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REACTIONS OF AMEBA TO LIGHT AND THE EFFECT OF LIGHT ON FEEDING.

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INTRODUCTION.

The reactions of ameba-like organisms to light have been studied by a number of investigators in order to determine whether "naked" protoplasm is capable of responding to light waves. Whenever intense light was used as a source, amebas, pelomyxas and plasmodia reacted negatively, and as a consequence it was tacitly assumed that in general naked, "undifferentiated," protoplasm reacted negatively to light.

But speaking now only of experiments performed on amebas, for I do not wish to leave the impression that in my opinion what is true of the behavior of amebas toward light holds also for plasmodia and pelomyxas, the first to be recorded appear to be those of Verworn ('89). He projected white light and various spectral colors of various intensities perpendicularly through the microscope slide, and observed no change of reaction as the ameba moved from one intensity to the other, or from one color to the other. With the experiment similarly staged, Davenport ('97) came to the same conclusions as Verworn. Amebas moved from a field of very weak light into one of very strong light, apparently without change of behavior, even when the change of intensity was sharp and abrupt. But Davenport showed that when a light beam is projected horizontally against an ameba, the ameba orients so as to flow away from the source of light. Harrington and Leaming ('00) showed that intense white, violet, or blue light, flashed on a moving ameba, arrested its movement momentarily; but red light was without any decisive effect. Mast ('10), experimenting under conditions similar to those under which Verworn and Davenport worked, confirmed Davenport's findings when horizontal beams of light are thrown against an ameba, and also concluded from his experiments that when an ameba finds a perpendicular beam of intense light in its path, it avoids the light in many cases. Mast also confirmed the general conclusions of Harrington and Leaming.

None of these investigators observed any but negative behavior, though Mast presumably looked for positive responses, for he says "I was unable to obtain positive reactions in *Stentor cæruleus*, *Amœba*, and fly larvæ" ('11, p. 270). The reason Mast failed to get positive responses was because the beams of light which he used were too large and the light was too intense. And further his apparatus was perhaps defective. He says: "The beam of light was produced by focusing a limited area of a luminous Welsbach mantle on the slide by means of *the mirror* and an Abbe condenser" ('11, p. 78). If he used an ordinary microscope mirror, as he seems to have, a very faint subsidiary beam as well as the very intense primary beam, was projected through the slide. The one is produced, of course, by the front surface of the glass, and is very readily overlooked; the other is produced by the surface of the silvering. Since the subsidiary image shows only on one or two sides of the main image, if square, the chances are about even that the ameba came into contact with the subsidiary image first, and therefore the likelihood of a positive response is increased. From my own experience I have found that whenever mirrors are necessary, it is absolutely essential that they are silvered on the front surface; otherwise subsidiary images will result, and in case monochromatic spectral light is used a considerable degree of impurity may occur. If Mast used back surface mirrors in his work on the light reactions of ameba, some of his experimental results are, therefore, incompletely described and consequently inconclusive; and his inability to observe positive responses was due, in part, to improper staging of the experiment for this purpose.

The great majority of the light experiments in this paper show positive reactions; a few are indifferent, and a few negative. This proportion resulted from the manner in which the experiments were set, and from the fact that observations were based upon the behavior of amebas *before* they came into contact with the beam of light as seen by the eye, instead of after, as previous observers did. But in a great many cases negative behavior did not result even after the ameba came into contact with the light; but if the beams had been larger it is not improbable that the proportion of negative reactions would have been larger.

These experiments on the reactions of ameba to light were performed not only for the purpose of testing the sensitiveness of these organisms to light by itself, but especially to see whether differences in intensity, quality or direction of light rays are capable of causing changes in the behavior of amebas while feeding. In order to let the results speak as definitely as possible, a number of experiments were performed first with beams of light only as stimuli. Later, particles of food were presented in connection with the beams of light. By comparing these two sets of experiments with each other and with the results of previous experiments on the feeding habits of ameba (see Bibliography for references) the effect of light on feeding may be readily observed.

Several investigators, as already pointed out, made observations on the reactions of ameba to light, but all of them made use of large areas of very intense light. But for purposes of comparison with other sources of stimuli it was thought essential, in my own work, to reduce the areas of light to a size comparable with the size of food and other objects which were placed at the disposal of the ameba. By throwing the beams of light vertically through the slide on which the amebas were placed, the area of the cross section of the beam of light used for stimulating the ameba could be varied within the desired limits.

As sources of light the Welsbach gas mantle and the Leitz Lilliput arc were used. The gas light was passed through five cm. of distilled water containing a very small quantity of an ammoniacal solution of copper sulphate to absorb the excess of green and yellow rays and the distinctive heat rays. Between the water and the microscope was interposed an opaque screen with several small, clear-cut pinholes in it. By moving the screen away from or toward the microscope, and focusing with the substage condenser, the size of the projected beams of light through the microscope could be easily controlled. The ordinary mirror of the microscope was discarded and a front surface mirror placed in its stead, in order to avoid reflection from the front surface of the glass and so producing a subsidiary image on the slide. The great sensitiveness of ameba to light makes this precaution absolutely necessary.

When spectral light was used, the arc was employed as a source, and either prism or grating interposed between clear distilled water and the screen.

The amebas were placed on large, clear thin cover glasses in clear culture fluid, without a cover glass over them. Usually in these light experiments the beams of light were left stationary while the coverglass containing the ameba was shifted, whenever shifting was necessary for experimental purposes. Both intermittent and continuous beams of light were employed. Intermittent light was produced by moving an opaque object up and down between the screen and the microscope. In a general way, continuous and intermittent light had about the same effect on the ameba. After orientation, it is worth noticing, the ameba advanced as definitely and as uniformly toward intermittent as toward continuous light.

The work was done in a dark room in which there was very little diffuse light. Some light was necessary in order to make the camera lucida drawings illustrating the experiments. But this light was confined, as far as possible, to the paper on which the drawings were made, and no more light was used than was necessary. Extra precautions were taken to prevent any but vertical beams from reaching the ameba.

Experiments with Light.

White Light.—Two beams of white gas light were projected in front and to the right of an *Amœba dubia* flowing along in spatulate form—Fig. 1. The ameba flowed straight past the small spot of light and also passed the larger beam a short distance, when two side pseudopods were thrown out directly toward the larger beam. The pseudopod nearer the light spot enlarged until it had flowed over the light, then it was arrested and the other pseudopod became the main one through which the ameba moved away. The slide was then moved so that the small beam lay in front of the ameba—Fig. 5. Two pseudopods were formed on the left side. The main pseudopod moved into contact with the small beam of light, then sent out on the left a pseudopod which moved directly into contact with the larger beam. At the same time the more posterior of the previously formed pseudopods sent out on its right a pseudopod which also moved into contact with the larger beam while the more anterior pseudopod was withdrawn, but this pseudopod was finally withdrawn as the one which previously moved into contact with the light, moved on over the light spot—8. These experiments show very clearly that small beams of white light attract amebas before they actually come into contact with the beams.

An *Amœba proteus*[1] was placed so that a small beam of white light lay to the right of its path—10. Two pseudopods were thrown out, one on the left and one on the right, but both were quickly retracted. The tip of the ameba then turned sharply to the right and directly toward the light—14. The ameba then moved forward in the original direction for a short distance

[1] The name *Amœba proteus* of Pallas and Leidy as used in this paper also includes the species *A. discoides* Schaeffer, discovered to be distinct from *A. proteus* after the experimental work recorded in this paper was done (for preliminary descriptions see Schaeffer, '16*b*).

—16. Finally a pseudopod was thrown out on the right partly encircling the light—19. After breaking up into a number of pseudopods the ameba moved away. The ameba was shifted with the beam of light on the left—22. Movement became somewhat uncertain at first, then streaming was reversed—25. As the ameba moved forward in the new direction, it gradually turned to the right until it was flowing directly toward the beam of light—29. After partly surrounding the beam—35—the ameba moved away.

Summary.—From the foregoing experiments it is clear that ameba responds positively to white light under the given conditions. The ameba is attracted from a distance of sixty-five microns or more. Since the beam is projected vertically, the question at once arises: How does the ameba become aware of the beam of light? Is some light reflected horizontally by particles of solid matter in the water; or is some of the light energy transformed into heat or other form of energy which, being radially propagated, stimulates the ameba? These are very important questions in sense perception, but they must remain unanswered for the present. The light apparatus at my disposal was too crude to attempt a solution of them.

In most cases the ameba reacts positively until it comes into contact with the beam of light, when negative behavior usually sets in. This difference in behavior may be due to differences in the intensity of the stimulus. Both *proteus* and *dubia* react positively to white light.

It is quite clear from these experiments that light of the intensity used does not tend to inhibit directly the formation of pseudopods, nor does it seem to have any other direct effect on the movement or form of ameba.

