terion for selection of high-yielding trees, we satisfy at the same time the desire of chocolate manufacturers for large, plump seeds.

References

1. POUND, F. J. *1st Ann. Rept. on Cacao Research.* Trinidad: I.C.T.A., 10 (1931).
2. ———. *2nd Ann. Rept. on Cacao Research.* Trinidad: I.C.T.A., 9 (1932).

Manuscript received August 22, 1952.

The Enzymatic Conversion of Lactose into Galactosyl Oligosaccharides

John H. Pazur

Department of Biological Chemistry, University of Illinois College of Medicine, Chicago

The enzymatic synthesis of oligosaccharides from the disaccharides, sucrose and maltose, has been reported from several laboratories (*1–5*). Experiments with labeled substrates (*6, 7*) have shown that these oligosaccharides arise through transfructosidation and transglucosidation reactions. In this communication, we shall report preliminary studies on the enzymatic conversion of lactose into a series of galactosyl oligosaccharides. Evidence from tracer experiments indicates that a transgalactosidation mechanism is involved in the synthesis of the new oligosaccharides. From a consideration of the products of partial acid hydrolysis of the oligosaccharides and their aldonic acid derivatives, it appears that two of the new compounds are disaccharides (glucose-galactose and galactose-galactose) and two are isomeric trisaccharides (glucose-galactose-galactose).

Ten g CP lactose in 100 ml of water was treated with 100 ml of a 2% solution of a yeast enzyme preparation.[1] At the end of 24 hr the digest was heated in a boiling water bath for 5 min. The products in the digest were resolved by paper chromatography (*8*). Examination of the paper chromatogram (Fig. 1) revealed the presence of the new oligosaccharides. These oligosaccharides were not synthesized from glucose and galactose since an enzymatic digest of these substrates contained no new compounds. Further, a blank of the enzyme and the lactose showed that the oligosaccharides were not present in the original solutions. It is noted, however, that the enzyme blank contained two monosaccharides (fructose and glucose) as contaminants. Dialysis of the enzyme removed not only the monosaccharides but also some essential cofactor of the enzyme.

The new oligosaccharides (I, II, III, and IV) were isolated by paper chromatographic procedures previously described (*7*). Hydrolysis of the pure compounds in 0.1 *N* HCl showed that Compounds I, III, and IV are composed of glucose and galactose residues and that Compound II is composed of galactose residues. As judged from the intensity of the spots on the paper chromatogram, the glucose-galactose ratios appeared to be 1:1 for Compound I and 1:2 for Compounds III and IV.

[1] The yeast concentrate "Lactase B" was kindly supplied by Rohm & Haas Co., Philadelphia, Pa. This concentrate possessed the transferring activity described in this report, as well as hydrolytic (lactase) activity.

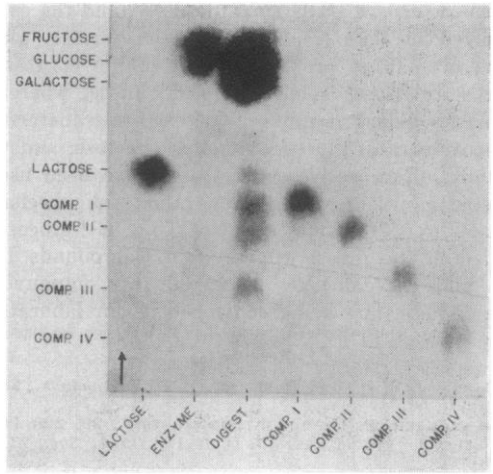

FIG. 1. A multiple ascent paper chromatogram of the lactose digest, of lactose and enzyme controls, and of the pure galactosyl oligosaccharides.

Two further lines of evidence point to the structure of the oligosaccharides. First, the apparent R_F values of the pure compounds (Fig. 1) are typical of oligosaccharides composed of two or three monosaccharide units joined through 1,4 or 1,6 glycosidic bonds (*7, 9*). Second, partial acid hydrolysis of the compounds and their aldonic acids yields the reducing products listed in Table 1. These hydrolytic products have been tentatively identified by a comparison of their R_F values with those of pure reference compounds. The structures suggested by these findings are: glucose-6,1-galactose for Compound I, galactose-6,1-galactose for Compound II, glucose-4,1-galactose-6,1-galactose for Compound III, and glucose-6,1-galactose-6,1-galactose for Compound IV.

There is a marked similarity in the mode of action

TABLE 1

REDUCING PRODUCTS OBTAINED ON PARTIAL ACID HYDROLYSIS OF THE GALACTOSYL OLIGOSACCHARIDES AND THEIR ALDONIC ACID DERIVATIVES

Oligosaccharides	Reducing products
Compound I	Glucose, galactose
Aldonic acid of Compound I	Galactose
Compound II	Galactose
Aldonic acid of Compound II	Galactose
Compound III	Glucose, galactose, lactose, Compound II
Aldonic acid of Compound III	Galactose, Compound II
Compound IV	Glucose, galactose, Compound I, Compound II
Aldonic acid of Compound IV	Galactose, Compound II

of the *A. oryzae* transglucosidase (*7*) and the enzyme involved in these reactions. In the former, glucose units of maltose are transferred to the cosubstrates glucose, maltose, isomaltose, and panose, whereas in the latter galactose units of lactose are transferred to the cosubstrates glucose, galactose, lactose, and Compound I. Radioactive cosubstrates have been used to substantiate the proposed transgalactosidic mechanism of action of the new enzyme. Thus C^{14}-glucose included in the digest appeared in Compounds I and IV, and C^{14}-galactose appeared in Compound II. These studies are being continued in our laboratories.

