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.

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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.

led to the quantitative recovery of a highly pure, stable, antigenic toxoid which should prove suitable for clinical use (*6*).

The purified toxoid is not precipitated by an anti-*C. diphtheriae* rabbit serum and is relatively free of porphyrin. It gives the usual protein reactions, and in general its characteristics are almost identical to those of the purified toxin prepared by Pappenheimer (*3*). The details of the purification procedures, as well as the characteristics of the purified toxoid, will be reported later.

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A Simple Anaerobic Method of Obtaining Plasma

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By the method described here one can obtain plasma for gas analysis without the inconvenience and uncertainty of transferring the blood from the syringe into which it is drawn and

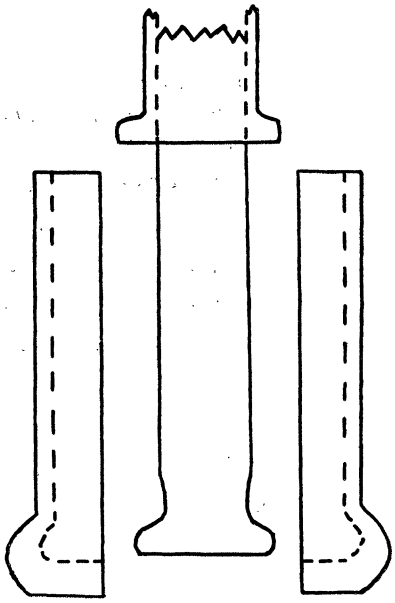

Fig. 1

without the use of oil or mercury. The method is described for a 5-ml. syringe, but syringes of any size can be used.

About 5.5 ml. of blood is drawn into a 5-ml. syringe containing some heparin solution and greased with stopcock lubricant. The needle is removed, and about 1 ml. of blood is delivered for estimation of the hematocrit, pH, etc. A short piece of thick-walled rubber tubing is slipped over the nozzle of the syringe. The two halves of the plastic spacer shown in Fig. 1 are placed around the plunger and held with rubber bands, the plunger then being pushed in until stopped by the spacer. The length of the spacer is such that the syringe contains about 4 ml. of blood. The lumen of the rubber tubing is now full of blood; and while the spacer is held firmly against the barrel of the syringe a glass plug is inserted into the lumen, displacing the blood. The syringe is centrifuged, plunger down, at about 1,500 r.p.m. in a 50-ml. cup fitted with a reducing ring.

When centrifugation is finished, the spacer and the glass plug are removed. The tip of a blood-gas pipette is inserted into the lumen of the tubing, and, by means of gentle twisting pressure on the plunger, plasma is delivered directly into the pipette.

"Braking" Pipettes

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A "braking" pipette is an extremely important tool in many microtechniques. A good braking pipette, because of its slow, controlled rate of flow of air, enables the investigator to pick up several single cells in a measured amount of fluid as small as 0.5 mm.[3]

The conventional type of braking pipette, constructed by pulling a fine, hair-like constriction in a capillary pipette, is very unsatisfactory. If any moisture collects in the constriction, it is almost impossible to clear it. Even if it is baked out, the residue left in the constriction usually changes the characteristics of the pipette, *i.e.* the rate of flow. It is unusual to make two of these with the same characteristics, since a good one is usually the result of a happy accident. An improvement over the constriction pipette has been described by Linderstrom-Lang and Holter (*1*), but their construction does not solve the problem of condensation in the brake, because they use a continuous capillary system such as that used in previous constriction pipettes.

The following two types of braking pipettes have proved easy to make; characteristics of one type can be duplicated in any number of pipettes, and both types are satisfactory in operation.[1]

Type A: The chief advantage of this type, in which a replaceable glass capillary brake is used, is that the brake can be replaced when necessary. For instance, if the pipette is calibrated, and a change in rate of flow is required, the brake can be replaced without recalibrating the tip. Furthermore, the possibility of moisture collecting in the brake is eliminated, in as much as the latter is not part of a continuous capillary system.

A piece of heavy pyrex capillary tubing, 6 cm. long and with an internal diameter of 0.5 mm., is joined to a capillary tube of the same length and external diameter but with an internal bore of 1.0-mm. diameter. The end of the 1.0-mm. capillary tube is flared as shown in Fig. 1a. A 0.3- to 0.5-mm. capillary

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