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

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September, 1952

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.

(*) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

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

sucrose + $H_3PO_4$ $\rightleftharpoons$ α-D-glucose-1-phosphate + 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

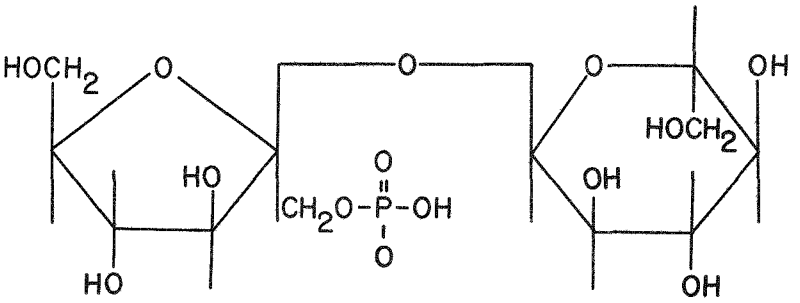


Fig.1

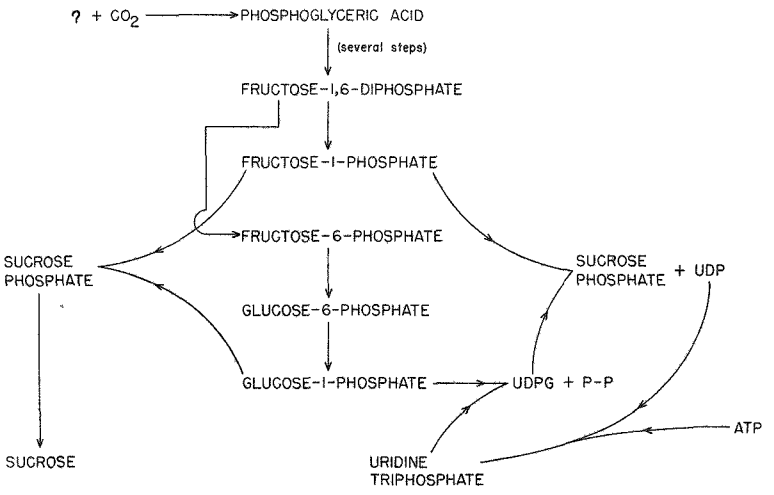


Fig. 2. The Path of Sucrose Synthesis from $CO_2$

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,

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.

