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THE PATH OF CARBON IN PHOTOSYNTHESIS. XIX THE IDENTIFICATION OF SUCROSE PHOSPHATE IN SUGAR BEET LEAVES

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Berkeley, California

THE PATH OF CARBON IN PHOTOSYNTHESIS. XIX

THE IDENTIFICATION OF SUCROSE PHOSPHATE IN SUGAR BEET LEAVES*

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Abstract

The recognition and characterization of a sucrose phosphate as an intermediate in sucrose by sucrose synthesis by green plants is described. A tentative structure for this phosphate is proposed and its mode of formation suggested.

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The synthesis of sucrose in green plants has for long been a problem to plant biochemists. The sucrose phosphorylase enzyme from Pseudomonas saccharophila will catalyse the reaction

$$\text{sucrose} + H_3PO_4 \rightleftharpoons \alpha\text{-D-glucose-1-phosphate} + \text{D-fructose}$$

and Hassid, Doudoroff and Barker[1] have isolated sucrose synthesized by this enzyme. It appears, however, that this is not the mechanism by which sucrose is synthesized in the green plant. It has not been possible to show the presence of this enzyme in higher plants. In Part IV[2] of this series it was shown that when Chlorella were allowed to photosynthesize in radioactive carbon dioxide, sucrose was the first free sugar to be formed. This was interpreted to mean that in sucrose synthesis in higher plants, only phosphorylated derivatives of sugars were involved, probably yielding a sucrose phosphate as the first sucrose-containing product. There is reason to suspect that there is a naturally occurring sucrose phosphate in nature from work on the utilization of sucrose by microorganisms and that it is the fructose moiety which phosphorylated. More recently, Putman and Hassid,[3] working with leaf punches, have obtained evidence for the formation of a phosphorylated sucrose derivative in sucrose synthesis.

We have examined the "hexose monophosphates" produced during photosynthesis in $C^{14}O_2$. These were treated with an invertase-free phosphatase preparation and subjected to paper chromatography. In several cases, there were only minute traces of sucrose formed by this treatment, but in sugar beet (5 minutes in $C^{14}O_2$) there was an appreciable quantity. It was identified by co-chromatography, and enzymatic hydrolysis to glucose and fructose, themselves identified by co-chromatography.

When this "hexose monophosphate" sample was subjected to chromatography in t-butanol/picric acid/water, radioactive areas corresponding to glucose-6-phosphate, fructose-6-phosphate, sedoheptulose and mannose phosphates, and

sucrose phosphate were obtained. The sucrose phosphate gave sucrose on phosphatase treatment, and on acid hydrolysis, glucose and a fructose phosphate were produced. The latter did not co-chromatograph with fructose-6-phosphate.

The sucrose phosphate in sugar beet leaves has the probable structure:

(Figure 1)

It would seem that in sucrose synthesis in green plants there are two possible mechanisms. Glucose-1-phosphate might react with fructose-1-phosphate to give sucrose phosphate, which would be dephosphorylated to sucrose. Alternatively, sucrose phosphate synthesis might be envisaged to occur through uridine diphosphate glucose,[4] which becomes labeled shortly before sucrose in kinetic experiments with $C^{14}O_2$.[5] The uridine diphosphate glucose may be formed from glucose-1-phosphate by a series of reactions analagous to those proposed by Kornberg.[6] These alternative schemes are summarized in Figure 2.

Although it has not been possible to find appreciable quantities of the sucrose phosphate in many of the plants studied, we believe that this is probably due to its reservoir size being very small and that the mechanism of sucrose synthesis is very similar in green leaves and in algae.

Experimental

The chromatographic techniques were those described in Part V.[7] The solvent t-butanol-picric acid was that of Hanes and Isherwood,[8] as modified by Moyle and Mitchell[9] to avoid crystallization of the picric acid on the chromatogram during chromatography: t-butanol (80 cc.), water (20 cc.), picric acid (2 g.). The enzyme preparations used were "Polidase-S" (Schwarz Laboratories, Inc.) and "Phosphatase" (General Biochemicals, Inc.). Both were used in 1% solution, without buffer. The incubations were carried out

