selected received 40,000 units in the next 24 hours in graded doses of 5,333, 2,666, 1,333 and 666 units each.

At the end of 96 hours the tumors were growing in all the mice. Therefore, two mice were selected from those that had received 8,000 units in the first 72 hours and 5,333 units in the following 24 hours. They were injected during a period of 37 hours with 20,000 units each, amounting to 540 units an hour.

From the above results it can be seen that the 32 mice received from 33,333 units each to 1,000 units each. At the end of seven days all the treated and the untreated mice had tumors of approximately the same size. Fourteen days after the experiment was begun two of the treated mice were dead and the others, both the controls and the treated ones, were moribund.

When it was found that the purified penicillin failed to retard the growth of sarcoma *in vivo* we prepared roller tube tissue cultures similar to those used by Ivor Cornman.[3] In these we found that the purified penicillin failed to damage the sarcomatous or the normal cells even when 120 and 160 units were added to the roller tube culture.

We then decided to compare the action of a more highly purified colorless sodium salt of penicillin (Merck Lot 4R263, 14,000 Oxford units in 9.216 mg) with that of a yellow sodium salt of penicillin (Squibb, Lot 87225).

The pieces of tissue were arranged in test-tubes in a row as follows: kidney, sarcoma, spleen, sarcoma, muscle, sarcoma and heart followed by sarcoma. When the tissue had grown for 24 to 30 hours the medium was withdrawn, fresh medium added and then into each tube was added a known number of units of penicillin dissolved in normal salt solution.

Twenty-four hours later the sarcomatous and the normal tissue were growing abundantly in the cultures that had received 20, 40, 53, 60, 80, 100, 120 and 160 units of the highly purified penicillin. In some experiments the tubes were given a second dose of 80 or of 160 units; nevertheless, the cells continued to grow for a number of days. In the tubes that received the yellow penicillin both the normal and the sarcomatous cells were growing abundantly in the tubes containing 20, 40 and 53 units, respectively, but in the tubes containing 60 and 80 units the tumor cells were damaged while the normal cells were growing. In the tubes that had received 100 and 120 units the tumor cells were killed and the normal cells were damaged.

The tube tissue culture experiments were repeated several times and in each instance they showed that the highly purified penicillin failed to damage the growing normal or the growing sarcomatous cells while the yellow penicillin used in proper dosage damaged the growing sarcomatous cells without injuring the normal cells. These experiments confirm the observations of Ivor Cornman.[3]

From our studies we surmise that the factor present in the less purified sodium salt of penicillin which damaged the sarcoma cells is lost in the highly purified product.

In addition to our studies with the sodium salts we tested one calcium salt. One hundred thousand units of a somewhat yellow colored calcium salt of penicillin, prepared by the Commercial Solvents Corporation, were tested on four mice that had been implanted 48 hours previously with grafts of sarcoma. The four mice tolerated injections of 250, 500, 500 and 750 units, respectively, every two and one-half hours for 28 doses. As the tumors were growing the injections were increased to 1,000 units every two hours. Totally the mice received 11,500, 24,000, 24,000 and 31,000 units, respectively, during 38 injections over a period of 5 days. At the end of 8 days the growing tumors were equally large in the 4 treated and the 4 untreated control mice.

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[3] SCIENCE, 99: March 24, 1944.

SYNTHESIS OF TWO NEW CARBOHYDRATES WITH BACTERIAL PHOSPHORYLASE

THE synthesis of two carbohydrates, which appear to be new analogues of sucrose, has been effected by the use of a phosphorylase preparation from the bacterium *Pseudomonas saccharophila*. This enzyme preparation catalyzes the reversible reaction:

sucrose + inorganic phosphate $\rightleftharpoons$ glucose-1-phosphate + fructose,

and has previously been shown to have no action on trehalose, maltose, raffinose, glycogen or starch.[1,2,3,4] Attempts to substitute phosphoric esters of fructose or aldose sugars for fructose have met with no success, nor could any reaction be observed between fructose and maltose-1-phosphate.[5] However, when either *l*-sorbose or *d*-ketoxylose (crude mixture of xylose and ketoxylose) was added together with glucose-1-phosphate to the enzyme preparation, there was evidence of a reaction similar to that observed in the synthesis of sucrose and characterized by the liberation of inorganic phosphate accompanied by a decrease in the

[1] M. Doudoroff, N. Kaplan and W. Z. Hassid, *Jour. Biol. Chem.*, 148: 67, 1943.
[2] M. Doudoroff, *Jour. Biol. Chem.*, 151: 351, 1943.
[3] W. Z. Hassid, M. Doudoroff and H. A. Barker, *Jour. Am. Chem. Soc.*, 66: 1416, 1944.
[4] H. A. Barker, W. Z. Hassid and M. Doudoroff, SCIENCE, 100: 51, 1944.
[5] The maltose-1-phosphate was recently synthesized chemically by W. R. Meagher and W. Z. Hassid (unpublished).

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.

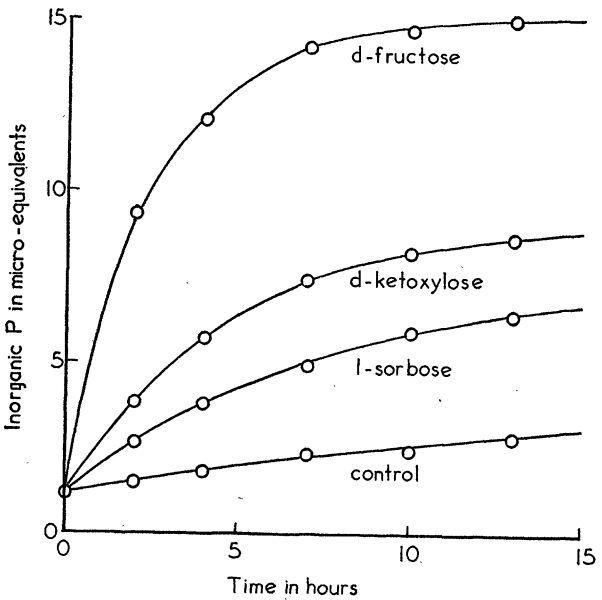

FIG. 1. Liberation of inorganic phosphate during the reaction between glucose-l-phosphate and ketose sugars.

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,