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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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.
(2) J. Larner and C. M. McNickle, *J. Biol. Chem.*, **215**, 723 (1955).
(3) J. Larner, THIS JOURNAL, **77**, 6385 (1955).
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(5) M. L. Wolfrom and A. Thompson, THIS JOURNAL, **77**, 6403 (1955).
(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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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-

(1) Aided by a grant from the National Foundation for Infantile Paralysis.
(2) H. Fraenkel-Conrat and Robley C. Williams, *Proc. Natl. Acad. Sci.*, **41**, 690 (1955).

ular weight of the bulk of these two fractions was approximately 100,000 and 250,000. At *p*H 6 a mixture of these almost non-infective components (10:1) was shown to reconstitute active virus. Typical TMV lesions were produced in susceptible plants, indicative of regeneration of up to 1%, and in more recent experiments of 1–5%, of the original infectivity. Electron micrographs showed rod-shaped particles 15 mμ in diameter. Many of these were 300 mμ long and appeared indistinguishable from the original TMV particles. These rods contained about 5% nucleic acid, as a central core.

It is the purpose of this communication to report on mixed reconstitution experiments performed with protein and nucleic acid fractions isolated from different strains of TMV. Native protein has been obtained only from common TMV and the masked (M) strain, active nucleic acid from these as well as yellow aucuba (YA) and Holmes ribgrass (HR). Five of the six possible combinations of these have given active virus which in each case, has produced on Turkish tobacco plants only those symptoms which are characteristic of the strain supplying the nucleic acid.

Of particular interest was the "hybrid" virus reconstituted from TMV-protein with HR-nucleic acid, because HR protein differs markedly from TMV protein in amino acid composition and in antigenic specificity.[3] When this hybrid was treated with anti-TMV serum its infectivity was largely neutralized while anti-HR serum did not appear to affect it. Immunologically, therefore, the hybrid resembled TMV, the virus which has supplied the protein coat, while its symptoms in the plant were only those of the HR-strain, which supplied the nucleic acid core. This finding of a viral activity showing properties characteristic of each of the parent strains appears to rule out any interpretation of the mode of virus reconstitution other than the *in vitro* production of active particles from the two components. The progeny produced from this hybrid (TMV-protein + HR-nucleic acid) in the plant, and in particular its protein were very similar to, if not identical with, HR virus and protein. The progeny protein, like HR and unlike TMV,[3] contained methionine and histidine, and much tyrosine, but quantitative analyses remain to be done. Thus the ribonucleic acid seems to represent the main genetic determinant even for the progeny protein in the TMV strains.

In attempts to free the original protein and nucleic acid fractions from any traces of undegraded virus, assays at high concentrations (up to 0.05%) in a very sensitive variety of *nicotiana* test plants indicated the presence of no more than about 0.0003% of active virus in either. Yet, when the nucleic acid was tested in a plant variety less sensitive to TMV (Holmes necrotic), sufficient lesions were sometimes produced to indicate the presence of about 0.1% of TMV. This residual infectivity differed from the virus in its relative activity on different hosts and at different levels, in its marked instability, and in its suceptibility to ribonuclease. Virus rods could not be found to account for this residual infectivity and it is therefore now regarded as a characteristic property of the nucleic acid itself. However, it is found only in those fresh preparations which are also able to combine with virus protein to give, with at least a 200-fold increase in activity, a much more stable and virulent infectious agent, the reconstituted virus.

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D-XYLOSE-3-PHOSPHATE

Sir:

Levene and Raymond[1] have recorded unsuccessful attempts to synthesize D-xylose-3-phosphate. Recently, Watson and Barnell[2] have suggested that this compound might be formed from X-xylose-5-phosphate as a result of a phosphoryl group migration from the 5- to the 3-hydroxyl group. In this communication we wish to report the synthesis and characterization of D-xylose-3-phosphate and to show that the change in the optical rotation of the solutions of xylose-5-phosphate observed by Watson and Barnell is due to the formation of D-xylulose-5-phosphate rather than to a phosphoryl group migration.

The crystalline 1,2-isopropylidene-D-xylofuranose-5-diphenylphosphate[3] (2.11 g., 5 mM.) was treated with a mixture of 2 *N* sodium hydroxide (15 ml.) and dioxane (5 ml.) at room temperature. The conversion to 1,2-isopropylidenexylofuranose-3,5-cyclic phosphate (I) was complete in 72 hours and the excess of alkali was then removed by the addition of Dowex 50 (H^+) resin. The solution of the sodium salt of the six-membered cyclic phosphate (I) was passed through a cyclohexylammonium-Dowex 50 column and the effluent evaporated to dryness. The cyclohexylamine salt crystallized from a mixture of acetone and ether in a yield of 85%; m.p. 217–219° with dec. $[\alpha]^{21}D$ +14.1 (*C* 1.9 in water) *Anal.* Calcd. for: $C_{14}H_{26}O_7NP$: C, 47.86; H, 7.46; N, 3.99. Found: C, 47.79; H, 7.50; N, 3.61. Potentiometric titration showed the absence of a secondary phosphoryl dissociation. The cyclic phosphate was hydrolyzed quantitatively by 1 *N* sodium hydroxide at 100° in 20 hours to, presumably, a mixture of II and III. An aqueous solution of the free acids (II and III), prepared by passing the alkaline solution through a Dowex 50-H^+ column, was heated at

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