Specimens of *Squalus acanthias* with the spinal cord pithed posteriorly from the level of the sixth vertebra were used. Simultaneous dorsal and ventral aortic blood pressures were recorded by means of two mercury manometers and two cannulas; one inserted in one of the first branches of the ventral aorta (thus leaving the second, third and fourth holobranchs on that side and all the gills on the other side for respiration) and the other inserted in the coeliac artery close to the dorsal aorta. Adrenalin chloride (Parke, Davis & Co.), diluted in urea-saline solution, was injected into the portal vein.

Adrenalin, in doses of one or two c.c. of 1:1000 to 1:500,000, produced long-sustained rises of ventral and dorsal aortic blood pressure, both systolic and diastolic, persisting for 30 minutes or more. Subsequent doses of adrenalin following doses stronger than 1:500,000 produced small temporary increases of systolic pressure due to increase in pulse pressure, with no change in diastolic pressure. Control injections of urea-saline solution gave results which were similar to second doses of adrenaline. It is probable, therefore, that such subsequent doses of adrenalin have little or no effect on the blood pressure. However, after doses of two c.c. of 1:500,000 subsequent doses of adrenalin were effective. The increase of blood pressure following initial doses of adrenalin may be associated with slowing or with no change in heart rate, suggesting a peripheral vasoconstrictor action of adrenalin. Perfusion of the blood vessels of the spiral valve with adrenalin solutions or microscopic observation of the minute vessels of the tail during the injection or direct application of adrenalin gave no evidence of vasoconstriction. Further work is necessary to locate the point of action of adrenalin in producing a rise of blood pressure.

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PHYSIOLOGY OF THE LOWER ORGANISMS

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During the year 1930-31 the following contributions based upon work done at the Mount Desert Island Biological Laboratory were sent to press:

"Locomotion in Amoeba proteus." S. O. Mast, *Protoplasma.*

"Localized Stimulation, Transmission of Impulses and the Nature of Response." S. O. Mast, *Physiol. Zool.*

"Movement and Response in Difflugia with Special Reference to the Nature of Cytoplasmic Contraction." S. O. Mast, *Biol. Bull.*

"Orientation in Light from Two Sources and Its Bearing on the Function of the Eyespot." S. O. Mast and Percy L. Johnson. *Zeitsch. f. vergl. Physiol.*

The work of the 1931 season consisted largely of a continuation of that done last season. It concerned directly or indirectly the processes involved in metabolism, locomotion and response in the lower organisms. It may be divided into several fairly distinct sections as follows:

I. ORIENTATION IN LIGHT FROM TWO SOURCES AND ITS BEARING ON THE FUNCTION OF THE EYESPOT.

Numerous observations by various investigators have been made on orientation of a considerable number of different plants and animals in light from two sources. The conclusions reached vary greatly. Some investigators maintain that the relation between the direction of orientation and the ratio of the intensities of the light received from the two sources, is such that the tangent of the angle between the direction of orientation and the rays from the stronger source is equal to the intensity of the weaker source divided by that of the stronger, and that this shows that the response is quantitatively proportional to the stimulus.

We ascertained in light from two sources of various relative intensities the direction of locomotion in *Euglena rubra, Gonium pectorale* and *Volvox minor* and found that *Euglena* follows fairly closely the "tangent rule," but that *Gonium* and *Volvox* do not.

The results obtained indicate that in *Euglena* the photosensitive substance is a thin layer in the concavity of the pigmented portion of the eyespot, that this layer is practically parallel with the direction of locomotion and that the amount of light absorbed by it varies from a maximum when the incident rays are perpendicular to it, to zero when they are parallel with it, so that the amount absorbed in all symmetrically opposite positions assumed in the process of rotation is equal, no matter what the ratio of intensities received from the two sources may be. The results indicate that this does not obtain in *Gonium* and *Volvox* but that the light is brought to a focus in the eyespots, that the location of the focal point varies with the direction of the incident light and that the stimulating effect of a given amount of light absorbed at this point varies with its location, being in *Gonium* about nine times as great for the central part of the eyespots as for the posterior part. This would indicate that the control part is about nine times as sensitive as the posterior part.

The fact that the direction of locomotion in *Euglena* is in accord with the "tangent rule" merely indicates that if the organism goes in any other direction it is unequally stimulated in different positions assumed in the process of rotation and that this results in responses which change its direction of locomotion until it is no longer unequally stimulated. It has no bearing on the problem concerning the quantitative relation between the stimulus and the response.

