theory of DNA structure[12] is provided by the constancy of molecular weight throughout the structural changes which we have described. In addition, however, we are led to wonder whether the observed phenomena do not point to the participation of water in stabilizing the structure of DNA. With this end in view, we are investigating the behavior of DNA in a variety of organic solvents with different hydrogen-bonding characteristics.

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SEGREGATION COEFFICIENTS OF VARIOUS IMPURITIES IN A SILICON TETRAIODIDE MATRIX

Sir:

The demand for transistors and other semiconductor devices has stimulated considerable research in the preparation of ultra-pure silicon. It was found that zone-refining techniques[1] were inadequate for the removal of certain impurities, *e.g.*, boron, in a silicon matrix,[2] and it was decided to approach the problem by preparing a suitable compound of silicon, subjecting this to such purification techniques as recrystallization, sublimation, and zone purification and ultimately decomposing it to elemental silicon. Thermodynamic calculations indicated that of the four tetrahalides, silicon tetraiodide decomposed most readily and that it lent itself best to zone-melting techniques because of its relatively high melting point (121.5–122.5°). Furthermore, it could be expected that the segregation coefficients for the various impurities in silicon tetraiodide would differ from those found in silicon.

Silicon tetraiodide was prepared by passing iodine vapor over Coleman and Bell Company ninety-eight per cent. pure silicon at 800° and the product was then crystallized from toluene. This material was used to fill Pyrex ampoules nine millimeters in diameter and thirty centimeters long. It was then densified, the tube sealed, and zone-purification was effected by vertical passage at the rate of five centimeters an hour. The zone width was two and one-half centimeters, and the temperature of the molten zone was about 135°. A small molten zone was passed through the charge only once in order to maintain impurities at spectrographically detectable levels. Spectrographic analyses of the successive two and one-half centimeter zones permitted preliminary calculations to be made based on Pfann's original equation.[1] The results of these calculations gave plots of C/C_0 *versus* x/l (where C is the concentration of an impurity in a solid frozen from a mother zone, C_0 is the mean concentration of the impurity before zone refining, x is the distance that the zone has traveled along the tube, and l is the zone length) which indicated that certain impurities can be efficiently removed by this technique. In the case of boron where the concentration level was below the limit of spectrographic detectability, a mathematical extrapolation was employed based on the minimum spectrographically detectable concentration found. Using this value in conjunction with the expression:

$$C_z = K[C_0 + (C_{z-1}/K - C_{z-1})]$$

where C_z is the concentration of an impurity frozen into the zth zone, C_{z-1} is the concentration of the impurity frozen into the previously frozen zone, k is the segregation coefficient of the impurity, and C_0 is as described above, the maximum value for the k of boron was determined. The values of the k's determined for various metallic impurity species in the SiI_4 matrix are: boron, 0.16 ± 0.07, aluminum, 0.88 ± 0.04, sodium, 0.07 ± 0.01, magnesium, 0.58 ± 0.06, copper 0.63 ± 0.05.

It is apparent from the above that the impurities listed can be removed to levels below the one part per million range by a suitable number of passes. Work on this project, and the method and/or methods of preparing pure silicon tetraiodide, as well as the preparation of the elemental silicon, is continuing.

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(2) J. A. Burton, *Physica*, **20**, 845 (Nov., 1954).

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RECEIVED JANUARY 9, 1956

GLUCOSIDURONIC ACID SYNTHESIS BY β-GLUCURONIDASE IN A TRANSFER REACTION

Sir:

Certain biological phenomena have been correlated with the activity of the enzyme β-glucuronidase. Among these are glucuronidogenesis,[1] action of gonadal hormones,[2,3,4,5] human cancer,[6,7,8] genetic control in mouse tissues,[3,9,10] and effects of pituitary interstitial cell stimulating hormone.[11] In attempts to arrive at an interpretation of the function of the enzyme *in vivo*, we have found it difficult to explain the findings on the basis of a purely hydrolytic action of the enzyme or its simple reversal. It was postulated that β-glucuronidase participates as a member of a multicomponent system concerned with glucosiduronic

(1) W. H. Fishman, *J. Biol. Chem.*, **136**, 229 (1940).
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(10) L. W. Law, A. G. Morrow and E. M. Greenspan, *ibid.*, **12**, 909 (1952).
(11) W. H. Fishman, G. Benjamin and S. Green, *J. Clin. Endocrinol.*, **15**, 876 (1955).

acid synthesis[12] and it was suggested[13,14] that the enzyme may catalyze a transfer reaction analogous in nature to the ones reported for a number of other so-called hydrolytic enzymes. We now wish to report direct experimental evidence for this type of reaction by β-glucuronidase.

β-Glucuronidase is capable of catalyzing the transfer of glucuronic acid from a donor substrate (*e.g.*, phenolphthalein or menthol glucosiduronic acids) to a suitable acceptor molecule, in a manner indicated by the equation:

$$\underset{\text{donor substrate}}{ROC_6H_9O_6} + \underset{\text{acceptor}}{R'OH} \xrightarrow[\beta\text{-glucuronidase}]{} \underset{\text{new glucosiduronic acid}}{R'OC_6H_9O_6} + \underset{\text{aglycone of donor substrate}}{ROH}$$

An example of the experimental data is given in Table I.

