tion between strength of dosage and rate of recovery. In one series, for example, where the time of exposure was always 5 sec, animals became motile in less than 1 min after 0.5 v, in 1–2 min after 1.0 v, in 2–5 min after 2.5 v, in 5–10 min after 5.0 v, and after 20–30 min shocking at 10 v. These data suggest a roughly straight-line relationship between dosage and time necessary for recovery. Significantly, all these swimming larvae survived, even at the higher dosages.

If used judiciously, electronarcosis of amphibian larvae and certain other small aquatic larvae is a valuable tool for the laboratory experimentalist. It is a quick, convenient substitute for chemical anesthesia. Stages of *Amblystoma* prior to completion of gastrulation are particularly susceptible to injury by electric shock. Subsequent to this time, however, animals become increasingly resistant and are able to tolerate a fairly wide range of dosages. It is possible to select dosages that will immobilize swimming larvae for roughly predictable periods without apparent permanent injury.

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Spectrophotometric Determination of Hemoglobin and Oxyhemoglobin in Whole Hemolyzed Blood[1]

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The difficulty of performing spectrophotometric analysis anaerobically has prevented the wide application of this method to determination of oxyhemoglobin (*1*). In view of this fact a lucite cuvette,[2] allowing for anaerobic analysis, was built to fit the Beckman D. U. spectrophotometer. It consists (Figs. 1, 2) of two lucite plates, a metal gasket 0.01 cm thick, and supporting plates of nickel-plated brass. The metal gasket and plates contain 4 apertures (so that when they are clamped in place 4 chambers are formed between the lucite plates, which are 0.01 cm thick and conform in shape and area to the standard Beckman cuvette holder). For filling and cleaning purposes, 8 L-shaped holes, one to serve as an inlet and one as an outlet channel for each of the 4 chambers, are drilled through one of the lucite plates so as to end on each side of a lateral wall of each chamber. These L-shaped holes, which are hidden in a frontal view by the metal plates of the cuvette, may be seen in a side view (Fig. 2). Filling of the chambers with blood is performed by inserting into the inlet channel a 20-gauge rubber-tipped needle attached to a syringe containing the blood sample. Coagulation is prevented by filling the dead space of the syringe with an anticoagulant solution (heparin, 10 mg/ml) before withdrawal of the blood sample. Hemolysis of the blood, which is necessary in order to obtain a clear solution (*1–3*), is produced anaerobically by adding to the blood sample 1/100 of its volume of saturated solution of saponin Merck. This procedure is performed by inserting into the sampling syringe a rubber-tipped needle fitted to the tuberculin syringe containing the saponin.

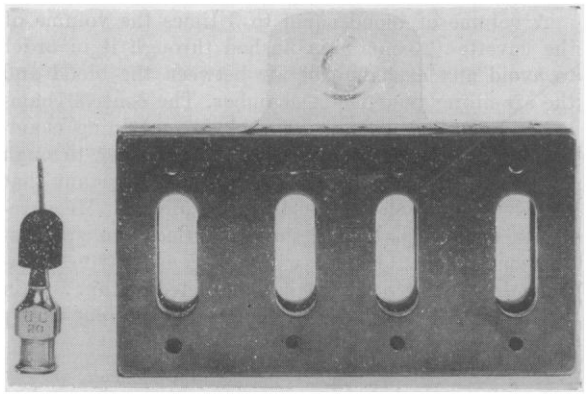

FIG. 1. Front view of cuvette with needle used for filling chambers.

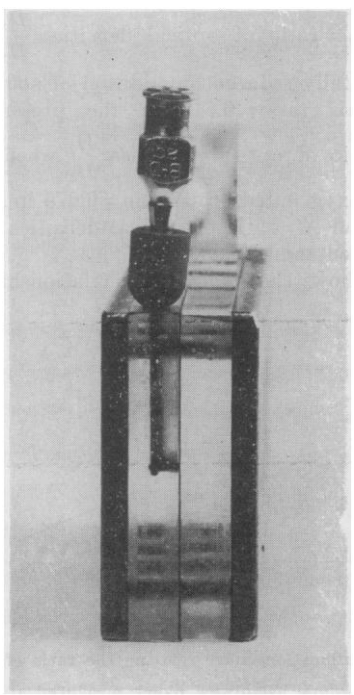

FIG. 2. Side view of cuvette with filling needle in place.

[1] The author wishes to acknowledge the cooperation of E. H. Wood, under whom this study was made, of M. H. Power, and of the Section on Engineering of the Mayo Clinic.

[2] Manufactured by the Waters Conley Company, Rochester, Minn.

A volume of blood equal to 3 times the volume of the cuvette (20 mm^3) is flushed through it in order to avoid any exchange of air between the blood and the air filling the cuvette chamber. The control chamber is left filled with air. The blood-containing chambers are cleaned by flushing distilled water through them, and are dried by suction. It is important that the determination be performed quickly. Measurements of optical densities on the Beckman spectrophotometer should start within 3–5 min after withdrawal of the blood and be completed within 3 min (*3*). The slit width used during the present study varied from 0.015 to 0.020 cm.