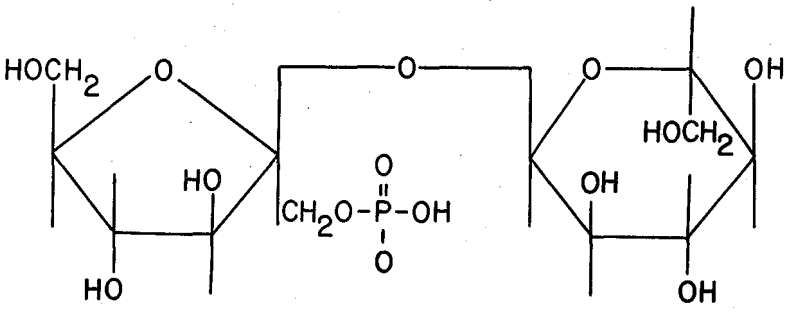

Fig.1

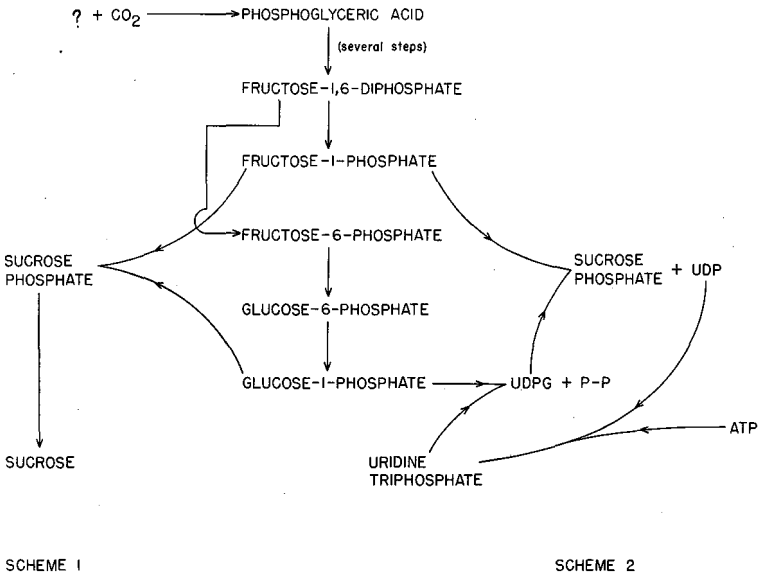

Fig. 2. The Path of Sucrose Synthesis from CO_2

at 35° C. under toluene, with 200 μg. of enzyme, for periods of 24-72 hours. The "Phosphatase" was shown to be devoid of invertase activity.

Detection of a Phosphorylated Sucrose Derivative

The source of radioactive compounds was an extract from sugar beet leaves which had photosynthesized for 5 minutes in $C^{14}O_2$. The radioactive area used was that designated "hexose monophosphates" on chromatograms in the standard solvents (Phenol: n-butanol-propionic acid).

The "hexose monophosphate" area was extracted and hydrolyzed with phosphatase. On rechromatography, besides the usual monosaccharides, there appeared a spot in a position characteristic of a disaccharide. Figure 3.

In one experiment, the unknown spot was rechromatographed with carrier sucrose (100 γ). After exposure to film, the chromatogram was sprayed with aniline-trichloroacetic acid and heated at 100° for 5 minutes. It was then sprayed with orcinol-trichloroacetic acid (orcinol (0.5 g.), trichloroacetic acid (15 g.), t-butanol (90 cc.), water (10 cc.)) and heated in the same way as before. The brown spot which appeared was completely coincident with the darkened area on the radioautograph.

In another experiment the disaccharide spot was cut out and treated with "Polidase-S" (known to have invertase activity). The hydrolysate was chromatographed with glucose and fructose carrier. After exposure to film, the chromatogram was sprayed with ammoniacal silver nitrate (5% $AgNO_3$ in methanol) and heated. The black spots were coincident with the dark areas on the film. (Figure 4)

The Separation of the Sucrose Phosphate from Monosaccharide Phosphates

The "hexose monophosphate" area was extracted and treated with a small quantity of the acid form of Dowex-50. Glucose-6-phosphate (250 γ of Ba salt,

treated with acid Dowex-50) was added as carrier and the solution chromatographed as a band in t-butanol-picric acid. Four bands were produced. (Figure 5) The slowest moving band was extracted and chromatographed in phenol to remove the picric acid and any free sugars which might be present. The only free sugar observed had the same R_f value in phenol as glucose. When the phosphate was extracted from the phenol chromatogram and treated with "Phosphatase," followed by chromatography, three sugars, with the chromatographic coordinates of glucose, fructose and sucrose were produced. (Figure 6)

In another experiment, using the same sample of radioactive hexose monophosphates, the sucrose phosphate, freed from picric acid by chromatography in phenol after partial separation from the other phosphates in t-butanol-picric acid, was divided into two parts (a) and (b). Sample (a) was rechromatographed in two dimensions: phenol (first direction), t-butanol-picric acid (second direction). Under these conditions, three major spots were produced (Figure 7). One co-chromatographed with glucose-6-phosphate, and another with fructose-6-phosphate. The other, the most radioactive of the three, behaved as sucrose phosphate.