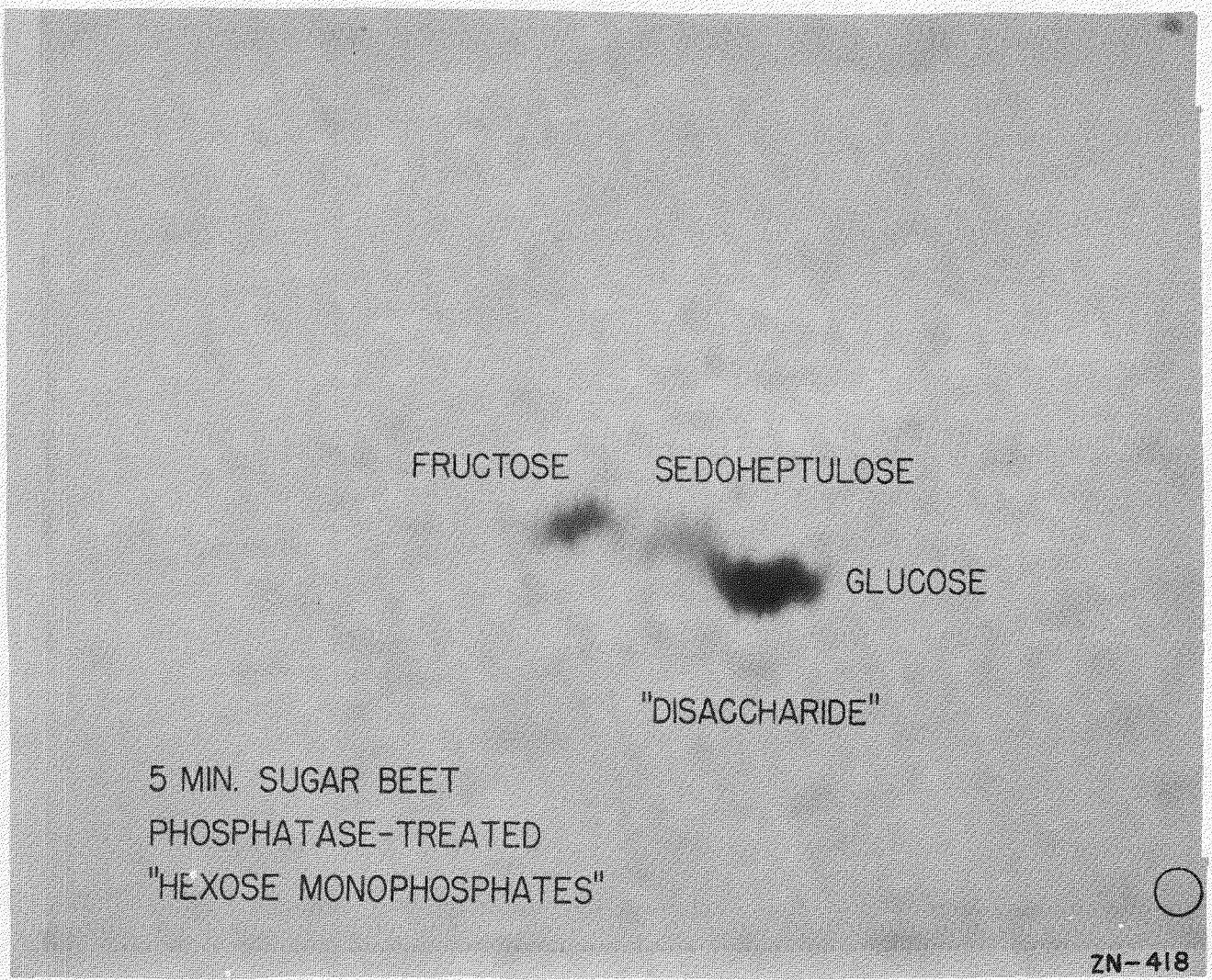
FRUCTOSE
SEDOHEPTULOSE
GLUCOSE
"DISACCHARIDE"
5 MIN. SUGAR BEET
PHOSPHATASE-TREATED
"HEXOSE MONOPHOSPHATES"
ZN-418

