Extraction of T-1824 in the Presence of Gross Hemolysis and Lipemia. (18417)

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Those who use the dye-hematocrit method (1) find that its many advantages are nullified by hemolysis and lipemia(2). For this reason a number of procedures have been devised for the more or less separate measurement of dye(3-8). The sorption of the blue dye, T-1824, has been described(9) as this occurs on aluminum and magnesium oxides, but the recovery is inconsistent largely because of instability at very low pH.*

T-1824 has been separated from plasma by adding an excess of anionic detergent and extracting with a Cellophane foil in which the optical density was measured(10). The dye was stable, but the routine was laborious. Instead of small foils a column of shredded paper can be used. The dye is sorbed on the column, thoroughly rinsed, then eluted and measured with ease.

Materials and procedures. The sorption column can be prepared from almost any type of cellulose. A dozen sheets of tissue paper† are shredded by brief agitation in a Waring Blendor containing 0.9 g% sodium chloride. The pulp is stored in a flask from which portions are transferred to Büchner funnels having a floor made of a coarse fritted glass disc. The pulp fiber is firmly but not forcibly packed with a plunger, only the excess fluid is drained through and then the head is levelled off by gentle tamping. The column—1.0 cm in length, 1.2 cm in diameter and 0.13 g in dry weight—is ready to receive the dye-tinged plasma which more than 5 minutes previously has been mixed with one-half of its volume of 15 g% sodium "Lorol" sulfate PT.‡ The mixture is either left to drain slowly or else sucked through in about 7 minutes. The test tube and column are rapidly rinsed with 3 successive 2 ml portions of 0.9 g% sodium chloride. Just as the last of the rinse is receding into the column, the funnel is removed from the aspirator flask and inserted into a 10.0 ml volumetric flask. The dye is eluted with 9.5 ml of a 1:1 volume mixture of acetone and distilled water. The first 3 to 4 ml are rapidly forced through by air pressure supplied from a reservoir flask connected through check valves to a hand-bulb pump. During the next one-half or more hours eluent is allowed to seep through the column. The remainder is then forced through, brought to volume, and is ready for measurement of optical density using a Beckman spectrophotometer.

In order to test for consistency of extraction it is necessary to know the purity of the T-1824 preparation. The aqueous solution of dye in lot no. 7 ampules§ contains 4.51 mg dry weight per ml. In this solution there is

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* Observed by J. C. Williams, first year medical student working in this laboratory on a summer research fellowship.

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† Cellulose Wipes, Johnson and Johnson.

‡ "Recrystallized from technical grade of sodium dodecyl sulfate made from a mixture of alcohols—contains some sodium tetradecyl sulfate and less than 0.2% of either sodium chloride, sodium sulfate, or unreacted alcohols," Fine Chemicals Division, E. I. du Pont de Nemours and Co.

§ Furnished by the Warner Institute for Therapeutic Research, New York City.

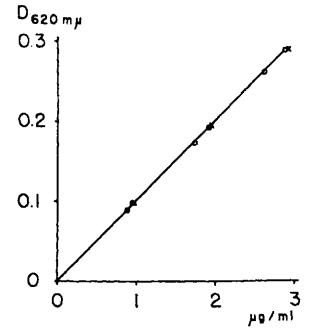

FIG. 1.

Optical density at 620 mμ of various concentrations in μg T-1824 per ml, not corrected for 0.5% impurity. *Crosses*, in 1:1:acetone:water; *circles*, in acetone:water containing 5 volumes % of 0.9 g % sodium chloride. Cuvette depth, 1.000 cm. Spectral interval, 4.8 mμ.

a red impurity(11) which washes through in the rinses and accounts for only 0.49% of the maximum absorption of light. The remainder is accounted for by the T-1824 which is eluted by 30 ml of acetone: water. The ampuled T-1824 when diluted in acetone:water has a mean optical density of 0.1005 per μg per ml. This result was obtained by using an accurate means of making known dilutions. A drop of the T-1824 solution was weighed to the nearest 0.1 mg in a volumetric flask and then brought to the 10.0 ml mark by addition of acetone:water. Three volumetric dilutions were prepared from each of three flasks. The optical density of the various concentrations is illustrated in Fig. 1. It is also shown that the optical density is not affected by the presence of 5 volumes % of saline, which corresponds to the relative volume in the effluent. Since 0.5% of the optical density is caused by impurity, it is concluded that in a 1.000 cm cuvette 1.00 μg of T-1824 per ml of effluent has an optical density of 0.100 at 620 mμ wave length of light with a spectral interval of 4.8 mμ. The variance of measurement has a standard deviation of 0.0011.

