the animals on the high carbohydrate diet. Apparently, diet is a factor influencing the ability of the organism in maintaining a more or less constant concentration of the blood as well as of some of its specific constituents. In spite of our inability at present to suggest the mechanism involved in the reactions reported, the practical bearing on standardization of experimental material is of sufficient importance to justify prompt publication.

9255 P

Determination of Sulfanilamide in Blood and Urine.

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Recently a method was described for the estimation of sulfanilamide (para-aminobenzenesulfonamide) in blood and urine.[1] In the rabbit and human subject this drug is partly excreted in the form of an acetylated derivative.[2] In developing a method for determining this conjugated derivative in blood, we have been able to improve the original procedure.

The present modification possesses the advantages of being more sensitive, of giving a more stable color, and of requiring fewer manipulations. Sulfanilamide can be determined very accurately by this method, and in addition one can obtain a fairly good estimate of the conjugated form present in blood. The present method consists of preparing a blood filtrate with toluenesulfonic acid, utilizing the acidity of the precipitant to perform diazotization and coupling with the amine. In determining the conjugated sulfanilamide in blood, the acidity of the blood filtrate is sufficient for hydrolysis on heating.

One volume of oxalated blood is measured into a flask, diluted and laked with 7 volumes of 0.05% saponin solution.* After laking is complete (1 or 2 minutes) 2 volumes of para-toluenesulfonic acid

[1] Marshall, E. K., Jr., Emerson, Kendall, Jr., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

[2] Marshall, E. K., Jr., Cutting, W. C., and Emerson, Kendall, Jr., *Science*, 1937, **85**, 202.

* Laking can be accomplished by diluting with water instead of saponin solution at least 15 minutes before adding the protein precipitant.

solution (20 gm. dissolved in water and diluted to 100 cc.) are added with shaking. After 5 minutes' standing, the mixture is filtered, and 10 cc. of the clear filtrate (a smaller amount can be used with proportionate reduction of the nitrite and dimethyl-α-naphthylamine reagents) is measured into a small flask and 1 cc. of 0.1% freshly prepared† sodium nitrite solution is added. After 3 minutes' standing, 5 cc. of a solution of dimethyl-α-naphthylamine (1 cc. in 250 cc. of 95% ethyl alcohol) is added from a burette. Ten cc. of a standard solution of sulfanilamide containing toluenesulfonic acid (18 cc. of the 20% solution per 100 cc.) is measured into a flask and treated like the blood filtrate. A 1 mg. % standard is satisfactory for bloods containing from 5 to 20 mg. % of sulfanilamide. The solutions can be compared in a colorimeter at any time from 10 to 60 minutes after the naphthylamine has been added, so that several blood filtrates can be read against one standard.

To determine the conjugated sulfanilamide (probably para-acetylbenzenesulfonamide) in blood, 10 cc. of the toluenesulfonic acid blood filtrate is placed in a tube graduated at 10 cc. and heated in a boiling water bath for 90 minutes. The solution is then cooled and diluted to 10 cc. to replace water loss by evaporation. The procedure is now exactly the same as that used in determining free sulfanilamide. The difference between the values obtained after and before hydrolysis gives the amount of conjugated compound (calculated as sulfanilamide).

If a determination of the sulfanilamide only is desired and toluenesulfonic acid is not available one can use trichloracetic acid to precipitate the blood proteins. One volume of blood is measured into a flask, diluted and laked with 8 volumes of 0.05% saponin solution, and precipitated by adding one volume of 20% trichloracetic acid. The filtrate is treated exactly like that obtained with toluenesulfonic acid, and the standard contains 8 cc. of 20% trichloracetic acid per 100 cc.

For the estimation of the sulfanilamide content of the blood of patients receiving the drug, plasma or serum can be used instead of whole blood as there is no essential difference in the values obtained.

† We prepare the nitrite solution fresh each day. It is advisable to use a very pure sodium nitrite. We have found that Kahlbaum's sodium nitrite obtained from Akatos in New York is satisfactory. We have purchased the dimethyl-α-naphthylamine (No. 1060) and the para-toluenesulfonic acid (No. 984) from the Eastman Kodak Company, Rochester, New York. As stated before it is essential to use all glass cups in the colorimetric comparison as the ordinary cup with a detachable glass bottom in a metal frame may give inconsistent and erroneous results.

Urine is diluted so that it contains about one mg. % of sulfanilamide. Ten cc. of this diluted urine is measured into a flask, one cc. of 20% toluenesulfonic acid added, and then one cc. of 0.1% sodium nitrite. After 3 minutes, 5 cc. of dimethyl-α-naphthylamine solution (1 cc. in 250 cc. of 95% ethyl alcohol) are added. A standard solution of sulfanilamide is treated in the same way. After 5 to 10 minutes, the solutions can be compared in the colorimeter. To determine the acetyl-derivative in urine, one cc. of urine is mixed with 2 cc. of N hydrochloric acid, heated in a boiling water bath for 30 minutes, neutralized with 2 N sodium hydroxide, and diluted to appropriate volume. The determination of total sulfanilamide is then made as described above.

9256 P

Neoplasms in Albino Rats Resulting from the Feeding of Crude Wheat Germ Oil Made by Ether-Extraction.

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The effectiveness of thymus and pineal extract in controlling the rate of growth and development in the young when administered through successive generations of rats led us to attempt the same procedure with the various vitamins. In administering vitamin E we have employed an ether-extracted crude wheat germ oil† prepared by the extraction of fresh wheat germ with ether. All animals fed this preparation of wheat germ oil eventually developed tumors.

The rats used were placed on the stock diet of the Institute, containing the following ingredients: rolled oats 15 parts, cracked corn 60, dried meat scraps 14, powdered milk 10, sodium chloride 1. To each 100 gm. was added 1.25 cc. of cod liver oil. Additional nutriment consisted of green vegetables once a week.

From July 3, 1934, four animals were fed one cc. daily of wheat germ oil, the oil being mixed with the food. On October 29, 1934,

* From the Samuel Bell, Jr., Laboratory.

† This oil was especially prepared for us and kindly donated by E. R. Squibb & Sons. It is not their refined wheat germ oil (Zygon) designed for human use. We wish to express our appreciation to Dr. John F. Anderson for his cooperation in preparing this special oil, and in other ways.