THE ROLE OF CHANGES IN ENZYME ACTIVITIES IN THE REGULATION OF CARBOHYDRATE DISSIMILATION IN POTATO TUBER TISSUE AFTER WOUNDING OR INFECTION WITH GIBBERELLA ZEAE

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SUMMARY

The activities of hexokinase, phosphoglucomutase, phosphoglucoisomerase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase have been determined in the tissue at various distances from the wounded or inoculated surface of potato tuber halves after 1 or 2 days of incubation. Phosphoglucomutase activity was very high when compared to hexokinase activity, and is probably present in great excess. Phosphoglucoisomerase was 3 times as active as glucose-6-phosphate dehydrogenase which was about 4 times as active as 6-phosphogluconate dehydrogenase. The enzyme activities in healthy tissue and their changes when metabolism was activated by wounding or infection, suggest that the increase of carbohydrate dissimilation and of the pentose phosphate pathway operation might not primarily be governed by the amounts of enzymes in the tissue. The possible role of the nicotinamide adenine nucleotides in the regulation of carbohydrate dissimilation is discussed.

1. INTRODUCTION

In wounded or diseased plant tissues many metabolic and physiological changes accompany the increase of respiration rate. Several reports, mainly dealing with leaves inoculated with obligate parasites, suggest a shift of carbohydrate breakdown in the infected host tissue from the glycolytic sequence in favour of the pentose phosphate pathway (AKAZAWA & URITANI 1962; DALY *c.s.* 1961; SCOTT & SMILLIE 1966; SHAW & SAMBORSKI 1957). A marked rise in TCA-cycle activity and pentose phosphate pathway activity occurs after wounding of potato tuber tissue and accompanies the development of induced respiration (AP REES & BEEVERS 1960; LATIES 1964; ROMBERGER & NORTON 1961).

So it is clear that marked changes in carbohydrate breakdown occur after wounding and infection. A study of the activities of some key-enzymes of glycolysis and pentose phosphate pathway in the course of the incubation might help to elucidate the regulatory mechanism of carbohydrate metabolism. In an extensive study on wheat leaves infected with *Puccinia graminis*, LUNDERSTÄDT (1964) tried to explain the changes in carbohydrate metabolism from shifts in enzyme activities during early stages of infection. However, the metabolic pattern of the infected leaves was complicated by changes in photosynthesis, and the interpretation of the results was difficult due to the problem of distinguishing between host and parasite metabolism. The greatest part of the increase of hexokinase activity observed in extracts from infected leaves might have been the result of fungal hexokinase (LUNDERSTÄDT 1966).

In storage tissue photosynthesis is absent and mycelium-free diseased tissue from parts adjacent to the tissue invaded by a facultative parasite can be studied (VERLEUR 1960). This paper deals with experiments with potato tuber tissue in which the *in vitro* activities of some enzymes involved in the metabolism of glucose-6-phosphate have been determined either after wounding or after inoculation with *Gibberella zeae*.

2. MATERIAL AND METHODS

Potato tubers, *Solanum tuberosum* L. var. Bintje, were externally sterilized and cut perpendicular to the length axis into two halves, one of which was inoculated over the whole surface of the cut with a suspension in sterile water of conidiospores of *Gibberella zeae* (Schw.) Petch. The halves were separately incubated at 25 °C in sterile glass jars. After incubation, tissue samples from the central parts of the tuber halves were collected at 0.5–1.5, 1.5–2.5, 2.5–3.5 and 4.5–5.5 mm from the wounded, non-inoculated surface or from the inoculated surface after removal of the parasitically invaded cell layers.

2.1. Respiration rates

Respiration at 25 °C was determined by the Warburg manometric technique in samples of 10 disks (1 mm thick, 6 mm diameter) cut out of the slices which composed the tissue samples described above.

2.2. Enzyme extraction

Tissue samples of 10 g. (fresh weight) were homogenized with 15 ml ice-cold medium during 1 min. in a Waring blendor alternately at high and at low speed for 2–3 seconds. The medium contained 0.05 M triethanolamine buffer pH 7.6, 0.1 M KCl, 0.001 M EDTA and 0.1 % sodium-isoascorbate. Addition of bovine serum albumine did not improve the results. Sodium-isoascorbate was added to prevent browning of the extracts and enzyme inhibition by the oxidation products of phenolic compounds. Cysteine which has the same effect, could not be used as antioxidant here, as hexokinase activities in cysteine containing extracts were roughly 30 % lower than when cysteine was replaced by sodium-isoascorbate, which might be due to effects of cysteine on the SH-groups of hexokinase (FASELLA & HAMMES 1963). The homogenate was pressed through cheese cloth and centrifuged at 30,000 *g* for 10 min. The supernatant was carefully decanted and recentrifuged for 30 min. The resultant supernatant was filled up to 30 ml with homogenizing medium and used as the extract for assays. All procedures were performed at temperatures close to 1 °C.

