existence of the plant depends upon them." Maclean ('18) fed one group of hens on a normal diet and another group on a diet free from fats and lipins and concluded that the "lipins play an

important, if as yet unknown part in the history of fat metabolism" (p. 172).

Some workers (Kingsbury, '12, Cowdry, '17, etc.) have expressed the view that the mitochondria are concerned in the processes of cell-respiration, but there has not been sufficient experimental work to support this view. Maclean ('18) points out some very interesting relations between lipins and oxydative processes and mentions the work of Stanewitch who found that wheat embryos treated with solvents which extracted most of the lipins showed a respiration energy which was lower than normal.

It would, I think, be premature to make any definite statement as to the function or significance of the mitochondria in organisms until we know more of the biological significance of the lipins. Maclean says (p. 107):

"From what has already been said regarding the unsatisfactory state of our knowledge of the lipins, it follows that their exact function in the animal and vegetable economy is necessarily obscure. Their great importance is proved by their general occurrence in every cell, but little or no direct experimental proof indicating their specific function has yet been obtained. When we consider the obscurity in which the chemistry of the lipins has been shrouded and the fact that even now, in many cases, is not satisfactorily established, it is easy to understand that many of the properties and functions ascribed to these bodies are based on little more than the imagination."

For the present, all we can say regarding the function of the mitochondria is that they form the major part of the lipoid constitution of organic cells, and when we know more of the biological significance of lipins, we shall know more concerning the rôle of the mitochondria in cell economy. Obviously, further progress in this direction must come along experimental lines.

F. Summary.

1. The chromosome number in all male diploid groups of *Cicada* (*Tibicen*) *septemdecim* is 19, and in the female diploid groups 20.

2. The diploid chromosome groups are characterized by the

presence of one large pair of chromosomes (the macrochromosome pair, *AA*) and two pairs of somewhat smaller chromosomes (*BB*, *CC*). The other 13 chromosomes show no size differences.

3. There is no variation in chromosome number or in their form and arrangement in any of the diploid groups studied.

4. In the spermatocytes there are two ring tetrads of the *Stenobothrus* type, which are derived from the *AA* and *BB* chromosome pairs of the spermatogonia. These tetrads divide reductionally in the first maturation division.

5. An odd chromosome is present which persists as a nucleolus in the growth stages of the spermatocyte. It passes undivided to one pole in the first maturation division and divides in the second division.

6. The tetrads are always grouped characteristically in the metaphase of the first maturation division and the same grouping is found in the metaphase of the second spermatocytes.

7. The synaptic stages in the oöcyte were studied, giving evidence that the chromosomes pair side-to-side (parasynapsis).

8. Two chromatin nucleoli are present in the preleptotene stages of the oöcyte. These disappear in the synaptic stages and reappear in the post-synaptic stages. These nucleoli are interpreted to represent the two sex-chromosomes of the female which go through a synaptic phase like the autosomes.

9. Mitochondria are found in the spermatogonia in the form of granules localized at the end of the cell bordering on the cyst cavity.

10. The first evidences of a degeneration of a spermatogonium is shown by an agglutination of the mitochondria. As degeneration continues the mitochondria continue to agglutinate forming large lipoid globules in the cell, evidently yielding a fatty degeneration.

11. In the spermatocytes the mitochondria are filar; they surround the spindle peripherally at the time of the maturation divisions and become divided by the cell-constriction. The mitochondria of the spermatid form the round compact Nebenkern which later becomes drawn out as a sheath surrounding the axial filament of the spermatozoon.

12. The ovaries of *Cicada* are typically Hemipteran in struc-

ture. The egg-strings of the oöcytes pass up into the nurse chamber and serve to carry the nutrient materials to the oöcytes. In the ovaries of the adult, the nurse-cells are ingested at the upper end of the egg-string and their ingested products pass down into the oöcyte as nutrient materials.

13. Mitochondria are found in the young oöcytes as a deeply staining mass of granules lying in the cytoplasm at one pole of the nucleus. In the later stages the mitochondria gradually extend around the nucleus forming a perinuclear zone of mitochondria.

14. The mitochondria increase greatly in numbers during the postsynaptic stages still retaining their perinuclear arrangement.

15. When the cytoplasmic volume of the oöcyte has reached its maximum, the perinuclear arrangement of the mitochondria becomes lost and the mitochondria become dispersed toward the periphery of the oöcyte.

16. At the periphery of the oöcyte, the mitochondria become transformed into yolk-spherules. First vacuoles are found surrounding the mitochondria, these structures resembling the "pseudo-nuclei" of Blochmann. The substance of the vacuoles at first takes the plasma stain lightly and as it grows in size, it becomes more and more deeply staining. The globules increase greatly in size and show a marked affinity for the basic stains.

17. The relation between the perinuclear zone of mitochondria and such structures as yolk-nuclei, etc., is pointed out.

18. The zone of the cytoplasm immediately surrounding the nucleus is taken to be the locus in the cell of the formation of mitochondria through the chemical action of the nucleus upon the products of assimilation taken in by the cytoplasm.

LITERATURE CITED.

Athias, M.

'19 Recherches sur les cellules interstitielles de l'ovaire des Cheiroptères. Arch. de Biol., T. XXX.

Boring, A. M.

