hydrodynamic factors. There was no significant difference between sucrose and heavy water as solvents, and since Sharp and Beard (*5*) found the same density in heavy water and albumin, it follows that the density is the same, within experimental error, in all three solvents. The same densities were obtained in the angle and swinging tube rotor. The value obtained for the density of the latex sample used, 1.055, is in fair agreement with the reported values of 1.052, 1.053, and 1.054 obtained by other methods.

The density gradient method should, when applied to purification problems, lead to a separation of particles having different densities, irrespective of their size.

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Prolongation of Thiopental Anesthesia in the Mouse by Premedication with Tetraethylthiuram Disulfide ("Antabuse")[1]

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It has been our purpose in this work to investigate certain substances, commonly encountered in clinical practice or occurring naturally, that are potentially capable of prolonging the anesthesia produced by thiopental. Brodie *et al.* (*1*) have shown that thiopental undergoes incomplete side-chain oxidation to a carboxylic acid in its metabolism by the normal mammal. The compounds that fell in our sphere of interest, therefore, have been those that might interfere with such oxidation. Reducing agents such as ascorbic acid and cysteine have, in fact, been found to potentiate thiopental anesthesia, presumably through this mechanism (*2*).

Tetraethylthiuram disulfide ("Antabuse") has achieved some clinical usefulness in the treatment of chronic alcoholism, probably by virtue of its blocking of the oxidation of ethyl alcohol at the acetaldehyde stage (*3*). It has been shown, further, that this inhibition by Antabuse is antagonized by "Ferroascorbin" solution[3] (*4*). The recent report by Richert and co-workers (*5*) that Antabuse can inhibit rat liver xanthine oxidase stimulated our interest in this compound. It seemed possible that Antabuse might prolong thiopental anesthesia through this inhibition. Therefore, the effect of Antabuse on thiopental anesthesia in the mouse, and the influence of Ferroascorbin on this interaction, were studied.

TABLE 1

INFLUENCE OF ANTABUSE ON THIOPENTAL ANESTHESIA IN THE MOUSE AND FAILURE OF FERROASCORBIN TO INHIBIT THIS INFLUENCE

Group	No. animals	Mean duration of anesthesia (min)
1 Thiopental control	20	4.7
2 Antabuse plus thiopental	10	256.2
3 Antabuse plus thiopental plus Ferroascorbin	5	221.5

Three groups of adult male albino mice (Carworth Farms), weighing 23–25 g, were selected at random. The anesthesia time in all three groups was determined for 30 mg thiopental/kg given intravenously. The criterion for recovery from anesthesia was the return of the righting reflex. The three groups were given the following medication prior to the determination of the anesthesia time:

Group 1: No premedication.

Group 2: Premedication with Antabuse.[4] Each animal received a daily oral (by intubation) dose of 25 mg Antabuse for 3 such doses.

Group 3: Premedication with Antabuse, and postmedication with Ferroascorbin. Each animal received Antabuse as in Group 2. On the third day, the injection of thiopental was immediately followed by 0.1 ml Ferroascorbin solution given intravenously to each animal.

The results of these experiments are summarized in Table 1.

This sixtyfold increase in thiopental anesthesia time produced by Antabuse is by far the most powerful effect of this type observed by us in our studies. Determinations of anesthesia time in Group 2 at random during the premedication period revealed no prolongation until after 3 days of the premedication period had elapsed. The ferrous iron and ascorbic acid solution, it may be observed, failed to prevent the Antabuse effect to any significant extent. A 2-week postanesthetic period of observation revealed no toxic abnormalities in the behavior and growth of the experimental animals.

Richert's analysis of his data (*3*) is of especial interest in the light of this work. He has concluded that Antabuse inhibits the oxidase action of xanthine oxidase, which action is responsible for reoxidation of the reduced enzyme by atmospheric oxygen. Appar-

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[3] "Ferroascorbin" solution contained 27.5% $FeSO_4 \cdot 7H_2O$ and 1.25% ascorbic acid in distilled water.