Fig.3

11

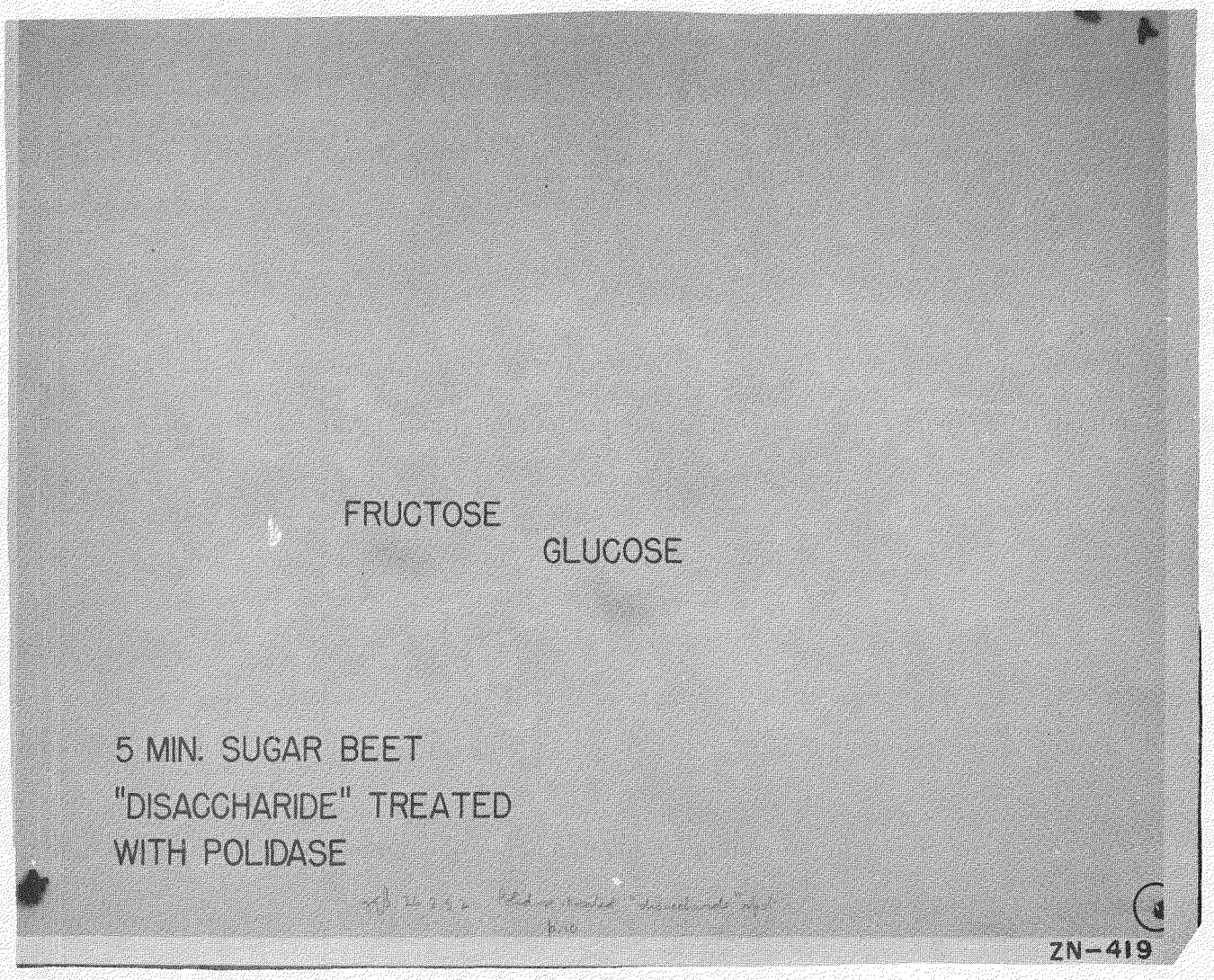
FRUCTOSE
GLUCOSE
5 MIN. SUGAR BEET
"DISACCHARIDE" TREATED
WITH POLIDASE
ZN-419

Fig.4

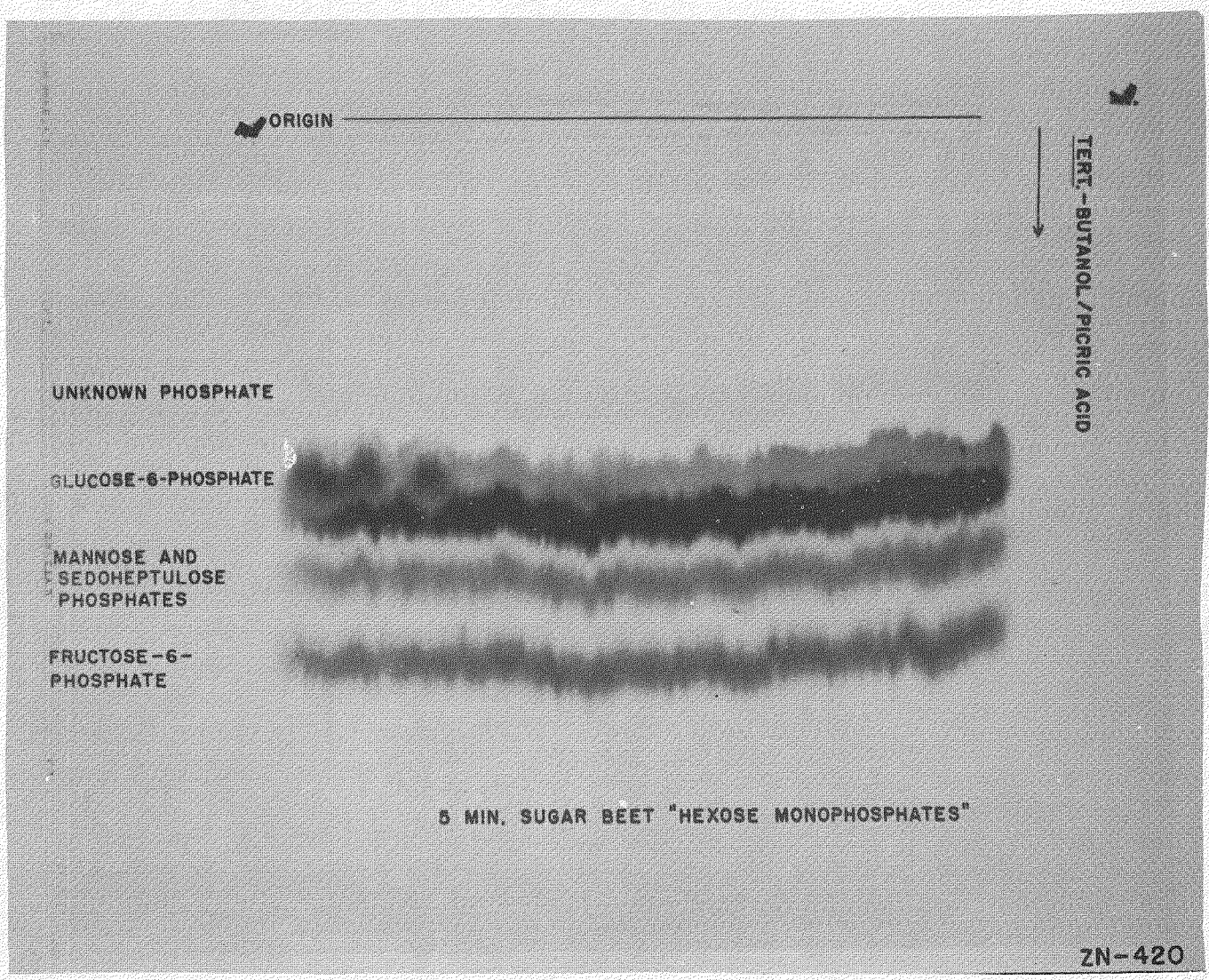
ORIGIN
TERT.-BUTANOL/PICRIC ACID
UNKNOWN PHOSPHATE
GLUCOSE-6-PHOSPHATE
MANNOSE AND
SEDOHEPTULOSE
PHOSPHATES
FRUCTOSE-6-
PHOSPHATE
5 MIN. SUGAR BEET "HEXOSE MONOPHOSPHATES"
ZN-420

