

The values obtained with polarimetric determinations are considered to be more accurate than those calculated from the amounts of reducing sugars formed. For the micro sugar determinations, the solutions must be highly diluted and the accuracy of the determinations might be impaired by this procedure.

Varying amounts of enzyme solutions were used for the same total volume of reaction mixture in order to investigate whether dilution of the enzyme decreases the effect of the inhibitor of "invertase" as claimed by Schwimmer *et al.*⁵ In the present investigation an increase of activity by dilution of the enzyme was also observed. The higher amount of enzyme used caused a slower hydrolysis of the sucrose, *cf.* Table 1.

It should, however, be mentioned that some of the isolated enzyme preparations, even from sprouted control tubers, failed to give any "invertase" activity. The reason for this fact could perhaps be that the "invertase" inhibitor, if present, is more or less developed in the tubers. "Invertase" originating from other biological materials is extremely sensitive to inhibitors, *e.g.* metal ions.⁷ The extent of inhibition depends on the degree of purity of the enzyme. However, as only a crude enzyme preparation was used in the present investigation, the possibility is not excluded that small variations in the isolation procedure might influence the activity.

No "invertase" activity, determined by method B, could be observed in the "transferase" preparation isolated from potato tubers as reported in Ref. 4.

It is obvious that the activity of "invertase" in non-irradiated tubers increases with prolonged storage, whereas the "transferase" activity decreases. In irradiated tubers, the "invertase" is inhibited, but the activity of "transferase" is increased. It is undoubtedly quite feasible that the sucrose synthesizing enzyme and the competitive sucrose hydrolyzing enzyme are respectively influenced in opposite directions by prolonged storage and by irradiation of the potato tubers.

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Influence of γ -Irradiation on UDPglucose-Fructose Glucosyltransferase in Potato Tubers

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Several authors¹⁻⁴ have shown that UDPglucose is involved in the biosynthesis of sucrose in higher plants. It is well known that the sucrose concentration in potato tubers is influenced by several factors, *e.g.* changes of the temperature, humidity, chemical treatments, and ionizing radiation.

Schwimmer and Rorem⁵ reported that the potato tuber is a rich source of UDPglucose-fructose glucosyltransferase ("transferase"). These authors found the highest amount of sucrose to be synthesized at pH 8.1. They pointed out, however, that this pH is perhaps not the true optimum pH of "transferase". The accumulation of sucrose was supposed to be maximal at pH 8.1, while the activity of β -fructofuranosidase ("invertase"), catalyzing the hydrolysis of sucrose, sharply decreased at this pH.

In order to investigate if the marked increase of sucrose in potato tubers, caused by ionizing radiation,⁶⁻⁹ could be related to changed activity of "transferase", this enzyme was isolated from γ -irradiated, and corresponding non-irradiated, tubers. The activities of the isolated enzyme preparations were determined.

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Experimental. The potato varieties King Edward, Bintje, and Early Puritan were used for this study. The irradiation dose was 14–15 kilorad (^{60}Co -rays), the dose rate 100–150 r/h. The isolation and determination of “transferase” was performed according to Schwimmer and Rorem,⁵ with slight modifications, *e.g.* using centrifugation at 10 000 *g* instead of filtration of the ammonium sulphate precipitated protein. The retentate obtained by dialysis in 20 h was also centrifuged at 10 000 *g*, the supernatant discarded and the precipitate suspended in redistilled water. The temperature throughout the isolation procedure was maintained at 0–2°C. The content of protein nitrogen was determined according to the Kjeldahl micro method.

The tubers used for the investigation were previously stored at about + 4°C. From control tubers the isolation of “transferase” was performed at different times after harvest, and from irradiated tubers immediately after the irradiation and then after 2, 7, 15, 30, 120, 210, and 750 days.

The activity of “transferase” was determined in a medium containing 9 μmoles UDPglucose, 24 μmoles fructose, 6 μmoles MgCl_2 , 12.6 mmoles Tris (2-amino-2-hydroxymethylpro-

pan-1,3-diol) buffer pH 8.1, and 2.04 ml enzyme suspension. The total volume of the reaction mixture was 2.40 ml and the incubation temperature 37°C. A few determinations of activity of “transferase” were performed at pH 6.9. The reaction time was 1 and 2 h, respectively. In order to save UDPglucose (Sigma), which is expensive, about half of the experiments was carried out in one third of the reaction mixture and the 1 h reaction time was omitted.

