Phytochemistry, 1977, Vol. 16, pp. 1495–1498. Pergamon Press. Printed in England.

RESPIRATION, ATP LEVEL, AND SUGAR ACCUMULATION IN POTATO TUBERS DURING STORAGE AT 4°

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(*Revised received* 25 *April* 1977)

Key Word Index—*Solanum tuberosum*; Solanaceae; potato; starch–sugar interconversion; sweetening; low temperature storage; adenosine triphosphate.

Abstract—A kinetic study was made of the relationship between respiration rate, sugar content and ATP levels, in fresh and aged potato tubers stored at 4°. The ATP content in tubers rose rapidly immediately after the chilling stress, while respiration rate decreased below the initial rate and sugar accumulation was not detected. After 4 days of storage, the ATP level declined and the sugars started to accumulate. The typical increase in respiration rate that usually follows chilling stress, appeared only in fresh tubers (at about the 6th day of storage). In dinitrophenol-treated tubers, the ATP level remained below the initial level and sugar accumulation was blocked completely. The evidence presented suggests that ATP elevation is not generated by the respiration burst.

INTRODUCTION

Starch degradation and sugar accumulation is a general response of potato tubers to environmental stresses, such as storage at low and high temperature, or exposure to ethylene, and to cyanide treatments [1–3].

A marked increase in respiration rate concomitant with sugar accumulation is a frequent result of low temperature storage. Barker [4] has shown that up to a certain sucrose level, there was a positive correlation between sucrose content and respiration rate. Other workers, however reported that the respiration rate of the tubers was independent of sugar concentration [5–6]. Sugar added exogenously to potato discs did not effect the respiration rate [5].

It was recently shown that the respiration burst at low temperature generates ATP and it is the latter that is utilized in sucrose synthesis [2, 7, 8]. This finding suggests that ATP level in potato tubers may in part control starch–sugar conversion [2]. We have recently reported that sprouting of potato tubers stored at 17° could be prevented by a simple chemical treatment [9], thus enabling one to study the effect of long term storage at 17° (aging) on starch–sugar conversion in comparison with freshly harvested tubers. Preliminary experiments showed that aged potatoes did not exhibit the typical respiration burst at 4°, although they did accumulate sugars. This observation led to the question of the role of respiration rate and ATP level in sugar accumulation at 4°.

The object of the work presented here was to determine the relationship between respiration, sugar accumulation and ATP level, in fresh and aged potato tubers stored at a low temperature.

RESULTS

Effects of aging on respiration, sugar accumulation and ATP level during storage of tubers at 4°

The results of a kinetic study of the changes in respiration rate and sugar content occurring storage at 4° in both fresh and aged tubers are shown in Fig. 1. The respiration rate of both fresh and aged tubers declined during the first 3–7 days of storage at 4° (Fig. 1). In fresh tubers, this initial decline was followed by a marked increase in respiration, reaching a maximum of 5.2 mg CO_2/kg/hr after 8–9 days. From the 9th day on, the respiration declined to about the original level. Aged tubers had the same initial respiratory rate as fresh tubers, but then decreased to below the initial level within 7 days. The respiration burst, the typical response of fresh tubers to cold storage, did not occur in aged ones. In fresh tubers, the reducing sugar level remained fairly constant during the first 4 days of storage, increased by *ca* 10 fold by the 14th day, and remained unchanged thereafter. The sucrose level also remained nearly constant during the first 4 days of storage at 4°, then rose to a maximum at about the 9th–10th day, and declined to the original level by the 14th day (Fig. 1a). In aged tubers (stored at 17° for 3 months) the rate of increase of reducing sugar and sucrose was much slower than that in fresh tubers (Fig. 1b). It should be emphasized that comparable levels of reducing sugars were reached in both groups of tubers; within one month in aged tubers, compared with two weeks in fresh ones.

