purity (specific activity 50,000[16,17]). (3) The manner in which the transfer reaction is influenced by *p*H, substrate concentration, temperature and time resembles markedly the effects of these variables on hydrolysis measured in companion digests lacking acceptor. (4) The specific β-glucuronidase inhibitor, saccharate, is a potent inhibitor of the transfer reaction.

We have been impressed by the fact that, under optimal conditions, from 60 to 89% of the transferable glucuronic acid appears in the new glucosiduronic acid. Furthermore, it can be detected in systems where the donor to acceptor ratio is close to 1. Other monohydric, dihydric, trihydric and aromatic alcohols can serve as acceptors.

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CANCER RESEARCH AND CANCER CONTROL UNIT
TUFTS UNIVERSITY SCHOOL OF MEDICINE
BOSTON 11, MASSACHUSETTS WILLIAM H. FISHMAN
S. GREEN

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HYDROLYSIS OF NIGEROSE BY INTESTINAL EXTRACTS[1]

Sir:

Extracts of intestinal mucosa have been shown to contain, in addition to maltase, an enzyme, oligo-1,6-glucosidase, which specifically hydrolyzes the α-1,6 linkages of the oligosaccharides isomaltose, panose and isomaltotriose, and thus allows essentially complete intestinal digestion of starch to occur.[2,3] These preparations have essentially no activity against gentiobiose.[4] Wolfrom and Thompson have recently reported the isolation of the α-1,3 linked disaccharide nigerose from acid hydrolysates of waxy maize amylopectin.[5] We should like to report the enzymatic hydrolysis of this disaccharide by intestinal extracts.[6]

By means of a spectrophotometric assay specific for glucose, hydrolytic activity was demonstrated by a rapid, linear rate of optical density increase at 340 mμ on addition of an intestinal extract to a reaction mixture containing hexokinase, glucose-6-phosphate dehydrogenase, ATP, TPN, Mg^{++}, and glycyl glycine buffer.[2] Rate of TPN reduction under these conditions was proportional to amount of extract added. Hydrolytic activity was demonstrated in addition by increase in reducing power and identification of glucose by paper chromatography. With a preparation containing 155 units of oligo-1,6-glucosidase activity[2] (3.5 mg. protein) 7 mg. of nigerose was hydrolyzed to completion in 110 minutes at 30°.

Enzymatic activity has been determined in fractionated intestinal extracts with maltose, isomaltose, and nigerose as substrates under conditions in which activity is proportional to amount of enzyme added (Table I). Widely differing ratios indicate that these are three separate enzymatic activities. It is of interest to note that the rate of hydrolysis of nigerose is greater than that for isomaltose in the initial extract in spite of the fact that nigerose has been isolated from waxy maize amylopectin in much smaller quantity than isomaltose.[5]

TABLE I

HYDROLYSIS OF DISACCHARIDES BY INTESTINAL FRACTIONS

Fraction[a]	Enzyme activity[b] (1) Isomaltose, units/ml.	(2) Nigerose, units/ml.	(3) Maltose, units/ml.	Ratio of activities (2) ÷ (1)	(3) ÷ (2)
Initial extract	268	568	1840	2.1	3.2
0.3–0.8 saturated ammonium sulfate	815	1370		1.7	
Supernatant from alumina adsorption	368	616		1.7	
46–59% ethanol fraction from acetone powder extract	10[c]	131	2100	13.1	16.0

[a] Prepared from frozen hog intestine.[4] [b] Activity determined as previously described[2] with the following modifications; buffer concentration decreased from 0.083 *M* to 0.055 *M*; hexokinase 0.05 ml.; glucose-6-phosphate dehydrogenase (1% solution) 0.1 ml.; total volume 0.9 ml.; run in cylindrical 1-ml. cells, light path 1 cm. [c] Estimated from an optical density change of 0.006 in four minutes.

A value of 3×10^{-4} *M* has been obtained for the K_m of nigerose at *p*H 6.9 with the spectrophotometric assay. Under similar conditions, average K_m values of about 3×10^{-4} *M* and 7×10^{-4} *M* have been obtained for maltose and isomaltose, respectively. At acid *p*H up to and including *p*H 6.9, K_m values for maltose and isomaltose are essentially independent of *p*H.[7]

The presence of an enzyme in intestinal extracts capable of hydrolyzing nigerose constitutes additional evidence for the presence of this linkage in starch type polysaccharides.

(1) Supported in part by a grant from the National Science Foundation.
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(6) We are indebted to Drs. Thompson and Wolfrom for the gift of the nigerose sample and for kindly informing us of their results prior to publication.
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DIVISION OF BIOCHEMISTRY
NOYES LABORATORY OF CHEMISTRY
UNIVERSITY OF ILLINOIS JOSEPH LARNER
URBANA, ILLINOIS R. E. GILLESPIE

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THE ROLE OF THE NUCLEIC ACID IN THE RECONSTITUTION OF ACTIVE TOBACCO MOSAIC VIRUS[1]

Sir:

Preparations of native protein and ribonucleic acid have been isolated from tobacco mosaic virus (TMV) by treatment with *p*H 10.5 buffer, and sodium dodecyl sulfate, respectively.[2] The molec-

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(2) H. Fraenkel-Conrat and Robley C. Williams, *Proc. Natl. Acad. Sci.*, **41**, 690 (1955).