Red Light.—The apparatus for producing monochromatic light of the various wave-lengths was a Leitz Lilliput arc light, fifteen cm. of distilled water, a piece of grating with a slit two millimeters wide, a screen of heavy drawing paper with a pinhole about one half mm. in diameter with clear-cut blackened sides, front surface mirror and condenser. The screen was blackened on the far side and lighted on the near side with just enough light from a Welsbach mantle to barely see the amebas. The spectrum on the screen was about two cm. long. By means of the pinhole therefore only about one fortieth of its length was projected at any one time. The apparatus was rather crude and somewhat unsatisfactory, but it seemed to be sufficiently reliable for exploratory work.

On account of the impracticability of testing spectroscopically the beams of light which were thrown into the microscope, no accurate values in wave lengths can be given for blue, red, yellow, etc., so there may have been, for example, a few "rays" of orange or green in the yellow when that color is specified; but such mixtures cannot have been very significant since the size of the pencil of light was very small, roughly one fortieth of the length of the visible spectrum. As a matter of fact some rays of all wavelengths were mixed with the pencil of monochromatic light, but this defect could not be remedied, for some diffuse light (though this of course might be monochromatic spectral light) is necessary in order to see what is going on. But if any change in behavior takes place with reference to a pencil of monochromatic spectral light, the change must be caused by the more intense monochomatic light, since the diffused white light is equally intense all over the field.

A small beam of red spectral light was projected in the path of a *proteus*—37. The ameba moved forward a short distance, then sent out a pseudopod on the left—40. From this pseudopod another was sent out on the right—45—which moved directly toward and over the red beam. The pseudopod was soon withdrawn and the ameba then moved on.

Another *proteus* was brought into view with the light on the right—49. The ameba turned sharply to the right, then to the left, and finally moved over the red light. Presently a pseudopod was thrown out on the right through which the ameba moved away.

In the next experiment the light was placed on the left of a *proteus*—53. The ameba moved forward for a short distance, then threw out a pseudopod on the left—56—through which the ameba moved off without coming into contact with the light. The ameba was then shifted with the red beam lying on the left —59. As the ameba flowed forward toward the light spot a pseudopod was thrown out on the right, through which the ameba finally moved off. The ameba was then shifted so that the light lay on the right—63. The ameba turned to the left and moved on. The ameba was again shifted with the light lying on the left—65. As the ameba moved forward a small pseudopod which was thrown out on the left moved directly into contact with the light. The next four experiments—69–93—all show that the beam of light was sensed before the ameba came into contact with it, and also that the light produced more or less definite positive behavior, followed by indifferent or negative behavior.

A beam of red light was projected in the path of another *proteus*—94. A pseudopod was thrown out toward the light. After it came into contact with the light—97—the ameba moved away through a pseudopod thrown out on the left—99. The ameba was then shifted with the beam of light on the left—101. The ameba moved forward, then turned slightly to the right—104. A small pseudopod was then sent out toward the red light—107—but after covering half the distance it was retracted while a pseudopod was thrown out on the same side, but further forward. From this pseudopod still another was sent out on the left. The ameba thus partly encircled the red light. The ameba was shifted again with the red light lying in front of it—111. The ameba threw out a pseudopod on the right and passed on—111–113—but when the tip of the main pseudopod extended beyond the light, it broke up into four pseudopods—115—of which the left posterior ultimately became the main pseudopod through which the ameba moved away.

Another *proteus* was then brought into the field with a beam of red light lying to the right—120. The ameba moved on a short distance, then threw out two pseudopods on the right—122—one of which moved into contact directly with the red light—125—but which was retracted as the ameba moved on. The ameba was then shifted with the light lying on the left—125. Pseudopods were sent out on both sides but the one on the right finally became the main one leading the ameba away. The ameba was again shifted with the red light lying on the right—128. A pseudopod was thrown out on the right directly toward the light. When it came into contact with the light it forked, the limbs moving forward with the light spot between them —133. The right limb became the main pseudopod through which the ameba moved off.

A beam of red light was projected to the right of another *proteus*—167. The tip of the ameba turned slightly toward the left, then resumed its original direction. The ameba was then shifted with the red light on the left—170. There was at first a tendency for pseudopods to form on the right, but, as the ameba moved forward, two were formed on the left in the region of the light. One of them moved a considerable distance toward the light—174—but was then retracted as a pseudopod on the right was thrown out to become eventually the main pseudopod. The ameba was then shifted with the red light lying on the left—176. As the ameba moved forward a large pseudopod was thrown out on the left, directly toward the light. This pseudopod became the main one through which the ameba moved away.

Summary.—Red spectral light produces about the same changes in behavior as white light. The vertical beam of red light is sensed at a distance, and in almost all cases produces positive behavior. In some few cases an ameba may behave indifferently or even negatively; but if the experiments are repeated several times a positive reaction is almost sure to occur. Amebas are therefore not negative or positive permanently with respect to beams of red light, but the behavior may readily change from the one to the other aspect. Red light of the intensity used does not seem to stimulate the ameba disagreeably when it moves into direct contact with the beam of light, for in a number of cases the ameba moved on over the light without visible change of behavior. In some respects the ameba tended to encircle the source of light in the same manner in which it sometimes encircles solid objects.

Blue Light.—A beam of impure[1] blue spectral light was projected to the right of a *proteus*—135. The ameba turned to the

[1] Between the grating and the arc was placed twenty cm. of distilled water containing a very little ammoniacal copper sulphate. The copper salt gave rise to a subsidiary faint yellowish spot of light not quite coinciding with the blue. The yellow image disappeared when the copper salt was omitted from the distilled water. The yellow image was probably due to fluorescence of the copper sulphate.

left and moved away, a decided negative reaction. The light was shifted so that it lay directly ahead of the ameba—139. The ameba threw out three pseudopods on the left, but almost immediately retracted them. Another was sent out on the right, but it also was soon retracted. The ameba then moved forward, and in passing the blue light sent out a little pseudopod toward it—143. The ameba finally turned to the left and moved on. The ameba was shifted again with the blue light on the right—145. After the tip of the ameba had passed by the blue light it turned to the right. A small pseudopod was also sent out toward the light—148. The ameba was then shifted with the blue light lying directly in front—150. As the ameba moved forward a small pseudopod was thrown out into contact with the light—152. Then the ameba moved on. The ameba was shifted again with the blue light directly ahead—154—but a decided negative reaction set in. But when shifted again with the blue light ahead—158—the tip of the ameba turned away from, and then toward the light, but finally moved on in the original direction. The ameba was shifted again with the blue light directly ahead—162. The resulting behavior was indefinite.

Another *proteus* was then brought into the field with a beam of blue light directly ahead—181. The ameba then threw out a pseudopod on the left and from this one another on the right, and from this last one still another on the right, so that the light was partially encircled. The ameba then moved off through a pseudopod on the left. The ameba was shifted with the blue light on the right—186. A pseudopod was thrown out on the right directly toward, and into contact with, the beam of light. The ameba then flowed away through another pseudopod on the right. The ameba was again shifted with the light lying on the right—190. Two pseudopods appeared on the right, one of which was directed toward the light spot. Both pseudopods were presently retracted and the ameba moved on to the left. Very peculiar behavior was observed when the ameba was shifted again—194. The behavior was at first negative, the ameba moving away to the right—195—but streaming was then reversed, and as the ameba passed by the light, two small pseudopods were sent out toward it—200. They were retracted however as the ameba moved on. When shifted again with the blue light to the left—201—the ameba turned toward the light and then passed on to the left. The ameba again reacted positively when shifted with the light straight ahead—204. When shifted again the ameba reacted positively but rather uncertainly—209. In the next experiment, with the beam of blue light on the left, negative behavior was induced—216.

A beam of pure (see footnote p. 11) blue spectral light was projected to the right of a *dubia*—219. The ameba moved past the light spot for a considerable distance without any change in behavior. Then two pseudopods were sent out: one directly toward the light, and the other near the tip, but also on the right side—220. As the pseudopods enlarged, the tip of the ameba also turned sharply to the right—221. When the posterior pseudopod came into contact with the light, the pseudopods on the right were retracted, and two others thrown out on the left —222—but these also were retracted after a few seconds, and the ameba then moved on in the original direction. The ameba was then shifted with the blue light lying directly ahead—223. The tip of the ameba (only the tip of the ameba is shown) turned to the left—224—but a pseudopod was thrown out on the right toward and into contact with the light—225. The ameba flowed partly over the light—226—but withdrew from it later and moved off through a pseudopod on the right.

Summary.—There is no marked difference between the reactions toward red light and those toward blue. Blue light induces positive behavior in as marked a degree as red, though when all the experiments are considered, red light seems to be somewhat more attractive than blue. Blue light, like red and white, induces both negative and positive reactions. Blue light can also be sensed at a distance.