'07 A Study of the Spermatogenesis of Twenty Species of the Membracidæ, Jassidæ, Cercopidæ and Fulgoridæ. Jour. Exp. Zoöl., Vol. IV., No. 4.

'13 The Chromosomes of the Cercopidæ. Biol. Bull., Vol. XXIV., No. 3.

Browne, E. N.

'13 A Study of the Male Germ Cells in *Notonecta*. Jour. Exp. Zoöl., Vol. 14, No. 1.

'16 A Comparative Study of the Chromosomes of Six Species of *Notonecta*. Jour. Morph., Vol. 27, No. 1.

Buchner, P.

'09 Das accessorische chromosome in spermatogenese und ovogenese der Orthopteren, etc. Arch. f. Zellf. Bd. 3.

Calkins, G. N.

'95 Observations on the Yolk-nucleus in the Eggs of *Lumbricus*. Trans. N. Y. Acad. of Sci., Vol. XII.

Conklin, E. G.

'02 Karyokinesis and Cytokinesis, etc. Jour. Phila. A. N. S., Vol. 12.

'06 Sex Differentiation in *Dinophilus*. Science, Vol. XXIV., p. 294.

'05 The Organization and Cell-lineage of the Ascidian Egg. Jour. Phila. Acad., N.S., Vol. 13.

'17 The Share of Egg and Sperm in Heredity. Proc. Nat. Acad. Sci., Vol. 3.

'17 The effects of Centrifugal Force on the Eggs of Crepidula. Jour. Exp. Zoöl., Vol. 23.

Cowdry, E. V.

'16 The General Functional Significance of Mitochondria. Am. Jour. Anat., Vol. 19, No. 3.

Cowdry, N. H.

'17 A Comparison of Mitochondria in Plant and Animal Cells. BIOL. BULL., Vol. XXXIII., No. 3.

Crampton, H. E.

'99 Studies upon the Early History of the Ascidian Egg. Jour. Morph., Vol. 15, Supp.

Davis, H. S.

'08 Spermatogenesis in the Acridæ and Locustidæ. Bul. Mus. Comp. Zoöl. Harvard Coll., Vol. 53.

Duesberg, J.

'08 Sur l'existence des mitochondries dans l'oeuf et l'embryon d'Apis mellifica. Anat. Anzeig., Bd. XXXII.

'10 Nouvelles recherches sur l'appareil mitochondrial des cellules seminales. Arch. f. Zellf., Bd. 6, Heft. 1.

'12 Plastosomes, "Apparato reticolare interno," und Chromidial-apparat. Ergeb. der Anat. u. Entwick., Bd. XX.

'18 Chondriosomes in the Testicle-cells of *Fundulus*. Am. Jour. Anat., Vol. 23, No. 1.

'19 On the Present Status of the Chondriosome Problem. BIOL. BULL., Vol. XXXVI., No. 2.

Fauré-Frémiet, E.

'08 Evolution de l'appareil mitochondrial dans l'oeuf de Julus terrestris. C. R. Soc. de Biol. T. 64.

'10 Etude sur les mitochondries des Protozoaires et des cellules sexuelles. Arch. d'Anat. Micr. T. 11.

Foot, K. and Strobell, E. C.

'11 Amitosis in the Ovary of *Protenor belfragei* and a Study of the Chromatin Nucleolus. Arch. f. Zellf., Bd. 7, No. 2.

Gajewska, H.

'19 Ueber den sogenannten Dotterkern der Amphibien. Arch. f. Zellf. Bd. 15, 1st Heft.

Giardina, A.

'04 Sull 'esistenza di una speciale zona plasmatica perinucleare nell'oocite. Publicazioni de Laboratorio di Zoologica e. di Anat. Comp. dell Università di Palermo.

Goldschmidt, R.

'09 Das skelett der Muskelzelle von Ascaris nebst Bemerkungen ueber Chromidialapparat der Metazoenzelle. Arch. f. Zellf., Bd. IV.

Govaerts, P.

'13 Recherches sur la structure de l'ovaire des Insectes. Arch. de Biol., T. XXVIII.

Hegner, R. V.

'14 The Germ Cell Cycle in Animals. Macmillan, N. Y.

Kingsbury, B. F.

'12 Cytoplasmic Fixation. Anat. Rec., Vol. 6.

Kornhauser, S. I.

'14 A Comparative Study of the Chromosomes in the Spermatogenesis of *Enchenopa*, etc. Arch. f. Zellf., Bd. XII., No. 2.

Korschelt, E.

'86 Ueber die Enstehung und Bedeutung der verschiedenen Zellenelemente des Insektovariums. Zeit. f. Wiss. Zool., Bd. XLIII.

'89 Beitrage zur Morphologie und Physiologie des Zellkernes. Zool. Jahrb., Bd. 4.

Löwschin, A. M.

'13 "Myelinformen" und Chondriosomen. Ber. Deutsch. Bot. Gesell., Bd. 31.

Loyez, M.

'08 Les "noyaux de Blochmann" et la formation du vitellin chez les Hyménoptères. C. R. Assoc. Anat. 10 Réunion, Marseilles.

Maclean, H.

'18 Lecithin and Allied Substance. The Lipins. Monographs on Biochemistry, London.

McClung, C. E.