The enzyme reaction was stopped by deproteinizing the mixture according to Somogyi.¹⁰ The sucrose contents in the centrifuged solutions were determined by the method of Rorem *et al.*³ (using sodium borohydride to eliminate the colour of reducing sugars). The sucrose was also determined by thin layer chromatography.

Results and discussion. Of the three potato varieties investigated only Bintje and King Edward gave active preparations of “transferase”. From the tubers of Early Puritan, it was impossible to prepare an enzyme which could synthesize sucrose in detectable amounts.

Table 1. Sucrose synthesized by UDPglucose-fructose glucosyltransferase preparations from potato tubers of Bintje and King Edward varieties. I = irradiated tubers, C = control tubers.

Potato variety and treatment	Interval between irradiation and analysis, days	Interval between harvest and analysis, months	Number of enzyme isolations	mg N/ml enzyme suspension	Sucrose synthesized, $\mu\text{-moles}$ in 2 h; mean \pm error of mean	Sucrose synthesized, $\mu\text{-moles}$ per ml protein N
Bintje C		2–6	15	2.01	0.79 \pm 0.01	0.39
» C		12–15	11	2.17	0.41 \pm 0.03	0.19
» I	0	} 0–6	4	1.87	0.81 \pm 0.03	0.43
» I	2		5	1.95	0.72 \pm 0.03	0.37
» I	7		3	2.05	0.96 \pm 0.07	0.47
» I	15		6	1.97	1.18 \pm 0.02	0.59
» I	30		10	1.91	1.61 \pm 0.02	0.84
» I	120		4	1.83	1.49 \pm 0.02	0.81
» I	210		4	2.01	1.43 \pm 0.02	0.71
King Edward C		2–6	5	2.35	0.65 \pm 0.01	0.28
» C		12–16	3	2.24	0.28 \pm 0.04	0.13
» I	30	3	5	2.08	1.38 \pm 0.04	0.66
» I	750	28	4	1.84	2.90 \pm 0.09	1.58
» I	750	28	4	1.84	2.95* \pm 0.06	1.60
» C		17	3	1.89	0.33 \pm 0.05	0.17
» C		17	3	1.89	0.21* \pm 0.08	0.11

* pH of the reaction mixture 6.9.

The results obtained with "transferase" prepared from Bintje and King Edward are recorded in Table 1. In control tubers there is a pronounced decrease of enzyme activity after prolonged storage. The tubers stored for 12 to 15 months were well sprouted, in contrast to the control tubers stored for 2 to 6 months. In irradiated tubers, however, particularly one month or more after the irradiation, the activity of the isolated "transferase" was about twice the activity of this enzyme from the corresponding control tubers.

Special interest was devoted to a batch of King Edward tubers stored for more than two years after irradiation, in which an extraordinarily high increase of sucrose had been noted. The preparation of "transferase" from this batch of tubers showed an activity, which was about six times the activity of "transferase" from control tubers (see Table 1).

One might feel tempted to suppose that the irradiation caused an activation of "transferase". In many cases, however, such an activation is, in fact, caused by a removal of an inhibitor. Thus, in this case, the competing enzyme "invertase", which is also found in potato tubers,^{11,12} could have been inactivated by the irradiation and thus the "transferase" could have attained predominance over the "invertase". An inactivation of "invertase" in potato tubers has been shown to be caused by γ -irradiation.¹³

Some authors have shown that "invertase", isolated from other materials, e.g. intestines¹⁴ or yeast,¹⁵ is inhibited by Tris. In the reaction mixture, "invertase" should, according to Schwimmer *et al.*,⁵ counteract "transferase" at pH 7.1 causing decreased sucrose synthesis at this lower pH. In the present investigation, using the same reaction system, the reduction of pH to 6.9 did not result in decreased synthesis of sucrose. In order to examine whether Tris, even in this system, could have an inhibitory effect on "invertase", supposed to be included in the enzyme preparation (*cf.* Ref. 5), experiments at a

Tris concentration, 1/10 of that used by Schwimmer *et al.*,⁵ were also carried out. Only a slightly higher activity of "transferase" was observed at the lower molarity of Tris.

In experiments with the "transferase" preparation as enzyme and sucrose as substrate, performed at pH 4.4 and 5.3 in acetate buffer, no "invertase" activity could be observed. This latter investigation is described in detail in Ref. 13.

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