Fig. 5

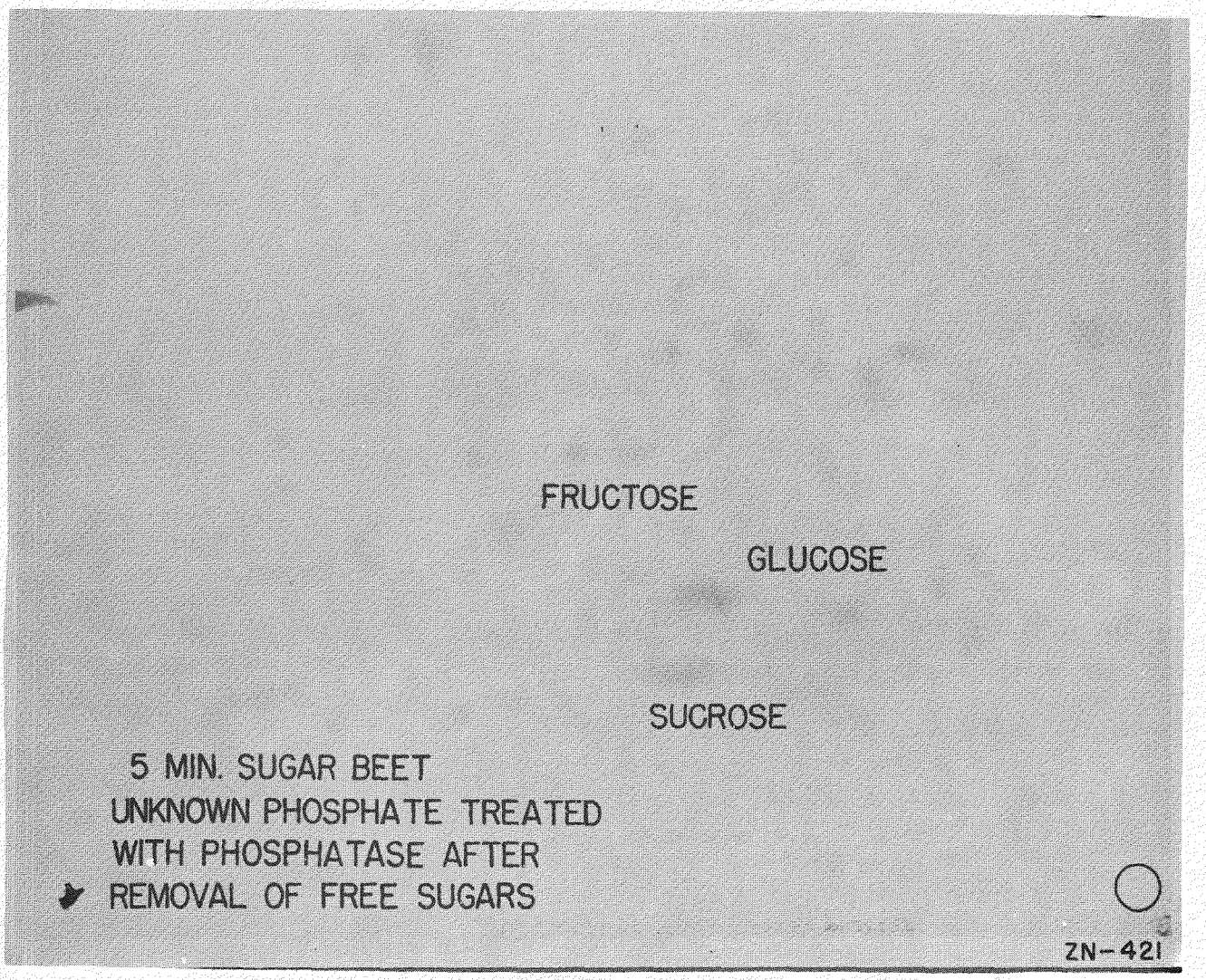
FRUCTOSE
GLUCOSE
SUCROSE
5 MIN. SUGAR BEET
UNKNOWN PHOSPHATE TREATED
WITH PHOSPHATASE AFTER
REMOVAL OF FREE SUGARS
ZN-421