[4] We are indebted to C. E. Glud, formerly of the Department of Pediatrics, Yale University School of Medicine, for supplying us with this material.

ently the dehydrogenase action of xanthine oxidase is not interfered with by Antabuse. In view of these facts, in order for Antabuse to have an effect in the whole organism one might expect some time to elapse in order to allow all the dehydrogenase function to be reduced, before any true inhibition may be observed. This was well borne out by our studies, since we found that a period of 3 days of Antabuse dosing was necessary before the thiopental potentiation became evident. It might be argued that the 3 days were necessary because of the cumulative dosage, but this hardly seems pertinent since the individual doses were massive, the 25 mg/mouse representing about 1,250 mg/kg.

Finally, these data lead to the inference that xanthine oxidase is one enzyme functioning in the metabolism of thiopental to thiopental carboxylic acid. Further work designed to substantiate this inference will be reported later.

It is concluded that Antabuse, possibly through its inhibitory effect on xanthine oxidase, has the ability to prolong markedly the anesthesia induced in the mouse by intravenous thiopental. Pending investigations in higher mammals, it is suggested that thiopental be administered with caution to individuals who are undergoing treatment with Antabuse.

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Infrared Microspectrum of Living Muscle Cells[1]

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The absorption spectra of many animal and plant tissues have been reported in the literature. For the ultraviolet, several authors (*1–3*) have reported successful microspectra of tissue cells and parts of cells, both living and fixed. For the infrared the macrospectra of dried tissues (*4, 5*) and the microspectra of individual dried cells (*6*) have been reported. Preliminary spectroscopic measurements on living muscle cells in the wavelength region from 1 to 15 μ were undertaken by the author to establish the feasibility of using infrared spectroscopy as a tool in studies of living tissues. These measurements are still incomplete, but the data so far collected are presented to show that the difficulties recently reported (*7*) in measuring useful microspectra of living tissues are not insurmountable.

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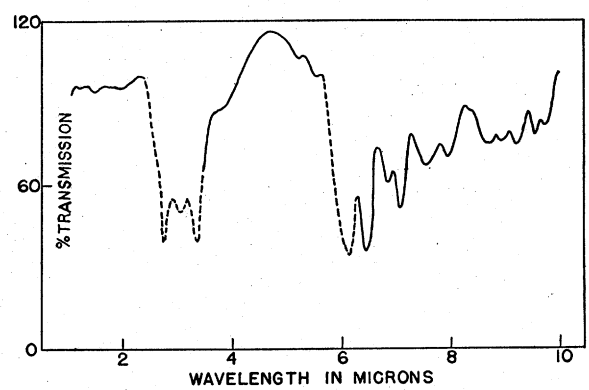
It seemed desirable to use the individual cellular unit of muscle for this work, both because of the difficulty of preparing suitable specimens in any other form, and because in some cases a variation is known to exist in the nature and function of different cells from the same muscle. For successful spectroscopy of such small specimens (20 μ–180 μ diam) in the infrared, it was necessary to use the microspectrometer described elsewhere (*8*), which was originally designed for studies of small fibers.

The specimens were prepared as follows: From the freshly isolated muscle a fiber was teased out under Ringer's solution. The isolated fiber was mounted in Ringer's between transparent plates (fluorite or silver chloride) and placed in the radiation beam of the microspectrometer. The spectrum was then recorded. If the fiber was too small to give a satisfactory spectrum, from 1 to 4 fibers were laid side by side to form a larger absorbing area. The spectra were considered to be representative of the living tissue if the fiber (or fibers) could be made to contract visibly on electrical stimulation after the spectrum had been measured. It was not always possible to measure the whole spectrum before the contractility of the fiber was lost. The interesting region from 6.5 to 10 μ could be examined conveniently in two sections, although it was possible in some cases to get through the whole region without losing contractility.

The principal difficulty in measuring the infrared spectrum of any living tissue is the large ratio of water to protoplasm. It is well known that water is a very strong absorber of infrared radiation. As a result, the absorption bands of the muscle are nearly obliterated by the very intense absorption of the water. The difficulty was resolved in this case by using a spectrometer of maximum sensitivity and measuring the absorption of the cells relative to the absorption of the aqueous suspension medium. The ratio of energy transmitted through the cell to that transmitted through the same thickness of Ringer's solution next to the cell was plotted against wavelength to obtain the spectrum.

The spectrum from 1 to 10 μ for a fiber from the gastrocnemius of a frog is shown in Fig. 1. It was



SPECTRUM OF A MUSCLE CELL FROM THE FROG GASTROCNEMIUS

FIG. 1.