The experiments with the *dubia*—219–222—are interesting inasmuch as a pseudopod was thrown out at the tip of the ameba on the side on which the light lay, some time after this part of the ameba had passed the light. It may be noted also that the tip of the ameba turned strongly in the same direction. It appears quite unlikely that the light acted as an efficient cause on this region of the ameba at the time of the formation of the pseudopod, for the effect of the light was continually decreasing in intensity as the ameba moved away from it. The light would therefore be expected to have the maximum effect at maximum intensity, which was when the tip of the ameba was closest to the light. It is improbable therefore that the throwing out of a pseudopod and the bending of the tip to the right were caused by the impinging of the light rays at that region at that time. It is possible that this behavior is the result of the cumulative effect of the light rays while the ameba was passing the beam. There was formed a tendency toward a positive reaction some few seconds before it expressed itself in visible change of behavior, and when this tendency "came to a head" it resulted in exaggerated behavior; for two pseudopods were thrown out on the stimulated side and at the same time the tip of the ameba was turned to the right. This feature of ameban behavior—the formation of two pseudopods on the stimulated side, one near the anterior end and the other opposite the stimulating object after the tip of the ameba has passed the stimulating object—is frequently observed and is of great interest. It indicates several things. First, it effectively disposes of the hypothesis that the movement of pseudopods toward an object is directly induced by the object. Second, it shows that there is some sort of a coördinating or integrating agency at work in the ameba so that the larger part of it, at least, tends to react in a coördinated manner, even if there are separate centers of reaction. When the posterior pseudopod came into contact with the light, negative behavior set in suddenly. The pseudopods on the right were promptly withdrawn and two others were rapidly projected on the left. Nevertheless, the ameba finally moved on in the original direction.

Violet Light.—The violet light that was selected was as near to the end of the visible spectrum as possible. A beam of violet light was projected on the right of a *proteus*—230. A small pseudopod was thrown out on either side—232—the one on the right being directed toward the light. The ameba moved away however through the pseudopod on the left. The ameba was shifted with the light again on the right—235. The tip of the main pseudopod turned to the right and moved into contact with and then on over the light. The ameba was shifted with the violet light on the left—241. As the ameba moved forward past the light a small pseudopod was sent out toward the light, but it was withdrawn before it came into contact with the light.

When shifted again with the light on the right—245—the ameba sent out a pseudopod anterior to the light—249—but it curved backwards toward the light as the ameba moved forward—251. On the next trial—253—the ameba first turned away from the light then sent out a pseudopod directly into contact with it; then another pseudopod was sent out on the side and anterior to this one.

Summary.—Amebas react positively, negatively, or indifferently toward violet light. The greater number of changes of behavior produced by violet light were positive. No definite differences could be observed between the effects of violet light and those of any other spectral light thus far described.

Green Light.—A beam of green spectral light was projected to the right of an *Amœba dubia*—259. As the ameba moved forward the tip of the main pseudopod moved to the left. A small pseudopod was formed on the right toward the light. The ameba then turned toward the right and at the same time threw out a pseudopod on the right near the tip of the main pseudopod. Both pseudopods were withdrawn as the ameba moved on. (Compare the behavior of this ameba with that illustrated in Figs. 219–222.)

The beam of green light was then projected to the right of a *proteus*—262–265. As the ameba moved forward, a large pseudopod which was thrown out on the left, was soon retracted, the ameba moving on in a straight path. The ameba was shifted—266–270—with the green light to the right. As the ameba moved on past the light, a small pseudopod appeared on the right near the light, but it was retracted before it had developed to any extent, as the ameba flowed on.

These few experiments indicate that the effect of green spectral light is similar in a general way to that of white, red, blue, etc. Although the positive reactions in these experiments are slight, they are nevertheless definitely positive. If I had made as many experiments with green light as with red or blue, I have no doubt that more decided reactions would have been obtained.

Yellow Light.—A beam of yellow spectral light was projected straight ahead of a *proteus*—271. The ameba moved on without any definite change of behavior and passed over the beam of light. The change in the direction of movement—275–277—indicates that the yellow beam had a disagreeable effect after the ameba came into contact with it. When the ameba was shifted—278—the tip of the main pseudopod turned to the left—a negative reaction continued from the previous experiment. But while passing the beam of light the negative condition gave way to a positive as is shown by the turning of the tip of the ameba toward the light—282. A pseudopod was then thrown out on the left on the convex side, and from this one another on the left through which the ameba moved on, again a negative reaction. The ameba was shifted again—285—with the yellow light ahead. The ameba turned sharply to the right, but as it passed by the light a pseudopod was thrown out on the left directly toward the light—287. This pseudopod became the main one through which the ameba flowed on over the light. As the ameba came nearly into contact with the light, a pseudopod was thrown out on the right—290—an indication of a negative reaction, but it was soon retracted.

To summarize: Amebas respond positively, negatively or indifferently to beams of yellow spectral light. As far as my experiments go, yellow light has about the same effect as red or blue or the other spectral colors which have so far been considered.

Orange Light.—A beam of orange light was projected to the left of a *proteus*—294. The ameba turned to the left and moved directly into contact with the light. When the ameba came into contact with the light, a pseudopod was started on the right, but it was soon retracted and the ameba flowed on over the light without further change of behavior. The ameba was then shifted with the orange light on the left—301. The tip of the ameba turned to the left, then broke up into two pseudopods of which the left one turned still further to the left and finally became the main pseudopod through which the ameba flowed away. The ameba was shifted again with the orange light slightly to the right—306. A pseudopod which was thrown out on the right elongated as it turned to the left. When the tip of the pseudopod had passed the light, a new pseudopod was thrown out on the left near the light—309. This pseudopod moved straight forward for some distance, when another pseudopod was sent out on the left—312—but this one was finally retracted as the ameba moved away.

Orange spectral light induced positive reactions in the ameba of this series of experiments, though they were wholly positive only in the first experiment. In the other experiments the tendency was toward positive behavior, but the source of stimulation was not definitely sought. The beam of light attracted the ameba only mildly after the first encounter, and the tendency to move forward (Schaeffer, '14*a*) may be presumed to have been about as strong as the tendency to move toward the beam, hence the partial encircling of the beam in the last two experiments. In my laboratory notes there is recorded one experiment with orange light in which the behavior was wholly negative.

Experiments with Dark Beams.

When it was seen that white light and spectral light of various wave-lengths had essentially the same effect on ameba, it seemed likely that these results were due to differences in intensity between the beam of light and the diffuse light on the field. The suggestion then presented itself whether a decrease in intensity of light in a small area produces a similar result. A dark beam was therefore projected into the microscope. The source of the dark beam was a hole in the screen, leading into a blackened light tight box fastened to the back of the screen. The sides of the hole were blackened to prevent as far as possible the reflection of light. The rest of the screen was illuminated by diffuse light, as in the other experiments, but more brightly so as to increase the contrast between the field and the hole. The hole as viewed through the microscope appeared as a very dark gray spot.

A *proteus* was shifted so that the dark spot lay directly ahead of the ameba—314. The tip of the ameba broke up into two pseudopods, one of which turned to the right and the other to the left of the dark spot, indicating a negative reaction. As the right pseudopod moved forward it turned to the left until it came into contact with the dark spot—a positive reaction. The ameba then moved on through this pseudopod and partly over the dark spot. The ameba was again shifted so that the dark beam lay directly ahead—319. A pseudopod which was thrown out toward the right led the ameba away, a definite negative reaction.

Another *proteus* was then brought into the field with the dark spot directly ahead—322. The ameba turned to the right and moved on, avoiding the dark beam. The ameba was then shifted with the dark spot straight ahead—325. The ameba became irregular in its streaming at the anterior end, indicating that the ameba sensed the dark spot and that there was present a tendency to react negatively; but the tendency to negative reaction was weak, for the ameba started presently to move over the dark area. The ameba, in irregular shape, was shifted again with the dark spot directly ahead—329. When the ameba came into contact with the outer edge, the tip of the main pseudopod forked, the right prong becoming the main pseudopod through which the ameba moved away. Negative behavior is again shown here. The ameba was shifted with the dark spot directly ahead—333. A pseudopod was thrown out on the left as the ameba moved into contact with the dark spot—334—indicating a tendency to negative reaction, but it was withdrawn as the tip of the ameba proceeded for some distance beyond the further edge of the dark area—336. The ameba was moved again so that the dark spot lay slightly to the right—340. The ameba moved into contact with the dark area, then sent out a pseudopod on the left, but it was soon withdrawn and at the same time another was sent out on the right. The ameba finally moved on in the original direction. Here we have first positive behavior in the turning of the ameba toward the dark spot; then negative behavior in the formation of the pseudopod on the left; then again positive behavior in the resumption of forward movement and the formation of the pseudopod on the right. In the next trial the ameba was moved with the dark spot slightly to the right—346. The ameba turned slightly further to the left, then directly toward the right and toward the dark spot—348. When the ameba came into contact with the dark spot, a pseudopod was thrown out on the left, but it was withdrawn as the ameba moved on.