The fact that aged tubers lacked a burst in respiration during storage at 4° and yet had a sugar content like that of fresh tubers (Fig. 1b), led us to compare the ATP level in both groups. Fig. 1 shows that in both cases, during the first 2–3 days of storage at 4°, there was a sharp rise in ATP content to about six-fold above the basal level. In fresh tubers, the ATP level then declined sharply and reached the initial level on the 8th day. In aged tubers, maximum ATP level was retained for about 6 days and then declined, reaching the initial level on about the 30th day of storage. It should be noted that in both fresh and aged tubers, the sharp rise in ATP content occurred at a time when the respiratory activity was near its minimum value (below the basal level). The respiration burst occur-

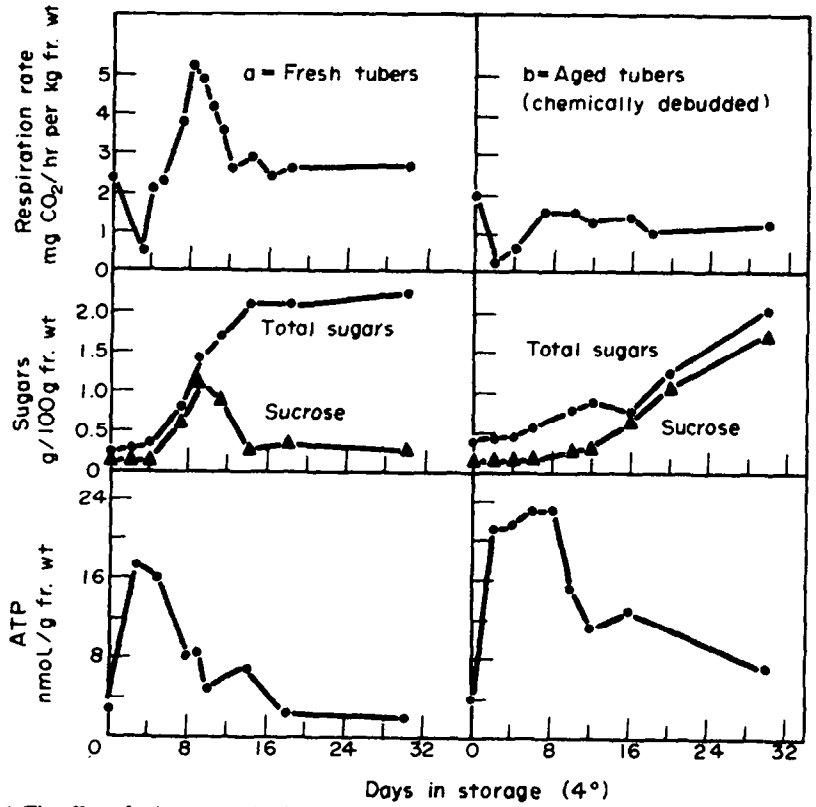

Fig. 1. The effect of aging on respiration, sugar accumulation and ATP level during storage of tubers at 4°. (a) Freshly harvested tubers were stored at 17° for two weeks and then transferred to 4°. (b) Chemically debudded tubers (stored for 92 days at 17°) and then transferred to 4°. Zero time refers to when the tubers were transferred to 4°.