II. RELATION BETWEEN ENVIRONMENTAL FACTORS AND PROCESSES INVOLVED IN LOCOMOTION IN AMOEBA PROTEUS.

(*a*) The observations made last season on the relation between salt concentration and rate of locomotion and on the relation between temperature and the gel/sol ratio were repeated and extended.

We confirmed the conclusion reached that as the temperature increases the gel/sol ratio decreases, becoming minimum at about 33°. We found that over the entire range tested (9 - 33°) the rate of decrease is fairly uniform with the exception of a sharp rise at about 15° and a sharp drop at about 23°. This indicates that these temperatures are extraordinary in their effect.

(*b*) The rate of locomotion in LiCl, NaCl, $MgCl_2$ and $CaCl_2$ respectively in various concentrations was ascertained. In all as the concentration decreased the rate increased to a maximum and then decreased to zero. The maximum in all occurred in N/2000. In this concentration at 24° the rates in mm. per minute were as follows: LiCl, .265; MgCl, .235; NaCl, .212; $CaCl_2$, .180. The rate obtained for lithium is however not strictly comparable with those obtained with the other salts, owing to the fact that the observations were made at a different time and on specimens from a different culture.

III. METABOLISM IN AMOEBA.

To understand locomotion and response in *Amoeba* it will be necessary to ascertain the processes involved in the transformation of energy associated with metabolism in the organism. We have consequently undertaken a study of various phases of metabolism. This study was largely confined to Amoeba proteus. Some of the more important results obtained follow:

(1). *Feeding and digestion.*

Amoeba proteus feeds almost exclusively on living organisms. It usually takes in one organism at a time, but sometimes it takes in several. Thirteen specimens of *Chilomonas paramecium* were seen taken at a time in a single food vacuole. Specimens of *Colpidium* and *Chilomonas* were frequently seen in the same vacuole. However, whenever more than one of either or both was found in a vacuole the vacuole soon divided into as many or more vacuoles as there were specimens. In this process colpidia were frequently divided into several pieces, one piece going to each of several vacuoles.

A rather unique series of observations was made on the feeding of *Amoeba proteus* on *Monas punctum*. This flagellate frequently appears in abundance in old cultures in which *Colpidium* and *Chilomonas* are scarce. Under these conditions *Monas* is readily eaten. Usually one individual is taken in at a time but the vacuole containing this individual soon unites with other vacuoles. This continues until huge vacuoles are formed. Several were found, each of which contained more than 1,000 of these flagellates closely packed together. They are not digested and are usually voided after 24 hours or more without

having been serious injured. The non-digestibility of *Monas* is doubtless due to the fact that it is very impermeable to various substances, *e.g.*, fat stains which penetrate *Amoeba* and *Chilomonas* in a few minutes require more than an hour to penetrate *Monas*. The fusion of the vacuoles is probably associated with the non-digestibility of these flagellates.

(2) *Changes in structure.*

Considerable time was devoted to microchemical studies concerning the content of *Amoeba proteus* changes in it during starvation and transformations that occur in the food. Numerous important facts were established but these studies are as yet so incomplete that we do not at present wish to make any definite statements concerning them. We would like to say, however, that we found mitochondria in the form of fibers and rods as well as granules, whereas they have heretofore been found only in the form of granules; and that their form depends upon the fixative used. If acid is used, there are only granules; if alkaline, there are fibers, rods and granules.

IV. ENVIRONMENTAL REQUIREMENTS FOR LOWER ORGANISMS.

Our aim in work on the environmental factors necessary for growth in the lower organisms was set forth in the report of last year. During this season the same methods were used. The observations made last year on *Gonium, Volvox, Chilomonas* and *Colpidium* were repeated and extended and several additional organisms were studied.

(1) *Gonium, Chilomonas and Colpidium.*

The results obtained with these organisms were point for point like those recorded in our last report.

(2) *Volvox minor.*

The effect on *Volvox* of four different salt solutions each ranging in hydrogen-ion concentration from pH 6.2 to pH 7.6 was ascertained. Of these it was found that the best growth was obtained in the following solution, known as Schreiber solution: $Ca(NO_3)_2$, .25 g + $MgSO_4$, .06g + KNO_3, .06g + K_2HPO_4, .06g + F_2SO_4, trace + H_2O, 1000 cc. + enough KOH to make the hydrogen-ion concentration pH 7.6. In this solution *Volvox* developed fairly rapidly for a few weeks, but it died at the end of a month. There is evidently something wrong with this solution for continuous development.

(3) *Pandorina morum and Endorina elegans.*

Pandorina grew well throughout the entire season in Schreiber solution. Growth does not seem to be very closely correlated with the amount of light received. *Endorina* grew well for about 6 weeks and then died out. It appears to require a specific amount of direct sunlight.