Hemoglobin concentration is determined by measuring the optical density D_1 of a sample of blood at 505 mμ, an isobestic point for oxyhemoglobin and reduced hemoglobin. The formula derived from the Lambert-Beer laws of absorption is then applied:

$$C = D_1 \cdot \frac{1}{KL}$$

where C = concentration of hemoglobin (g/ml); D_1 = optical density of blood at 505 mμ; K = extinction coefficient of hemoglobin and oxyhemoglobin at 505 mμ; and L = depth of cuvette in cm. In order to calculate the constant, K, it is necessary to make one simultaneous determination of hemoglobin concentration by a standard method.

The analysis of oxygen saturation depends on the difference in optical densities D_1 and D_2 of hemoglobin and oxyhemoglobin at 505 mμ and 605 mμ. The following formula is used:

$$\% \text{ saturation} = \frac{X_{Hb} - X}{X_{Hb} - X_{HbO_2}} \times 100$$

where X_{Hb} = ratio of optical densities $\frac{D_2}{D_1}$ when the blood is totally reduced by addition of sodium hydrosulfite (this ratio = 0.515 in the present study); X_{HbO_2} = ratio of optical densities $\frac{D_2}{D_1}$ when the blood is totally oxygenated (this ratio = 0.075 in the present study); and X = ratio of optical densities of the unknown blood sample.

Fig. 3 shows the straight-line relationship obtained between % oxygen saturation of blood and the ratio of optical densities. The significance of this relationship is that the slope represents the constant difference of the ratios of optical densities of a completely reduced sample to a completely oxygenated sample.

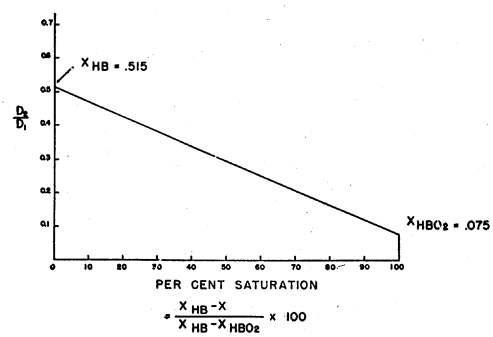

FIG. 3. Calibration curve relating the ratio of optical densities $\frac{D_2}{D_1}$ in whole hemolyzed blood, measured at 505 mμ and 605 mμ, to % oxygen saturation.

TABLE 1

COMPARISON OF SIMULTANEOUS MEASUREMENTS OF OXYGEN SATURATION AND HEMOGLOBIN CONCENTRATION OF BLOOD, BY MANOMETRIC AND SPECTROPHOTOMETRIC METHODS

No. of samples	Analysis	Mean	Range	Mean difference from Van Slyke analysis	Standard deviation of differences
37	Hemoglobin* (g/100 ml)	16.7	13.3–25.4	0.08	0.5
33	Oxygen saturation (%)	81.0	21.5–100.0	0.2	1.9

* Calculated by dividing oxygen capacity (*4*) in vol % by 1.34.

The intercept on the ordinate represents the ratio of optical densities in a sample of completely reduced blood. The procedure allows, therefore, an independent method of calibration in the measurement of oxygen saturation. The standard deviation of the differences between these photometric determinations and the Van Slyke determinations of oxygen capacity and % oxygen saturation of 33 blood samples (varying from 13.3 to 25.4 g/100 ml hemoglobin) was 0.5 g Hb/100 ml and 1.9% saturation, respectively, as shown in Table 1 and Fig. 4.

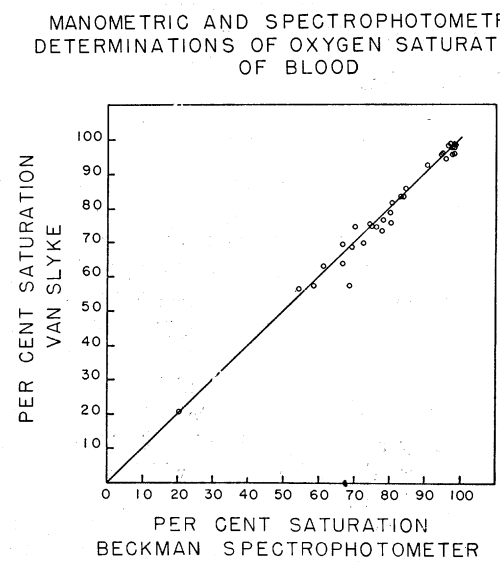

FIG. 4. Comparison of manometric and spectrophotometric determinations of oxygen saturation carried out on 33 samples of blood from human beings.