'14 A Comparative Study of the Chromosomes in Orthopteran Spermatogenesis. Jour. Morph., Vol. 25, No. 4.

'17 The Multiple Chromosomes of *Hesperotettix* and *Mermiria*. Jour. Morph., Vol. 29.

McGill, C.

'06 The Behavior of the Nucleoli during the Oögenesis of the Dragon-fly, etc. Zool. Jahrb., Bd. 23.

Marlatt, C. L.

'98 The Periodical Cicada. U. S. Dept. Agri. Div. Ent., Bull. 14, N.S.

Marshall, W. S.

'07a Contribution towards the Anatomy and Embryology of *Polistes pallipes*, II. Zeit. f. Wiss. Zool., Bd. 86.

'07b The Cellular Elements of the Ovary of *Platyphlax designatus*. Zeit. f. Wiss. Zool., Bd. 86.

Mathews, A. P.

'15 Physiological Chemistry. Wm. Wood and Co., N. Y., 1040 pp.

Montgomery, T. H.

'11 The Spermatogenesis of an Hemipteran, *Euschistus*. Jour. Morph., Vol. 22, No. 3.

Morgan, T. H.

'08 The Production of Two Kinds of Spermatozoa in the Phylloxerans, etc. Proc. Soc. Exp. Biol. and Med., Vol. 5, No. 3.

'09 A Biological and Cytological Determination of Sex Determination in Phylloxerans and Aphids. Jour. Exp. Zoöl., Vol. VII, No. 2.

Munson, J. P.

'99 The Ovarian Egg of *Limulus*. Jour. Morph., Vol. 15.

Nakahara, W.

'18 Some Observations on the Growing Oöcytes of the Stone-fly, *Perla immargenata* Say, etc. Anat. Rec., Vol. XV.

Payne, F. W.

'12 A Further Study of the Chromosomes of the Reduviidæ. Jour. Morph., Vol. XXIII.

'14 Chromosomal Variation and the Formation of the First Spermatocyte Chromosomes in the European Earwig, *Forficula* (sp.?). Jour. Morph., Vol. XXV., No. 4.

'16 A Study of the Germ Cells of *Gryllotalpa borealis*, etc. Jour. Morph., Vol. 28, No. 1.

Russo, A.

'12 Aumento dei granuli protoplasmici, etc. Arch. f. Zellf., Bd. 8.

Schafer, F.

'07 Spermatogenese von Dytiscus. Zool. Jahrb., Bd. 23.

Scott, W. J. M.

'16 Experimental Mitochondrial Changes in the Pancreas in Phosphorus Poisoning. Amer. Jour. Anat., Vol. 20, No. 2.

Shaffer, E. L.

'17 Mitochondria and other Structures in the Spermatogenesis of *Passalus cornutus*. BIOL. BULL., Vol. XXXII., No. 5.

'20 A Comparative Study of the Chromosomes of *Lachnosterna*. BIOL. BULL., Vol. 38, No. 2.

Stevens, N. M.

'05a A Study of the Germ Cells of *Aphis rosæ*, etc. Jour. Exp. Zoöl., Vol. 2, No. 3.

'05b Studies in Spermatogenesis with Especial Reference to the "Accessory" Chromosome. Carneg. Inst. Wash. Pub. No. 36, Part 2.

'06 Studies in Spermatogenesis. Part 2. Carneg. Inst. Wash. Pub. No. 36.

Sutton, W. S.

'02 On the Morphology of the Chromosome Groups of *Brachystola magna*. BIOL. BULL., Vol. 4.

Vander Stricht, O.

'98 Contribution à l'etude du noyau vitellin de Balbiani dans l'oocyte de la femme. Verhand. der Anat. Gesell., Jena.

'04 La couche vitellogène et les mitochondries de l'oeuf des Mammiferes. Verhand. der Anat. Gesell. Jena.

Vander Stricht, R.

'11 Vitellogénèse dans l'ovule de chatte. Arch. de Biol., T. XXVI.

Van Bambeke, Ch.

'95 Contribution à l'histoire de la constitution de l'oeuf. II. Arch. de Biol., T. XIII.

von Winwarter, H. et Sainmont, G.

'09 Nouvelles recherches sur l'ovogenèse et l'organogenèse de l'ovaire des Mammiferes (chat). Arch. de Biol., T. XXIV.

Wenrich, D. H.

'16 The Spermatogenesis of *Phryontettix magnus* with Especial Reference to Synapsis and the Individuality of the Chromosomes. Bull. Mus. Comp. Zoöl. Harvard Coll., Vol. LX., No. 3.

Wieman, H. L.

'10 A Study in the Germ Cells of *Leptinotarsa signaticollis*., Jour. Morph., Vol. 21, No. 2.

Wilcox, E. V.

'95 Spermatogenesis of *Caloptenus femur-rubrum* and *Cicada tibicen*. Bull. Mus. Comp. Zoöl. Harvard Coll., Vol. XXVII.

Wildman, E. E.

'13 The Spermatogenesis of *Ascaris megalocephala* with Special Reference to Two Cytoplasmic Inclusions, etc. Jour. Morph., Vol. 24, No. 3.

Wilke, G.

'13 Chromatinreifung und Mitochondrienkorper in der spermatogeneses von *Hydrometra paludim*. Arch. f. Zellf., Bd. X.