Another *proteus* was then brought into the field with the dark spot slightly to the left—354. The tip of the main pseudopod broke up into two pseudopods of which one moved directly toward and over the dark area. But when the ameba came into contact with the dark spot, a large pseudopod was thrown out on the right—356—but it was withdrawn as the ameba moved on over the dark spot. The ameba reacted in effect positively throughout the experiment, but a strong tendency to react negatively is shown by the breaking up of the main pseudopod into two pseudopods—355—and by the appearance of the pseudopod on the right when the ameba came into contact with the dark spot —358. The ameba was then shifted with the dark spot slightly to the left—359. The ameba moved forward a short distance, then the tip of the main pseudopod spread out, and then the protoplasmic stream was suddenly reversed and the ameba moved away to the right through a vestige of a previous pseudopod—a decided negative reaction. But the ameba was then moved with the dark spot directly ahead—363. After the ameba had moved forward a short distance the tip forked broadly, and the ameba moved off through the left prong, again a decided negative reaction.

Summary.—Amebas become aware of dark spots before they come into contact with them, as seen through the microscope with the eye, just as they become aware of beams of light before encountering them. In most cases the tendency is to react negatively, but in some instances the first change in behavior is positive. Usually when the ameba first comes into contact with the dark beam there is a tendency toward negative behavior, as is shown by the formation of pseudopods which, if they became main pseudopods, would lead away from the dark area. These pseudopods are usually withdrawn as the ameba moves forward over the dark spot. The behavior is seldom wholly positive or wholly negative; in most cases there is some vacillation between negative and positive reactions. The reactions on the whole were not so pronounced as those toward light. The actual stimulating quality is very likely to be looked for in the difference in light intensity between the dark spot and the field.

Reactions toward Solid Particles when Stimulated at the Same Time by Beams of Light or of Darkness.

A grain of globulin was placed over a small beam of blue spectral light, and arranged so that the illuminated globulin lay in the path of an *Amœba proteus*—365. The ameba moved in spatulate form directly toward the globulin-blue light until it came into contact with the globulin, when a pseudopod appeared on the right. The ameba however ingested the globulin in typical manner and then quieted down over the blue light for over twelve minutes.

A grain of globulin was placed in the path of a *proteus* with a beam of green spectral light between the ameba and the globulin —373. Through a pseudopod thrown out on the right the ameba moved away from the light-globulin—374. The ameba then broke up into four pseudopods of which the left one of the middle pair became the main pseudopod. The ameba moved forward through this pseudopod toward the globulin in a curved path, apparently, so as to avoid the light, pushed the globulin ahead a short distance, and then ingested it in an imperfect food cup. This is an interesting experiment. The beam of green light stimulated the ameba negatively when contrasted with the globulin. The ameba made a detour around the light to get to the globulin. This experiment should be compared with Figs. 1–13 in a recent paper (Schaeffer, '17*b*) in which very similar behavior is recorded as an ameba moved toward a grain of globulin with a grain of silicic acid lying immediately in front of the globulin.

A grain of globulin was placed to the left of a *proteus* with a beam of green spectral light between the globulin and the ameba —386. The ameba moved forward a short distance, then bifurcated, the right prong being directed backwards while the left prong was directed toward the globulin—388. The ameba moved toward the light at first—389—but presently the tip of the ameba broke up into two pseudopods. The one thrown out on the right enlarged rapidly as it moved in a slight detour around the light toward the globulin—392, 393. After rolling the globulin along the surface for a short distance it was ingested in a typical food cup. This experiment as well as the preceding, shows that a beam of green light acts as a disturbing factor when an ameba is stimulated at the same time by globulin.

A grain of globulin and a beam of yellow spectral light were placed to the right of the path of a *proteus* with the light between the globulin and the ameba—397. As the ameba moved forward it turned to the right and directly toward the yellow light. The ameba moved over the light, then turned to the left and moved into contact with the globulin, which the ameba rolled around a short distance before ingesting it in a normal food cup. The yellow light did not disturb the ameba when stimulated simultaneously by globulin.

A grain of globulin and a beam of yellow light arranged as in the preceding experiment were placed in the path of another *proteus* —408—but the behavior observed was negative, due doubtless to lack of hunger in the ameba. The ameba was in Y-shape at the beginning of the experiment—408. The ameba responded negatively by bending the right prong to the right and flowing along it. A pod was thrown out on the right, indicating the presence of a tendency to a positive reaction. The ameba was then shifted with the yellow beam straight ahead and the globulin a little to the left—412. The tip of the ameba forked, the axes of the limbs coinciding with the same straight line, and nearly perpendicular to the rest of the ameba—413. The right prong turned toward the yellow light—415—and presently two pseudopods were sent out a short distance toward the globulin—416 —but both were withdrawn as the ameba moved away through a pseudopod thrown out on the right—417. The ameba was shifted with the globulin straight ahead and the beam of yellow light to the left—419. The ameba moved forward a short distance when a pseudopod was thrown out to the left—421. This pseudopod became the main one, and after it flowed ahead some distance it turned to the right and moved toward the globulin —423. The tip of the ameba then turned to the right still more strongly and at the same time a pseudopod was thrown out in the direction of the stimulating objects—424. The posterior end now became activated but only for a short time—425. Several new pseudopods were formed indicating uncertainty in behavior, of which the one on the right extending toward the globulin became for the moment the main pseudopod—427. After moving toward the globulin for a short distance a pseudopod was thrown out on the right leading the ameba away from the test objects—429–432. Presently however the main pseudopod bent strongly to the left—433, 434—with the formation at the same time of two pseudopods on the convex side of the main pseudopod—434, 435. The pseudopod pointing toward the globulin moved forward a short distance—436—but this pseudopod was retracted as the other one of the two formed on the convex side became reactivated leading the ameba away from the globulin and light.

Since it was evident that the ameba reacted negatively to both light and globulin when these substances stimulated the ameba at the same time, two further tests were made on this ameba in which each test substance was used by itself.

The piece of globulin was laid some distance ahead of the ameba —438. The ameba moved toward the globulin a short distance then threw out a pseudopod on the left—440—through which the ameba moved forward with the globulin on the right—442. A pseudopod was then thrown out on the right toward the globulin while the tip of the main pseudopod turned strongly toward the left—444. When the pseudopod on the right came into contact with the globulin the main pseudopod was retracted —447, 448. The globulin was only partly surrounded—449. One of several pseudopods formed on the right led the ameba away, leaving the globulin behind.

After a few minutes the beam of yellow light was projected in front of this ameba—453. As the ameba moved forward several pseudopods were thrown out on either side of the main pseudopod, giving the ameba a very irregular shape. When the ameba came nearer the light—458—it advanced definitely forward passing the beam immediately to the right—459. The main pseudopod then swerved a little to the side as two pseudopods were formed on the right, through the lower one of which the ameba finally moved away from the light.

This ameba then reacted definitely positively to globulin but much less definitely positively to yellow light, when presented separately, but decidedly negatively when presented together.

The experiment shows that yellow light was the cause of the negative behavior when both it and globulin were presented together; or perhaps one ought to say yellow light, *in combination*, possessed deterring qualities which were absent when it stimulated the ameba alone. Here again we have another case of where the milder of two positively stimulating objects when encountered alone becomes negative when encountered simultaneously with the more strongly stimulating object (Schaeffer, '17*b*).

A dark spot with a piece of carbon lying in it was then placed in the path of a *proteus*—463. As the ameba moved forward it turned slightly to the left at first—467—but the tip of the ameba then turned to the right and moved directly toward the dark beam and carbon—471—until within about ten microns of the beam, when the ameba suddenly turned to the right—472. The ameba moved along in this direction without coming into contact with either the dark spot or the carbon. That this decided negative reaction was caused by the dark spot is evidenced by the next experiment in which the carbon was omitted—481. The ameba moved forward toward the dark beam for a short distance, then became very uncertain in its behavior. First it turned to the right, then formed a pseudopod on the left through which it moved forward with the dark spot on the right—484. A pseudopod was thrown out on the left but it was promptly retracted—487. At the same time the tip of the pseudopod turned very sharply to the right and moved directly toward the dark spot—488. The ameba continued moving toward the dark spot until within thirty microns of it—489—when the main pseudopod was retracted and the ameba then moved away to the left through another pseudopod which had been slowly forming while the ameba was moving toward the dark beam. A dark beam with a piece of globulin lying in its centre was placed to the right of the ameba with a pseudopod already turned toward the dark spot—492. The ameba flowed on through the pseudopod directly toward the dark spot. When within about eighty microns of it, the tip of the main pseudopod forked—495—indicating a tendency to negative behavior. The left prong which became the main pseudopod, moved directly forward until it came nearly into contact with the dark beam when a slender pseudopod moved into the dark area, and another, indicating a tendency to negative behavior, moved to the right —498. But when the ameba came into contact with the globulin the pseudopod on the right was withdrawn and then the globulin was ingested, the ameba quieting down over the dark spot. The experiments with this ameba show that the dark beam may be sensed at a distance of at least 150 microns, and that the globulin may be sensed as well apparently when lying on a dark spot as when illuminated.

