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The Culture of *Spirostomum ambiguum.*

By

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Spirostomum ambiguum, one of the largest ciliates, is frequently used as experimental and demonstration material. This is partly due to its large size, remarkable contractility, and unique structure, but mainly to the great numbers found at some seasons in natural waters. Such sources, however, never provide a uniform supply of animals for extensive investigations and it is often difficult, if not impossible, to procure any. It is, therefore, highly desirable to have a method by means of which this organism can be cultured in the laboratory.

Miss Bishop maintains (1923) that thick cultures of this protozoan can be grown on a wheat extract in deep vessels, e. g. test tubes, which have a relatively small free surface for the medium. Reports from dealers in stock cultures of protozoa indicate, however, that great difficulty has been experienced in the culture of this form and they seem to show that Miss Bishop's method has not been successful.

The culture of *Spirostomum ambiguum* was begun in this laboratory with the view of providing large numbers of animals for respiratory measurements. The wheat extract prescribed by Miss Bishop was tried with the following variations: pond water plus hay and wheat in different proportions, and spring water plus hay and wheat in different proportions. Cultures of each kind were placed in crystallizing dishes and tall jars, and were also inoculated at different times after being set up.

The usual infusion cycle of changes in bacterial flora and accompanying changes in hydrogen-ion concentration occured in these cultures but the yield of spirostoma was unsatisfactory. Division of the organisms was never observed and the number of individuals did not visibly increase. In those cultures inoculated a long time after being set up the animals remained alive for some time but in the others they died off at a rate proportional to the acidity of the medium. The dimensions of the free surface of the media in relation to their depth had no influence upon these results.

WEATHERBY (1929) found considerable quantities of ammonia in collections of spirostoma which had been standing in the laboratory for some time. The author of the present paper found, in the course of some respiratory measurements on freshly collected spirostoma, that ammonia is actually produced by them. With these facts in mind, and on the suggestion of Prof. E. A. ANDREWS, fresh[1]) cow manure was added to the series of cultures briefly outlined below:

1. Spring water plus hay and wheat in different proportions plus cow manure in different proportions.

2. Spring water and pond water plus cow manure in different proportions.

3. Balanced salt solution[2]) plus each of four strains of bacteria isolated from cow manure and grown on beef extract.

4. Horse manure was substituted for cow manure in a few cultures similar in other respects to those of 1.

As before, cultures of each kind were inoculated at different times and also kept in both flat and tall containers.

The best growth of *Spirostomum ambiguum*, on the basis of rapidity of development and stability of supply was obtained in a medium prepared in the following manner: Add one percent timothy hay and one percent wheat to spring water, boil long enough to stop germination of the wheat, then add to each liter of this solution, after it is cool, one tablespoonful fresh cow manure and, in two or three days, a pipet full of an old culture containing spirostoma.

It was found that the hydrogen-ion concentration of the media containing a hay-wheat infusion plus cow manure rapidly adjusted itself to about p_H 7.6 and remained there for several weeks. In a week to ten days after inoculating these cultures at a hydrogen-ion

[1]) Collected while still plastic and kept in a closed jar to prevent drying. Old manure which has been dried and leached by rain does not give satisfactory results.

[2]) CHALKLEY's five salt solution (see W. F. HAHNERT, Studies on the Chemical Needs of *Amoeba Proteus*: A Culture Method., Biol. Bull. 62 (2) p. 205—210).

concentration of p_H 7.0, or greater, flourishing stocks were obtained. The shape of the containing vessels had no influence upon this result; the animals grew equally well in crystallizing dishes and tall jars.

Cow manure without hay and wheat does not provide a suitable substrate for whatever food is needed by the spirostoma. Its action is rather in supplying some substance which, through its breakdown, keeps the culture alkaline, that is, as a buffer of great capacity but small osmotic value. Presumably, the bacteria of the manure break down the nitrogenous substances in the latter to ammonia and thus maintain the proper chemical environment for *Spirostomum*. That this condition of alkalinity is necessary to growth and rapid division, as well as to survival [1]), of this ciliate is indicated by the fact that, when after some time the cultures begin to produce fewer animals the addition of a small amount of cow manure to each rapidly restores the former rate of multiplication. Original cultures, nearly a year old to date of writing, are still yielding, under this treatment, a plentiful supply of spirostoma.

Literature cited.

BISHOP, A. (1923): Some Observations on Spirostomum ambiguum EHRBG. Quart. J. Micr. Sci. Vol. 67 p. 392—434.

SAUNDERS, J. T. (1924): The Effect of the Hydrogen-ion Concentration on the Behavior, Growth and Occurrence in Spirostomum. Proc. Camb. Philos. Soc., Biol. Sci. Vol. 1 p. 189—203.

WEATHERBY, J. H. (1929): Excretion of Nitrogenous Substances in the Protozoa. Physiol. Zool. Vol. 2 p. 375—394.

[1]) SAUNDERS (1924) states that hydrogen-ion concentration of p_H 7.4—7.8 is most favorable for *Spirostomum ambiguum* and is the main factor in its conditions for survival. PETER's medium, as listed below, fulfils this condition and is recommended for the handling of spirostoma in experimental work.

$CaCl_2$—0.00055 M, $NaNO_3$—0.00015 M, $MgSO_4$—0.00015 M, K_2HPO_4—0.00015 M.

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