2.3. Protein in extracts

The sodium-isoascorbate present in the extracts appeared to interfere seriously with protein determinations with the Lowry method (LOWRY *c.s.* 1951), but did not disturb determinations using the Biuret method (COLOWICK & KAPLAN

1957). Extinction was measured at 546 mμ. The amounts of protein were calculated in units Bovine serum albumine (BSA) using a calibration curve which was linear for 0–10 mg BSA per ml test solution.

2.4. Abbreviations used

NADP and NADPH: oxidized resp. reduced nicotinamide adenine dinucleotide phosphate; G6P glucose-6-phosphate; G1P glucose-1-phosphate; F6P fructose-6-phosphate; 6PG 6-phosphogluconate; ATP adenosine triphosphate; G6PDH glucose-6-phosphate dehydrogenase; 6PGDH 6-phosphogluconate dehydrogenase.

3. ASSAY OF ENZYMES

The activities of hexokinase (E.C.2.7.1.1), phosphoglucomutase (E.C.2.7.5.1), glucose-6-phosphate dehydrogenase (E.C.1.1.1.49), 6-phosphogluconate dehydrogenase (E.C.1.1.1 44) and phosphoglucoisomerase (E.C.5.3.1.9) were determined at 25 °C as soon as possible after extraction. These enzymes show some differences in pH value at which they exhibit optimal activity (BERGMEIJER 1962). Yet, pH 7.6 was selected for all enzymes in order to allow the enzymes to be assayed in samples of the same extract within reasonable time after extraction. Storage of extracts in a refrigerator during one night, or dialysis against homogenizing medium in the cold room did not affect the activities of phosphoglucomutase and phosphoglucoisomerase. G6PDH and 6PGDH activities seemed to decrease a little during storage, while hexokinase activity was completely lost after dialysis.

The enzyme activities were determined spectrophotometrically at 366 mμ by estimating the rate of extinction increase during the first 10 min of reaction due to the reduction of NADP. The G6PDH (1 mg per ml) added to the reaction mixtures in the assays of phosphoglucomutase, phosphoglucoisomerase and hexokinase for coupling the reactions to NADP reduction which accompanies the oxidation of the glucose-6-phosphate formed by the enzyme reactions, had been obtained from C. F. Boehringer & Soehne. The activities were calculated as extinction increases during 10 min per volume of extract representing 1 g original tissue: ΔE 10 min per gram.

3.1. NADPH-oxidase activity

Changes in extinction could not be observed in mixtures of 2.6 ml 0.05 M triethanolamine buffer pH 7.6, 0.2 ml 0.1 M $MgCl_2$ and 0.1 ml 0.04 M NADPH to which either 0.2 ml extract or 0.2 ml distilled water had been added. This demonstrated that NADPH-oxidase activity was absent and could not affect the results of the enzyme assays.

3.2. G6PDH and 6PGDH activities

Usually G6PDH activity is determined by measuring NADP reduction in the presence of G6P as the substrate (BERGMEIJER 1962; LUNDERSTÄDT 1964).

However, additional NADP reduction might occur due to the 6PGDH activity of the extracts which oxidized the 6PG formed by G6PDH action. As a matter of fact, the rate of extinction increase was but slightly higher when an excess of 6PGDH has been added to the reaction mixture (*fig. 1*), indicating that in the usual G6PDH assay a large portion of the 6PG formed from G6P was further oxidized by the 6PGDH activity of the extract. Since preliminary experiments demonstrated that the presence of 6PG in concentrations equal to or lower than the concentration of G6P did not inhibit NADP-reduction due to G6PDH activity, the following procedure could be adopted for the assay of both enzymes.

The total activity of both enzymes together was measured in a total volume of 3.1 ml composed of 2.4 ml 0.05 M triethanolamine buffer pH 7.6, 0.2 ml 0.1 M $MgCl_2$, 0.1 ml 0.013 M NADP, 0.1 ml 0.023 M G6P, 0.1 ml 0.023 M 6PG and 0.2 ml extract. In a parallel reaction cell in which G6P was omitted and replaced by 0.1 ml buffer the 6PGDH activity could be observed (*fig. 2*), after which the G6PDH activity could be calculated. The reactions were started by the addition of extract. A mixture without substrates served as the control.