Sample (b) was mixed with the same carriers as (a) and hydrolyzed with 0.1 N HCl at 100° for 5 minutes. The hydrolysate was then chromatographed in the same way as above. Four major (and several minor) spots were visible on the radioautograph: glucose-6-phosphate, fructose-6-phosphate, glucose (all identified by co-chromatography) and another phosphate close to but not coincident with the fructose-6-phosphate. (Figure 8) Only a very small amount of radioactive fructose was present, although spraying of the chromatogram showed that practically all of the carrier fructose was present. Table I shows the relative radioactivities of the compounds (approximate).

Table I

	c.p.m.	
Compound	(a)	(b)
Sucrose phosphate	180	0
Glucose-6-phosphate	80	80
Glucose	20	120
Fructose-6-phosphate	110	110
Fructose-1-phosphate(?)	0	90
Fructose	0	20

The glucose-6-phosphate and the fructose-6-phosphate probably arises from a small amount of cross contamination between them and sucrose phosphate on the original picric acid chromatogram. Since practically all of the sucrose phosphate had been hydrolyzed and only half of the radioactivity originally present in it appears as glucose, almost all of the rest being in the new phosphate appearing next to fructose-6-phosphate, it follows that this phosphate is a fructose phosphate different from fructose-6-phosphate. The amount of free fructose present is roughly about 1/10 of that of the fructose phosphate suggesting that the rate of hydrolysis of this fructose phosphate corresponds to that of fructose-1-phosphate. This would lead to the tentative structure for the sucrose phosphate shown in Figure 1.

References

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2) M. Calvin and A. A. Benson, Science, 109, 140 (1949).

3) E. W. Putman, Thesis, Ph.D., University of California, June, 1952.

4) J. G. Buchanan, et al., "Phosphorus Metabolism, II", Johns Hopkins Press, 1952.

5) A. A. Benson, S. Kawaguchi, P. Hayes and M. Calvin, J. Am. Chem. Soc., in press.

6) A. Kornberg, "Phosphorus Metabolism, I", Johns Hopkins Press, 1951.

7) A. A. Benson, et al., J. Am. Chem. Soc., 72, 1710 (1950).

8) C. S. Hanes and F. A. Isherwood, Nature, 164, 1107 (1949).

9) Moyle and Mitchell

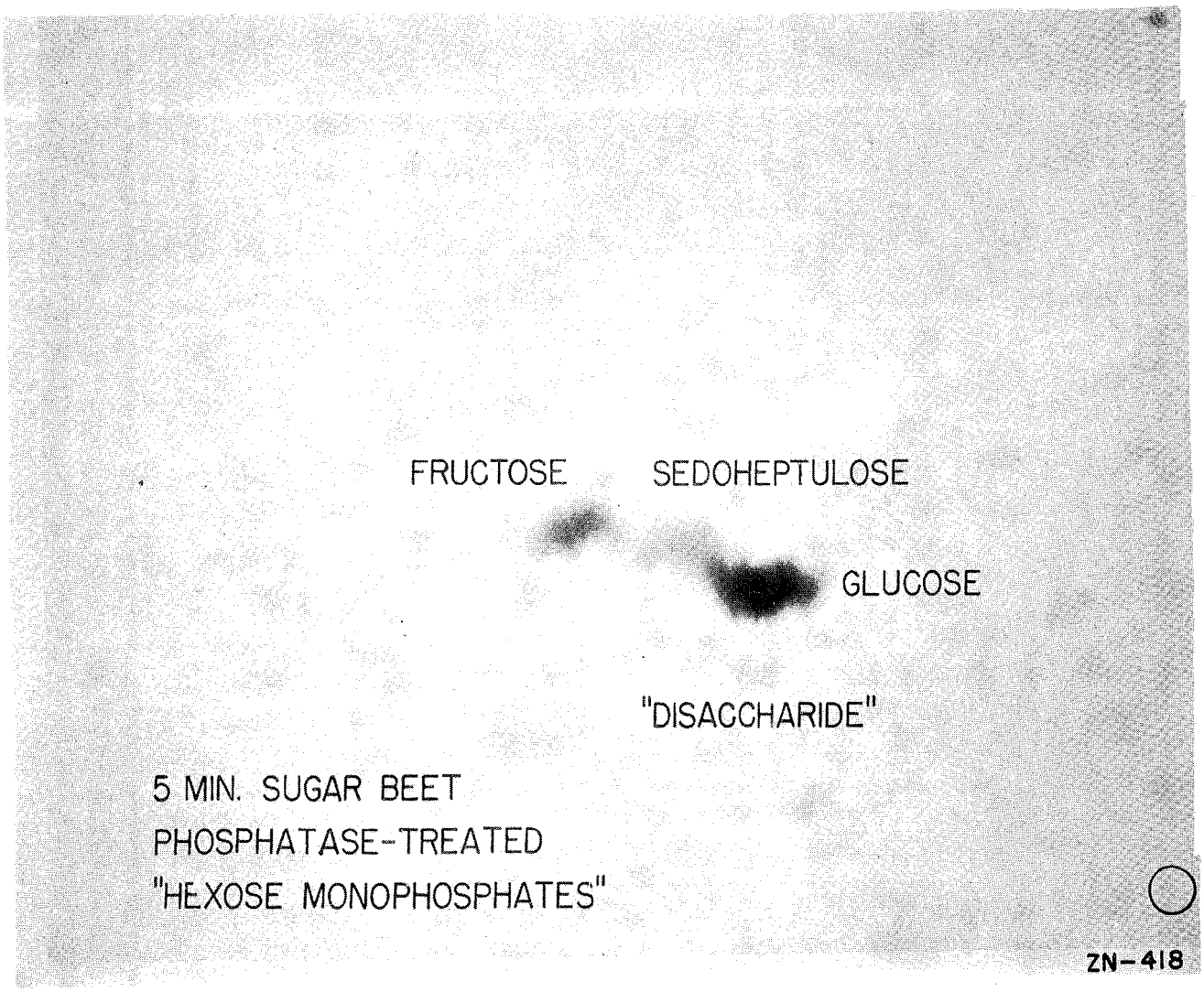
FRUCTOSE
SEDOHEPTULOSE
GLUCOSE
"DISACCHARIDE"
5 MIN. SUGAR BEET
PHOSPHATASE-TREATED
"HEXOSE MONOPHOSPHATES"
ZN-418