TABLE I

FORMATION OF NEW GLUCOSIDURONIC ACID IN β-GLUCURONIDASE DIGESTS CONTAINING PROPYLENE GLYCOL

Three digests were prepared. One (control) contained 0.5 ml. of 0.01 molar phenolphthalein glucosiduronic acid (PGA) solution, 50 Fishman units[15a] (0.5 ml.) of purified liver β-glucuronidase (S.A. = 5000) and sufficient 0.1 molar acetate buffer *p*H 4.5 to attain a final volume of 5 ml. In the second digest, 0.75 ml. of propylene glycol was substituted for an equal volume of acetate buffer to give an acceptor concentration of 15% (v./v.). The third digest was identical to digest 2, except that a boiled enzyme solution was used. These digests were incubated at 38° in a waterbath for 15 minutes. The reaction was stopped by immersing the tubes in boiling water for one minute. Duplicate samples of one ml. were taken for phenolphthalein measurements.[18]

Digest	Phenolphthalein liberated, μM/ml. digest	Free glucuronic acid,[a] μM/ml. digest	(Unextracted) glucosiduronic acid synthesized Calculated[b] μM/ml. digest	Found μM/ml. digest	Mole transfer,[c] %
1 Control	0.0812	0.0853	0	0	0
2 Propylene glycol	0.1600[d]	0.0645	0.0955	0.0870	60
3 Propylene glycol plus boiled enzyme	0	0	0	0	0

[a] A 2-ml. aliquot was acidified with 0.2 ml. of 6 *N* H_2SO_4 made up to 10 ml. with water and then extracted four times with 10-ml. portions of ethyl acetate to remove unhydrolyzed PGA. The aqueous phase was made alkaline with a drop of concentrated NaOH and restored to a volume of 10 ml. Analyses were performed on the aqueous phase for total glucuronic acid and (unextracted) glucosiduronic acid, from which data figures for free glucuronic acid were computed.[15] [b] A calculated quantity derived from the molar difference between total glucuronic acid (or phenolphthalein liberated) coming from the donor substrate and free glucuronic acid present, it being assumed that this moiety has been transferred to propylene glycol to form its glucosiduronic acid. [c] Mole transfer is defined as the ratio of micromoles of glucosiduronic acid formed to micromoles of phenolphthalein liberated. [d] The presence of propylene glycol produced a greater release of phenolphthalein from the substrate.

In the case of the acceptor, propylene glycol, there appears in the digest a glucosiduronic acid which differs from phenolphthalein glucosiduronic acid in that it is not extractable by ethyl acetate at acid *p*H. The amount of unextracted glucosiduronic acid agrees closely with the molar difference between the amounts of phenolphthalein and free glucuronic acid in the digest. In addition, it has been possible to isolate the product of the reaction. It is completely hydrolyzable by β glucuronidase under conditions different from those of the transfer reaction (Fig. 1), contains propylene glycol but no phenolphthalein. The quantitative release observed of the glucuronic acid present in the product by the enzyme is strong evidence for glucosiduronic acid.[11] For identification purposes, the tetraacetyl methyl ester of the product was prepared; the glucosiduronic acid content is 98% of the theoretical value computed for the tetraacetyl methyl ester of propylene glycol glucosiduronic acid.

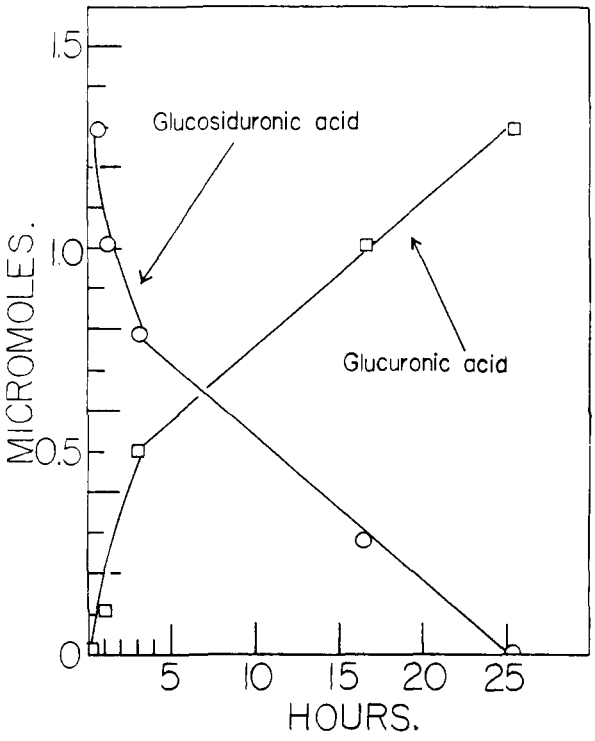

Fig. 1.—A series of identical digests were prepared with the following composition: 1 ml. of the new glucosiduronic acid (1.285 micromoles), 1.5 ml. of 0.1 molar acetate buffer *p*H 4.5 and 0.5 ml. of a purified liver β-glucuronidase preparation (2500 Fishman units) and incubated at 38°. At each indicated time interval, the reaction was stopped in a digest and analyses were performed for total glucuronic acid and glucosiduronic acid.[15]

The following evidence supports the concept that it is one enzyme, not two, which accounts for both hydrolysis and transfer. (1) Liver β-glucuronidase of varying specific activities from four animal species catalyzed transfer in proportion to its hydrolytic activity. (2) The transfer reaction is observed in β-glucuronidase preparations of high

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(14) W. H. Fishman, in "Advances in Enzymology," Vol. XVI, 1955, p. 361.
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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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RECEIVED DECEMBER 12, 1955

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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(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).