A dark spot with a fragment of carbon lying on it was placed to the right of an ameba—503. There was no definite response. The ameba turned toward the right and moved toward the test objects in a more or less uncertain manner—504, 505. A pseudopod was then sent out on the left—506. After it had attained to considerable size it was withdrawn and the ameba moved off through a pseudopod thrown out on the right—508. The ameba was shifted with the carbon-dark spot lying on the right—509. As the ameba moved forward it turned toward the right—510—a small pseudopod being formed, as frequently happens, on the convex side, but a pseudopod was presently thrown out on the left—513—through which the ameba moved away. To show that it was the dark spot and not the carbon which produced the negative behavior, the results of the next experiment are appended—514. The ameba moved directly into contact with and on over the carbon without any sign of a negative reaction. A grain of globulin lying in a dark beam was then placed at some distance to the right of the ameba—520. An accidental jar displaced the globulin so that it lay near the anterior edge of the dark spot. The ameba changed its direction of motion and moved straight toward the globulin and the dark beam—521. (It may reasonably be doubted whether the ameba sensed the globulin at this distance.) The globulin was then moved to the further side of the dark beam—522. The ameba kept on moving forward for some distance, then its behavior became somewhat uncertain. The ameba moved slightly to the left—524. A pseudopod which was thrown out on the right—525—became later the main pseudopod. The ameba now moved directly toward the dark spot until within about twenty microns—528—when the protoplasmic current was reversed and the ameba moved off through a pseudopod on the left near the posterior end—529. Soon this pseudopod was withdrawn and the ameba flowed into another pseudopod (perhaps the vestige of the former main pseudopod) which led the ameba to the right (to the left of the dark spot)—533. After moving some distance in this direction, a pseudopod was thrown out on the left directly opposite to the dark spot—534. Two more pseudopods were then formed on the right through the more posterior of which the ameba moved on —535–537. A pseudopod was then formed on the left which led the ameba out of range of the dark spot—538. The ameba was then shifted with the dark spot on the right and the grain of globulin just in front of it—540. The ameba turned to the left—541—and sent out on the convex side a pseudopod through which the ameba moved forward with the dark spot on the left—543. As the ameba moved on it turned toward the dark beam but presently two pseudopods were thrown out from the middle of the ameba, one on either side—546. (The pseudopod on the left was evidently formed to enable the ameba to move at once into contact with the globulin; the one on the right was formed without assignable cause, although opposite pseudopods are frequently formed under conditions similar to these.) The pseudopod on the left moved through a curved path to the left into contact with the globulin—547–550. When the ameba came into contact with the globulin, it was pushed into the dark area. A slender pseudopod followed it while the tip of the main one remained stationary for the moment, which indicates that the dark area had a deterrent effect on the ameba—551. A food cup was however soon formed and the globulin ingested.

Conclusions.

From these experiments it may be concluded that white light and all the visible spectral colors cause positive responses; but whether all are equally attractive cannot be definitely stated, for experiments would have to be staged differently to produce accurate results. Nevertheless the red end of the spectrum seems to be somewhat more attractive than the blue.

What is of considerable interest in the behavior of ameba toward light is that the character of the response may vary rapidly. See Figs. 22 to 36; 37 to 48; and a number of other experiments. A negative reaction may be followed by a positive and vice versa. There is no definite relationship between ameba and light, on account of which the ameba is always either positive or negative or indifferent. Stimulation from light produces the same general character of reaction as stimulation from glass or carbon. The only observable difference is a quantitative one; light beams are sensed at a greater distance than particles of glass or carbon. This difference may however be due to a difference in intensity of the stimuli.

Ameba reacts to dark spots in much the same way that it does to beams of light. The reactions are either positive, negative or indifferent. But they are negative in much the greater number of cases. But no sooner does one observe the reactions of an ameba to perpendicular beams of light and of darkness than the question arises as to the transfer of the stimulus to the ameba as well as the nature of it. How can an ameba sense a beam of light or darkness which never comes nearer to it than 100 or 150 microns? It is possible that small particles suspended in the water reflect light from a beam of light so as to reach the ameba in much the same way that man can observe a beam of light in a dark room because of the dust particles in the air. But if so the ameba, being eyeless, is wonderfully sensitive to light. But as to beams of darkness the case is entirely different. Is it conceivable that an ameba can sense a beam of darkness at a distance because not as much light is reflected from the particles in the dark beam as from those more brightly illuminated surrounding the beam? If one did not know of reactions to beams of darkness, one might adopt the hypothesis of the reflection of light from particles in the beam; but since similar behavior is observed toward beams of darkness, this explanation is obviously not the right one. Some disturbance is created by the beams which is then radially transmitted; so much is certain. But just what is the nature of the disturbance is not clear.

In a preceding paper (Schaeffer, '16*c*), in which the reactions of ameba to particles of glass, carbon, and similar materials were described, it was concluded that the nature of the stimulus which enabled amebas to react to these substances at a distance also remains unrecognized. Now it is possible that the nature of the stimulus which makes reaction at a distance possible is the same for all these various test objects, since the reactions are very similar. If so, the nature of the stimulation must be simple and fundamental, such as differences in electrical potential which give rise to electrical currents. But if the nature of the stimulation should be electrical, the quantities of current arising from the various test objects must be infinitesimally small, and very great if not insurmountable difficulties would be encountered in demonstrating the presence of such small currents.

To show the general reactions of ameba to globulin, carbon, etc., when stimulated simultaneously by beams of light or of darkness, the experiments may be classified as follows.

1. Food objects (grains of globulin) were laid *over* a beam of intense light so that the food should be very brightly illuminated —365–372. Blue spectral light was used in the experiments recorded, for blue light has been regarded as more disagreeable than other spectral colors. The globulin was sensed at a distance and the ameba moved toward it and ingested it. There was no definite indication that the blue light had any effect in modifying the behavior unless the pseudopod to the right in Fig. 368 is to be regarded as expressing a deterrent effect of the light. The ameba, in effect, reacted as if no spectral blue light was present.

2. The food substance was laid *some distance from* the green or yellow light, and in various positions with respect to the ameba and the beam of light—373–437.

(*a*) When the green light lay between the ameba and the globulin, the light had a slight disturbing and deterring effect —386. The ameba made a slight detour around the green light. In another test with the experiment similarly staged, the disturbing effect of the green light was more pronounced—373. The ameba made a wide detour around the light and moved into contact with the globulin without coming into contact with the green beam. In both experiments green light, which is positive when sensed alone, became negative in contrast with the more strongly (or differently) positive globulin.

(*b*) In the experiment with yellow light—397–407—the ameba moved straight toward the light after the globulin was within sensing range, then moved over the beam of light, after which the direction of motion was changed so that the ameba moved directly toward the globulin. The globulin was eaten in a typical food cup. The yellow light was not deterrent in this case. But another ameba reacted negatively to both yellow light and globulin, when presented simultaneously, but positively when presented separately. The ameba was satiated or sick, for the globulin was only partially surrounded.

3. Grains of globulin and carbon were laid over beams of darkness.

(*a*) An ameba moved toward a dark spot on which lay a grain of carbon until it came within about thirty microns of the dark spot, when negative behavior set in. The ameba moved away to the right—463. In the succeeding test the ameba reacted at first positively to the dark spot alone, and after that decidedly negatively.

(*b*) A piece of globulin was laid on the dark spot, to the right of the ameba. The ameba moved directly toward the dark spot —globulin—though it seemed to have been slightly deterred by the dark area, for the ameba broke up into two pseudopods—495 —and just when the dark beam was reached a little later, a small side pseudopod appeared. The globulin was however finally ingested. In another experiment the globulin was placed near the far edge of the dark spot—522. The behavior of the ameba became very irregular as it moved near the dark beam. Soon a pseudopod was sent out straight toward the globulin, but it was presently retracted and the ameba moved off to the left, veering to the right. There can be no doubt of the strongly deterrent effect of the dark beams. There can also be no doubt of the strongly attractive effect of the globulin.

Summary.

1. Ameba senses beams of light of twenty microns' diameter that pass no nearer to the ameba than 100 microns or 150 microns. In nearly all cases under these conditions the ameba moves directly toward the beam. When the ameba comes into contact

with the beam it either flows over it indifferently, or it reacts negatively to the beam by changing its direction of movement.

2. Beams of spectral light and of white light have approximately the same general effect. It appears however that spectral light at the blue end is somewhat less attractive than that at the red end.