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Colloidophagy in the Human Thyroid Gland[1]

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Various investigators have observed invasion and ingestion of colloid by macrophages (colloidophagy) in the thyroids of animals. The first to describe this process were Leo Loeb and Gray (*1*). Thurston (*2*), after injection of pituitary extract, saw phagocytosis of colloid in the thyroid of guinea pigs, rats, and pigeons.

According to Eggert (*3*), phagocytes play an important role in the resorption of colloid. After giving large doses of thyrotropic hormone he saw, in the thyroids of lizards, wandering cells enter hypertrophic follicles, ingest colloid, and carry it into the interfollicular vessels. R. G. Williams (*4*) studied living thyroid follicles in transparent chambers inserted in rabbits' ears. He saw in the colloid of some activated follicles 1–20 wandering cells. Their number changed from day to day in the same follicles, eventually disappearing. Thyrotropic hormone increased the activity of these colloidophages; there was no evidence of degeneration of the epithelium in the invaded follicles.

In human thyroids, this process of colloidophagy has never been studied. Although cells have been described repeatedly in follicular lumens, they were regarded as desquamated, degenerated cells of the follicle wall.

During a study of microscopic slides from 435 goiters removed by operation and from 619 thyroids obtained by autopsy, I often found cells lying in colloid—i.e., in 63.9% of surgical goiters and in 16.2% of normal thyroids obtained by autopsy. Almost without exception we noticed groups of lymphocytes in the area surrounding the involved follicles.

These intrafollicular cells varied in number from 1 to 20 in one lumen; they were of large size, their cytoplasm was eosinophilic, and their nucleus was oval or kidney-shaped. They did not resemble thyroid cells at all, and there was no evidence of degeneration of the involved follicles. To discover the nature of these cells, small pieces of fresh surgical goiters were teased with dissecting needles on slides. They were stained supravitally with neutral-red (1:10,000 physiologic salt solution) and examined ½ hr later under the microscope. In 11 of the 23 cases typical macrophages

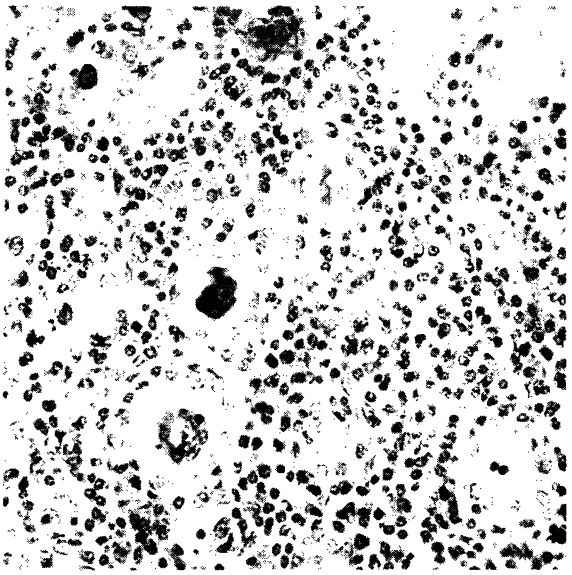

FIG. 1. Normal human thyroid with macrophages in follicles. (Colloidophages, × 400.)

filled with large salmon-red granules were seen within the lumen of thyroid follicles, and in 10 cases they were also present in the interfollicular tissue; the follicle epithelium remained unstained or showed only fine granules.

Although under normal conditions the macrophages, after ingestion of colloid, reenter the blood vessels, two pathological phenomena may result from colloidophagy in human goiter. First, in many exophthalmic and lymphadenoid goiters the macrophages within the follicular lumen fuse together, forming a large multinuclear syncytial mass (Fig. 1); second, macrophages loaded with colloid do not reenter the blood vessels

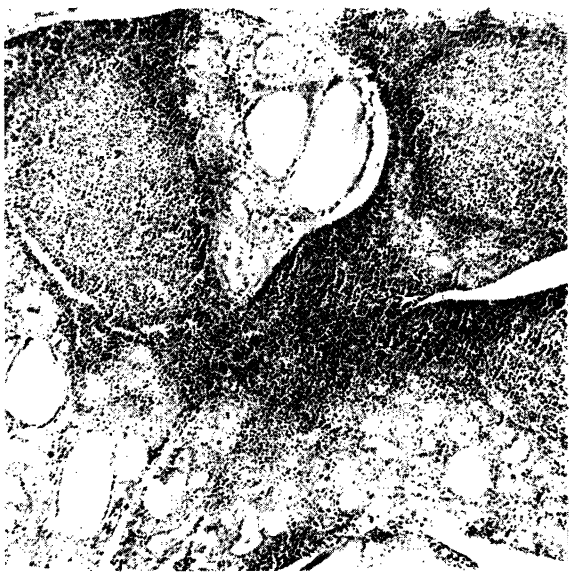

FIG. 2. Human thyroid with lymph follicles containing colloid; so-called chronic thyroiditis. (× 100.)

[1] The electron microscopic study of colloids was aided by a grant from the American Cancer Society.