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

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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.

ciently immobilize, we found it excellent for observing physiological processes.

Various stages of feeding can be observed, including the ciliary beat and currents into the gullet, and the formation of the food vacuole, its elongation and pinching off, and its course through the cytoplasm. The principle of cyclosis is well illustrated by the movement of the beautifully colored food vacuoles in the cytoplasm. Inasmuch as congo red is a chemical indicator, it gives information regarding the chemical changes occurring in the food vacuoles. Usually the food vacuole is bright red when it is first formed, then changing to blue, which indicates an acid reaction (pH 3.0 or below), then to purple in the intermediate stage, and finally to an orange-red, which appears at a pH of 5.2 or above. Actually, the food vacuole is known to be alkaline in the later stages of digestion.

Even the beginning biology student with little interest in biology and inexperienced in microscopy finds it exciting to observe the pulsating vacuole, gullet, undulating membrane, cyclosis, changes in the color of the food vacuoles, defecation, trichocysts, and other features of physiology and structure. In fact, it has been reported that these studies of paramecia create so much interest and enthusiasm for beginning students that they are a common topic for dormitory discussion.

References

1. Brown, R. B. *Science*, 1944, **100**, 62.
2. Buck, J. B. *Science*, 1943, **97**, 494.
3. Marsland, D. A. *Science*, 1943, **98**, 414.
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The Purification of Diphtherial Toxoid[1]

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Highly purified diphtherial toxoid has been separated in this laboratory by methods similar to those employed for the crystallization of tetanal toxin (*7*) and for the purification of tetanal toxoid (*4, 5*). The purified diphtherial toxoid contains between 2,000 and 2,200 Lf/mg. of protein nitrogen. This value agrees with that reported by Pappenheimer (*3*) and by Eaton (*2*) for purified diphtherial toxin. The only diphtherial toxoid of comparable purity which contained 2,000 Lf/mg. of nitrogen is that reported by Boivin (*1*), who employed precipitation with trichloracetic acid. However, Pope and Lingood (*8*) were unable to confirm Boivin's results.

The parent toxoid employed for purification was prepared by detoxifying potent diphtherial toxin produced on a deferrated semisynthetic medium by a special, highly toxigenic strain of *Corynebacterium diphtheriae* (supplied by Lederle Laboratories). The methods employed for the purification are summarized briefly in Tables 1 and 2.

By balancing precisely the charged condition of the toxoid and the solvent effect of the electrolytes with the precipitating action of methanol at low temperatures, diphtherial toxoid separates from nearly all of the culture medium constituents and bacterial products. In fact, as shown in Tables 1 and 2, a single precipitation at pH 5, ionic strength of 0.09 in 40 per cent methanol at −5° C., yields a product containing 900 Lf/mg. of nitrogen. In Method I, the porphyrins which are present in the parent toxoid were removed from Fraction 3P by adsorption on asbestos fibers at pH 6.6. Fraction 4P was dried from the frozen state and dissolved to a syrup in 0.04 M sodium acetate, the pH adjusted to 5.6 with acetic acid, and the precipitate discarded. The supernatant was adjusted to pH 5.3 at

TABLE 1

Preparation	Conditions of separation				Lf/mg. N	Kf* (min.)	Yield (% of parent toxoid)
	pH	Methanol (%)	Ionic strength	Temperature (C.)			
Parent toxoid	—	—	—	—	10	45	—
1 P†	5.0	40	0.09	−5	900	30	100
2 P	5.35	25	0.11	−5	1,280	25	95
3 P	5.85	40	0.11	−7	1,440	25	95
4 P	5.4	40	0.06	−5	1,600	20	85
5 S‡	5.6	—	0.04	0	1,740	20	75
6 S	5.3	—	0.03	0	2,090	20	70

* Tested at 35 Lf units.
† P indicates precipitate.
‡ S indicates supernatant.

0° C. and the precipitate again discarded. Fraction 6S contains between 2,000 and 2,200 Lf/mg. of nitrogen and exhibits a much shorter Kf than that of the parent toxoid when tested at identical Lf concentration. On standing at 0° C., poorly defined protein crystals formed in Fraction 6S. The precise conditions necessary for crystallization are being investigated further. An alternative purification procedure, given in Table 2, eliminates both the adsorption of the porphyrins on asbestos fibers and multiple dryings from the frozen state. In this method, the porphyrins and bacterial proteins are largely removed as a precipitate at pH 4, ionic strength of 0.075 in 5 per cent methanol at −2° C. The toxoid principle is then precipitated as indicated under 4P and 5P. Whereas the remaining bacterial proteins are soluble under these conditions, the toxoid

TABLE 2

Preparation	Conditions of separation				Lf/mg. N	Kf* (min.)	Yield (% of parent toxoid)
	pH	Methanol (%)	Ionic strength	Temperature (C.)			
Parent toxoid	—	—	—	—	10	55	—
1 P	5.0	40	0.09	−5	880	40	100
2 P	5.8	40	0.09	−7	1,260	40	95
3 S	4.0	5	0.075	−2	1,840	20	70
4 P	4.1	30	0.05	−6	1,980	20	65
5 P	5.35	25	0.075	−5	2,120	20	65

* Tested at 50 Lf units.

principle precipitates, resulting in a final product containing over 2,100 Lf/mg. of nitrogen. The decreased flocculation time of the purified toxoid as compared with the parent toxoid indicates that no damage to the toxoid principle occurs under the above-stated conditions.

The employment of stabilizing agents and of special precautions in filtration and in drying from the frozen state has

[1] Aided by a grant from the Lederle Laboratories.