3. Beams of darkness are also sensed at a distance like beams of light. They are usually negative. That is, the ameba usually avoids the beams before coming very near them.

4. It is the change of light intensity that determines changes in reactions. Neither high nor low intensities seem to be either negative or positive in themselves. Movement from a region of low light intensity into a region of high intensity frequently occurs if the contrast is not too great; but movement toward a region of lower light intensity (dark beams) is seldom seen.

5. No explanation is suggested for the sensing of beams of light and of darkness at a distance. The nature of the stimulus and the means of its transfer in such cases is not known.

6. Grains of globulin illuminated by perpendicular beams of light seem, on the whole, to be at least as attractive as when not more brightly illuminated than the field. But when globulin grains are laid in large dark beams, the ameba frequently shows unmistakable signs of a tendency to react negatively.

7. Both light beams and globulin grains are positive when stimulating the ameba separately; but when a grain of globulin and a beam of light, placed a small distance apart from each other, stimulate the ameba simultaneously, the more weakly positive object—the beam of light—becomes usually strikingly negative.

8. An ameba is positive, negative or indifferent to beams of light depending upon circumstances.

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EXPLANATION OF PLATES.

The figures are camera lucida drawings of sample experiments taken from the laboratory notes without alterations. The camera lucida was attached to the right-hand tube of a long-arm Zeiss binocular microscope, which was used in connection with the stage and condenser of a compound microscope. Eyepieces number 4 and objective a_3 were used, giving a magnification of 65 diameters. A scale by means of which the size of amebas and light beams can be estimated is shown on Plate V.

The figures are numbered serially from 1 on, for reference. The numbers are placed inside the figures. They are to be looked upon as labels only. They have no other significance. An x following a number, as 21x, indicates the end of the experiment illustrated by Figs. 10 to 21x inclusive. A new experiment starts with Fig. 22 and ends with Fig. 36xx, and so on. If a number is followed by xx, it means that the next experiment was performed upon a different ameba. Thus Figs. 1 to 9xx represent the result of a single experiment upon an ameba. With Fig. 10 a new ameba was employed, and so on. The order in which the figures were drawn is represented by the serial numbers for all the figures in any one experiment, and in nearly every case for all the experiments performed on any one ameba.

The time of the beginning and the end of each experiment is given in hours and minutes. In some cases the time of drawing of each figure is also given, and where it is not given it may easily be computed—the figures in such case being spaced equally in time.

The arrows show the direction of active protoplasmic streaming. The larger arrow in the last figure of each experiment denotes the direction the ameba took in moving away from the test object.

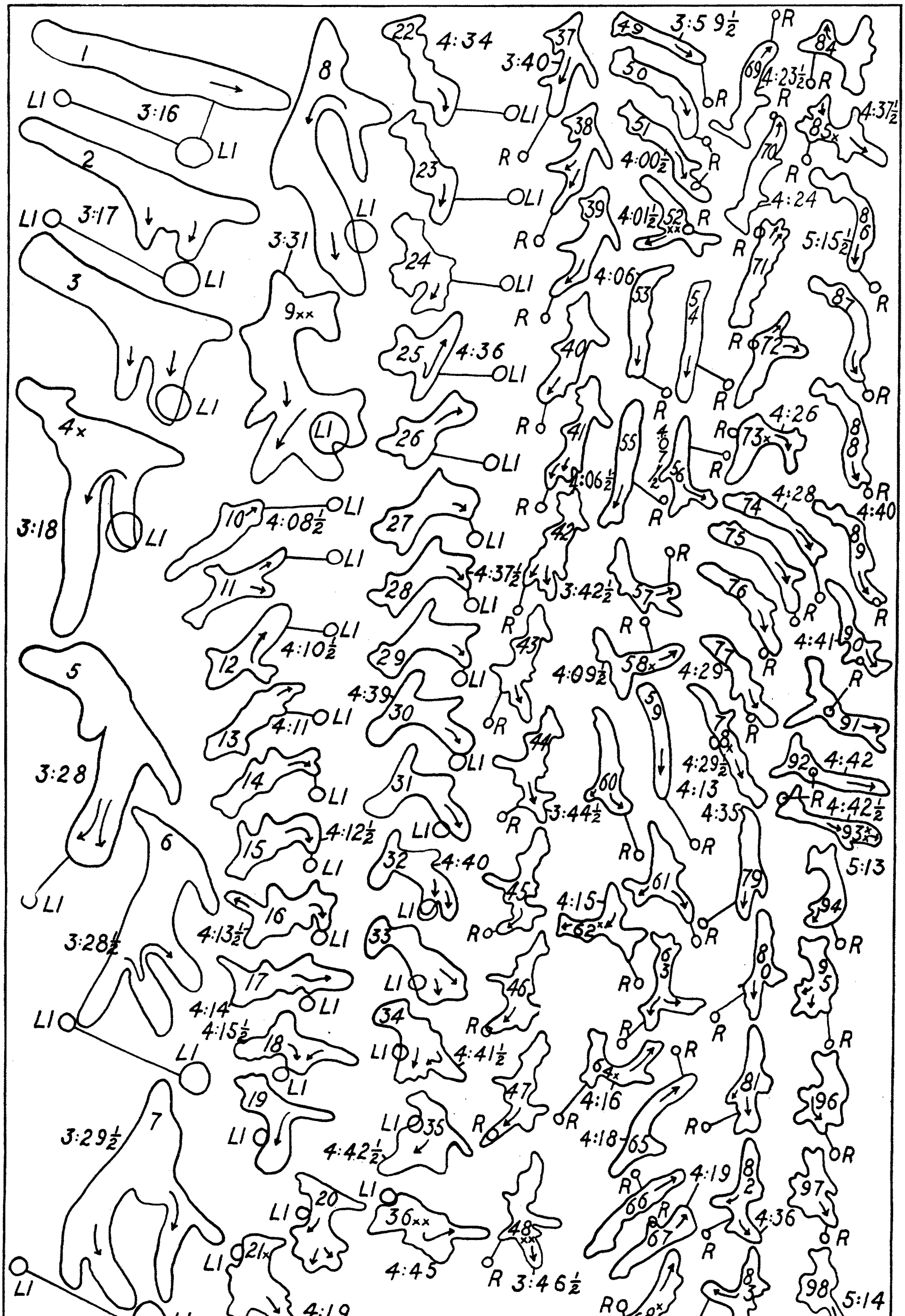
The light beams, etc., are labelled in abbreviated form. See table of abbreviations on below. For quick and correct reference these test objects are connected with the proper ameba by leader lines. These lines have no other significance.

It will be noted that there are slight differences in the size and shape of the same light beam or other test object as drawn in the figures of any single experiment. The explanation for this difference lies in the speed with which the drawings had to be made in order to catch important items of behavior. As a rule the parts of the ameba lying nearest the test object received the most careful attention and were drawn first; the posterior parts of the ameba and the test object were drawn last.

For detailed explanation of figures see pages 49–67 of the text.

Table of Abbreviations.

B, blue light.	*LI*, white light.
CA, carbon.	*OL*, orange light.
D, dark beams.	*R*, red light.
G, globulin.	*V*, violet light.
GN, green light.	*Y*, yellow light.