Fig.3

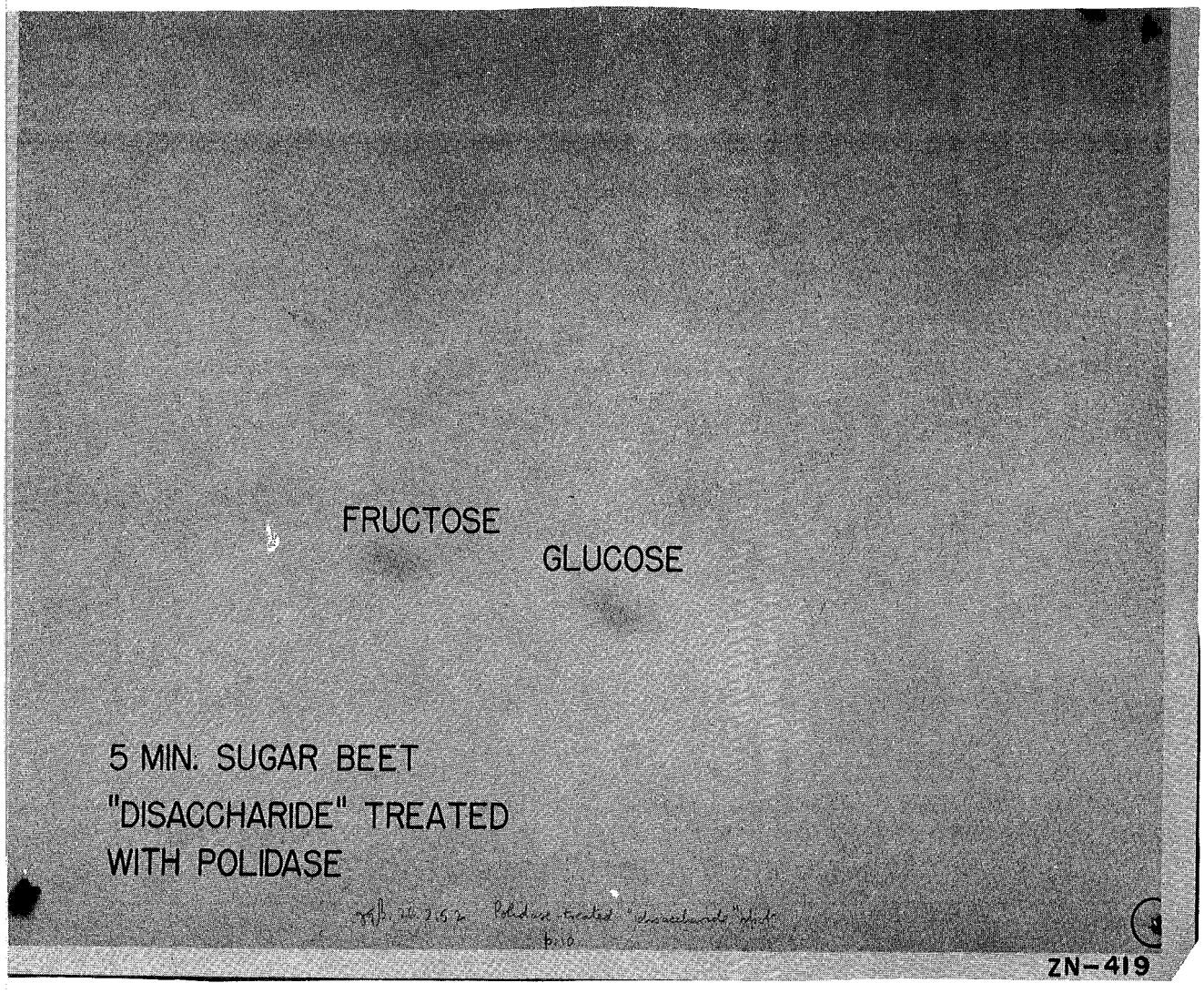
FRUCTOSE
GLUCOSE
5 MIN. SUGAR BEET
"DISACCHARIDE" TREATED
WITH POLIDASE
ZN-419

Fig.4

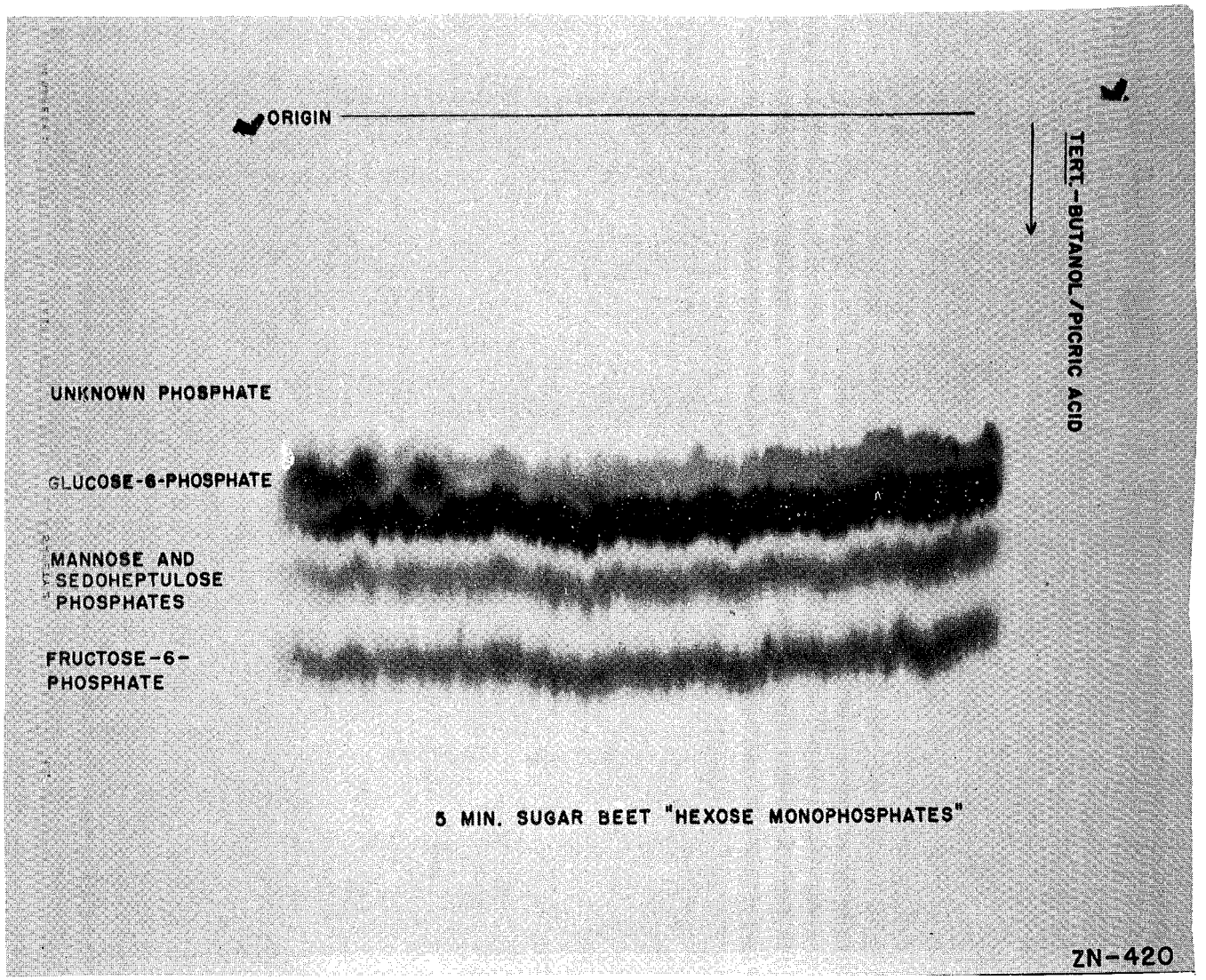
ORIGIN
TERT.-BUTANOL/PICRIC ACID
UNKNOWN PHOSPHATE
GLUCOSE-6-PHOSPHATE
MANNOSE AND
SEDOHEPTULOSE
PHOSPHATES
FRUCTOSE-6-
PHOSPHATE
5 MIN. SUGAR BEET "HEXOSE MONOPHOSPHATES"
ZN-420

