of the phospholipid molecule, hydrolecithin should not be susceptible to auto-oxidation.

The hydrolecithin was used as a substrate with the enzyme preparation previously described. The results of typical experiments are recorded in Table II.

Discussion. Since acetoacetic acid is not metabolized by the homogenized rat liver preparation, and there is no inhibition of acetoacetic acid formation when octanoate and phospholipid are added to the same preparation, the failure of the oxidizing phospholipid to produce acetoacetic acid indicates that free fatty acids are not liberated from the phospholipid in the preparation employed.

It was found that the rat liver homogenate was able to dehydrogenate phospholipid in the presence of ATP, as measured by the Thunberg technic. The aerobic reaction, however, did not result in an increased phospholipid iodine number, and it was found impossible to determine what changes might have occurred in the fatty acids of the phospholipid as a result of the oxidation. Nevertheless, the fact that both free fatty acids and phospholipid are oxidized by the same enzyme preparation prompts the suggestion that this new oxidative reaction of phospholipid is somehow related to intermediary fatty acid metabolism.

Summary. Beef brain phospholipid and beef lung hydrolecithin are oxidized by rat liver homogenates in the presence of adenosine triphosphate. Although this preparation oxidizes free fatty acids with the production of acetoacetic acid, the phospholipid oxidation does not result in such a product.

17031

Effects of Glucose Fermentation Products on Determination of Mannitol by Periodate-Titrametric Method.

A. B. KENDRICK, W. P. SWISHER, AND R. A. FORREST. (Introduced by R. W. Keeton.)

From the Department of Medicine, College of Medicine, University of Illinois, Chicago.

When mannitol clearance and maximal glucose reabsorptive capacity are measured simultaneously in studies of renal function,[1,2] it becomes necessary to determine mannitol in the presence of high concentrations of glucose in both plasma and urine. Satisfactory recoveries of mannitol under these conditions have been reported by others,[2] but certain discrepancies have been noted by us. This report deals with a) the extent of interference of glucose fermentation products with the determination of mannitol, b) a correction factor, and c) the nature of the interfering substance.

Glucose was added to water in varying concentrations from 0 to 1250 mg %. The increases were made in steps of 50 mg %. The solutions were then analyzed for mannitol by the method of Smith, Finkelstein, and Smith[3] except that $CdSO_4$-NaOH[4] was used instead of $ZnSO_4$-NaOH as the precipitating reagent, and the time of fermentation was extended to 2 hours. The method entails fermentation of glucose from the samples by yeast, removal of yeast by centrifugation, precipitation of the proteins, oxidation of the mannitol in the filtrate with periodic acid, and the determination of the excess periodic acid, together with the iodic acid formed, by titration with sodium thiosulfate. All of the filtrates used in our determinations were analyzed quantitatively for glucose[5] to make cer-

1 Smith, H. W., *J. Mt. Sinai Hospital*, 1943-44, **10**, 59.

2 Klop, C., Young, N. F., Taylor, H. C., Jr., *J. Clin. Invest.*, 1945, **24**, 117.

3 Smith, W. W., Finkelstein, N., Smith, H. W., *J. Biol. Chem.*, 1940, **135**, 231.

4 Fugita, A., Iwatake, D., *Biochem.*, 1931, **242**, 43.

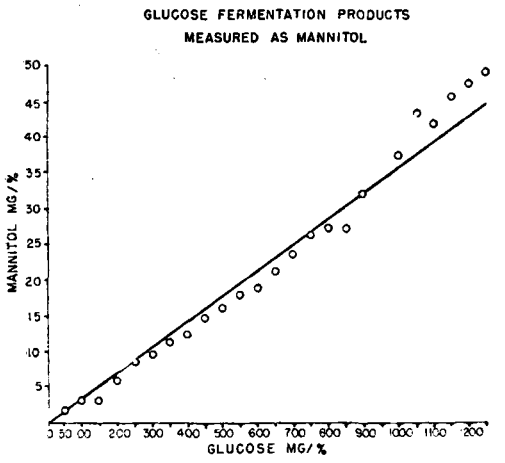

FIG. 1.

tain that the fermentation was complete. The titrations of the filtrates for mannitol were found to increase almost in direct proportion to the quantity of glucose removed. (Fig. 1). An analysis of the results revealed that 3.51% of the glucose fermented was measured as mannitol.

Attempts were made to control the formation of measurable glucose fermentation products by using a constant temperature incubator and increasing the temperature to 37°C. Recovery of apparent mannitol was thus increased to 4.9% of the glucose fermented, indicating that an increased and more variable production of the interfering substance had occurred.

Under optimum conditions for fermentation of glucose by yeast at 25 to 30°C and at a pH 5.6 to 5.8,[6] the glycerol formed is equivalent to 3 to 4% of the glucose destroyed.[7] Periodic acid, which is used for the oxidation of mannitol, will also oxidize the glycerol,[8] each cc of 0.005 N sodium thiosulfate being equivalent to 0.091 mg of mannitol or to 0.093 mg of glycerol. Para-aminohippuric acid is also oxidized by periodic acid, and hence interferes with the determination of mannitol. The method of Barker and Clark[9] prevents this interference, but not the reaction of periodic acid with the fermentation products of glucose.

When the effect of the glucose fermentation products are ignored, the mannitol clearance values determined simultaneously with the maximal glucose reabsorptive capacity are lowered, the degree depending upon the relative amounts of glucose and mannitol in plasma and urine. The amounts vary with the ratio of the mannitol clearance to the maximal tubular glucose reabsorptive capacity, the mannitol clearance value, and the plasma levels of mannitol and glucose. The details of the factors which affect the mannitol clearance are shown in the following equation:

$$\frac{U_M V + ((C_M P_G - T_m G) \times \text{Glucose Correction})}{P_M + (P_G \times \text{Glucose Correction})} = C_M \text{ Uncorrected Values.}$$

C_M = mannitol clearance, cc/min.
U_M = urine mannitol, mg/cc.
V = urine vol., cc/min.
P_M = plasma mannitol, mg/cc.
P_G = plasma glucose, mg/cc.
T_mG = max. glucose reabsorptive capacity, mg/min.

The mannitol clearance values are always lowered as the concentration of glucose in the urine is less than that of plasma due to the tubular reabsorption of glucose.

Summary. 1. Glucose fermentation products interfere with the periodate-titrimetric method of mannitol determination. 2. Failure to correct for this interference lowers the mannitol clearance values.

The advice of Dr. Robert W. Keeton, the technical assistance of Mr. Edward Eckert, and the generous donation of mannitol by Dr. J. Wm. Crosson of Sharp and Dohme, Inc., are gratefully acknowledged.

5 Nelson, Norton, *J. Biol. Chem.*, 1944, **153**, 375.

6 Harden, Arthur, Alcoholic Fermentation, Longman, Green and Co., 1923, third edition.

7 Anderson, C. G., An Introduction to Bacteriological Chemistry, Williams and Wilkins Co., 1946, pp. 310, 311.

8 Bradford, P., Pohle, W. D., Gunther, J. K., Mehlendbacher, V. C., *Oil and Soap*, 1942, **19**, 189.

9 Barker, Harold G., and Clark, John K., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 120.