complex configurations in the transient rupturing stress, such as those illustrated by Kies, Sullivan, and Irwin (*4*).

In either case, the fracture traverse exhibits marked characteristics, the interpretation of which must lead to important information concerning the nature of cohesion and dehesion in the solid state.

References

1. ZAPFFE, C. A., WORDEN, C. O., and ZAPFFE, C. *Am. Mineral.*, **36**, 202 (1951).
2. ZAPFFE, C. A. *Trans. Am. Soc. Metals*, **42**, 387 (1950).
3. *Ibid.*
4. KIES, J. A., SULLIVAN, A. M., and IRWIN, G. R. *J. Applied Phys.*, **21**, 716 (1950).

Density of Polystyrene Latex by a Centrifugal Method

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Particulate densities in solution or the reciprocal of the partial specific volume have been determined by a number of investigators by centrifugal means. The method used has involved the determination of the density of solvent for which the velocity of sedimentation of the particles is zero. This was done either by extrapolation of the velocity-density relation from low solvent densities to the iso-density point, by interpolation between low and high densities of solvent, or by experimentally adjusting the density of the solvent to the point at which no sedimentation occurred.

For a review of the work prior to 1942 the reader is referred to the article by Markham, Smith, and Lea (*1*). Similar investigations have since been reported (*2, 3*).

The idea occurred to us of arranging a density gradient in the centrifuge tube in such a manner that the sedimenting particles would concentrate in the central part of the tube at the position where the densities of the particles and solvent were equal. This would be accomplished by establishing a constant density gradient so that in the upper part of the tube the particles would be more dense than the solvent, whereas in the lower part of the tube the particles would be less dense than the solvent. A constant density gradient (linear density scale) between the extremities of the solution would permit by a simple distance measurement the determination of the density at any point in the tube, the density of solvent at the concentration band being the density of the particles.

As a trial material to test the method, polystyrene latex, lot 580G, Dow Chemical Company, was chosen. This has been reported to have a density of 1.052 (*4*),[1] 1.053 (*5*), and 1.054 (*6*).

For centrifugation, the standard 35° angle rotor holding 5-ml tubes and a swinging tube rotor (*7*) holding 1.5-ml tubes were used. The latex, .01% to .03%, was suspended in 5 solutions of graded density, first calculated from known concentrations and then checked by pycnometer measurements. The solvents used were sucrose and heavy water with 1% NaCl added. After centrifuging for 2–4 hr, depending on the gradient in density, the tubes were removed from the rotor and the position of the concentration band was measured. In some cases they were photographed using side illumination to obtain scattering with little direct transmission (Fig. 1).

[1] This value was obtained for material in the dry state.

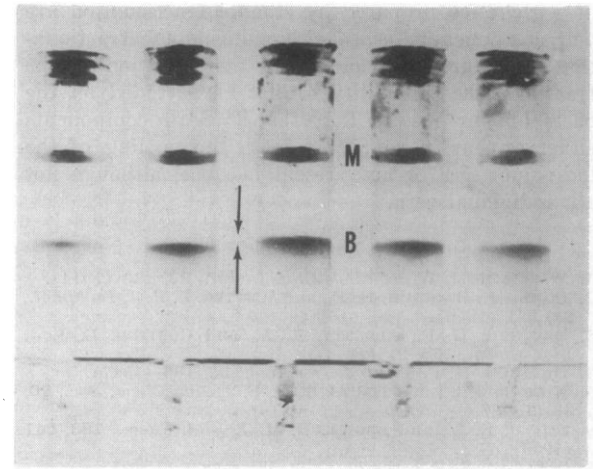

FIG. 1. Five tubes removed from angle rotor and mounted in holder. Tubes contain 5 ml .01% latex in sucrose + 1% NaCl, densities ranging from 1.030 at top to 1.099 at bottom of tubes; centrifuged 2 hr at 500 rps. Position of meniscus at *M* concentrated band of latex at *B* and apparent direction of sedimentation indicated by arrows.

In the swinging tube rotor 6 tubes with heavy water and NaCl as solvent were used, and in the angle rotor 14 tubes were used with sucrose and NaCl. The greatest density range used was 1.030–1.099, and the smallest was 1.052–1.061; the most satisfactory was found to be 1.048–1.065. With too large a range the concentration band is narrow and sharp, but the accuracy is small because a small error in the determination of the band position introduces a large error in the density. With too low a gradient the band is broad and diffuse, and the time of centrifugation must be unduly prolonged.

In the case where the iso-density point of the solvent extends over several millimeters a double band builds up. Each edge of the constant density region acts as a barrier for the sedimenting material, giving a high concentration at the edges of the constant density region and the original concentration in the center of the band, since no motion occurs within the region. The measurement under these conditions is made from the meniscus to the center of the double band.

The value obtained from six runs was $1.055 \pm .001$. No significant difference could be detected between sucrose and heavy water as solvents. The concentration of latex in the central band was of the order of ten times the original concentration.

The gradient density method is simple and rapid and is not appreciably affected by viscosity or other

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.

References

1. MARKHAM, R., SMITH, K. M., and LEA, D. *Parasitology*, **34**, 315 (1942).
2. SHARP, D. G., *et al. J. Biol. Chem.*, **159**, 29 (1945).
3. SCHACHMAN, H. K., and LAUFFER, M. A. *J. Am. Chem. Soc.*, **71**, 536 (1949).
4. WILLIAMS, R. C., and BACKUS, R. C. *Ibid.*, 4052.
5. SHARP, D. G., and BEARD, J. W. *J. Biol. Chem.*, **185**, 247 (1950).
6. SHARP, D. G. *J. Applied Phys.*, **21**, 71 (1950).
7. KAHLER, H., and LLOYD, B. J. *J. Phys. & Colloid Chem.* (in press).

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-

[1] This work has been supported by the James Hudson Brown Fund of Yale University Medical School.

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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.