Recovery from "clear" plasma. Small weighed amounts of the stock solution of dye were brought to a volume of 10.0 ml by addition of plasma in which before mixture the optical density was 0.025 in the dog plasma and 0.048 in the human plasma. The concentration of dye after correction for impurity was 21.20 μg per ml in dog plasma and 18.08 μg per ml in human plasma. An accurately graduated pipette was used in transferring small volumes, 0.10 ml to 2.00 ml of the dye-tinged plasma to test tubes containing one-half these volumes of 15 g% "Lorol" sulfate. Nine or 10 determinations were made on each solution, and the recovery of various amounts is shown by the circles in Fig. 2.

Recovery from lipemic plasma. Several hours after the subject had consumed gross amounts of fatty food, blood was withdrawn, mixed with heparin and centrifuged in order to obtain the plasma. As compared to water the optical density at 620 mμ was 0.438 in human and 0.231 in dog plasma. The lipemic plasma was mixed with dye, and the resulting concentrations were 21.09 and 23.98 μg per ml in the dog and in the human plasma. The recovery from lipemic plasma, as shown by the crosses (Fig. 2), is not significantly different from that of clear plasma.

Recovery from hemolyzed blood. Clear plasma was obtained from heparinized blood. A separate 4.0 ml portion of this blood was hemolyzed with 0.1 ml of "Lorol" sulfate solution. Then, 0.5 ml of the hemolyzed blood was added along with plasma to the flask used in preparing the dye solution. Although the dye concentration was 19.67 and 19.31 μg per ml in human and in dog plasma respectively, the usual strong blue color was

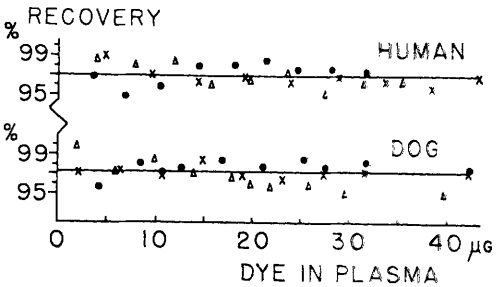

FIG. 2.

Recovery of T-1824 (corrected for impurity), 97.2% with standard deviation of 1.05% from human plasma, 97.0% with standard deviation of 1.11% from dog plasma. *Circles*, "clear" plasma; *triangles*, plasma and hemolyzed blood; *crosses*, lipemic plasma.

11. Leeson, D., and Reeve, E. B., *J. Physiol.*, 1949, v109, 170.

completely masked. The extent of contamination was found by separately diluting the hemolyzed blood and measuring the optical density at 505 mμ(12). The hemoglobin concentration of the dye-tinged dog plasma was 0.76 g% and that of the human was 0.73 g%. The most striking thing is not so much the recovery of dye (Fig. 2, triangles) as the removal of the hemoglobin. A sharp blue band about 1 mm thick is revealed at the top of the column within a few seconds after rinsing begins. From this band some 96.8% of the dye is recovered.

Extent of separation from plasma constituents. The spectral curves of the dye in effluent obtained from the 3 types of plasma were compared with that of dye in effluent obtained by sorbing dye out of saline solution. The maximum optical density always occurred at 620 mμ. The measurements at each indicated wave length from 475 mμ to 700 mμ are in excellent agreement. Between 400 mμ and 475 mμ the dye from plasma has a somewhat greater absorption of light than the dye which was extracted from saline solution. The spectral data from human plasma, which are identical with those of dog plasma, are shown in Fig. 3. These results indicate that the extent of separation is quite remarkable. In fact this procedure can even be employed in the recovery of T-1824 from other biological fluids, such as liver and gall bladder bile.

12. Drabkin, D. L., p. 40 in *Haemoglobin,* Interscience Publ. Inc., New York, 1949.

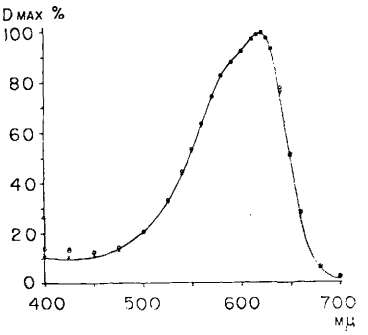

FIG. 3.
Spectral curves: relative absorption of light by T-1824 extracted from saline compared to that by T-1824 extracted from types of plasma denoted by symbols as in Fig. 2.

Summary. Consistent mean recoveries of 97% are obtained by use of a simple procedure for the stable extraction of T-1824. Dye-tinged plasma is treated with a modern soap and then quantitatively transferred to a column of paper fiber. Dye is sorbed on the paper, rinsed with saline solution and eluted with acetone:water. The various steps including the spectrophotometric measurement can be finished in one hour. Spectral curves of eluted dye show that interference from hemolysis and lipemia is removed in the rinse. This procedure improves the versatility and certainty of the dye-hematocrit method for estimation of blood volume.

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Constriction of Thoracic Inferior Vena Cava in Rabbits Following Two-Stage Portal Ligation.* (18418)

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Rapid or gradual constriction of the thoracic inferior vena cava in animals generally produces ascites. The fluid formed by such a procedure is believed to be the result of

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