(4) *Euglena* and related organisms.

Two unidentified species of *Euglena* were found to grow well in Schreiber solution, also in Pringsheim and in Chalkley solution: but *Euglena rubra* and *E. spirogyra* did not grow in any of these solutions and a number of other tried solutions; nor did *Leptocinclis teres, Phacus longicaudus, Trachelomonas armata.*

(5) *Amoeba.*

A considerable number of different salt solutions were tried as culture media for *Amoeba proteus* and *Amoeba dubia.* It was found that among those tried the following was the best for the growth of both *Amoeba dubia* and *Amoeba proteus*: $MgCl_2$, 5 mg; $Ca(NO_3)_2$, 10 mg; Na_2SO_4, 5 mg; NaCl, 15 mg; Na_2SiO_3, 80 mg; H_3PO_4, 70 mg; H_2O, 1000 cc. + 2 grains of wheat per 100 cc.

For some unknown reason silicon seemed to play a very important role in the growth of amoebae.

We are planning greatly to extend this work.

V. ECOLOGICAL SURVEY CONCERNING PROTOZOA.

During the season we continued the study of different types of amoebae found in this region made last year and extended it to other protozoa. The work was again pursued as a side issue. There was no attempt made systematically to cover any particular portion of the surrounding territory. We merely examined solutions collected here and there usually for other porposes.

Last year we found in this way 19 different types of amoebae and this year 16 additional ones, and about 150 other protozoans.

The results obtained show that this region is rich in protozoa of many different kinds.

VI. THE GENUS OURAMOEBA (LEIDY).

Last season we found in a well differentiated region on the shore of Upper-breackneck pond a considerable number of amoeboid specimens each of which contained a large filamentous caudal appendage. These specimens were in full accord with Leidy's description of *Ouramoeba.* We made a study of them and concluded that the caudal appendages consisted of parasitic mould hyphae which had developed from spores in the amoebae and that the specimens in which they appeared were *Amoeba proteus* (Leidy).

At various times during the present season we made a careful study of the same location but were unable to find any amoebae with appendages. This supported the conclusion reached last year, namely, that the caudal appendages observed by Leidy were parasitic fungi and that the genius *Ouramoeba* is not valid.

VII. ORIENTATION IN ANTS.

Detailed observations were made on ants carrying crumbs of bread and various dead insects. It was found that if the pieces carried are small, the ants pick them up with their jaws and carry them to their nest, taking a fairly direct route; but that if they are large, the ants drag or roll them and proceed backwards or sidewise. The important point is that in going backward or sidewise the ants go practically as directly toward their nest as they do when they go forward. Thus, given individuals were repeatedly observed in transporting pieces of substance to go alternately forward, sidewise and backward, without changing their direction of locomotion. That is, they continually went toward their nest when they traveled backward and sidewise as well as when they traveled forward. The fact that ants can go directly toward a fixed location in space no matter what their axial orientation may be or how this may change shows concluisvely that their direction of locomotion cannot be controlled by anything in the nature of equal or unequal stimulation of organs on opposite sides due to the action of external stimulating agents. It strongly indicates that the direction of locomotion is not directly controlled by the immediate action of anything in the environment and that the processes involved are somewhat similar to those involved in the control in higher organisms of direction of movement toward a given point in space.

THE RELATION BETWEEN RATE OF LOCOMOTION IN AMOEBA PROTEUS AND HYDROGEN-ION CONCENTRATION IN SOLUTIONS CONTAINING SODIUM, POTASSIUM, OR CALCIUM IONS IN DIFFERENT CONCENTRATIONS OR MIXTURES OF THEM

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As test solutions sodium, potassium, and calcium phosphate buffers with hydrogen-ion concentrations ranging from pH 4.2 to 8.0 were used. Constant cation concentration throughout a range of CH was obtained by adding to the primary phosphate of a given normality with respect to the cation (*e.g.*, N/100 NaH_2PO_4) a required amount of the corresponding hydroxide of the same normality (*e.g.*, N/100 NaOH) to give the desired hydrogen-ion concentration. Preliminary tests comparing phosphates and chlorides at the same hydrogen-ion concentrations seemed to indicate little or no effect due to change in the anion. The rate of locomotion was measured by camera lucida drawings of strictly monopodal specimens.

As found in previous investigations the rote of locomotion in a pure solution of a sodium, potassium, or calcium salt at constant hydrogen-ion concentration, decreases with increase in salt concentration from N/1000 to N/100. In sodium or potassium salts the rate of locomotion increases with increasing CH of the solution to an optimum and