References

1. BACON, J. S. D., and EDELMAN, J. *Arch. Biochem.*, **28**, 467 (1950).
2. BLANCHARD, P. H., and ALBON, N. *Ibid.*, **29**, 220 (1950).
3. PAZUR, J. H., and FRENCH, D. *J. Am. Chem. Soc.*, **73**, 3536 (1951).
4. PAN, S. C., NICHOLSON, L. W., and KOLACHOV, P. *Ibid.*, 2547.
5. WHITE, L. M., and SECOR, G. E. *Arch. Biochem. and Biophys.*, **36**, 490 (1952).
6. PAZUR, J. H. *J. Biol. Chem.*, **199**, 217 (1952).
7. PAZUR, J. H., and FRENCH, D. *Ibid.*, **196**, 265 (1952).
8. FRENCH, D., KNAPP, D. W., and PAZUR, J. H. *J. Am. Chem. Soc.*, **72**, 5150 (1950).
9. FRENCH, D. *Science*, **113**, 352 (1951).

Manuscript received August 20, 1952.

Chromatographic Separation of Estrone, Estradiol, and Estriol

Joel Bitman and J. F. Sykes

Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture, Washington, D. C.

The estrogens appear to be present in human and cattle blood in extremely small concentrations, of the order of 3–8 μg/l whole blood (*1, 2*). The determination of the three natural estrogens in blood requires quantitative methods for their separation and estimation. The object of this report is to present a method suitable for routine use for the quantitative separation of estrone, estradiol, and estriol at this microlevel. The following work has been carried out with pure crystalline estrogens, as a preliminary to the application of the method to the estrogens in blood.

Partition chromatography has been used for the separation of closely related compounds that show a difference in partition coefficients (*3*). Recently a method for the separation of estrone and estradiol by partition chromatography was reported (*4*). A NaOH solution adsorbed on Celite forms the stationary phase, and benzene the moving phase. A similar system, also using NaOH-Celite and benzene, was used earlier by another group (*5*) for the separation of certain estrogen isomers. The method proposed by the British workers proved to be more effective in our hands in accomplishing the estrone-estradiol separation.

It seemed valuable from the standpoint of the number of manipulations, time, and magnitude of losses, to effect a separation of all three estrogens on one column in one operation. Because of the more hydrophilic character of estriol, as compared with estrone and estradiol, it is not eluted from the NaOH-Celite phase by benzene. It was recognized that a more favorable partition system was necessary for the separation of estriol. This can be achieved by two methods: a change in the moving phase, and/or a change in the stationary phase. A separation of all three estrogens has recently been reported by the British group (*6*), utilizing a change in the moving phase to accomplish the separation of estriol. The eluent used was chloroform-butanol (3 : 1). This procedure, or the use of acetone, ethylene dichloride, or methanol as eluents, did not prove as satisfactory as the method reported below, because of the elution of a large amount of butanol-soluble material in the estriol fraction. Accordingly, the second approach, a change in the nature of the stationary phase, has been used in the technique developed in our laboratory. It was discovered that estriol could be eluted by benzene from a less alkaline stationary phase consisting of Celite to which water rather than NaOH had been added. It occurred to us that after use of the NaOH-Celite column to separate estrone and estradiol, it could be changed to the less alkaline water-Celite or $NaHCO_3$-Celite column by reaction with gaseous HCl or CO_2. Estriol is then readily eluted by benzene from these systems. These modifications were carried out successfully, and, because of the greater safety in handling CO_2, it has been used routinely to modify the partition characteristics of the column.

The chromatographic conditions of Swyer and Braunsberg (*4*) were used, and the columns were prepared according to the careful description of Haenni, Carol, and Banes (*7*) for preparations of this type. A West-type straight condenser (ID, 10.8 mm), with stopcock at the reciving end, was employed as a chromatographic tube. This allowed the temperature of the column to be controlled at 24° ± 2° C by circulation of water of the appropriate temperature through the condenser. The column is filled with benzene (CP benzene-thiophene–free is shaken 6 times with sulfuric acid and redistilled), and fine glass wool (Fiberglas, Corning) is packed into the constriction above the stopcock. Three g Celite 535 (Johns-Manville) and 25 ml benzene in a small beaker are thoroughly mixed with 2.4 ml 2.3 *N* NaOH for several minutes. The stopcock is opened enough to permit slow drainge, and the Celite mixture is transferred to the tube in small portions, with a spatula. A flocculent suspension is formed by slowly working the packing rod (a glass rod flattened at one end to a circular head with a clearance of 1.0 mm in the adsorption tube) up and down as a piston through the Celite mixture. The Celite is then gently compressed with the rod to form a uniform pack with a sharply defined level surface. The mixture fills the tube to a height of 125 mm ± 5 mm.

Suitable aliquots (containing 2–10 γ) of solutions