TECHNICAL PAPERS

An Alternative Method for the Culture of *Sciara* Larvae

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In the course of a study of chromosomes with the electron microscope it became advisable to have in the laboratory stock cultures of the so-called fungus gnat, genus *Sciara*. Certain features of the salivary gland of the larva of this insect made its culture desirable for this work. The following method of culture developed in our laboratory seemed to be somewhat more satisfactory than that which had previously been described (*1*). In the earlier method the food supply was largely powdered dry yeast and powdered dry mushrooms, whereas in this method it is a living fungus.

The first step in the procedure is to initiate the growth of the fungus culture. The customary half-pint milk bottles were filled to a depth of about 1½″ with Sabouraud's media. After autoclaving, the media was slanted before cooling to provide more surface for the fungal growth which served as food for the developing larvae. The agar surface was then streaked with the fungus material. Pure cultures[2] of the genera *Haplosporangia, Allescheria,* and *Chaetoconidia* were used. All of them provided a satisfactory nutrition for the *Sciara* cultures, but somewhat better success was obtained with the *Chaetoconidia.*

The fungus was allowed to grow several days before the *Sciara* flies were introduced. By the time the larvae appeared, usually about 7 days after the flies were introduced, the fungus had formed a luxuriant mat over the media surface. Apparently most of the feeding is done in the larval stage, for the larvae feed voraciously on the fungus, which then begins to disappear. The growth of the fungus is adequate to provide food throughout the larval stage. The life cycle of the *Sciara* flies occupies about a month, the egg stage taking 5–6 days; larva, 14–15 days; pupa, 3–4 days; adult, 5–8 days. A new fungus culture is necessary for each new generation of flies, and therefore the fungus culture must also be maintained in stock on the Sabouraud media.

The *Sciara* cultures can be kept in the laboratory at ordinary room temperatures provided the temperature does not rise above about 29° C, since it is known that a higher temperature is lethal if maintained for more than a short time (*1*). The larval stage is particularly sensitive to heat, and if it is convenient, the cultures should be kept in an incubator at 22°–24° C.

The advantages which this method seems to offer over the previous one are: (1) a greater biological constancy in the nature of the food supply, (2) elimination of the necessity of repeated periodic feedings during the larval stage and (3) greater ease in maintaining an adequate moisture supply.

[1] The technical assistance of Mr. Sam H. Stone is gratefully acknowledged.

[2] The initial fungus cultures were kindly provided by Prof. O. A. Plunkett, Division of Botany, University of California, Los Angeles.

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Discontinuities in Properties of Water as a Function of Temperature

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It was shown by the author long ago (*1*) that a plot of the densities of many liquids vs. temperature gives a curve that is not smooth, but exhibits inflection points, or kinks, at some intervals. This can be shown to be true for any liquid for which there exist accurate experimental data. But water seemed an exception since the temperature-density curve is perfectly smooth according to figures given in tables used at the present time.

These kinks are often ignored, one reason being that they are not predictable on the basis of current theories. Another is that the previous experimentalists were not aware of them and performed the measurements of densities at regular intervals of temperature. But the kinks, or places of deviation from the smooth path, frequently fall between the observed points, and thus remain unnoticed. Some may attribute these effects to conditions which cause experimental errors of more or less systematic character. This view, in the light of the present data, seems absurd.

It would not be easy to repeat the work of Ramsey and Sydney Young (*3*) for this requires complicated apparatus and takes much time. But there is an easy method of demonstrating these phenomena at moderate temperatures (*2*). The density of propyl alcohol, which had been purified by the procedure of Sidney Young, was determined in a pycnometer at various temperatures between 20° and 80° C. The curve so obtained showed a kink in the same place as it had appeared in the data of Sidney Young. Other liquids also show kinks in this region of temperature, e.g. benzene at 45° C. The temperature was carefully regulated during these experiments, and, by weighing a 25-cc pycnometer, the density can be determined to five decimal places. But this is not

needed since the third decimal is sufficient to demonstrate the kinks. If the curve is plotted on graph paper in such a way that a fraction of a millimeter represents the fourth decimal place, the experimental errors are not seen at all. Any deviations from the smooth path must be due to intrinsic causes.