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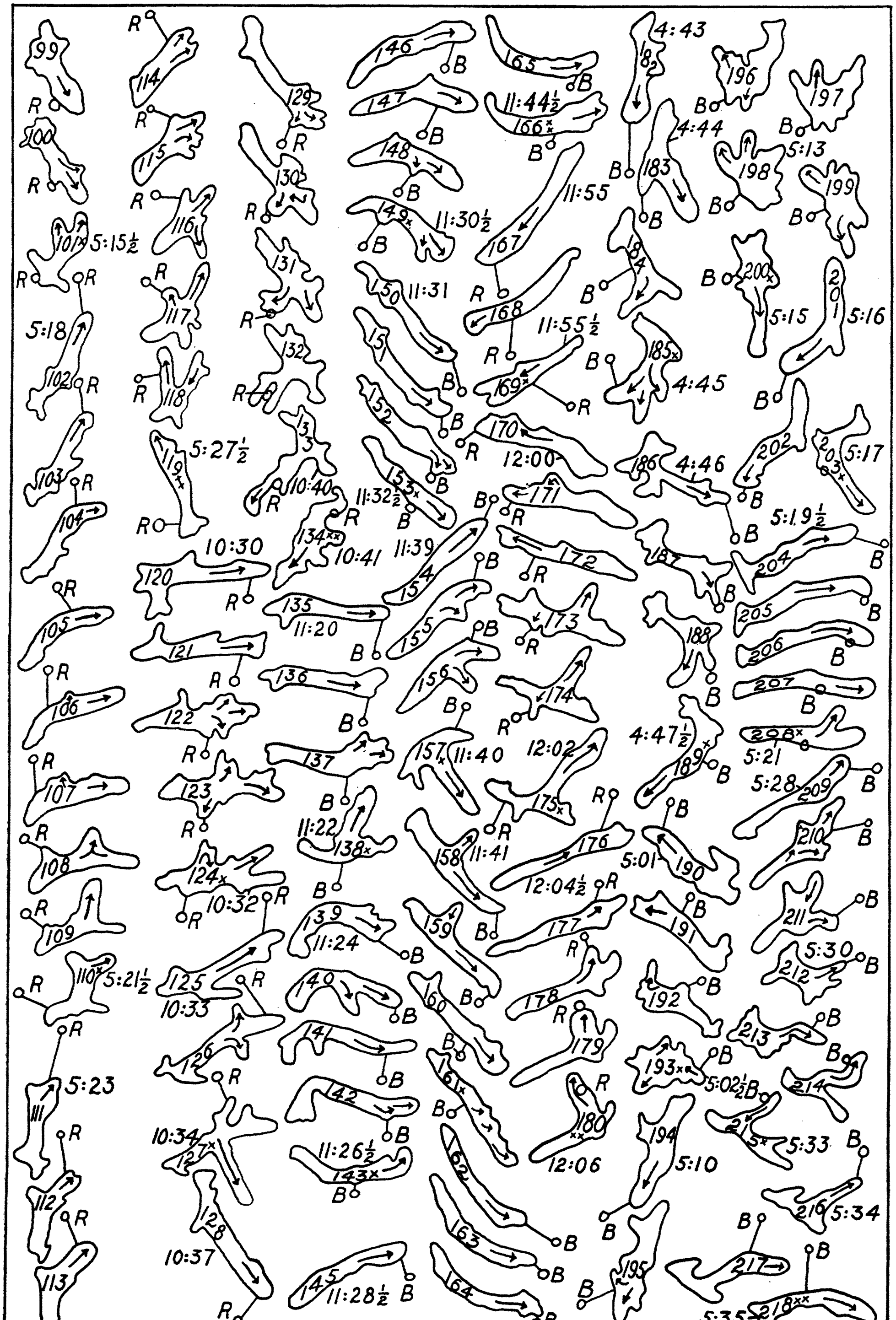

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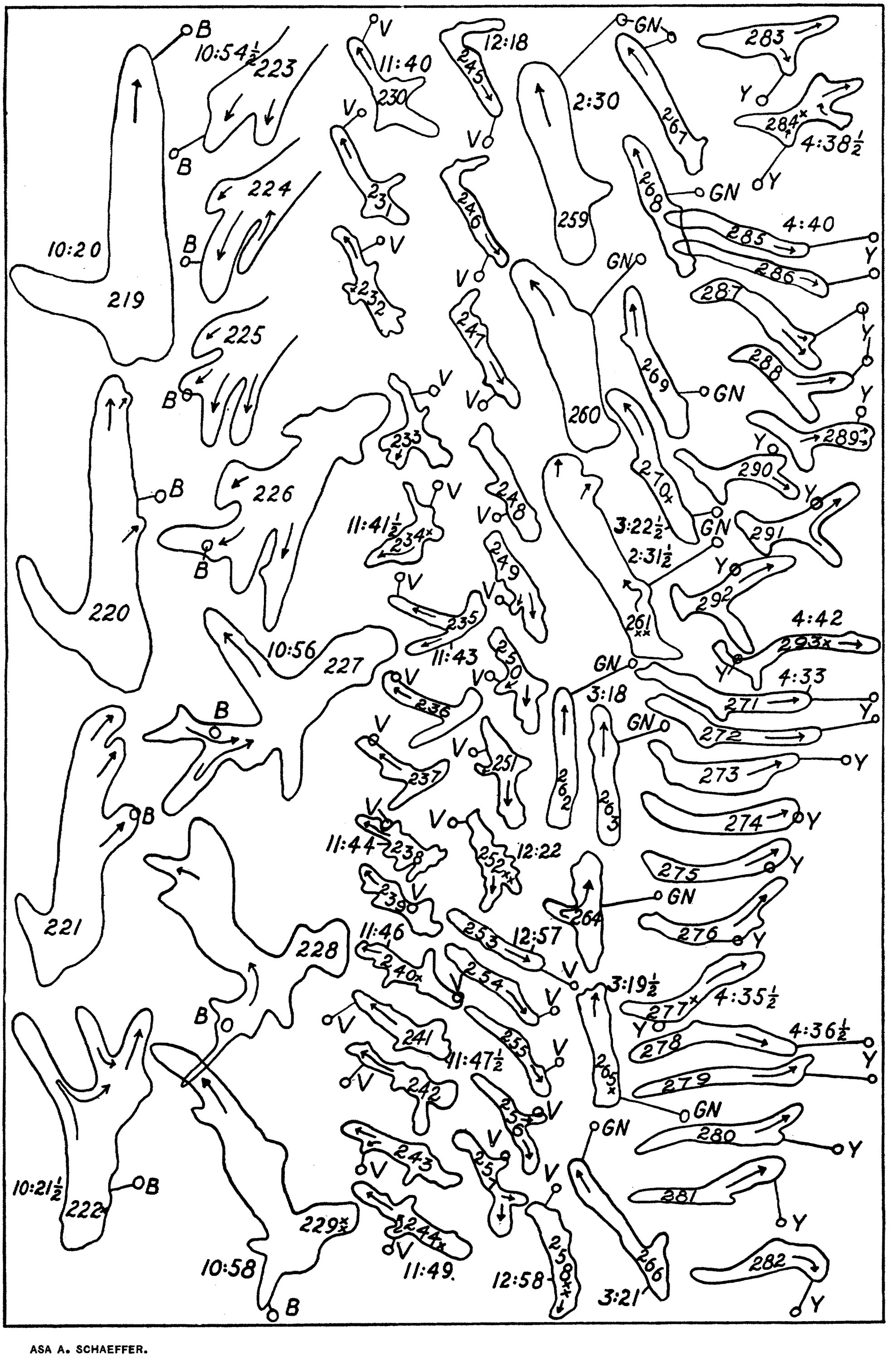

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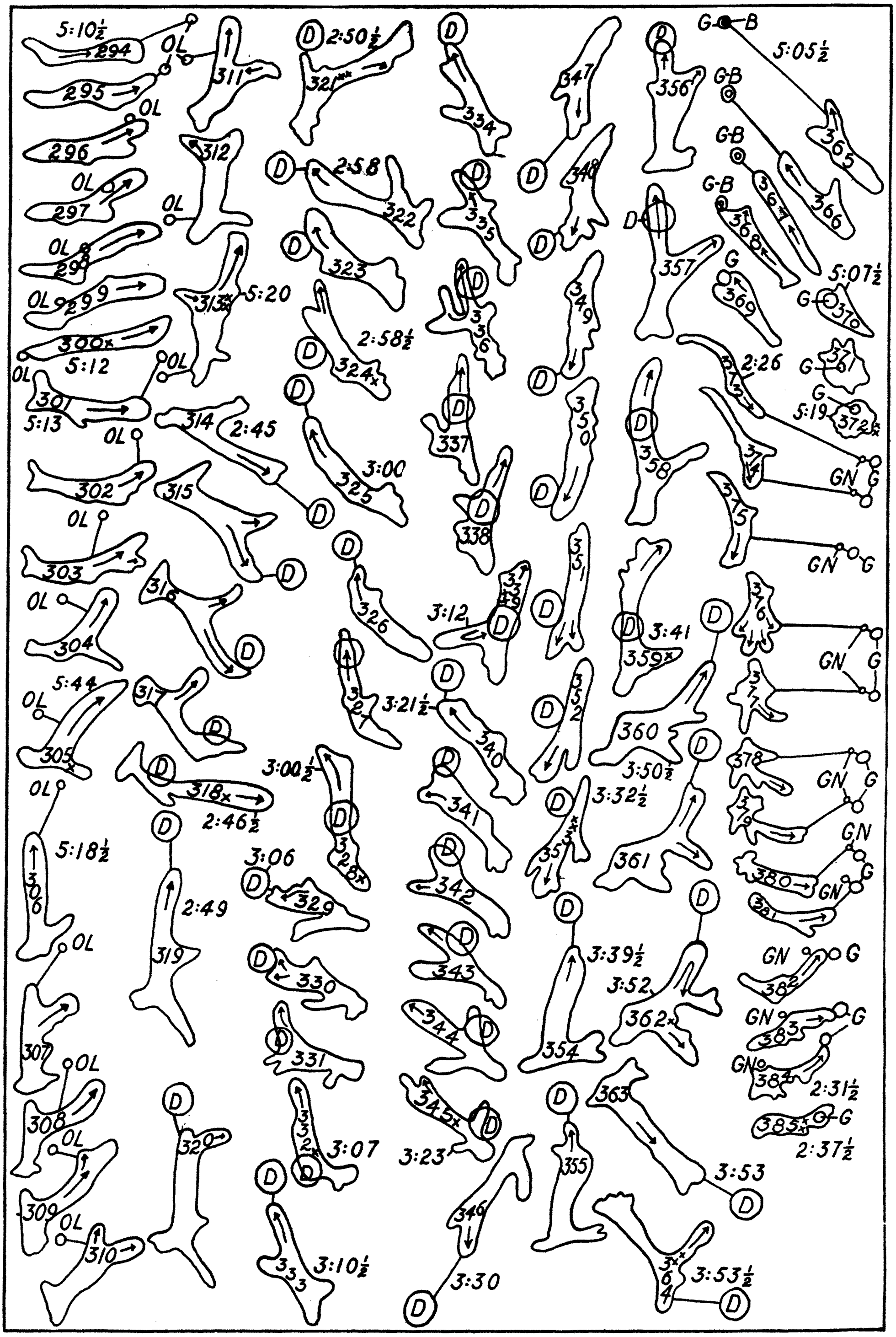