Wilson, E. B.

'06 Studies on the Chromosomes, III. Jour. Exp. Zoöl., Vol. 3, No. 1.

'13 A Chromatoid Body in *Pentatoma*. Biol. Bull., Vol. XXIV., No. 6.

ABBREVIATIONS.

AA, macrochromosome pair.
BB, *CC*, smaller chromosome pairs.
c., Centrosome.
cy., cytoplasm of oöcyte.
cr., chromatoid body.
cr.n., chromatin nucleolus of oöcyte.
e.s., egg-string of oöcyte.
f.e., follicular epithelium.
id., idiozome.
I.n.c., ingested nurse-cells.
N., Nebenkern of spermatid.
n.c., nurse-cell.
ncl., nucleus of oöcyte.
oocl., oöcyte.
pl., plasmosome.
S., spindle derivative.
X., sex-chromosome.

All drawings were made at table level with the aid of a camera lucida. Plates I, II, III, V (inc.) were made using a 1/12 oil immersion objective and a No. 12 ocular. They have been reduced $\frac{1}{4}$ in reproducing them here.

DESCRIPTION OF FIGURES.

PLATE I. (FIGS. 1 TO 12).

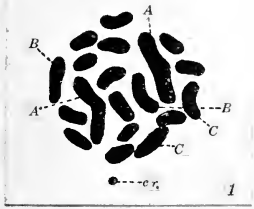
FIGS. 1, 2. Spermatogonial chromosome groups, showing macrochromosome pair (*AA*), the smaller chromosome pairs (*BB, CC*) and thirteen other chromosomes showing no size differences.

FIGS. 3, 4, 5, 6, 7, 8. Metaphase plates of follicle-cells of ovary, showing 20 chromosomes (female type). The chromosome pairs (*AA, BB, CC*) are easily distinguishable. Fig. 8 is late prophase.

FIG. 9. Metaphase plate from embryonic cell, showing 20 chromosomes hence of female type.

FIGS. 10, 11. Metaphase plates of ovarian follicle-cells (20 chromosomes) from follicles surrounding old oöcytes. Note that the chromosomes are poor in chromatin content; in Fig. 11 note the precocious longitudinal split of each chromosome.

FIG. 12. Late telophase of a follicle-cell division in which the macrochromosome, *A*, is recognizable in the daughter cells.



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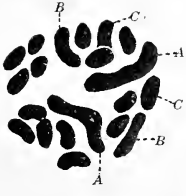

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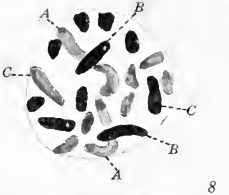

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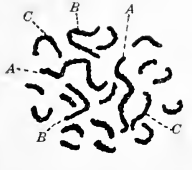

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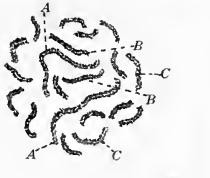

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PLATE II. (FIGS. 13 TO 22).

FIG. 13. Secondary spermatogonium with characteristic chromatin nucleolus; chromatoid body, and granular mitochondria localized at one end of the cell.

FIG. 14. Spermatogonium in early stage of degeneration. Mitochondria are larger due to agglutination, and nucleus is polymorphic.

FIGS. 15, 16. Later stages in degeneration of spermatogonia. Mitochondria continue to agglutinate and form large lipoid globules.

FIG. 17. Pachytene bouquet stage of spermatocyte, showing persisting sex-chromosome (*X*), the macrochromosome loop (*AA*) and the filar mitochondria localized about the idiozome (*id.*).

FIG. 18. Stages in the condensation of the ring tetrad of the macrochromosome pair (*AA*).

FIG. 19. Similar stages in the formation of the small ring tetrad of the *BB* chromosome pair.

FIG. 20. Early prophase of the first maturation division.

FIGS. 21, 22. Formation of first maturation spindle; mitochondria enveloping the spindle.

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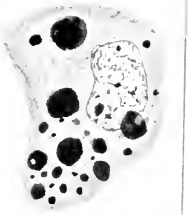

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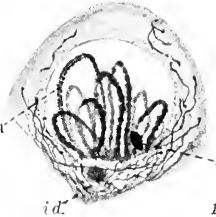

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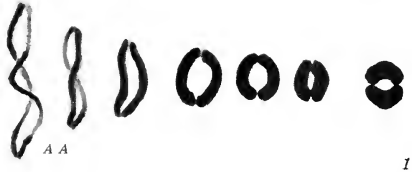

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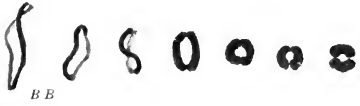

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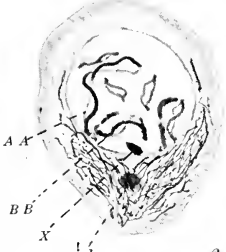

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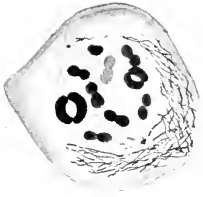

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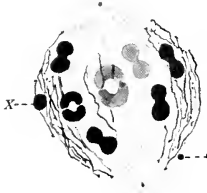

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PLATE III. (FIGS. 23 TO 33).