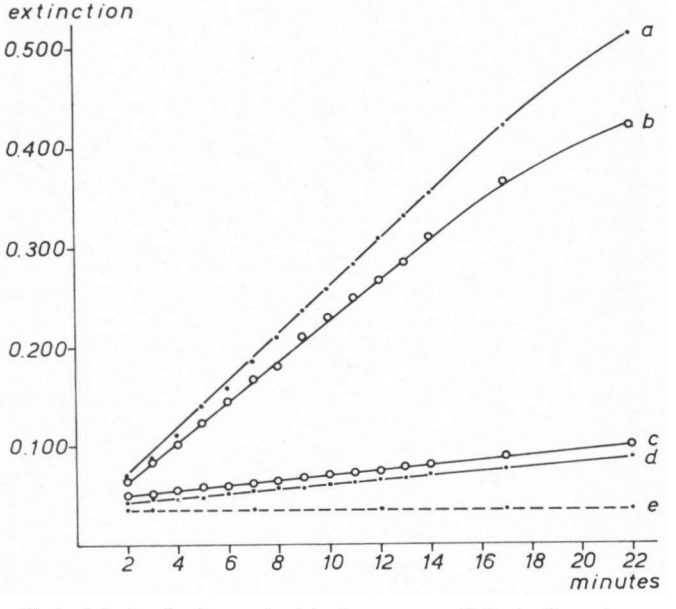

Fig. 1. The effect of 6-phophogluconate dehydrogenase activity in the extracts on the assay of glucose-6-phosphate dehydrogenase.

Reaction mixture: 0.2 ml 0.1 M $MgCl_2$, 0.1 ml 0.013 M NADP, 0.1 ml 0.023 M G6P and 0.2 ml extract, filled up with 0.05 M triethanolamine buffer pH 7.6 to a total volume of 3.1 ml.

a. complete system with extra 0.01 ml 6PGDH (Boehringer, 2 mg per ml)
b. complete system without further additions.
c. as in b, but without glucose-6-phosphate.
d. as in a, but without glucose-6-phosphate.
e. 0.2 ml extract in 0.2 ml 0.1 M $MgCl_2$ and 0.05 M buffer pH 7.6.

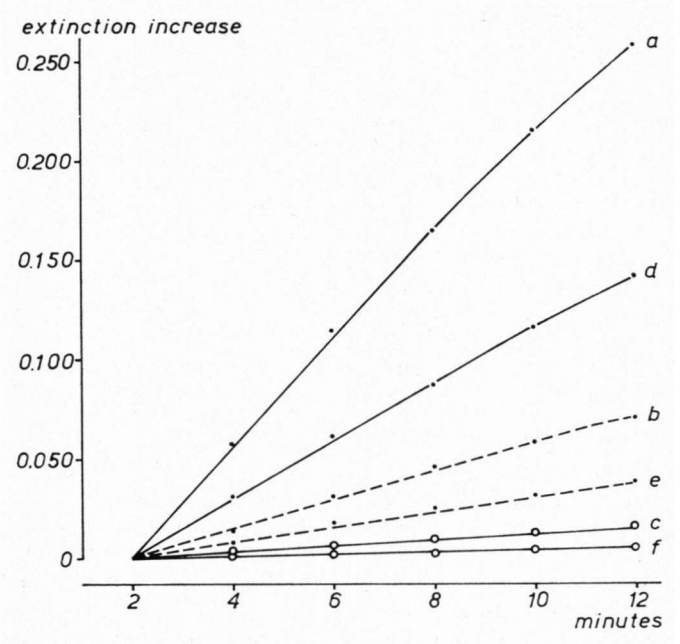

Fig. 2. The assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in tissue extracts. Proportionality to volume of extract.

Reaction mixture: 0.2 ml 0.1 M $MgCl_2$, 0.1 ml 0.013 M NADP, 0.1 ml 0.023 M G6P, 0.1 ml 0.023 M 6PG and extract, filled up with 0.05 M triethanolamine buffer pH 7.6 to a total volume of 3.1 ml.

a. 0.1 ml extract, both substrates added.
b. 0.1 ml extract, no G6P added.
c. 0.1 ml extract, both substrates omitted.
d. as in a with 0.05 ml extract.
e. as in b with 0.05 ml extract.
f. as in c with 0.05 ml extract.

3.3. Phosphoglucoisomerase

This enzyme was assayed with F6P as the substrate in a reaction mixture composed of 0.2 ml 0.1 M $MgCl_2$, 0.2ml 0.033 M F6P, 0.02 ml G6PDH (1 mg per ml), 0.1 ml 0.013 M NADP and 0.05 ml extract, filled up to a total volume of 3.1 ml with 0.05 M triethanolamine buffer pH 7.6. A system from which F6P had been omitted served as the control.

