flasks. A vacuum of 0.1–0.2 cm. is maintained in the lyophil by means of a Wegner vacuum pump.

As soon as the samples are frozen, dry ice is added to the methyl cellosolve in the condenser cone of the lyophil. The ice is added cautiously in order to obviate the excessive boiling which is otherwise encountered. No more ice than can be

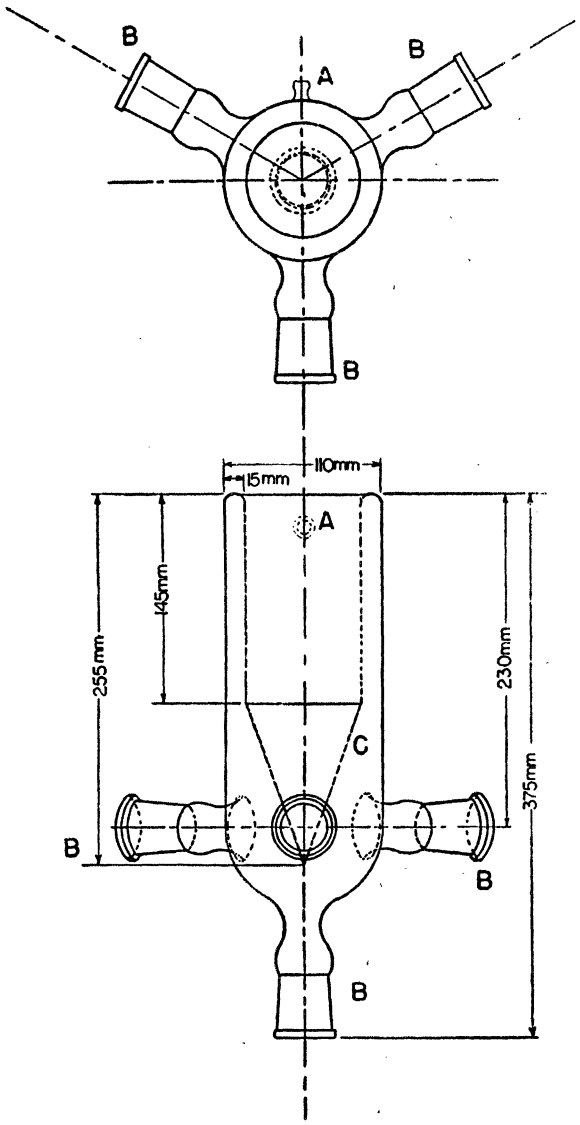

FIG. 1. Diagram of lyophil apparatus showing the side and cross-section views.

covered by the cellosolve is used at this stage, but the mixture is permitted to become cold before the flasks are put on. The ground-glass joints are well lubricated with a light vacuum grease.[2] The flasks are then placed quickly on the lyophil. Care is taken, however, to observe that each joint becomes transparent in order to be certain of good contact. A sealed-off standard taper is put in place on the joint at the bottom of the apparatus before the freezing is started. During the first 20–30 minutes the lyophil is watched rather carefully until the ice or frost begins to accumulate on the condenser cone. This occurs soon after the peak of foaming in the flasks is reached. Although the condenser cone must be kept as cold as possible during this time, it should not contain too much dry ice because considerable boiling occurs when the condensation begins. After the onset of condensation, the condenser cone should be kept full of dry ice.

Once the ice has formed on the outside of the flasks, the apparatus needs attention only at intervals of approximately one hour. The time of drying depends for the most part on the following factors: (1) size of the sample; (2) concentration of the sample; and (3) temperature and relative humidity of the room. The following results were obtained with urine taken to dryness:

Total volume of samples (cc.)	Specific gravity	Time of drying (hrs.)
100	1.095	9½
200	1.016	8
400	1.014	10
400	1.006	8
400	1.025	9

This method of concentrating or drying urine of varying concentrations is satisfactory from a number of standpoints: (1) The apparatus is simple and of moderate cost; (2) the process requires little attention once the initial steps are taken; (3) concentration of the urine is done at below-zero temperatures, which are unfavorable to chemical alterations in the constituents of the urine.

The application of the method to the extraction of steroids is now being studied.

Elementary Isotopic Analysis: Determination of Carbon

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We have recently described a new method for the determination of oxygen (*1*) in organic compounds by using heavy oxygen. Using the same method, higher precision is possible in the determination of carbon, since C^{13} is now available (from our two thermal diffusion plants in Marcus Hook) in much higher concentration (10–15 per cent) than the oxygen isotope (1.2 per cent).

In order to demonstrate the increased precision obtainable with our method when using higher isotope concentrations and to extend this method to carbon, we have made the following three carbon determinations on two representative pure compounds, namely, acetic acid (m.p. = 16° C.) and iso-octane (n_{20}^{D} = 1.3916). The $C^{13}O_2$ used had a concentration of 12 atom per cent C^{13}.

A known weight of substance (= a) was burned in a platinum tube with a slight excess of O_2, as described previously (*1*), and simultaneously equilibrated for 30–45 minutes with a known volume of pure isotopic CO_2 (= b). The acetic acid was

[2] Celvacene-Light Vacuum Grease, Distillation Products, Inc., Rochester, New York.

burned at about 500° C., the iso-octane at 750° C. The per cent carbon was calculated from the formula: $x = 100\ b(m - n)/an$, where m and n are, respectively, the initial and final atomic per cent *excess* of C^{13} in the CO_2.