FIGS. 23, 24. Metaphase plates of first spermatocytes showing characteristic grouping of chromosomes; macrochromosome tetrad in center surrounded by a circle of autosome tetrads with the sex-chromosome (*X*) lying outside the group.

FIGS. 25, 26. Successive sections of same cell. Anaphase of first maturation division showing separation of dyads of the *AA*, *BB*, *CC* tetrads. Mitochondria surrounding the spindle.

FIG. 27. Late anaphase of first maturation division. Sex-chromosome lags behind and appears bipartite; macrochromosome dyads (*A*, *A*) showing secondary split. Mitochondria are divided by cell-constriction.

FIG. 28. Daughter plates of second spermatocyte, one with 9 dyads the other with 9 dyads plus the sex-chromosome (*X*). All the dyads show the secondary split.

FIG. 29. Late anaphase of the second maturation division. Centrosomes adhere closely to the daughter nuclei; chromatoid body has passed to one of the daughter cells. Mitochondria are divided by the cell constriction.

FIG. 30. Normal spermatid. Centrosome (*c*) adheres closely to nuclear membrane.

FIG. 31. Giant spermatid, with all the structures of a normal spermatid, except that they are much larger.

FIG. 32. Transformation of the spermatid. Elongation of the Nebenkern pierced by axial filament. *Cr.*, chromatoid body passing out into tail.

FIG. 33. Stages in spermiogenesis. Elongation of Nebenkern to form sheath around axial filament.

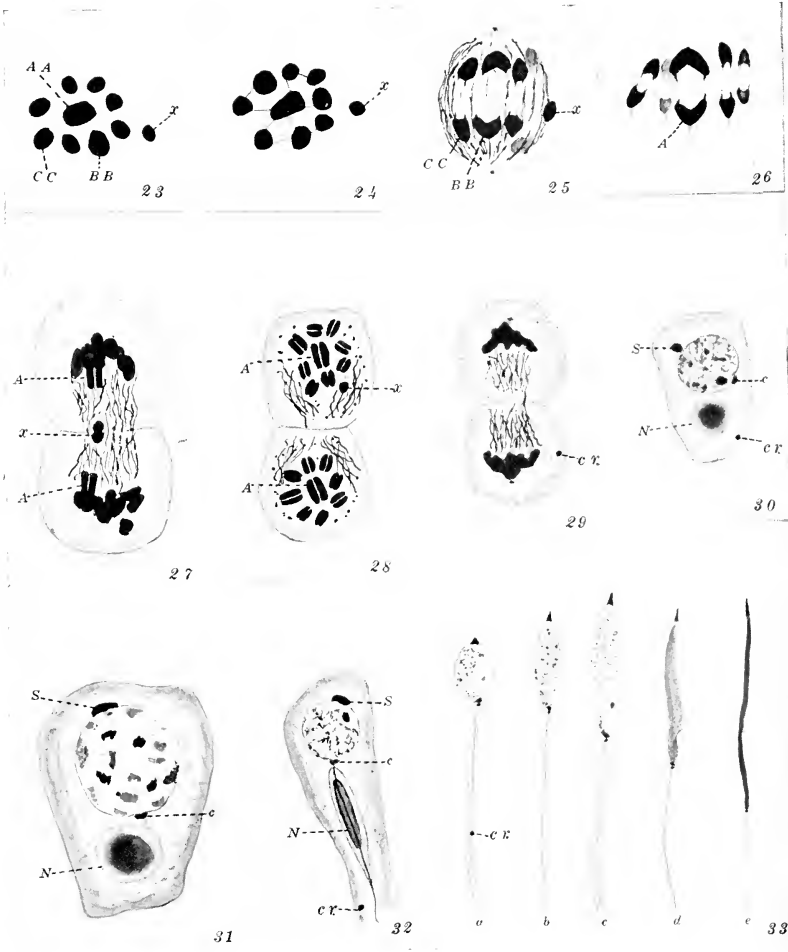
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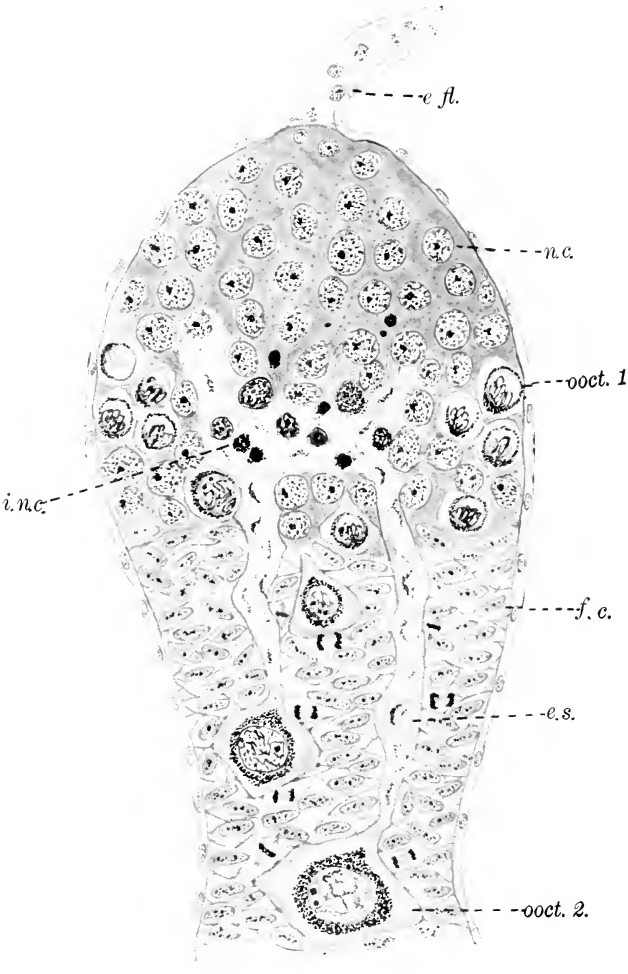
PLATE IV. (FIG. 34).