However, the commercial F6P (Boehringer) contained some G6P (<2%) which caused a rapid increase of the extinction during the first 2–3 min after addition of G6PDH and NADP to the reaction cell. In this period the G6P was completely oxidized (*fig. 3*). Therefore, the reaction was started by the addition of extract 5 min after mixing the other components of the reaction system. When G6P was added instead of extract, the strong increase of extinction could be observed again, indicating that the initial increase was caused by the G6P

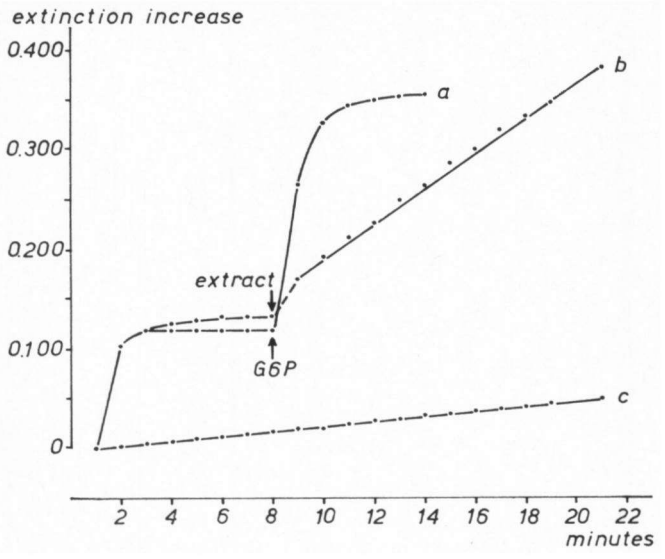

Fig. 3. The effect of the G6P contamination of the F6P on the extinction course in the phosphoglucoisomerase assay.

Reaction mixture: 0.2 ml 0.1 M $MgCl_2$, 0.2 ml 0.033 M F6P, 0.02 ml G6PDH (1 mg per ml), 0.1 ml 0.013 M NADP and 0.05 ml extract, filled up with 0.05 M triethanolamine buffer pH 7.6 to a total volume of 3.1 ml.
a. complete system without extract; addition of 0.115 μmoles G6P after 8 min.
b. complete system without extract; addition of extract after 8 min incubation.
c. reaction mixture without substrate.

contamination of F6P. The presence of 0.1 ml or more 0.033 M F6P in the reaction mixture resulted in extinction changes which were proportional to the volume of extract used up to 0.1 ml.

3.4. Hexokinase

The activity was measured with glucose and ATP as the substrates in a reaction mixture which contained 0.1 or 0.2 ml extract (dependent on the activity), 0.2 ml 0.1 M $MgCl_2$, 0.2 ml 0.016 M ATP, 0.1 ml 0.055 M glucose, 0.1 ml 0.013 M NADP, 0.02 ml G6PDH (0.1 mg per ml) made up to a total volume of 3.1 ml with 0.05 ml triethanolamine buffer pH 7.6. A mixture without substrates served as the control. However, there appeared to exist hardly any difference between the extinction course during the first 12 min of reaction between the complete system and the control (*fig. 4,1*). This was thought to be caused by the presence of ATP and especially glucose in the tissue extracts, by which the control system was not really a system without substrates. From previous data (VERLEUR 1960) it could be calculated that the extracts might contain glucose in concentrations to 10^{-4} M. After 20 min of incubation the rate of extinction increase of the control mixture had slowed down to a constant, relatively low value. Adding both substrates now, resulted in a rate of extinc-

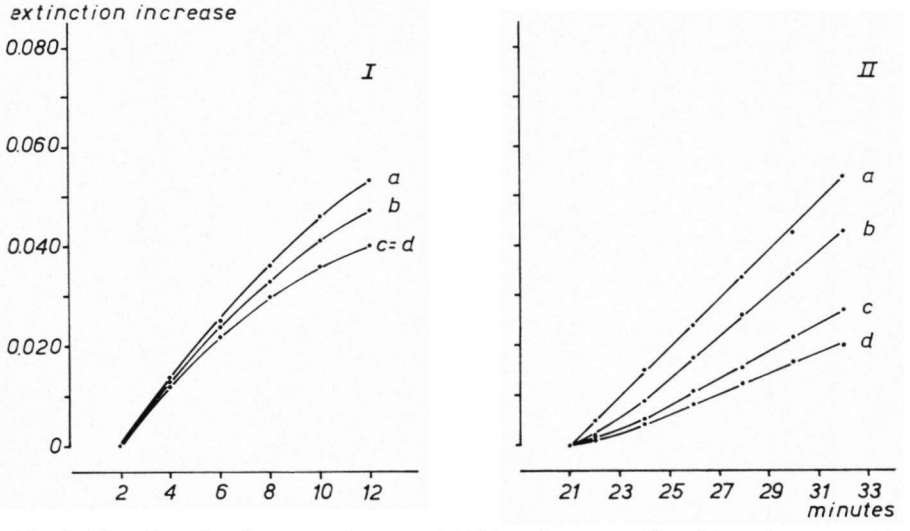

Fig. 4. The effect of endogenous glucose and ATP on the assay of hexokinase in extracts (I). Improvement of the assay by 20 min pre-incubation before addition of substrates (II).