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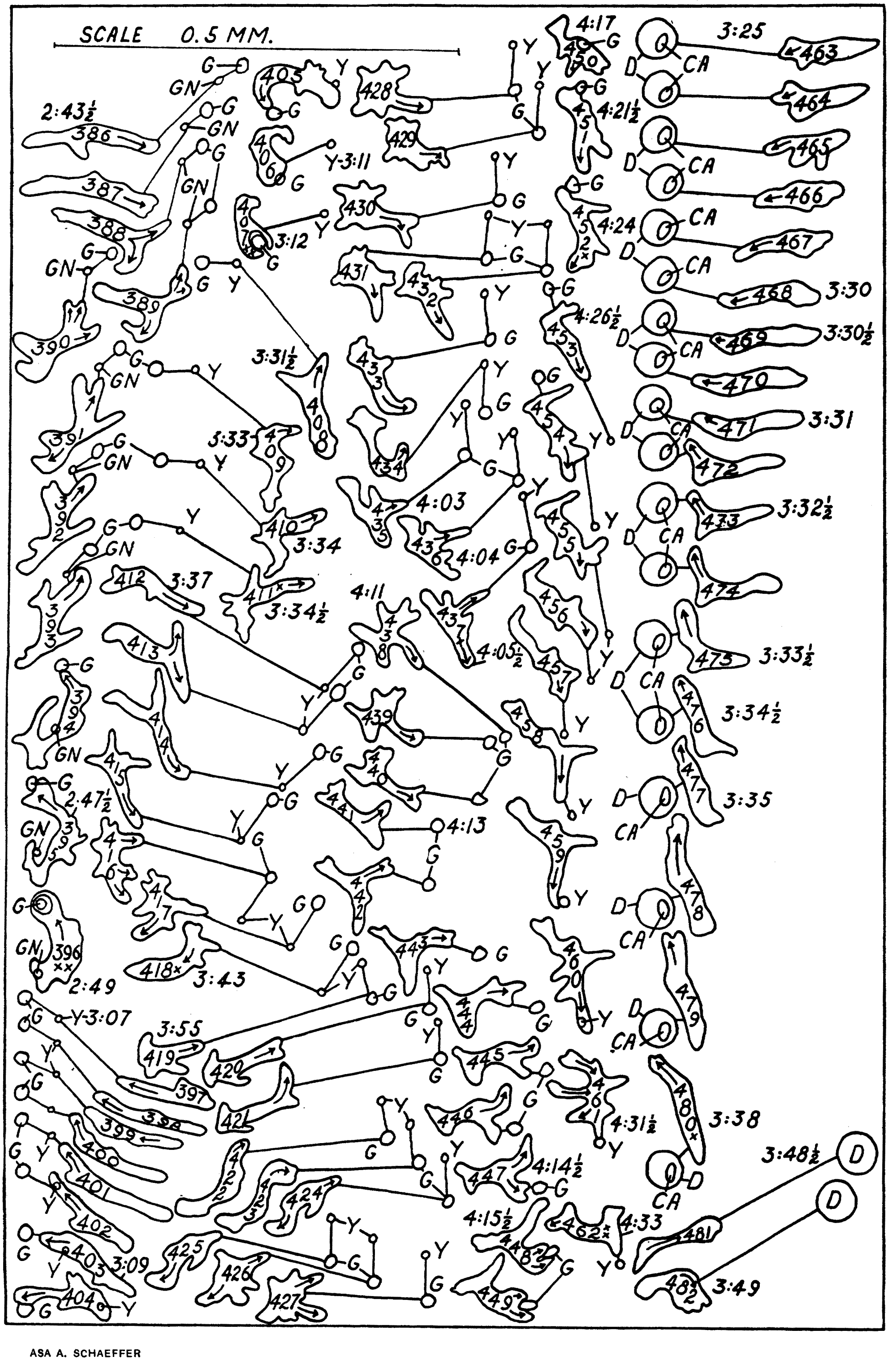

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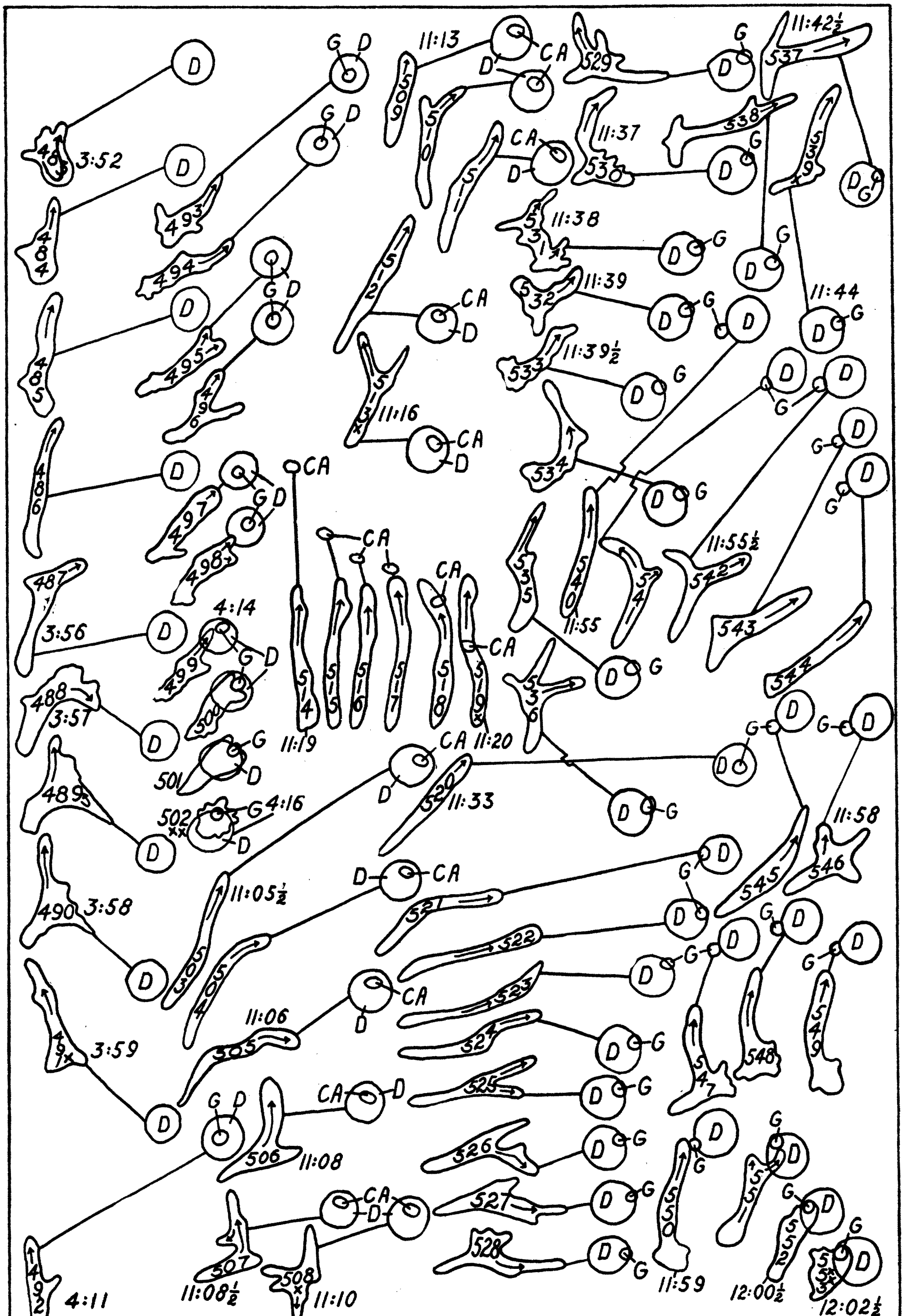

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