FIG. 34. Longitudinal section through proximal end of ovarian tubule, × 500. Nurse-cells (*n.c.*) in nurse chamber with deeply staining cytoplasm. At base of nurse chamber are found young oöcytes in various stages of synapsis. *l.n.c.*, region where ingestion of nurse-cells takes place. Products of this ingestion are seen passing down egg-string (*e.s.*) to the older oöcyte (*oöct. 2*), which also shows a perinuclear zone of mitochondria.


e fl.
n.c.
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i.n.c.
f. c.
e.s.
oct. 2.

PLATE V. (FIGS. 35 TO 43).

Synaptic Stages in the Oöcyte.

FIG. 35. Nucleus of very young oöcyte at beginning of growth period. Chromatin in form of a delicate network; two chromatin nucleoli are present (Protobroque nucleus).

FIG. 36. Deutobroque nucleus. Chromatin beginning to loose net-work appearance and individual threads become recognizable; two chromatin nucleoli present. Mitochondria in crescentic area closely applied to nuclear membrane.

FIG. 37. Leptotene stage. Polarization of threads; chromatic nucleoli absent Mitochondria spreading around nucleus.

FIG. 38. Zygotene stage. Leptotene threads are pairing parasynaptically. Mitochondria completely surround the nucleus.

FIG. 39. Pachytene stage. Bouin fixation, showing dissolution of mitochondria.

FIG. 40. Pachytene bouquet stage. Macrochromosome loop recognizable as largest loop.

FIG. 41. Section across bouquet stage showing pachytene threads on end view, 20 in number. Bouin fixation, showing effect on mitochondria.

FIG. 42. Release from bouquet stage; primary split evident in threads. Bouin fixation, 10 hours, showing artefacts produced by dissolution of mitochondria.

FIG. 43. Strepsistene stage. Synaptic threads separating and twisting about each other; two chromatin nucleoli are again present. Mitochondria increasing in numbers in perinuclear zone.

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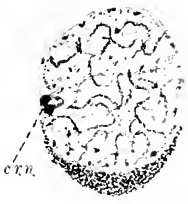

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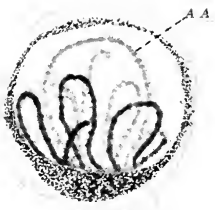

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PLATE VI. (FIGS. 44 TO 48).

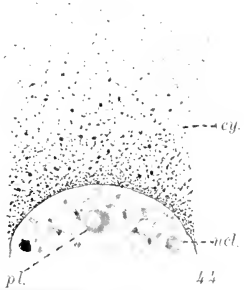
FIG. 44. Portion of nucleus (*ncl.*) and cytoplasm (*cy.*) of oöcyte showing migration of mitochondria from perinuclear region toward the periphery of the cell. × 1,200.

FIG. 45. Ingestion of nurse-cells by upper end of egg-string (*e.s.*). Products of ingestion are seen passing down egg-string into cytoplasm of oöcyte. Note perinuclear arrangement of mitochondria.

FIG. 46. Germinal vesicle of old oöcyte showing plasmosome (*pl.*) and several chromatic nucleoli. × 800.

FIG. 47. Portion of oöcyte and its follicle. Mitochondria arranged in periphery of cytoplasm. Persistence of egg-string. × 600.

FIG. 48. Stages in the transformation of mitochondria into yolk-sperules.



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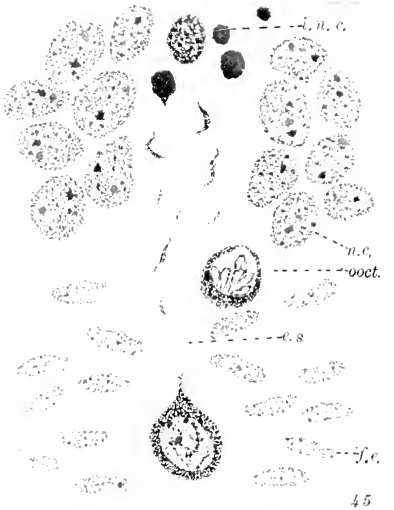

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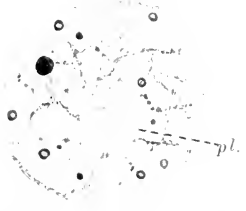

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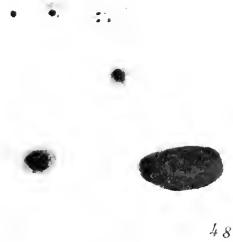

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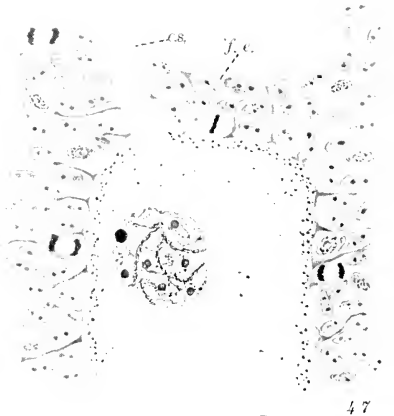

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Plates VII, VIII, IX.