Reaction mixture: 0.2 ml 0.1 M $MgCl_2$, 0.2 ml 0.016 M ATP, 0.1 ml 0.055 M glucose, 0.1 ml 0.013 M NADP, 0.02 ml G6PDH (0.1 mg per ml) and 0.2 ml extract, filled up with 0.05 M triethanolamine buffer pH 7.6 to a total volume of 3.1 ml. In assay II glucose and ATP were added after 20 min pre-incubation of the mixture.

a. complete mixture.
b. system without glucose.
c. system without ATP.
d. system without both glucose and ATP.

tion increase which was much higher than the rate in a system without substrates. As could be expected from the high glucose concentration in the extracts, the extinction changes in a mixture to which glucose had not been added, were but slightly lower than in a complete system. However, the extinction course of a mixture from which ATP had been omitted, approximated that of the control mixture without substrates (*fig. 4, II*). It was concluded that during the first 20 min of incubation the endogenous ATP had been exhausted.

Therefore, in the hexokinase assay the reaction cells containing the reaction system except ATP and glucose were incubated during 20 min, after which both substrates were added and the extinction changes measured during the next 10–12 min. A mixture to which buffer was added instead of substrates served as the control. This assay resulted in extinction changes which were proportional to the volume of extract in the range of 0.05–0.2 ml.

For coupling the hexokinase reaction to NADP reduction, the addition of G6PDH was necessary. In a series of assays in which 0.01–0.1 ml of the diluted G6PDH (0.1 mg per ml) was given to the reaction mixture, it appeared that high amounts of G6PDH inhibited the reactions. Maximal activity in terms of NADP reduction was obtained when 0.01–0.03 ml was applied. In the routine assay 0.02 ml was used.

Addition of 5×10^{-3} M NaF to the reaction mixture, in order to prevent other ways of G6P conversion, proved to result in lower rates of extinction increase than when NaF was omitted, possibly due to the interaction of NaF with the Mg^{++} in the mixture, which is a requisite for hexokinase action.

3.5. Phosphoglucomutase

The activity was measured by using G1P as the substrate. The reaction mixture was composed of 0.5 ml 0.1 M $MgCl_2$, 0.3 ml 0.01 M EDTA, 0.2 ml 0.073 M G1P, 0.1 ml 0.013 M NADP, 0.01 ml G6PDH (1 mg per ml), 0.05 ml extract and 0.05 M triethanolamine buffer pH 7.6 to reach a total volume of 3.1 ml. A mixture without G1P served as the control. Because of the high activity of this enzyme, rates of extinction increase could only be exactly measured with up to 0.05 ml extract. Yet, in assays with extinction changes of up to around 1.000 per 10 min there was a proportionality to the volume of extract supplied.

4. EXPERIMENTS

Respiration rates and enzyme activities have been determined in tissue samples cut at different distances from the wounded or inoculated surface of potato tuber halves after an incubation at 25 °C for 1 or 2 days. In one group of experiments phosphoglucoisomerase, G6PDH, 6PGDH and hexokinase activities have been separately measured in samples of the extracts, in a second group both hexokinase and phosphoglucomutase activities were assayed. The patterns of hexokinase activities in both groups were completely comparable. Therefore, the activity patterns of all 5 enzymes are considered as if they have been observed in the same tissue extracts.

Enzyme activities can be calculated either on the basis of the amount of tissue used for extraction or on the basis of the protein content of the extracts. In agreement with previously published results (VERLEUR *c.s.* 1966), the protein content of the extracts per gram of tissue used for extraction, was the same in all 4 samples cut at different distances from the surface of a tuber half, and appeared not to be affected by wounding or infection. Consequently, it is indifferent on which of the two bases enzyme activities will be calculated. In this paper, activites are represented as extinction increases per 10 min of reaction per volume of extract representing 1 gram original tissue: ΔE10 min per gram.

As can be seen from the data in *table 1*, the *in vitro* activities of the 5 enzymes varied rather much from tuber to tuber, whereas the variations within each tuber were rather small. Therefore, the best comparison between enzyme activities in tissues affected by wounding or infection to a varying degree, can be made between the activities in samples cut from the same tuber half at different distances from the surface of wounding or inoculation. Such tissue samples exhibit marked differences in respiratory activity (VERLEUR 1960).

