so that the inked needles pass through the ear and their tips penetrate the rubber pad. By releasing pressure the punch springs open. The operator then dabs the protruding needle points and removes the punch, at which time the needle points carry ink back into the holes as they are being withdrawn. Finally the operator brushes both sides of the ear vigorously in order to work the ink deeply into all the needle holes.

The chief difficulty encountered in preparing such a tatooing device is to have the needles fine enough and numerous enough that when a rat is tatooed at 3 weeks of age, the tatooed points will not separate enough to distort the numeral during subsequent growth of the pinna, because later the numbers increase about ⅓ both in length and breadth.

Likewise the numerals must be small enough that at least two of them may be placed side by side in each ear.

By using two numbers in each ear one may distinguish up to 10,000 individuals and by employing three numbers one may continue the series up to 1,000,000.

CLYDE E. KEELER

WISTAR INSTITUTE, PHILADELPHIA

A TESTED METHOD OF GROWING STENTOR COERULEUS

THE heterotrich protozoan *Stentor coeruleus* is valuable for many laboratory purposes but is difficult to maintain in culture. Several attempts were made to grow it according to methods described in the literature (Hetherington[1]; Turner,[2] Brandwein[2]), but none of these produced a satisfactory supply of specimens. Finally, a standard method was developed which has yielded specimens in abundance for more than a year. The method is described in the following paragraph:

Gallon jars are nearly filled with spring water, and to each is added 10 cc of an extract of lettuce, prepared by boiling 10 grams of fresh lettuce leaves in 100 cc of distilled water until about 50 cc has been evaporated. After 12 to 24 hours the water begins to appear cloudy due to bacteria. Then specimens of *Stentor coeruleus* and *Chilomonas paramecium* are put into the jars. Every few days, when the water shows signs of becoming clear, fresh lettuce extract is added. Whenever (usually two to four weeks) the stentors begin to multiply less rapidly, new cultures are started by transferring some stentors and chilomonads from the old cultures to jars of new medium. The jars are kept at room temperature in light of very low intensity or in darkness.

It is not known whether the stentors depend mainly for food upon the chilomonads or upon the bacteria. It is known, however, that the chilomonads are ingested readily. Rotifers which may be present in the cultures seem to cause no trouble, but paramecia must be kept out because they consume the bacteria so quickly that the stentors are deprived of their food supply.

The jars must be kept in light of low intensity or in darkness to prevent the excessive growth of unicellular green algae. Even in moderate light the algae gradually cause the culture medium to become green. If this condition is approached the stentors must be transferred to fresh medium.

No more than 10 cc of lettuce extract may be added to a jar at one time because an excess will cause bacteria and yeast cells to increase so much that the culture becomes too acid. The hydrogen-ion concentration of the cultures must remain between pH 6.2 and pH 7.8. These limits will ordinarily not be exceeded if the bacteria do not become too numerous.

Some difficulty may be experienced from the growth of aquatic fungi. The stentors tend to become entangled in the mycelia of these molds, making it difficult to remove them without injury. By stirring the cultures vigorously every few days, the mycelia are dislodged from the sides of the jars and settle to the bottom, where they cause no further trouble. If no lettuce infusion is added for some time, the stentors accumulate in the mats of mycelia on the bottom of the jars, but if more lettuce infusion is added they again collect near the surface of the water.

If it is desired simply to maintain a culture of stentors with a minimum of effort, fresh lettuce leaves may be put into the culture jars. But if this is done the stentors attach to the leaves. The advantage of using an extract of lettuce is that the stentors attach to the sides of the jars near the surface, whence they can be easily removed with a pipette.

W. H. BELDA
W. J. BOWEN

THE JOHNS HOPKINS UNIVERSITY

[1] A. Hetherington, *Archiv. für Protistk.*, 76: 118–129, 1932.

[2] J. P. Turner and P. Brandwein, "Culture Methods for Invertebrates," pp. 60 and 64. Ithaca, N. Y.: Comstock Publishing Company.

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