Fig.5

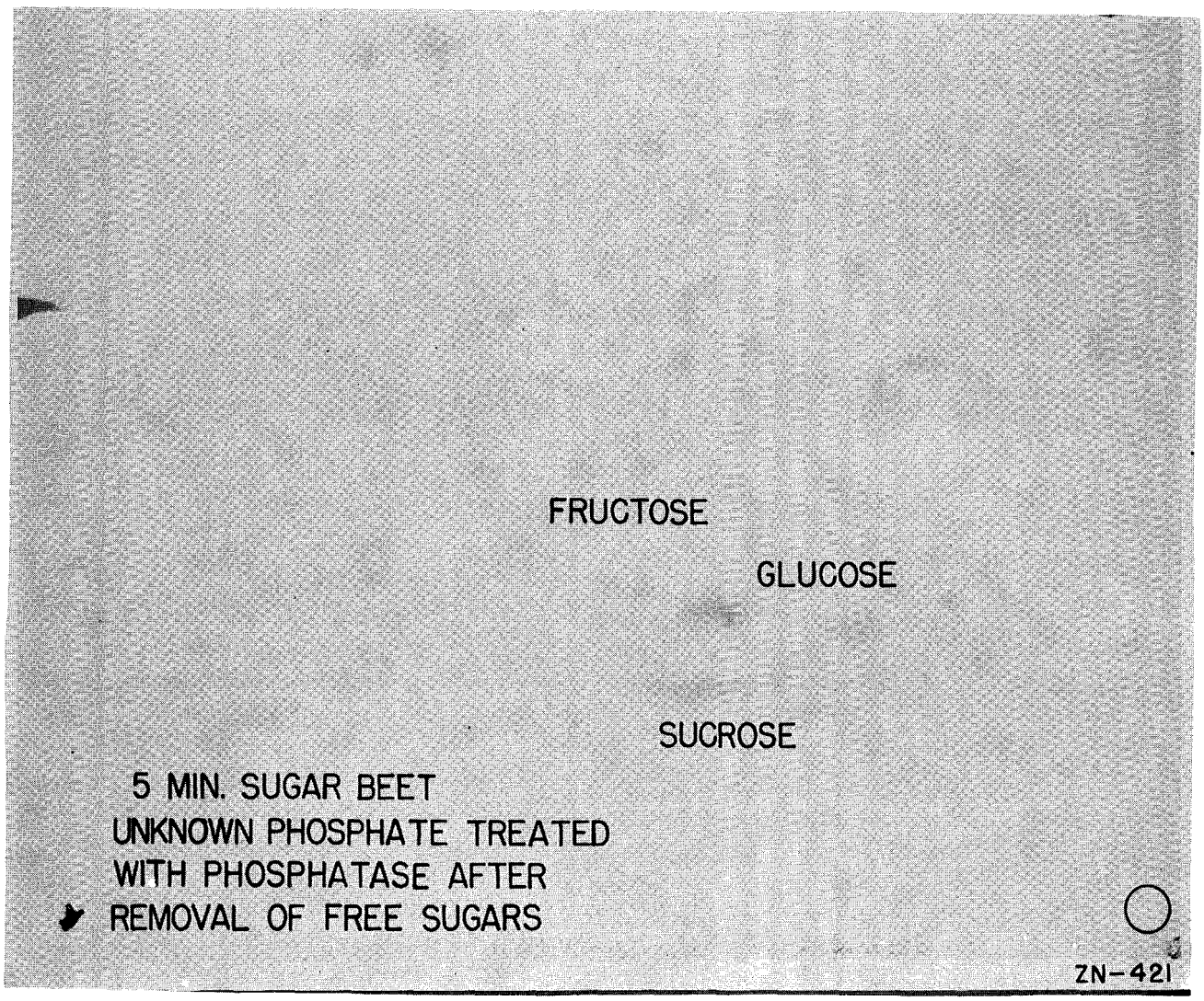
FRUCTOSE
GLUCOSE
SUCROSE
5 MIN. SUGAR BEET
UNKNOWN PHOSPHATE TREATED
WITH PHOSPHATASE AFTER
REMOVAL OF FREE SUGARS
ZN-421

