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Determination of Glucose, Galactose, and Lactose in a Mixture of the Three Sugars.

MARY SCOTT AND EDWARD S. WEST.

From the Department of Biochemistry, University of Oregon Medical School, Portland.

Harding and coworkers[1] have devised a method of estimating a number of the common sugars when present in a mixture. They utilized *Proteus vulgaris, M. krusei, S. marxianus,* and *M. tropicalis* for the differential removal of sugars in a mixture of glucose, fructose, galactose, sucrose, maltose and lactose. This procedure combined with reduction values before and after hydrolysis provided a reasonably satisfactory method of determining the individual sugars in the mixture.

The writers have found it possible to determine glucose, galactose, and lactose in a mixture by a similar, though simplified technique which avoids the use of organisms other than baker's yeast. An improved method for the hydrolysis of lactose was devised which gives complete decomposition as compared with the 72% hydrolysis by the method of Harding and Grant.[2]

Galactose or lactose mixed with glucose were satisfactorily recovered after fermentation with washed baker's yeast. Table I shows recoveries of different quantities of glucose, galactose and lactose from solutions of all three. The data are not selected and represent all determinations made. The recoveries of galactose and lactose are not quite as good as those for glucose as might be expected due to the greater number of procedures involved in their estimation. 1. The titration values for the mixture are determined by a Shaffer-Somogyi[3] reagent (79.5 gm. Na_2CO_3, 1 gm. of KI, and 21 gm. of $NaHCO_3$ per 1) both (A) before and (B) after fermentation using 5 cc. of a 15% suspension of washed baker's yeast[4] (Fleischmann) and 14 cc. of the sugar solution made first just acid to congo with a drop of H_2SO_4.

2. The original sugar solution is mixed with an equal volume of 2 N H_2SO_4 in a 25x200 mm. tube fitted with a rubber stopper car-

1 Harding, V. J., and Nicholson, T. F., *Biochem. J.*, 1933, **27**, 1092.

2 Harding, V. J., and Grant, G. A., *J. Biol. Chem.*, 1931, **94**, 538.

3 Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 696.

4 Somogyi, M., *J. Biol. Chem.*, 1927, **75**, 35. Starch was removed during centrifugation.

TABLE I.
Recovery of Glucose, Galactose and Lactose from Solutions of All Three.

Mg. in 5 cc.			Recovery, %		
Glucose	Galactose	Lactose	Glucose	Galactose	Lactose
1.25	1.25	1.25	101	100	98
"	"	"	103	98	97
1.00	1.00	1.00	103	99	107
"	"	"	104	101	108
"	"	"	100	97	110
0.50	0.50	0.50	100	102	100
"	"	"	100	100	102
"	"	"	96	100	112
"	"	"	98	104	102
"	"	"	99	112	98
"	"	"	98	94	106
0.50	0.50	1.00	102	100	100
"	"	"	99	114	100
"	"	"	100	102	100
0.50	1.00	0.50	104	108	99
"	"	"	102	104	98
1.00	0.50	0.50	98	109	102
"	"	"	102	104	96
		Aver.	100.4	102.6	101.9

rying a 12 cm. capillary tube. The mixture is heated for 2.5 hours in a boiling water bath, cooled, poured into an Erlenmeyer flask and about 0.5 gm. of solid ferric sulfate per 50 cc. of solution are added.* The solution is then neutralized with precipitated $BaCO_3$ (until solution does not redden blue litmus) and thoroughly shaken. It is then filtered, the filtrate made just acid to congo with drops of concentrated H_2SO_4, and again filtered.

Titration values on the final filtrate are run (C) before and (D) after fermentation. Since the hydrolyzed solution was diluted with an equal volume of acid the titration values must be doubled for comparison with the original solution.

In determining the titration values of the above solutions which are acid (B, C, D.), 5 cc. portions are accurately pipetted into sugar tubes, 2 drops of phenol red (0.04% in water) added, followed by drops of 0.5 N NaOH to the red color of the indicator. The sugar reagent is then added and the titration value determined in the usual way.

Calculations. Curves for the sugar reagent used were prepared from known concentrations of the sugars. They were essentially straight lines from which factors for the different sugars were calculated.

The calculations are given for 5 cc. of the original solution.

*Added to remove sulphide found in practically all $BaCO_3$.

1. (A-B) x glucose factor = glucose in the original solution.
2. (C-D) x 2 x glucose factor = original glucose + glucose from the hydrolysis of lactose.
3. D x 2 x galactose factor = original galactose + galactose from lactose hydrolysis.
4. (2-1) = glucose from lactose. This value ÷ 0.53 = lactose. Glucose from lactose is also equal to galactose from lactose.
5. (3-4) = galactose in original solution.

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Rapid Method of Preparing Solutions of Gonadotropic Substance of Pregnant Mares' Blood.

EDWIN L. GUSTUS, ROLAND K. MEYER AND OLIVER R. WOODS.
From the Research Laboratories, The Upjohn Company, Kalamazoo.

The method of purifying the gonadotropic substance in pregnant mares' blood by adsorption of the active material to specially prepared aluminum hydroxide followed by elution[1] is a difficult and time-consuming procedure. The method yields preparations directed to the requirements of clinical investigation but for studies on laboratory animals somewhat less purified solutions of the hormone are entirely satisfactory. For this purpose it would be desirable to have a rapid method of eliminating the bulk of the inert material from the serum or plasma of the mare and of concentrating the activity in a smaller volume.

Although there are many chemical and biological differences between the gonadotropic material present in pregnant mares' blood and that of the urine of pregnant women, we have found that the method of Katzman and Doisy[2] for the preparation of the gonadotropic substance of the latter by adsorption of the active material to benzoic acid may be successfully employed to effect a partial purification of the gonadotropic material in pregnant mares' blood. Solutions of the hormone prepared by this method contain much larger amounts of horse serum proteins than solutions prepared by adsorption to aluminum hydroxide.

One liter of citrated plasma from a pregnant mare[3] was diluted

1 Evans, H. M., Gustus, E. L., and Simpson, M. E., *J. Exp. Med.*, 1933, **58**, 569.
2 Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 745.
3 Cole, H. H., and Hart, G. H., *Am. J. Physiol.*, 1930, **93**, 57.