Of all substances known to the author, water seemed an exception in that it alone seemed free of these discontinuities. However, experiments performed recently

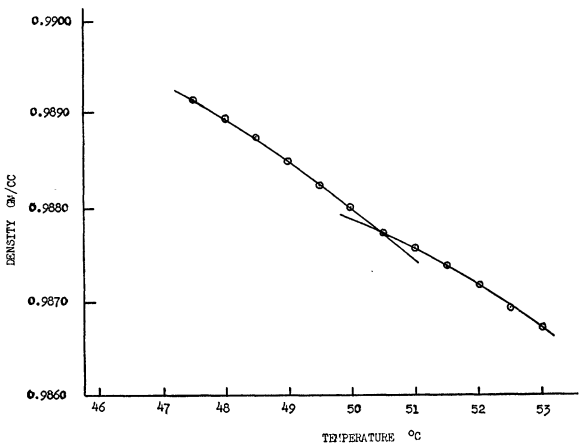

FIG. 1.

by the co-author show that this is not true, and that kinks are easily noticed in the density-temperature curve for ordinary distilled water. A 25-cc pycnometer was

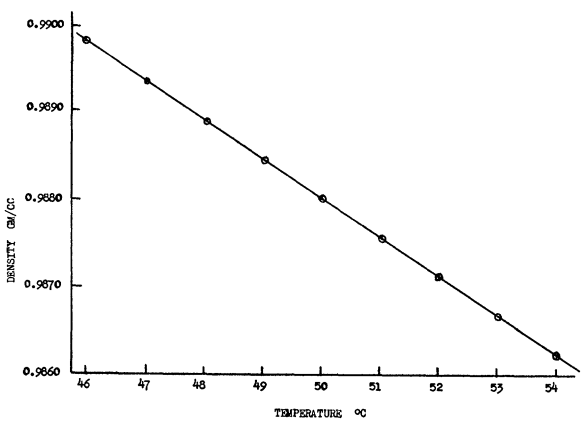

FIG. 2.

used in these measurements, and densities determined for the range of temperatures from 47° to 53° C in a thermostat regulated to .002° C. There is a smooth stretch of the curve between 47° and 50° and another between 50° and 53°. These intersect just a little above 50° C, giving a well defined kink (see Fig. 1). Below 47 another smooth stretch of the curve begins, which is not shown on the drawing. Fig. 2 was drawn from the data given in the Smithsonian Tables. As may be seen, the graph is perfectly smooth with only a slight curvature.

There can be no doubt that the experimental results were smoothed by applying the generally accepted rules for drawing a representative curve amidst erratic points due to experimental errors. In this case, however, it is not legitimate to do so because, as explained, the effect is well above the limits of experimental errors, and, on a drawing of the scale shown, the errors do not appear.

This is not the only case in which the figures given in tables are adulterated. There are some instances, especially in the study of liquids, in which the entire experimental work ought to be done afresh.

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A Study of Gastric HCl Formation[1]

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The mechanism whereby parietal cells secrete HCl against a concentration gradient has previously been investigated in preparations of isolated gastric mucosa (*6–10*). In the present study a modification of earlier apparatus has been introduced to permit simple continuous measurement of pH difference across the wall of the isolated stomach of the rat. The experimental procedure entailed the opening of the abdominal wall under barbiturate anesthesia and the injection, into the exposed stomach, of chilled phosphate buffer at pH 7.4. The entire stomach was then removed, opened along the lesser curvature, and rinsed with several changes of the solution with which the apparatus was to be filled. The rugated portion of the stomach was so clamped between the smoothly ground faces of two half-cells (Fig. 1) that it served as a membrane separating the apparatus into two compartments. Ten ml of solution (see below) was then pipetted into each half-cell and a glass electrode immersed on each side. O_2 saturated with water vapor was bubbled through both sides to effect oxygenation as well as mixing. The potential difference generated between the two glass electrodes was read on a Beckman pH meter (Model H). More stable readings were obtained when the cell was placed in a grounded metal box and the housing of the meter was grounded. It was repeatedly observed that the pH difference, as computed from the total potential difference, and as calculated from individual determinations of pH on both sides of the membrane, agreed within 0.2 pH unit. With the development of a pH difference, it was invariably found that the solution in contact with the mucosal surface became acid and simultaneously the solution in the serosal compartment became alkaline.

In every experiment the solutions introduced on both sides of the membrane were initially identical, and when

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