Photomicrographs taken at a magnification of about 1,000 diameters, except Nos. 71 and 72 which have been magnified 500. The reproductions here have not been reduced.

Plate VII. (Figs. 49 to 70).

Figs. 49, 50, 51. Metaphase plates of follicle-cells of ovary, showing 20 chromosomes among which the macrochromosome pair can be distinguished. Fig. 51 is at a greater magnification.

Fig. 52. Tripolar spindle in one of the cells of the adhesive gland in the female.

Figs. 53, 54, 55, 56. Metaphase plates of first spermatocytes from four different animals, each showing 10 bivalent chromosomes which are similarly grouped in each case.

Fig. 57. An oblique section through the metaphase plate of the 1st maturation division, showing the two halves of the macrochromosome in the center of the complex.

Fig. 58. Daughter plates of second spermatocytes, one with 9 dyads, the other with 9 dyads plus the sex-chromosome. Note that the grouping of the chromosomes is the same as in the first spermatocyte.

Fig. 59. Giant spermatocyte with a great many bivalent chromosomes and a large amount of mitochondria.

Fig. 60. Anaphase of first maturation division showing mitochondria surrounding spindle.

Figs. 61, 62, 63. Various forms of ring tetrad (macrochromosome tetrad) in the early prophases.

Fig. 64. Macrochromosome tetrad in late prophase.

Fig. 65. Late prophase of first spermatocyte, showing character of tetrads.

Fig. 66. Anaphase of first maturation division from smear preparation, showing separation of macrochromosome dyads.

Fig. 67. Ring tetrad in prophase of first spermatocyte; from smear preparation.

Fig. 68. First maturation division showing separation of macrochromosome dyads.

Fig. 69. Anaphase of first maturation division showing separation of the dumb-bell shaped tetrads.

Fig. 70. Late anaphase of first maturation division, showing lagging sex-chromosome.

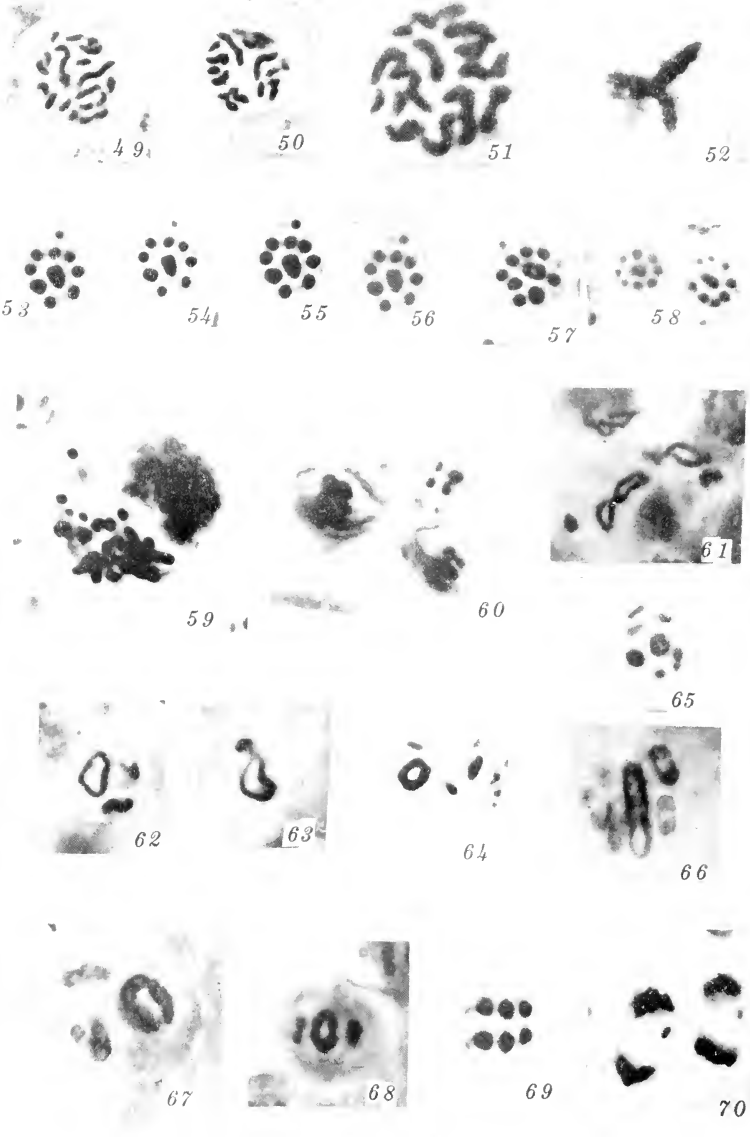
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PLATE VIII. (FIGS. 71 TO 81).

