amount of reducing sugar. No reaction took place with *d*-ketoxylose or *l*-sorbose alone, nor in mixtures of glucose-l-phosphate with either *d*-xylose or *d*-tagatose. Fig. 1 shows the changes in inorganic phosphate in the presence of the enzyme preparation with glucose-l-phosphate as the only substrate, as well as in combination with *d*-fructose, *d*-ketoxylose and *l*-sorbose, respectively.

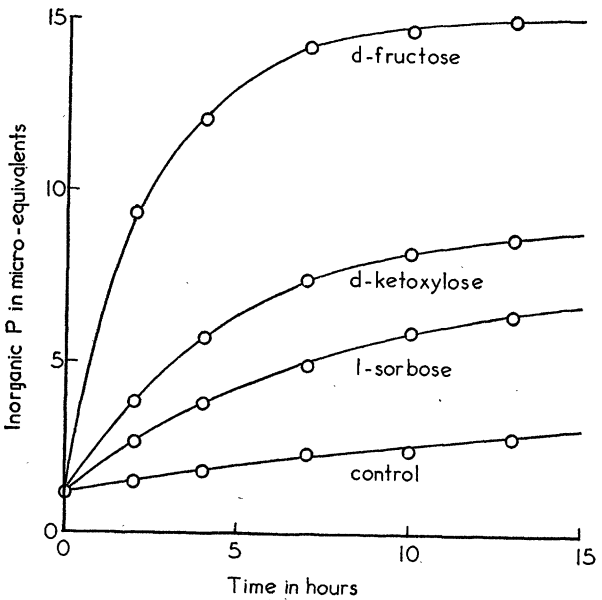

FIG. 1. Liberation of inorganic phosphate during the reaction between glucose-l-phosphate and ketose sugars.

In the hydrolytic decomposition of glucose-l-phosphate, one mole each of reducing sugar and inorganic phosphate is formed. In the reactions under consideration between gluocse-l-phosphate and the ketose sugars, one mole of inorganic phosphate is formed and one mole of reducing sugar disappears. This indicates a net utilization of two moles of sugar (one mole each of ketose and glucose) for each mole of inorganic phosphate liberated. The synthetic compounds therefore appear to be the disaccharides glucosido-sorboside and glucosido-ketoxyloside, respectively. No such disaccharides have ever been synthesized or found in nature. The ketoxyloside is of particular interest since a hexose-ketopentose disaccharide has never been reported.

It will be seen from Fig. 1 that at apparent equilibrium the amounts of phosphate liberated and hence of synthetic products formed are less when *l*-sorbose or *d*-ketoxylose is used than when the reaction involves fructose. Assuming the compounds to be disaccharides, the apparent equilibrium constants at pH 6.5 and 36°, expressed as

$$K = \frac{(\text{inorganic P})\ (\text{synthetic disaccharide})}{(\text{glucose-1-phosphate})\ (\text{ketose})}$$

were found to be 0.013 ± 0.004 and 0.004 ± 0.001 for the reactions with *d*-ketoxylose and *l*-sorbose, respectively.

Although neither of the two new sugars has as yet been obtained in pure form, a study of their properties in mixture with the parent ketoses has revealed that, like sucrose, they are very unstable in acid. Complete hydrolysis was effected by 5 minutes treatment with 1N HCl at 70° and with 0.1 N HCl at 100°. Unlike sucrose, neither compound is attacked by yeast invertase.

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ADSORPTION PHENOMENON OF BETA-CAROTENE

BETA-CAROTENE, dissolved in skellysolve B (65.5–70.5° C.), was adsorbed on activated alumina (200 mesh, Alorco) and the chromatogram was developed with chloroform (Merck's reagent or Baker's C.P.). Two pigment bands were formed. The top, narrow, orange band "T" moved slowly, and the lower, broad beta-carotene band passed rapidly down the column. Skellysolve B, benzene, acetone, ethylene chloride, carbon tetrachloride, trichlorethylene, ethyl acetate, methanol and ethyl ether were tried as developers but did not possess the resolving power of chloroform.

Five commercial, crystalline carotene preparations of different purity were dissolved in skellysolve B and immediately chromatographed. All preparations showed 3.0 to 4.9 per cent. of the T pigment to be present under these conditions. One preparation, declared to possess an extinction coefficient, $E^{1\text{ per cent.}}_{1\text{ cm}}$ (480 mμ) 2270, was considered the purest. A typical absorption curve of pigment T is presented as Fig. 1. In skellysolve B, beta-carotene has its maximum at 450; pigment T at 427 mμ.

