under such conditions have an increased plasma and urinary chloride concentration. Severe injury to the skin causes in a few hours almost a hundred per cent rise in plasma chloride, and a twenty-fold or greater increase in urine flow.

Urine from diuretic fish contains a much higher concentration of chloride, a smaller concentration of total nitrogen and inorganic phosphate, and about the same concentration of sulfate as "normal" urine. Preliminary work on the mechanism of skin injury in producting diuresis has been started, but no definite statements can be made at present.

1. Marshall: Amer. Journ. Physiol., *94*, 1, 1930.
2. Smith: Amer. Journ. Physiol., *93*, 480, 1930.
3. Marshall and Smith: Biol. Bull., *59*, 135, 1930.

13. REPORT CONCERNING WORK AT THE MT. DESERT ISLAND BIOLOGICAL LABORATORY, JUNE 15 TO SEPTEMBER 15, 1930

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Several closely correlated lines of investigations were carried on during the season. We shall present a few of the more important results obtained in each.

I. *Locomotion and Response in Difflugia pyriformis with Special Reference to the Nature of Protoplasmic Contraction.*

Difflugia pyriformis inhabits various ponds on Mt. Desert Island. We found it abundant and in excellent condition in a pond near Town Hill.

Locomotion in Difflulgia consists normally of extension of pseudopods, one after another and attachment to the substratum at the tip, followed by contraction which pulls the shell containing the body forward. The fleshy part of Difflugia is in structure much like an amoeba and the factors involved in the extension of pseudopods and in attachment to the substratum are essentially the same in the two forms. The contraction of the pseudopod is the outstanding distinguishing phenomenon in the process of locomotion in Difflugia and also in the process of response. This contraction is rapid and extensive and since it occurs in the simplest sort of cytoplasm, cytoplasm which has no fixed differentiation, it is of great interest.

Numerous observations on the contraction of the pseudopods in the process of locomotion and in response to mechanical stimulation, were made with the best optical equipment obtainable. It was found that contraction is associated with extensive increase in thickness of the plasmagel owing to gelation of the adjoining plasmasol.

If the shell is broken, Difflugia leaves, moves about and lives a week or more. Immediately after it leaves the shell movement is quite normal, but later the contraction of the pseudopods disappears and locomotion is essentially the same as it is in Amoeba.

II. *Response to Localized Stimulation in Amoeba*

Methods were devised by means of which any portion of an amoeba could be subjected to intense illumination.

It the illumination of the entire animal is increased all movement stops. If the hyaline cap, i. e. the very tip of the pseudopod, is illuminated there is no response. If the cap and the plasmagel sheet (a thin layer back of the cap) is illuminated movement in the entire pseudopod stops. If all excepting the cap and the plasmagel sheet is illuminated there is no response.

If half of the cap and the plasmagel sheet is illuminated, movement in this half stops but continues in the other half, resulting in marked deflection in the direction of the extension of the pseudopod.

These results show that the response consisting of cessation of movement is due to the effect of light on the plasmagel sheet. The cessation in movement is due to increase in the elastic strength of the plasmagel sheet, owing to gelation of the adjoining plasmasol produced by the action of the light.

If the tip of one pseudopod is illuminated cessation of movement does not occur in other pseudopods. The gelating effect of local illumination is not transmitted to other regions. This does not mean, however, that there is no transmission of any kind. An ameoba is an organized system and the local effect of localized stimulation results in action throughout the entire system, although local gelation produced by localized stimulation does not produce gelation elsewhere.

III. *Ecological Survey Concerning the Amoebae*

From time to time during the season we examined for amoebae some of the waters in various regions of Mt. Desert Island and the adjoining mainland. In this study we found 19 different types of amoebae and we made records for each type concerning form, size, structure, locomotion, habitat, associated organisms and hydrogen-ion concentration of the water in which they lived.

This study was superficial. It was in the nature of a diversion from more serious undertakings. The results obtained show, however, that the region is rich in varieties of amoebae and they indicate that a thorough systematic investigation along the line pursued is highly desirable.

IV. *Relation between Environmental Factors and Processes involved in Locomotion in Amoeba proteus.*

A comprehensive quantitative study was made of the effect of temperature, hydrogen-ion concentration and salt concentration ($CaCl_2$, $MgCl_2$, NaCl, KCl) on rate of locomotion, gel-sol ratio and frequency of rupture in the plasmagel sheet. The results obtained may be summarized as follows:

Temperature

As the temperature increases the following occurs:

(a) The rate of locomotion increases to a primary maximum at about 24° then decreases somewhat, after which it increases to a secondary maximum at about 30° and then decreases rapidly to zero at about 33°.

(b) The gel-sol ratio decreases to a minimum at about 33°, i. e. the amoebae become more fluid, then the organism suddenly coagulates entirely. This condition is not reversible. It results in death.

(c) The frequency of ruptures in the plasmagel sheet increases to a maximum at about 33° then suddenly drops to zero.