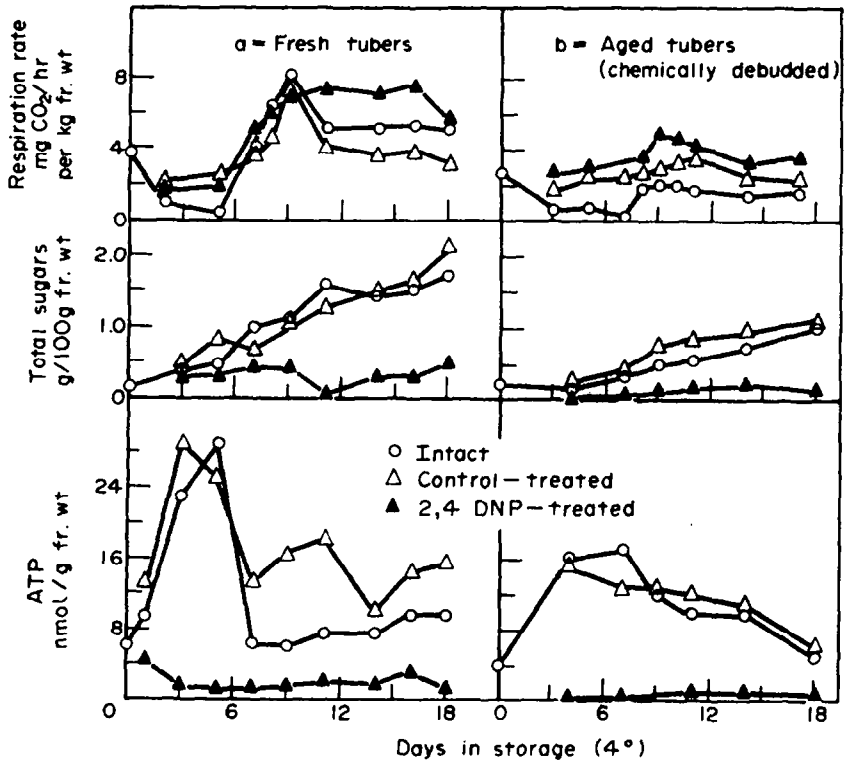

Fig. 2. The effect of DNP on the respiration rate, sugar accumulation and ATP level in fresh and aged tubers during storage at 4°. (a) Freshly harvested tubers were stored at 17° for 2 weeks and then transferred to 4°. (b) Aged tubers (chemically debudded tubers) stored for 107 days at 17°. 3 ml solution containing either control solutes or DNP (10mM) were then introduced into the tubers and 12 hours later transferred to storage at 4°. Sugar and ATP contents were measured in samples taken from the yellow region in DNP-treated tubers, and from comparable regions in control-treated and in intact tubers.

ring in fresh tubers after 10 days of exposure to 4° did not coincide with the ATP peak.

The effect of DNP on respiration rate, sugar content and ATP level

It was thought that treatments which would reduce the ATP level in the tissue might, in turn, reduce the sugar level. The effect of dinitrophenol (DNP), which acts as an uncoupler of oxidative phosphorylation, was therefore tested on fresh and on aged tubers; the results are shown in Fig. 2. Intact and control-treated tubers as well as DNP-treated tubers were all characterized by a drop in respiration (below the initial level) during the first 5 days of cold storage, followed by a rapid increase reaching a maximum on the 9th day of storage. DNP-treated tubers exhibited a plateau for the following 7–8 days, while the respiration in control tubers fell off markedly. The sugar level in fresh control tubers rose from 0.2% to 1.8% during 18 days of storage, while in DNP-treated tubers sugar accumulation was almost completely inhibited.

During the first 4 days of storage at 4°, the ATP level in DNP-treated tubers fell to below the initial level and remained there throughout the 18 days of storage. In control tubers, on the other hand, there was a sharp and immediate burst in the ATP level upon storage at 4°, reaching a maximum after 3–4 days and then declining rapidly. During the first two weeks of storage at 4°, the degree of sugar accumulation, respiration and ATP content was lower in aged tubers than in freshly harvested tubers but the pattern of changes in these parameters as a function of time of storage at 4° were generally similar in the two cases, except for the lack of respiration peak in intact aged tubers (Fig. 2). It should be noted that the DNP-treated region (stained yellow) remained viable even after 80 days of storage at 4°. At that time a small, but definite renewal of sugar accumulation occurred *in situ*, suggesting the recovery of the tissue from the uncoupling effect (data not shown).

DISCUSSION

A burst in respiration rate and sugar accumulation are typical responses of potato tubers to a variety of environmental stresses, including storage at 4° [3]. In tubers stored at low temperature, Barker found that, up to a certain sucrose level, there was a close correlation between sucrose accumulation and enhancement in respiration rate [4]. Recently, Isherwood [7, 8] and Salomos and Laties [2] emphasized the role of ATP in the starch–sugar interconversion in potatoes. According to their findings, the sugar accumulation in potato tubers under such stress is considered to result from the elevation in ATP level generated during the typical burst in respiration.

Our findings suggest that the respiration rise is not always the trigger for sugar accumulation. Fig. 1 demonstrates that in fresh tubers there is a peak in ATP level occurring immediately after exposure to 4°, about 6 days prior to the peak in respiration. Furthermore, in aged tubers there was a similar, but broader, peak in ATP level, yet there was no increase in respiration above the basal level. We suggest that the ATP elevation in tubers stored at 4° is not necessarily generated from the respiration burst, but may result from a temporary malfunction of tuber anabolism consequent to the perturbation of cellular organization by the low temperature [8, 10]. Figs. 1 and 2 also demonstrate that in fresh tubers, the accelerated increase in sugar level occurs concomitantly with the sharp decrease in ATP level. In aged tubers, the decrease in ATP level extended over a longer period of time and the rise in sugar level increases at a slow rate, over the same period of time. This may be attributed to a possible slowdown in the capacity of aged tissues to synthesize sucrose, followed by a low consumption of the available ATP.