Fig.6

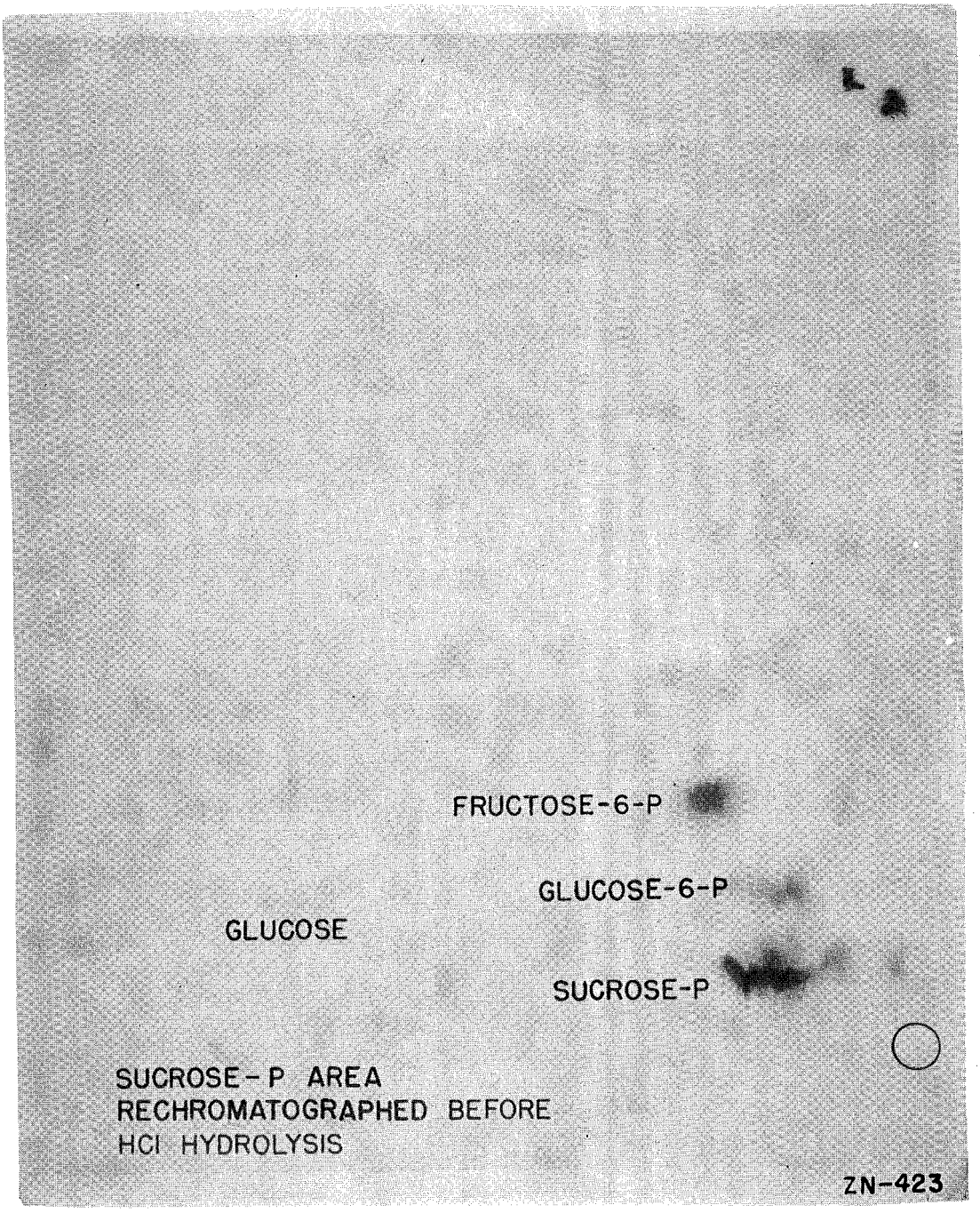
FRUCTOSE-6-P
GLUCOSE-6-P
GLUCOSE
SUCROSE-P
SUCROSE-P AREA
RECHROMATOGRAPHED BEFORE
HCl HYDROLYSIS
ZN-423

