prepare haem-albumin only when serum albumin was used.

6. Caffeine hemin can be prepared in liquid ammonia. It absorbs at 577 mμ and 548 mμ when it is dispersed in liquid ammonia.

7. Insulin reacts with choline chloride and hemin in liquid ammonia to form a conjugate in which the strong absorption band of insulin at 275 mμ is missing, yet this conjugate maintains a prolongation of lowered blood glucose of 5 times the duration obtained by insulin controls.

16519

Determination of Para-Aminosalicylic Acid in Blood.*

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Since para-aminosalicylic acid has been found to have a definite chemotherapeutic effect in experimental tuberculosis, a simple method for its determination in blood appears desirable. The sulfonamide method in general use[1] cannot be employed without modification. In view of several requests for a method for determination of this substance, and since the slight modification of the sulfonamide method found necessary may prove useful with other aryl amines, we are publishing this short note.

No color is obtained when a solution of p-aminosalicylic acid is subjected to the ordinary procedure for determining sulfonamides. If the acidity of the solution is increased a color is obtained. Maximum color is developed at room temperature when the solution is made about 3 N with hydrochloric acid. The results are, however, not consistent. Diazotization for one minute gives a more intense color than for 3 or 10 minutes. Obviously, either the diazo compound is unstable or a secondary reaction is occurring with the nitrous acid. Diazotization done with the solution at 1°C and made 1 N with hydrochloric acid yielded a more intense color than any other procedure which we have tried. The results under these conditions appear to be quite consistent and reproducible.

Reagents. 1. A solution of trichloroacetic acid containing 15 g dissolved in water and diluted to 100 cc.

2. A 0.1% solution of sodium nitrite.

3. An aqueous solution of N-(1-naphthyl)-ethylenediamine dihydrochloride containing 100 mg per 100 cc. This solution should be kept in a dark colored bottle.

4. 6 N hydrochloric acid.

5. A solution of ammonium sulfamate, containing 0.5 g per 100 cc.

6. A standard solution of p-aminosalicylic acid prepared by suspending 50 mg of the compound in water and dissolving in slightly more than the theoretical amount of sodium hydroxide, and diluting to one liter. Dilutions are made from this stock solution in order to prepare a calibration curve for the colorimeter.

Procedure. Blood or plasma is diluted 1:50 or, if very low values are expected, 1:20. For 1:50 dilution, use 1 part of blood or plasma, 39 parts of water and 10 parts of trichloroacetic acid solution. To 10 cc of the clear filtrate are added 2 cc of 6 N hydrochloric acid, and then the solution is cooled in ice (to about 1°C). One cc of the solution of sodium nitrite is added and after 3 minutes, 1 cc of the solution of sulfamate. The tubes are removed from the ice bath, and after 2 minutes, 1 cc of the solution of N-(1-naphthyl)ethylenediamine dihydrochloride is added. Read-

* This investigation has been aided by a grant from the U. S. Public Health Service.

[1] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

ings are taken with a photoelectric colorimeter after the solutions have stood for 20 minutes in the dark. The zero is set with 10 cc of 3% trichloroacetic acid to which the reagents have been added (6 N hydrochloric acid, nitrite, sulfamate and coupler).

Recovery. For a 1:50 dilution of whole blood, the average recovery of added p-aminosalicylic acid was 90% (86-95); for a 1:50 dilution of plasma, the average recovery was 95% (91-97); and for a 1:20 dilution of plasma, the average recovery was 93% (89-96).

16520

Distribution of 3,4-Dimethyl-5-sulfanilamidoisoxazole in the Body.*

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Sulfanilamide has been found to be distributed in all tissues and fluids of the body in approximately equal concentration.[1] Since the apparent volume of distribution of sulfanilamide is 87 to 98% of the body weight, which is somewhat in excess of the water content of the body (about 65%), it must be localized to a slight degree in tissue.[2] Sulfapyridine is distributed in a manner similar to that of sulfanilamide.[3,4] Sulfathiazole, sulfadiazine, sulfapyrazine and sulfamerazine are distributed in a volume corresponding to 45 to 55% of the body weight,[4] indicating that they penetrate tissues but to a lesser extent.† The data to be presented indicate that 3,4 dimethyl-5-sulfanilamidoisoxazole is distributed differently from any of the sulfonamides in clinical use; it appears to be confined to the extracellular water, with a volume of distribution of the order of magnitude of 25-30% of the body weight.

Methods. Determinations of the distribution of the drug were done on 3 dogs and 4 patients. The drug was injected intravenously; in the dog the sodium salt was used and in man, the lithium salt. About 50 mg per kilo were given in each case. Blood samples were taken at 0.5, 1, 1.5, 2 and 2.5 hours and urine was collected for the first hour after injection. The drug was determined in plasma and urine by the usual sulfonamide method.[5] In man both free and total sulfonamide were estimated; in the dog, no conjugated drug was found. The amount of conjugated drug in either the plasma or urine taken at 1 hour was 3% or less in 3 of the individuals and was about 10% in the other. All calculations have been based on the figures for free sulfonamide. No great error is involved.

Results. The apparent volume of distribution in per cent of body weight was calculated from the data obtained in two ways. The logarithms of plasma concentrations were

* I wish to thank Dr. C. Gordon Zubrod for aid in the studies on patients and Dr. Elmer L. Sevringhaus of Hoffmann-LaRoche, Inc., for supplying the drug used in this study.

1 Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. Pharm. and Exp. Therap.*, 1937, **61**, 196.

2 Waterhouse, A., and Shannon, J. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 189.

3 Marshall, E. K., Jr., and Litchfield, J. T., Jr., *J. Pharm. and Exp. Therap.*, 1939, **67**, 454.

4 Fisher, S. H., Troast, L., Waterhouse, A., and Shannon, J. A., *J. Pharm. and Exp. Therap.*, 1943, **79**, 373.

† In addition to data given in the literature for distribution of these drugs, I have unpublished experiments showing that in the dog sulfanilamide is distributed in 87% and sulfadiazine and sulfapyrazine in 48% of the body weight.

5 Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.