technique is useful in preparing filtrates of tissue extracts, etc. Further work is in progress and a more detailed report will be published elsewhere.

4299

A Method for the Preparation of Blood Filtrates for Analysis.

MICHAEL SOMOGYI.

From the Laboratory of the Jewish Hospital of St. Louis.

The use of zinc salts for the precipitation of blood proteins furnishes a method of preparing filtrates in which fermentable sugar (glucose) is the only substance to reduce alkaline copper solutions. The reducing substances other than sugar—responsible for the "residual reduction" of fermented blood filtrates—are precipitated along with the proteins so that sugar determinations in zinc filtrates give true blood sugar values.

In a simple method of deproteinization we employ the following 2 reagents:

Reagent I. 12.5 g. of zinc sulphate ($ZnSo_4 7H_2O$) are dissolved in water, 125 cc. of 0.25 normal sulfuric acid are added, and made up with water to one liter.

Reagent II is a 0.75 normal solution of sodium hydroxide.

The 2 reagents have to be in such a definite relation that when Reagent I is titrated against Reagent II in the presence of phenol phtalein, 25 cc. of I should require 3.35 to 3.40 of II to produce a permanent pink color.

Procedure: In order to prepare a 1:10 filtrate, lake one volume of blood in 8 volumes of Reagent I, add one volume of Reagent II, stopper tightly, shake well, filter after a few minutes through a dry filter paper. Centrifuge before filtration if a maximum yield of filtrate is wanted.

For the preparation of filtrates of concentrations other than 1:10, we use a somewhat different technique and pair of reagents. Reagent *A* is a 19.5% zinc sulphate solution, Reagent *B* a normal sodium hydroxide. One volume of blood requires one half a volume of each of these reagents for deproteinization. If, for instance, a 1:5 filtrate is desired, lake one volume of blood in 3 volumes of water, introduce one half a volume of Reagent *A*, mix, then add one half a volume of Reagent *B*, stopper tightly, shake well, filter

after a few minutes. The 2 reagents must be so adjusted that 10 cc. of *A* should require 11 cc. of *B* to produce a permanent pink color in the presence of phenol phtalein.

Filtrates prepared by either of the 2 procedures yield sugar values (Shaffer-Hartmann method, modified reagent) substantially identical with the true sugar values obtained from tungstic acid filtrates as the difference of apparent sugar (total reduction) and reducing non-sugars (residual reduction). The zinc filtrates are free from uric acid, glutathione and ergothioneine, do not consume iodine in the presence of acid, and possess no reducing power after treatment with washed yeast.[1] Pure glucose, added to fermented zinc filtrates, is quantitatively recovered. These facts support the view, advanced by the writer in a previous paper[2] that glutathione and ergothioneine represent the bulk of the non-sugar reducing substances in blood.

The non-protein nitrogen values are lower in zinc filtrates than in tungstic acid filtrates. The difference is 8 to 15 mg. per 100 cc. of blood, a figure closely corresponding to the aggregate amount of glutathione, ergothioneine and uric acid, the substances precipitated along with the proteins. The non-protein nitrogen values of zinc filtrates seem to represent urea with creatine and creatinine. Experiments concerning the partition of non-protein nitrogen of zinc filtrates are in progress in this laboratory.

4300

Growth of Rats on Diets Containing Sodium Benzoate.

WENDELL H. GRIFFITH.

From the Department of Biological Chemistry, St. Louis University School of Medicine.

The increase in weight of young male white rats during a 40 day experimental period on diets containing sodium benzoate has been determined. The food consumption was regulated so that all of the rats received equal quantities of food of equivalent calorific value. The increase in weight of rats receiving the basal diet plus 1.5, 2.0 or 2.5% sodium benzoate was practically the same as that of rats receiving the basal diet. On the diet containing 3% sodium benzoate one-third of the rats died and the growth of the remainder

[1] Somogyi, *J. Biol. Chem.*, 1928, xxviii, 9.

[2] Somogyi, *J. Biol. Chem.*, 1927, lxxv, 33.