Fig.6

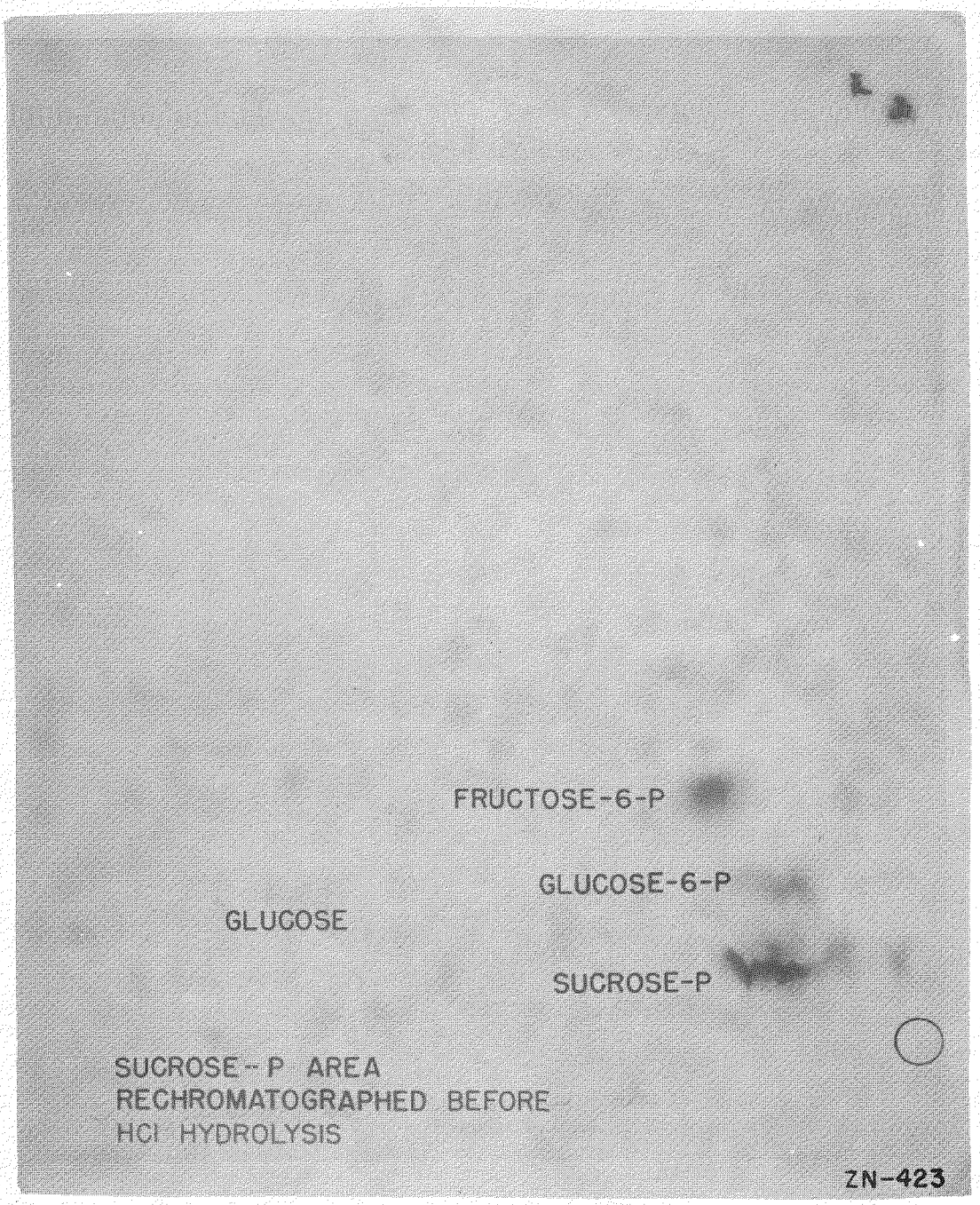
FRUCTOSE-6-P
GLUCOSE-6-P
GLUCOSE
SUCROSE-P
SUCROSE-P AREA
RECHROMATOGRAPHED BEFORE
HCl HYDROLYSIS
ZN-423