It was demonstrated that the T pigment is formed continuously. A beta-carotene preparation was first freed of any alpha-carotene present by adsorption on calcium hydroxide (325 mesh, Marblehead Lime Company) and development with skellysolve B. The beta-carotene band was eluted with a skellysolve B-methanol solution. The water washed and dried (anhydrous sodium sulfate) beta-carotene solution was adsorbed on activated alumina and developed with chloroform. The T band formed. The beta-carotene section of the column was immediately transferred without elution onto the top of a freshly prepared column and washed with chloroform,

whereupon the T band formed again. This transfer was carried out three more times with the same results. In another experiment the beta-carotene was eluted before its transfer to a new column. The same phenomenon was observed. In a third experiment the beta-carotene solution was introduced on the column and washed with chloroform until the two bands were 30–40 mm apart. The vacuum was released for 10 to 15 minutes. Development was resumed until the main band moved another 40 mm and the vacuum again released. Four separate bands of the T-pigment were produced on the same column by this method. It appears that the conversion goes on continuously if the T pigment is constantly removed.

Part of the chloroform was purified by washing successively with concentrated sulfuric acid, dilute sodium hydroxide and water. The dried (potassium carbonate) and distilled chloroform did not effect a separation. Experiments were carried out to ascertain whether the purified chloroform could regain its separatory power. Irradiation of the solution in an open vessel by a Mazda S–4 mercury vapor lamp from a distance of 22 mm for 30 minutes had no effect. The addition of as little as 0.2 per cent. methanol restored the resolving power of the chloroform. Ethanol appeared less effective; acetone, water or hydrochloric acid were of no value. The addition of the alcohols to skellysolve B did not improve the resolving power of skellysolve B.

Previous workers[1,2] judged similar phenomena to be due to isomerization. However, pigment T does not resemble the beta-carotene isomers[3] reported to form above beta-carotene on the column. The present authors believe that under the conditions encountered beta-carotene may undergo isomerization or some other spontaneous change. The chloroform-methanol solution separates the newly formed compound on the column by shifting the adsorption affinity of the compounds. The authors are not familiar with any previous reports demonstrating such a rapid change without the use of specific activation. Hence these brief observations are reported to facilitate further investigation. Specific experiments to establish the reversibility of this change were not carried out.

Resemblance of the absorption spectrum of the T pigment to a combined absorption curve pigment band C3a[4] separated from the carotene fraction of yellow corn, and beta-carotene is observed. The absorption curves of beta-carotene (dotted line) and of pigment C3a (dashed line) are presented as background in Fig. 1. Chromatographically, pigments C3a and T are identical by mixed adsorption on activated alumina

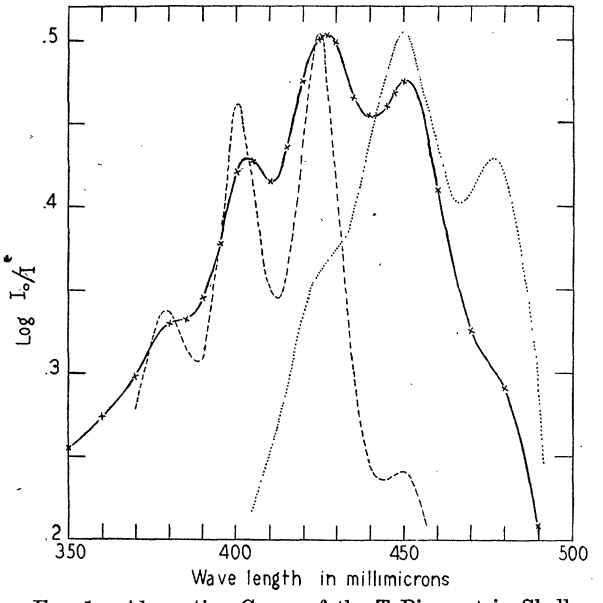

FIG. 1. Absorption Curve of the T Pigment in Skellysolve B. —x—x—x = Pigment T; - - - - - - - - = Pigment C3a; ..... = Beta-Carotene.

with non-treated chloroform. Spectrophotometrically, however, this identity is not confirmed.

Since pigment C3a is derived from the carotene fraction of corn its formation during the life of the corn plant, or during the extraction and saponification process may be postulated, in which case it may represent a final stage, whereas pigment T represents an intermediate one and in the stage of transformation. If pigment T is a mixture of beta-carotene and pigment C3a, a separation should have occurred by the adsorption procedure.

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ON THE OCCURRENCE OF A FLUORESCING POLYENE WITH A CHARACTERISTIC SPECTRUM

IT has been frequently observed by members of our group,[1] as well as by other authors,[2] that when some carotenoid containing plant extracts were

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