These observations are consistent with the view that sucrose biosynthesis is an effective pathway for extra ATP formation during environmental stresses [2]. This is further supported by the results obtained with the uncoupling agent DNP which caused inhibition of ATP production and a complete blockage of sugar accumulation.

EXPERIMENTAL

Plant material. Mature potato tubers (*Solanum tuberosum*, var. Up-to-Date) were harvested at the Gilat Experiment Station in the northern Negev in autumn and spring. Blemish free tubers weighing 150–200 g were washed, dried and stored at 17° and 90% relative humidity. The tubers of each harvest were divided into two groups (a) those left intact ('fresh tubers'), and (b) those in which the tuber buds were destroyed chemically ('chemically debudded'). Chemical debudding was achieved by immersion of the tubers in a mixture of EtOH–Me_2CO (1:1) for 4 hr followed by drying, as described in ref. [9].

Introduction of solutes into tubers. A hole 0.5 cm diam. was drilled (with a cork borer) in the tubers and 3 ml of 10 mM DNP (dissolved in 10 mM KOH) was injected into it. Control tubers were injected with 3 ml of 10 mM KOH in an identical manner ('control-treated'). The cutting area healed within a few days after infiltration of the sol.

Sugar determination. Samples of peeled potato tubers (10 g) were homogenized in the cold, with 50 ml ice cold 80% EtOH for 3 min. The sugars were extracted for 1 hr with 80% EtOH in a Soxhlet extractor (using a fibreglass thimble). Reducing sugars were determined as in ref. [11]. Sucrose was hydrolyzed to reducing sugars by invertase and the total amount of reducing sugars was determined [11]. The difference between total reducing sugar (after invertase treatment) and reducing sugar before enzyme hydrolysis, was used as a measure of sucrose content. Data presented regarding sugar content are the average of samples taken from 3 different tubers; the standard error of such triplicate assays was generally 4%.

Respiration rate was determined in sealed desiccators (8 l. capacity) containing 2 kg of potato tubers. A current of CO_2-free air (2 l./hr) was passed through the desiccators and respired CO_2 was trapped in 0.2 M KOH.

ATP determination. Cylinders (1 cm diam.) were removed from the central longitudinal axis of the tuber and sample of *ca* 3 g (in duplicate) was rapidly immersed in boiling H_2O (10 ml). During boiling for 3 min, the cylinder was sliced with a scalpel. After boiling, the sample, including the liquid, was immediately cooled, homogenized, and the homogenate was centrifuged (0°) at 15000 *g* for 20 min. ATP was determined on sample of the supernatant by the luciferin–luciferase system, with minor modifications of the procedures of refs [12] and [13]. Counting vials included; 0.6 ml M Na_2 $HAsO_4$ buffer containing 40 mM $MgSO_4$ (pH 7.4), 0.6 ml 0.1 M K-Pi buffer containing 4 mM $MgSO_4$ (pH 7.4) and 0.5 ml H_2O. Firefly lantern extract (Sigma FLE-50) (50 mg) dissolved in 10 ml H_2O, filtered, and 0.1 ml of this prepn added to each vial. The vials were equilibrated to the temp. of the scintillation counter and the supernatant of potato samples (prepared as above) was injected into vials and counted for 6 sec. A liquid scintillation counter (gain 30% discriminator 50–1000, coincidence switch 'off'), was used to measure the luminescence rate. The calibration curve of ATP dissolved in HEPES buffer and 0.025 M $MgSO_4$ pH 7.4 was linear in the range of 0 to 50 pmol ATP. Each tuber sample was assayed in duplicate and the results from 2–3 different tubers were averaged. The standard error of such assays was generally 14%. Tests

showed an almost complete recovery of ATP (10–50 pmol) added to the potato samples just prior to boiling. For comparison ATP was also extracted with $HClO_4$ by the method of ref. [14] for potato tubers. The ATP levels extracted by the two methods were very similar.

Acknowledgements—We thank Professor J. J. Blum, Dept. of Physiology and Pharmacology, Duke University, Durham, N.C., U.S.A., for his interest, encouragement and helpful discussions. The authors are indebted to Mrs. R. Parash and R. Zipori for their excellent technical assistance. This research was supported by a grant from the United States–Israel Binational Science Foundation (BSF), Jerusalem, Israel. Contribution No. 103-E, 1977 series, from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.

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