Fig.7

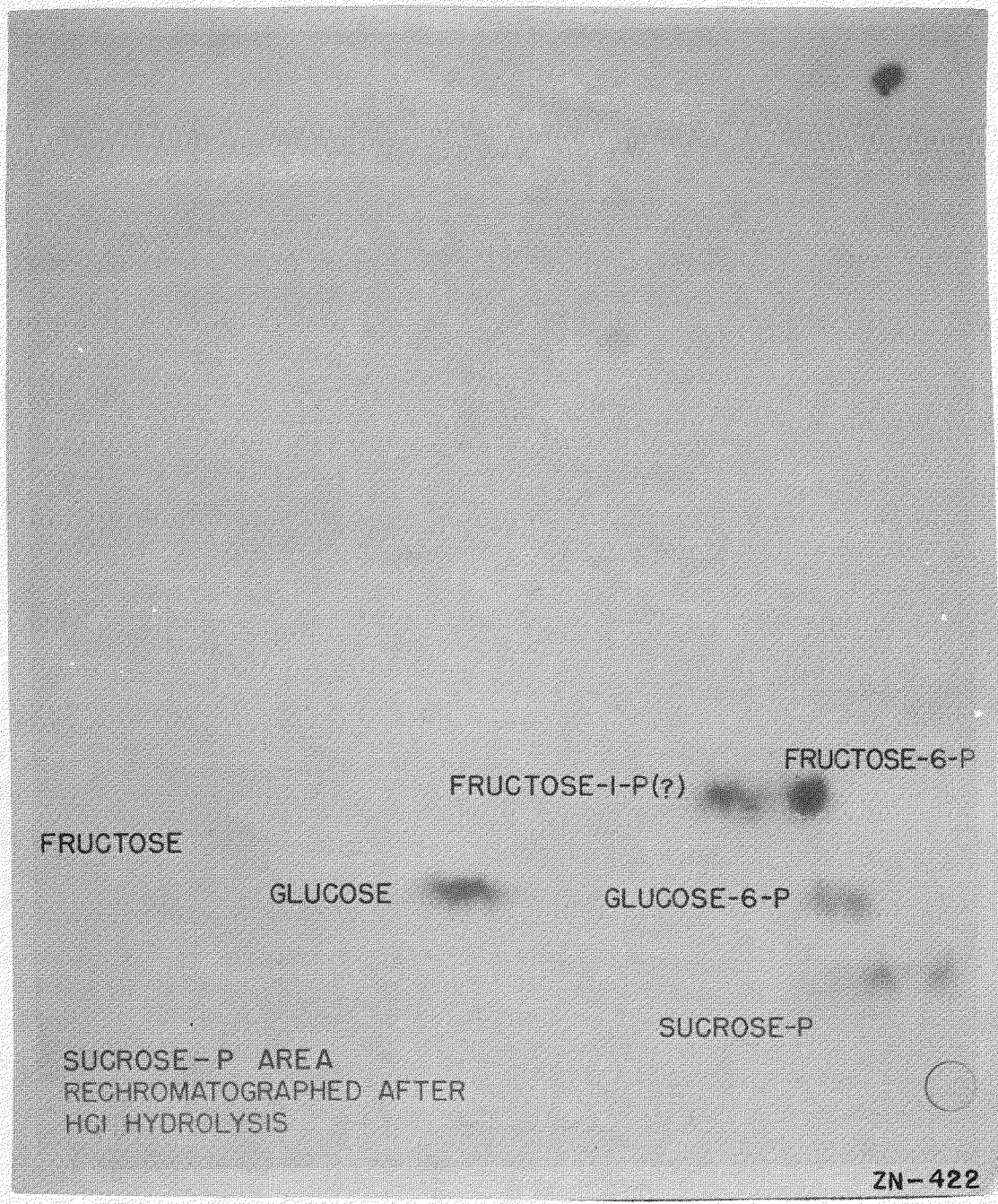
FRUCTOSE-6-P
FRUCTOSE-1-P(?)
FRUCTOSE
GLUCOSE
GLUCOSE-6-P
SUCROSE-P
SUCROSE-P AREA
RECHROMATOGRAPHED AFTER
HCl HYDROLYSIS
ZN-422

Fig.3