Fig.7

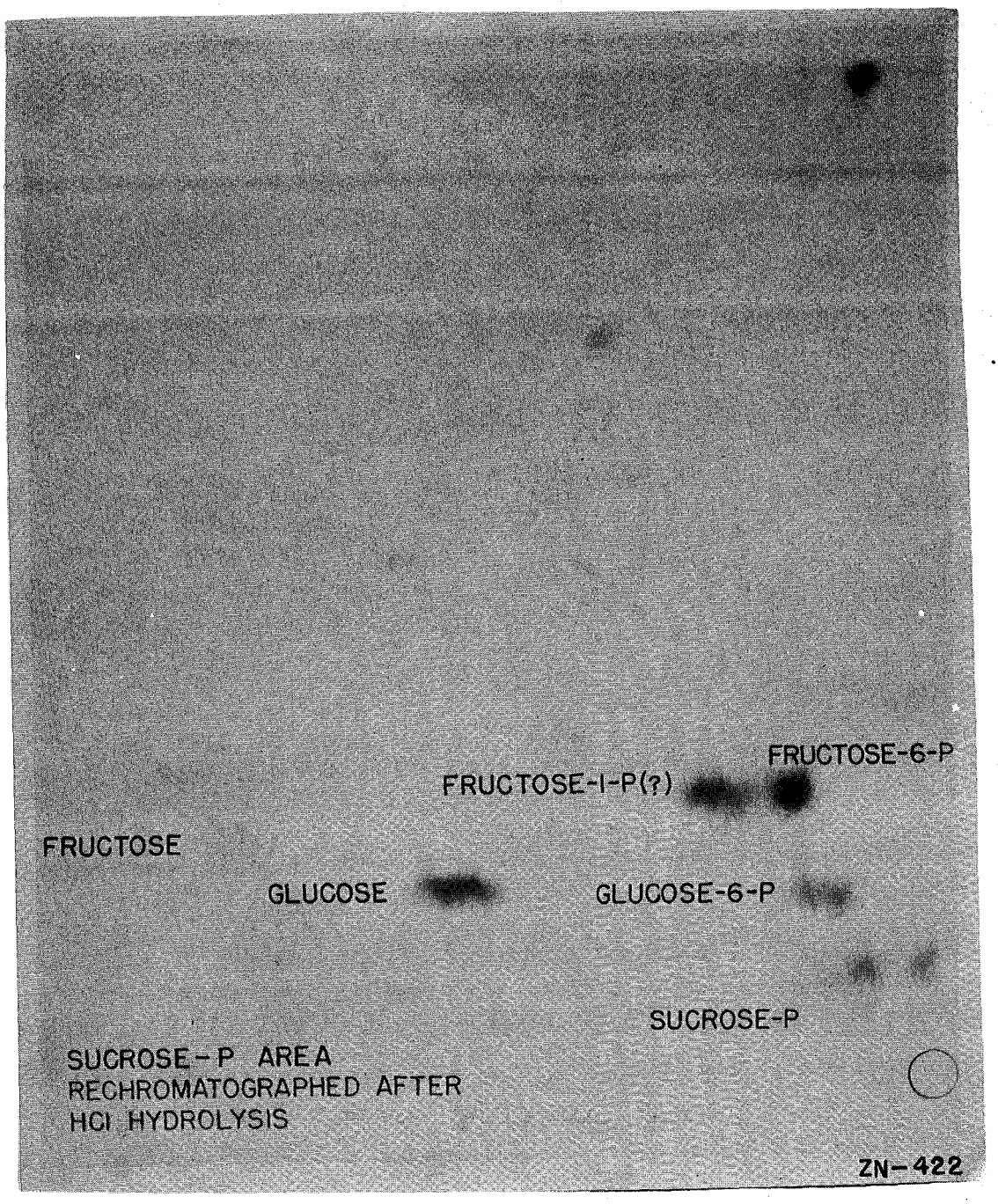
FRUCTOSE-6-P
FRUCTOSE-1-P(?)
FRUCTOSE
GLUCOSE
GLUCOSE-6-P
SUCROSE-P
SUCROSE-P AREA
RECHROMATOGRAPHED AFTER
HCl HYDROLYSIS
ZN-422

Fig.3