When the *in vitro* activities of the enzymes in healthy, not wounded tissue are compared with each other (*table 1*), the high phosphoglucomutase activity

Table 1. Range of enzyme activities observed *in vitro* in 5 different potato tubers, and variation of activities found in parallel determinations within each tuber. (Values calculated as ΔE per 10 minutes per gram of tissue.)

Enzymes	ΔE 10 min/gram in a series of tubers		ΔE10 min/gram variation per tuber	Ratios
	range	average		
phosphoglucomutase (PGM)	38.3 –45.2	42.0	± 1.0	PGM/HK = 54.5
hexokinase (HK)	0.63– 0.98	0.77	± 0.08	
phosphoglucoisomerase (PGI)	7.80–10.68	9.58	± 0.16	
glucose-6-phosphate dehydrogenase	2.54– 3.95	3.20	± 0.17	PGI/G6PDH = 3.0
6-phosphogluconate dehydrogenase	0.53– 1.03	0.87	± 0.07	G6PDH/6PGDH = 3.7

is striking, being more than 50 times the hexokinase activity. It is also clear that phosphoglucoisomerase activity was 3 times as high as G6PDH activity, while the latter enzyme proved to be nearly 4 times as active as 6PGDH.

As could be expected, after 1 day of incubation respiration rates of the tissue cut at various distances from the surface were high close to the surface and had, at greater distances, values similar to that of healthy, not wounded tissue (*fig. 5*). Respiratory increase was found mainly in the tissue up to 2.5 mm from the wounded surface, but could be demonstrated in the diseased tissue at greater distances. The wound-induced respiratory increase took place during the first 24 hours of incubation. In the diseased tissue a further increase occurred during the second day, except in the tissue very close to the infected parts, where respiration already declined. This respiration pattern clearly indicated that tissue metabolism was markedly activated after one day of incubation already, both after wounding and after infection.

In contrast to this, hexokinase activity in the diseased tissue had not changed during the first day of incubation (*fig. 5*). Some increase, not exceeding 50%, was observed 2 days after inoculation. In the wounded halves, no increase of hexokinase activity could be observed 2 days after wounding, although some activation has been found very close to the surface after 1 day. Phosphoglucomutase activity did not change or tended to decrease in the tissue closer to the surface.

As for the enzymes concerned in the further conversion of glucose-6-phosphate (*fig. 6*), the phosphoglucoisomerase activity as well as the activities of G6PDH and 6PDGH in the diseased tuber tissue were higher at shorter distances from the surface after 1 day of incubation, and increased further during the second day. However, in wounded tissue no or hardly any increase in activity could be demonstrated 24 hours after cutting the tubers, while the increase in 6PGDH activity still appeared to be slight 2 days after wounding.