Hydrogen-ion Concentration

As the hydrogen-ion concentration increases the rate of locomotion increases slowly to a secondary maximum at about pH 7.4, then decreases to a minimum of pH 7 after which it increases to a primary maximum of pH 6.8 and then rapidly decreases to zero at a point somewhat beyond pH 5.4.

The gel-sol ratio increases gradually over the entire range tested, pH 8 to 5.4. The frequency of rupture in the plasmagel sheet decreases over the entire range tested pH 8 to 5.4.

Salt Concentration

As the concentration increases the rate of locomotion in all of the four salts tested increases to a maximum, then decreases to zero. The optimum concentration and the rate of locomotion at the optimum concentration varies somewhat with the salt. The optimum concentration is highest for $CaCl_2$ and lowest for KCl. The rate of locomotion at optimum concentration is highest for NaCl and lowest for $MgCl_2$. In all four salts the gel-sol ratio increases with increase in concentration, but the increase is considerably greater for $MgCl_2$ and $CaCl_2$ than it is for NaCl and KCl. It is greatest for $MgCl_2$ and lowest for KCl.

The frequency of rupture in the plasmagel sheet in all the salts tested decreases to zero as the concentration increases.

In connection with the observations considered above the following facts were established:

(a) The rate of locomotion under constant environmental conditions depends upon the form of the amoebae. It is greatest in amoebae in monopodal form, less in those in bipodal form and least in those in multipodal form.

(b) The plasmagel in Amoeba increases in thickness from the anterior to the posterior end.

(c) In amoebae which have become stellate in form after having been transferred from culture fluid to distilled water, the plasmagel has practically disappeared, so that they are almost entirely fluid.

V. *Concerning the Genus Ouramoeba (Leidy)*

Leidy found in the neighborhood of Philadelphia a considerable number of amoebae which had at the posterior end long filamentous projections, forming a sort of caudal appendage. This appendage was essentially the same in all the amoebae which had it. In other respects some of the amoebae were like Amoeba proteus (L) and the rest were considerably smaller and differed in other respects. Owing to the presence of the caudal appendage Leidy held, however, that the amoebae which have it are generically distinct and he consequently (1874) grouped them together under the generic name Ouramoeba, i. e. amoebae with tails.

In July 1930 we discovered in a small pool adjoining Breakneck Pond, Mt. Desert Island, Maine, a considerable number of amoebae which had caudal appendages like those described by Leidy. We examined some of these amoebae very thoroughly under low and high power apochromatic objectives and compensating oculars and we found that, with the exception of the appendages, they contained all the essential characters of Amoeba proteus (Leidy). These amoebae were therefore like some of those described by Leidy We obtained evidence, however, which strongly indicates that the appendages are not part of the amoebae; some of this evidence follows:

1. The filaments in the appendages are connected with spherical bodies in the amoebae, which in size and structure resemble mould spores. Some little distance from the spherical bodies the filaments branch dichotomously, they do not change in form, they contain numerous vacuoles and in general look like mould hyphae.

2. The appendages play no part in locomotion or in attachment of the amoebae. They can be removed without in any way interfering with the activities of the amoebae and if they are removed no new ones develop.

3. The amoebae with appendages formed only a small percentage of the amoebae found in the pool. Among those without appendages there were numerous specimens like those with appendages in all essential respects except the appendages.

4. The specimens without appendages had numerous food vacuoles; those with appendages had none. Some of both kinds were isolated and put into culture media. Those without appendages lived and reproduced. Those with appendages died without dividing. This indicates that the specimens with appendages were in a pathologcal condition, produced by the appendages.

Taken as a whole the evidence presented practically proves that the appendages in question were mould hyphae which developed from spores taken in by the amoebae, and that there consequently is no foundation for the genus Ouramoeba.

VI. *Environmental Reqirements of Lower Organisms*

Our aim in the study of the environmental requirements of the lower organisms was twofold. We desired for experimental purposes relatively simple organisms which could be subjected for long periods of time to various known factors and we wished to ascertain in detail the chemical elements necessary for the synthesis of protoplasm in the lower organisms and the environmental conditions necessary for this synthesis.

This aim can best be attained, we think, by an ecological field study of the lower organisms accompanied with intensive cultural experiments in the laboratory. We have consequently entered upon investigation along these lines. Some of the more important results thus far obtained are presented in the following paragraphs:

1. *Gonium pectorale*

Gonium pectorale was found in abundance in a pond frequented by cattle. The water in which the organisms occurred contained considerable manure and other material and the hydrogen-ion concentration was pH6.

We obtained excellent cultures in the laboratory in solutions containing five salts $Ca(NO_3)_2$, KNO_3, $MgSO_4$, KH_2PO_4, and $FeCl_2$) in very low concentration. We will continue these experiments with the view of ascertaining the minimum requirements for growth and the effects of substituting other chemical elements and changes in light and temperature.

We are now carrying on some investigations with our laboratory cultures of these organisms concerning reactions to light and we shall use them in feeding experiments on carnivorous protozoa.