FIGS. 71, 72. Portions of the nurse chambers of the ovaries, showing fibrous appearance of central plasmatic mass in which the egg-strings of the oöcytes end. Nurse-cells in various stages of ingestion may be seen and the products of their disintegration may be seen passing down the egg-strings into the oöcytes. The oöcytes are distinguishable by their deeply staining perinuclear zone of mitochondria.

Figs. 73 to 75 are various stages of synapsis in oöcyte.

FIG. 73. Protobroque nucleus at beginning of synaptic period. Two chromatic nucleoli are present.

FIG. 74. Deutobroque nucleus of oöcyte. Treads become more evident; two chromatic nucleoli are present.

FIG. 75. Leptotene stage. Polarization of the threads. Chromatic nucleoli are absent.

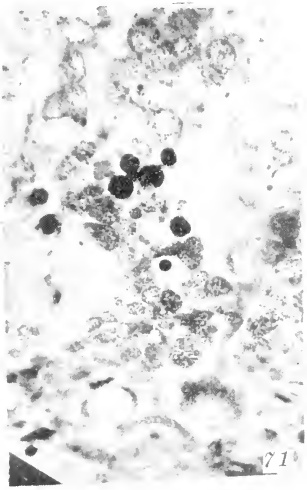
FIG. 76. Pachytene bouquet stage.

FIG. 77. Section across bouquet stage, showing 20 threads on end view.

FIG. 78. Strepsistene stage. Reappearance of the two chromatin nucleoli.

FIG. 80. Oöcyte from ovaries fixed in Bouin's fluid (10 hours) showing effect of acetic acid on mitochondria. Vacuoles, globules and nucleolar-like structures are found in cytoplasm due to the partial dissolution and agglutination of the mitochondria.

FIG. 81. Oöcyte after fixation in Bouin's fluid for six hours, showing perinuclear zone of mitochondria only partially destroyed. Two chromatin nucleoli are present in the nucleus.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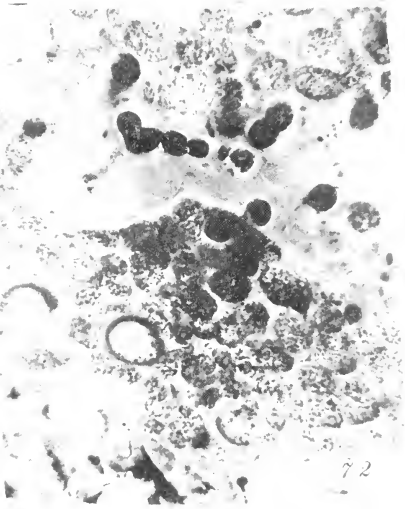
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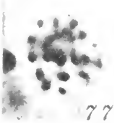
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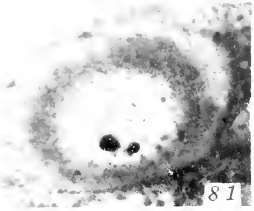
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PLATE IX. (FIGS. 82 TO 87).

FIG. 82. Oöcyte with its egg-string, from ovaries fixed in Bouin's fluid 15 hours showing the complete disappearance of mitochondria.

FIGS. 83, 84. Oöcytes from Flemming fixed material showing characteristic arrangement of mitochondria in a perinuclear zone sharply delimited from the rest of the cytoplasm.

FIG. 85. Oöcyte at the period when the cytoplasmic volume has reached its maximum. The mitochondria still surround the nucleus, but the zone is not so sharply delimited. Shrinkage of the nucleus away from the cytoplasm shows the nuclear membrane to be intact.

FIG. 86. Older oöcyte in which perinuclear arrangement of mitochondria is lost, the granules becoming scattered toward the periphery of the cytoplasm.

FIG. 87. Typical germinal vesicle of almost mature oöcyte, showing two chromatic nucleoli.

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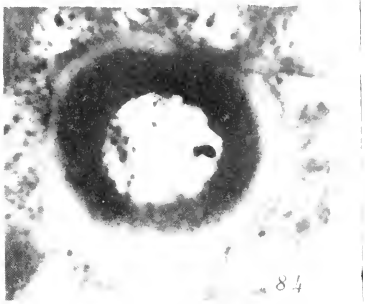

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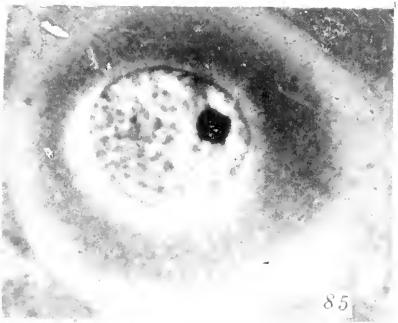

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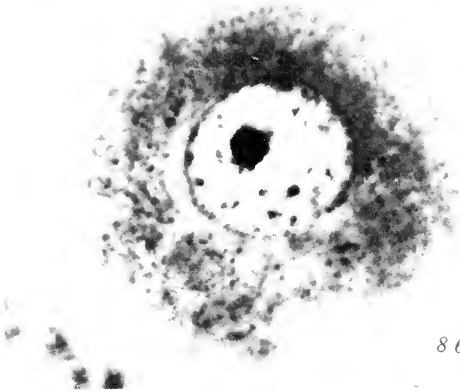

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