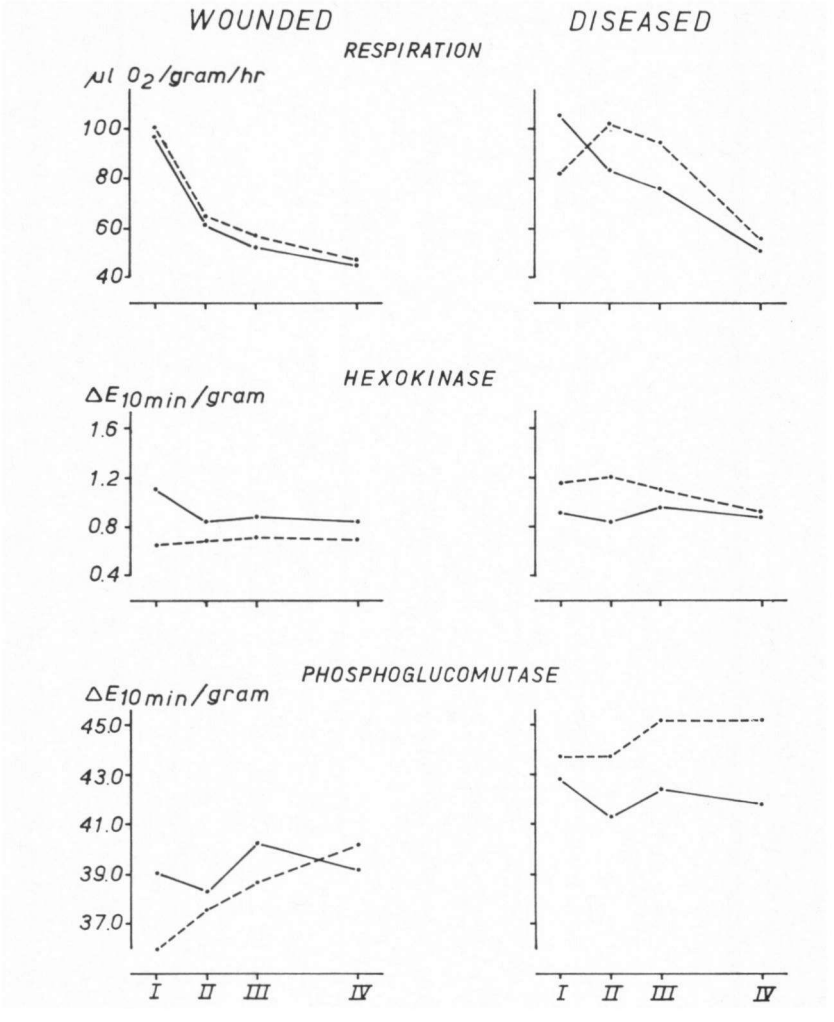

Fig. 5. Respiration rate, hexokinase and phosphoglucomutase activities in wounded and diseased potato tuber tissue incubated at 25 °C for 1 day (.———.) or 2 days (.– – – –.).
Tissue zones: I. 0.5–1.5 mm from the surface.
II. 1.5–2.5 mm from the surface.
III. 2.5–3.5 mm from the surface.
IV. 4.5–5.5 mm from the surface.

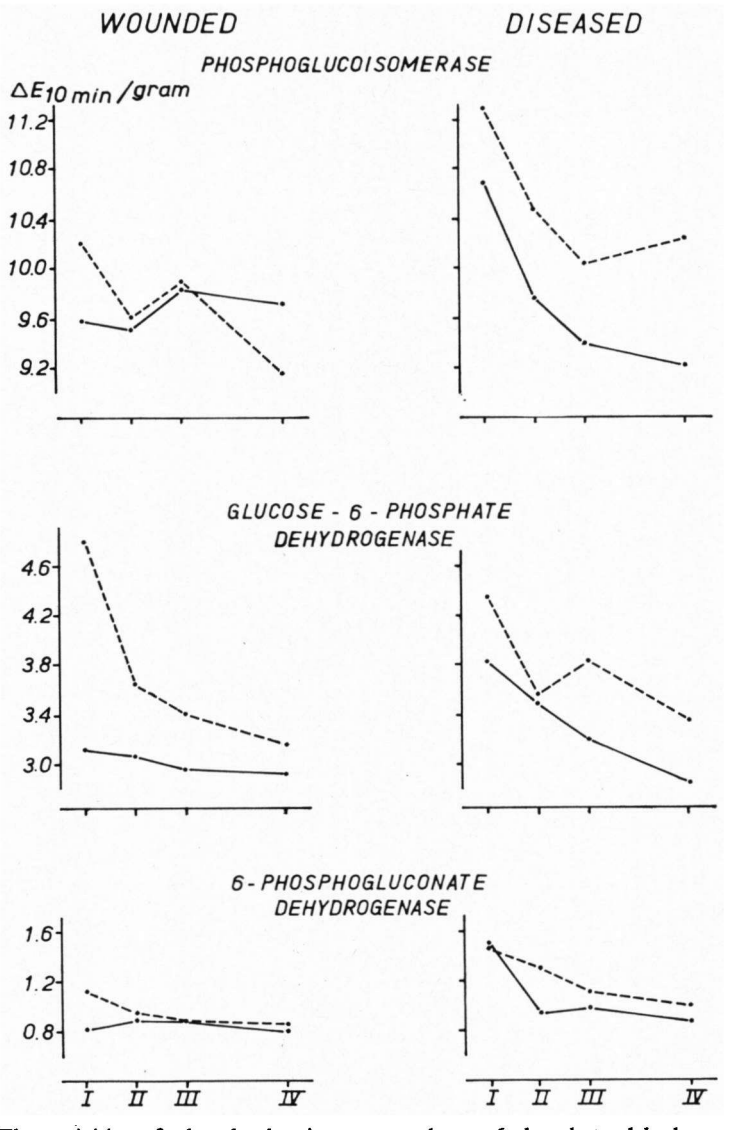

Fig. 6. The activities of phosphoglucoisomerase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in extracts from wounded and diseased potato tuber tissue incubated at 25 °C for 1 day (.———.) or 2 days (.- - - - - -.). Legends as in fig. 5.