The results were as follows:

	Acetic acid		Iso-octane (C_8H_{18})
	Preliminary run	Final run	
a, mg. substance	20.47	24.04	20.96
b, mg. C, added as CO_2 (using atomic wt. of C = 12.01)	7.90	11.76	11.10
m, % excess of C^{13} above normal (= 1.04%) in b	11.18	11.20	11.24
$C^{13}O_2$-content of equilibrium mixture	6.39	7.19$_5$	5.37$_0$
	6.41	7.19$_0$	5.40$_0$
	6.36	7.20$_5$	5.37$_5$
Average	6.39 ±0.02	7.19$_7$ ±.006	5.38$_1$ ±.012
n, avg. % excess of C^{13} in equilibrium mixt.	5.35	6.15$_7$	4.34$_1$
% C in substance, experimental	42.1	40.07	83.50
% C, theoretical	40.00	40.00	84.19

A mass spectrograph constructed by the Consolidated Engineering Corporation (Pasadena, California) was used. The average precision obtainable is ±0.3 per cent, based on the carbon content.

A detailed description of this work will be published elsewhere.

Reference

1. Grosse, A. V., Hindin, S. G., and Kirshenbaum, A. D. *J. Amer. chem. Soc.*, 1946, 68, 2119.

Immobilizing Paramecia for Study in the Introductory Course[1]

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A laboratory study of paramecia for the biology student may be highly instructive and of special motivational value if it comes early in the course. Because of its large size and clear organelles, the paramecium has long been a standard form for the study of protozoa in introductory courses. However, its constant, rapid locomotory activity is often discouraging to elementary students in their efforts to study its structure. Therefore, many methods have been suggested for its immobilization, most of which involve either (1) mechanical restraint, such as entanglement in cotton or lens-paper fibers, (2) immersion in some viscous solution such as agar, gelatin, tragacanth, and quince-seed jelly, or (3) narcosis with chloretone, isopropyl alcohol, and nickel chloride. The first method merely impedes locomotion; the other two either are unsatisfactory immobilizers or interfere with the normal physiology of the animal.

[1] Contribution No. 2 of the Department of Biological Science.

However, immobilizing paramecia has been made relatively easy in recent years with Methocel[2] (methyl cellulose), suggested by Marsland (*3*), or polyvinyl alcohol, described by Moment (*4*). The authors have made comparative studies of these two agents for quieting paramecia and prefer the former, chiefly for the reason that some difficulty was experienced in obtaining clear, homogeneous solutions of polyvinyl alcohol, whereas uniformly good success was enjoyed with Methocel.

Doubtless, Methocel has not been used as generally as it deserves to be by biology instructors, for during World War II it was available only for essential uses. Now, however, it is obtainable for all purposes.

Our technique differs somewhat from that of Marsland. Ten grams of Methocel, with a viscosity rating of 15 centipoises, are mixed with 90 ml. of distilled water. The viscosity of Methocel solutions in centipoises is based on the viscosity of a 2.0 per cent aqueous solution at 20° C. The centipoise values of the six viscosity types are: 15, 25, 100, 400, 1,500, and 4,000. The Methocel is dissolved most easily by first mixing it thoroughly with half the required water at boiling temperature and allowing it to soak for 20–30 minutes. The remaining water may be added as cold water. The mixture is then cooled to room temperature and stirred until smooth. Methocel solutions of maximum clarity are obtained by reducing the temperature to 10° C. after thoroughly wetting the Methocel fibers with hot water. Methocel is unique among the hydrophilic colloids in that solutions of it gel on heating.

Solutions may also be prepared by stirring Methocel in water at room temperature. If bubbles are observed, the mixture should stand until these disappear. The advantage of an initial hot-water treatment is that it hastens dispersion, and cooling to 10° C. insures a haze-free, colorless solution. Aqueous solutions of Methocel are stable on long standing and usually do not require a preservative.

After much experimentation with Methocel, we find that the most satisfactory method of making preparations for laboratory studies is as follows: Make a ring of Methocel about $\frac{1}{8}$ inch wide, with a diameter a little smaller than a $\frac{7}{8}$-inch cover glass; place a small drop of paramecium culture in the center of the ring; then add cover glass. This gives the observer an opportunity to see the unhindered movements of paramecia before they are slowed down by diffusion of methyl cellulose. As the Methocel diffuses, there is a progressive increase in viscosity of the culture medium which gradually slows the paramecia until they are practically immobilized. Brown (*1*) used 5 per cent Methocel for the ring on his slides, but he does not give the viscosity type used.

Under the conditions provided by a Methocel preparation, a paramecium can be observed leisurely and critically. The activities appear to be normal but much slower, and such preparations have the fascination of a slow movie for beginning students. The Methocel technique also makes possible an excellent demonstration of Brownian movement of protoplasmic granules in a paramecium.

Instead of using carmine to observe the passage of food vacuoles throughout the body, as Marsland (*3*) suggests, we use a mixture of yeast and congo red, recommended by Buck (*2*) for immobilizing paramecia. While we discontinued Buck's method of quieting paramecia, because it did not always suffi-

[2] Methocel used in our experiments was generously supplied by the Dow Chemical Company, Midland, Michigan.