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Modification of Colorimetric Method for Determination of Mannitol and Sorbitol in Plasma and Urine.

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In this note is presented a modification of the method of Corcoran and Page[1] for the determination of mannitol which, in our hands, has proven simpler and more accurate than the original procedure. The method is applicable for the determination of sorbitol.

Reagents. 1. Periodic acid reagent: potassium periodate 0.0075 M in 0.25 M sulfuric acid.

2. Stannous chloride reagent: 0.035 M stannous chloride, reagent grade, in 0.33 M HCl. This reagent retains its strength for 4 days if kept in the refrigerator. After this time it results in blanks of high and variable density.

3. Chromotropic acid reagent. 0.15 g of chromotropic acid, purified by the method of Boyd and Logan[2] are dissolved in 20 cc of approximately 9 M sulfuric acid in a 200 cc volumetric flask. The contents are then made up to volume with concentrated (18 M) sulfuric acid. The first 20 to 30 cc of the concentrated acid should be added slowly, and with the flask in a cold water bath, to prevent heating the reagent. The density of the final reagent is approximately 0.010 at 570 mμ. It remains stable for many weeks if kept in the dark in a refrigerator.

4. Sulfuric acid, approximately 10.5 M.

Procedure. Mannitol-containing solutions should be preserved by the addition of a small amount of benzoic acid and kept at room temperature. Preservation in a deep freezing unit at —20°C may affect the determination by production of more color in the chromotropic reaction than corresponds to the liberation of 2 moles of formaldehyde per mole of mannitol.

Plasma filtrates are prepared as described by Somogyi.[3] Urines are diluted with water. The 2.0 cc sample should contain 7.0 to 40.0 μg of mannitol or sorbitol. The reagent blank consists of 2.0 cc of distilled water and is carried through the same procedure as the mannitol-containing solutions.

The procedure is carried out as follows: 2.0 cc samples of unknown, standards and water are pipetted into test tubes of 25 cc capacity. To each tube is added 0.5 cc of the periodic acid reagent and the contents are immediately and thoroughly mixed with a stirring rod. The tubes are then allowed to stand for 8 minutes at room temperature. The subsequent steps in the procedure should be carried out without interruption or delay.

After 8 minutes, 0.5 cc of stannous chloride reagent is added to each tube, the reaction mixture again well mixed, and the tubes are placed in a cold water bath. To each 5.0 cc of chromotropic acid reagent are added and the contents are mixed. It is advisable to add the chromotropic acid reagent to the blank before adding it to the mannitol-containing solutions. The tubes, with stirring rods in place, are immersed in a boiling water bath for 30 minutes. After removal and cooling, 10.0 cc of 10.5 M sulfuric acid are added to each tube, the contents are thoroughly mixed and the stirring rods removed.

The solutions are read in a Coleman spectrophotometer against distilled water at 570 mμ. The color is very stable, no change in density occurring if the tubes are permitted to stand for several hours before reading.

Calculations. Glucose, under the conditions described, is oxidized to formaldehyde to a slight and constant extent. By com-

[1] Corcoran, A. C., and Page, I. H., *J. Biol. Chem.*, 1947, **170**, 165.

[2] Boyd, M. J., and Logan, M. A., *J. Biol. Chem.*, 1942, **146**, 279.

[3] Somogyi, M., *J. Biol. Chem.*, 1945, **160**, 69.

paring the densities of glucose-free mannitol standards with standards containing known amounts of glucose, the color production by glucose can be determined and correction for glucose content of the sample applied. In our experience the density per μg of glucose is 0.0024, as compared with 0.0142 per μg of mannitol. This value for glucose remains remarkably constant with varying proportions of glucose in the sample.

The density per μg of mannitol varies within $\pm$ 2% in the range of 10 to 30 μg of mannitol. For greater accuracy or for quantities beyond these limits, a calibration curve should be prepared.

Calculation of the mannitol content of the sample is as follows:

$$\frac{D_O - D_B - (K_G \times \mu g \text{ glucose in sample})}{K_m} = \mu g \text{ of mannitol in sample.}$$

Where D_O = observed density
D_B = density of blank
K_G = density per μg of glucose
K_m = " " " " mannitol.

Summary. A simple and accurate procedure for the determination of mannitol and sorbitol based on the method of Corcoran and Page has been described.

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Hemagglutinins in the Serum of Mice of Low and High Mammary Tumor Strains.

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The purpose of this communication is to report striking differences in the distribution of antisheep agglutinins in the serum of mice belonging to different inbred strains.

Preparatory to studying the response of inbred mice to antigenic stimulation, it became necessary to investigate natural hemagglutinins for sheep red cells, one of the antigens used. Gorer[1] reported the presence of isoagglutinins in mice, but no reference was found in the literature to natural heteroagglutinins in mice, although many investigations dealt with such natural antibodies in other species.[2]

Material. Healthy stock animals of 4 strains were used: 100 C57 black, 73 C3H, 123 dba, and 30 Marsh-albino animals.* The sex distribution was as follows: C57 black, 59 males and 41 females; C3H, 23 males and 50 females; dba, 32 males and 91 females; Marsh-albino, 9 males and 21 females. The age of the animals ranged from 10 weeks to 20 months, with the majority from 3 to 8 months. There was no apparent relation between the agglutinating ability of the mouse serum, and the sex or age of the animals, as represented in our material.

Technic. The animals were decapitated by a rapid stroke of sharp scissors. By holding the severed parts over a funnel, the blood was caught in small test tubes. The tubes were kept slanting until the blood clotted, left in the icebox for 18 to 24 hours, and then the serum was separated from the clots. The serum was inactivated in the water bath for 30 minutes at 56°C. The titration of the antisheep agglutinins followed the method used by the senior author in previous work.[3]

1 Gorer, P. A., *Cancer Research*, 1947, **7**, 634.

2 (a) Kolle and Wassermann, *Handbuch der pathogenen Mikroorganismen*, 1929, **3**, 351, 784; (b) Shimidzu, T., *Tohoku J. Exper. Med.*, 1932, **18**, 526.

* The animals were either bred in this laboratory from parent stock received 3 years ago through the courtesy of Dr. A. Tannenbaum, Michael Reese Hospital, Chicago, or were received directly from Dr. Tannenbaum.