5. DISCUSSION

The phosphoglucomutase activity in the extracts was very high, especially when compared with hexokinase activity. This is in agreement with the opinion that formation of G6P in potatoes through the phosphorylase-phosphoglucomutase reactions is more important than the production by hexokinase action (BARKER 1965). From the *in vitro* activities of phosphoglucomutase and hexokinase, the amount of G6P formed in a certain time interval and the O_2-uptake which would occur when this G6P is completely oxidized, can be calculated from the extinction changes in the enzyme assays, on the basis of an extinction of 0.207 per 0.1μmoles NADPH per 3 ml mixture in the reaction cells. This leads to the conclusion that the *in vitro* activities of phosphoglucomutase and hexokinase can produce amounts of G6P sufficient for an O_2-uptake in the order of 15000 mm^3 and 250 mm^3 per hour per gram respectively, while respiration rates of only 40–100 mm^3 O_2-uptake per hour per gram tissue have been observed in the tissue samples. Naturally one should realize that *in vitro* activities might be quite different from the real activity in the intact cell, but these calculations give an impression of the potential capacity of the enzyme system present in the cells. The high phosphoglucomutase activity and the absence of an increase after wounding or infection, together with the fact that hexokinase activity did not change parallel with respiratory increase, suggests that phosphoglucomutase might be present in the tissue in great excess, and the amount of enzyme might not be rate-limiting in carbohydrate catabolism.

The enzymes involved in G6P utilization also exhibit marked differences in activity. Phosphoglucoisomerase was 3 times as active as G6PDH and about 11 times as active as 6PGDH, this in contrast to the situation in wheat leaves, where phosphoglucoisomerase activity was slightly lower than G6PDH activity (LUNDERSTÄDT 1964). This suggests that, as far as the amounts of these enzymes present in the tissue are concerned, the conversion of G6P in potato tubers will take place mainly through the phosphoglucoisomerase reaction and glycolysis. It can be calculated as above from the *in vitro* activity that phosphoglucoisomerase activity is high enough to supply sufficient respiratory intermediates to give, when completely oxidized, an O_2-uptake in the order of 4000 mm^3 per hour per gram, which is many times higher than the respiratory O_2-uptake observed.

For the operation of the pentose phosphate pathway it might be of great importance to notice that 6PGDH activity was low compared with G6PDH activity. The same had been observed in wheat leaves (LUNDERSTÄDT 1964). Although both activities have been measured *in vitro*, this shows that one should be careful in concluding to an activation of the pentose phosphate pathway on the basis of an increase of G6PDH activity alone. This is emphasized by the fact that when G6PDH and 6PGDH activites increased after infection or after 2 days of wound reaction, the absolute increase of 6PGDH activity was much smaller than that of G6PDH, which raises the question of what reaction might be considered to be rate-limiting in the pathway. The low 6PGDH activity might even lead to an accumulation of 6PG, which can inhibit phosphoglucoisomerase action (PARR 1957; VEITCH 1964).

The results presented in *figs. 5* and *6* clearly show that in early stages of wound reaction and infection, metabolism can be affected and activated markedly despite the fact that the activities of the 5 enzymes studied had not yet increased. The activation of carbohydrate catabolism and the increase in pentose phosphate pathway activity after wounding and infection might not primarily be governed by the amount of enzymes present, but might primarily be achieved by an increased reaction velocity, for instance due to abolishment of inhibiting factors or by a higher availability of the nicotinamide adenine nucleotide co-enzymes. NADP is a requisite for both G6PDH and 6PGDH action, and is also functional with other enzymes such as isocitric dehydrogenase.

Preliminary results on the increase of NAD and NADP in wounded and diseased potato tuber tissue have been reported elsewhere (VERLEUR 1968). Increase of both co-enzymes could already be demonstrated 1 day after wounding or infection. In addition, NADP appeared to be increased in the mitochondrial fraction, isolated from these tuber halves. Since then, this has been in study in further detail. HAWKER & LATIES (1963) reported an increase of NAD and NADP concentration in potato tuber tissue aged by shaking in 10^{-4} M $CaSO_4$ solution at room temperature for 24 hours. In the meanwhile several authors published results on animal tissues and suggested that apart from enzyme synthesis the availability of nicotinamide adenine nucleotides might be another important factor to be considered in relation to metabolic levels (DENIS 1968; GUMAA *c.s.* 1968).

The differences in metabolic activities in the tissue samples cut at various distances from the surface of the incubated tuber halves might give an indication of the time course of metabolic alterations in reaction to wounding or infection, especially when this is combined with different lengths of the incubation periods.

Considering the experimental results as discussed above, one can imagine that the events in carbohydrate catabolism occurring after wounding or infection, primarily lead to an increased activity of the enzymes which are already present, due to the better availability of nicotinamide adenine nucleotide co-enzymes. This might result, among others, in an activation of the pentose phosphate pathway and the production of C-skeletons and "reducing power" in the form of NADPH for synthetic reactions. Then, the total capacity of the metabolic system in general, including the respiratory system, is brought to a higher level by the synthesis of enzymes, the formation of mitochondria (VERLEUR & URITANI 1965) etc.

The hypothesis that the co-enzyme availability might be essential early in the induction of metabolic activation and in the regulation of carbohydrate metabolism in potato tuber tissue, possibly has a wider application as a general principle in the regulation of metabolic changes occurring after other treatments.

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