2. *Volvox.*

Volvox was found in various ponds on Mt. Desert Island but not in abundance. The hydrogen-ion concentration in all of the ponds on the island is rather high. This may be the reason why this organism does not thrive in them.

The most favorable place found was in a small pool adjoining Breakneck Pond. A rather detailed study was made of this pond in reference to the relation between the distribution of Volvox in it and temperature, hydrogen-ion concentration and illumination. It was found that the colonies were continuously confined to a layer of water at the surface about 6 cm. thick and that in this layer they were continuously fairly uniformly distributed. The temperature and the illumination were considerably higher nearer the surface than at the bottom of the pool. The hydrogen-ion concentration was practically the same at different depths but it varied from about pH 5.9 in the morning to about 6.5 in the afternoon. We could not, however, definitely ascertain with which environmental factors the distribution of the colonies was correlated.

Considerable growth was obtained in various culture solutions in the laboratory. The most favorable of the solutions tested consisted of KNO_3, KH_2PO_4 $MgSO_4$, .25 gr. each, $Ca(NO_3)_2$, 1 gr. $FeSO_4$, trace and K_2CO_3 enough to make the hydrogen-ion concentration pH6.6.

In this solution the colonies reproduced rapidly sexually and appeared to be in excellent condition for from 3 to 4 weeks, then they became yellowish in color and began to produce eggs, after which they gradually decreased in number until all had disappeared.

These results raise a number of interesting problems which we hope to attack soon.

3. *Colpidium sp. and Chilomonas paramecium.*

After extensive experimentation involving numerous failures we found that Colpidium thrives very well and indefinitely in the following solution, provided it is renewed once every two weeks: $CH_2(NH_2)$ $COOH$, and $K(C_2H_3O_2)$, 2.5 gr. each, $MgCl_2$ .05 gr., K_2HPO_4 .05 gr. and H_2O, 1000 cc. Bacteria develop rapidly in this solution and the colpidia feed on them.

Chilomonas develops rapidly and continues indefinitely in the following culture medium, provided it is renewed from time to time: $CH_2(NH_2)$ $COOH$, $C_6H_{12}O_6$ and $Na(C_2H_3O_2)$, .5 gr. each, $MgSO_4$, .02 gr. KH_2PO_4, .002 gr. and H_2O, 1000 cc.

It will be seen that neither calcium, sulphur nor iron were added to the first solution and neither calcium nor iron to the second. The results therefore indicate that calcium, sulphur and iron are not necessary for the synthesis of protoplasm in Colpidium and that calcium and iron are not necessary for the synthesis of protoplasm in Chilomonas. This is of considerable importance for all of these elements have invariably been found in protoplasm. It should be emphasized however that our results do not definitely prove that the elements in question are not necessary for the growth of the organisms mentioned, for the methods used did not exclude the entrance of traces of them from the air. But this much is clear, if they are actually necessary, the amount necessary is exceedingly minute.

We expect to continue these experiments under more carefully controlled conditions.

4. *Amoeba*

We have for several years produced in culture media Amoeba proteus, Amoeba dubia and Amoeba dofleini, in great numbers. These culture media contained in addition to known salts a little wheat rice or timothy hay. Bacteria, Chilomonas, Colpidium and a water mould developed in the cultures. The amoebae fed largely if not entirely on Chilomonas and Colpidium. The specimens obtained in these cultures have been used extensively in experimental work, but it is evident that there were in them a considerable number of unknown constituents which made it impossible to ascertain the elements necessary for the synthesis of the protoplasm in the amoebae. We therefore attempted to eliminate this difficulty by replacing the wheat, rice, or hay with a known organic substance.

Numerous tests were made, but so far we have not been successful in raising amoebae in these solutions. We have, however, been able to raise both Chilomonas and Colpidium in them, and to raise amoebae if they were kept in a salt solution somewhat more complex and if specimens of Chilomonas or Colpidium were, from time to time, transferred from this culture medium to this solution, so as to serve as food for the amoebae. We consequently have here a method by means of which we will be able indirectly to ascertain within certain limits, the compounds and the elements necessary for the synthesis of protoplasm in amoebae.

Up to the present our experiments have been confined to Amoeba proteus and Amoeba dubia. We found that Amoeba dubia in a solution consisting of K_2HPO_4—KH_2PO_4, .02 gr., $MgCl_2$ .004 gr., $CaCl_2$ .06 gr., and H_2O, 100 cc., and fed on Colpidium raised in a solution consisting of CH_2 (NH_2) COOH, 2.5 gr., $KC_2H_3O_2$, 2.5 gr., $MgCl_2$, .15 gr., K_2HPO_4, .05 gr. and H_2O, 1000 cc. thrives but that Amoeba proteus does not. This indicates that the protoplasm in Amoeba dubia can be synthesized without sodium, sulphur and iron and it shows that this form is markedly different from Amoeba proteus.

We hope greatly to extend this work using numerous other combinations of chemical compounds and various other types of amoebae.