CHANGES IN THE TRICARBOXYLIC ACID CYCLE ACIDS IN POTATOES AFTER ANAEROBIOSIS

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Abstract

Potatoes were subjected to anaerobiosis and then returned to air. Citrate was estimated in three experiments and malate in one. Citrate increased slightly in air after nitrogen in two experiments but not in the third. There was also a small increase in malate. From these results no firm conclusion was drawn as to whether the turnover rate in the tricarboxylic acid cycle had increased. However, the lost lactate which was unaccounted for as increased CO_2 output during recovery from anaerobiosis was less than the observed increases in citrate and malate contents in two experiments.

An increase in acetate and acetaldehyde contents was observed during recovery from anaerobiosis.

I. Introduction

A number of workers have shown that the tricarboxylic acid cycle (TCAC) is the pathway of respiration in potatoes (Barker and Mapson 1953, 1955, 1963; ap Rees and Beevers 1960; Barker 1963). Barker and Mapson (1953, 1963) observed that lactate, which had accumulated in potatoes stored in nitrogen, was lost when the potatoes were returned to air. This loss was accompanied by an increase in CO_2 output and in the amounts of pyruvate, α-ketoglutarate, and oxaloacetate.

If the TCAC is operative in potatoes, it would be expected that the increase in α-ketoglutarate, oxaloacetate, and CO_2 production in potatoes kept in air after nitrogen may be followed by increases in the amounts of other acids of the TCAC. In the present study the changes in citrate, isocitrate, malate, acetate, and acetaldehyde concentrations were investigated in potatoes stored in nitrogen and then in air.

II. Methods

Potatoes, cv. King Edward VII, were stored until required at 10°C. Experimental samples were placed in sealed glass jars through which was passed either CO_2-free air or nitrogen at rates of 2·2–2·8 litres/hr.

In all, 14 samples were used in experiments 1 and 2, eight being transferred to nitrogen at 0 hr and the remaining six being maintained in air. After 12 days, six samples were returned to air from nitrogen and two were taken for analysis together with two samples which had been kept in air. Other samples were extracted for analysis at 24-hr intervals. Experiment 3, consisting of 20 samples, was performed in the same way, with eight samples being kept in air, 12 transferred to nitrogen for 12 days, and 10 being returned to air after that time.

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Thin slices were cut from each half of longitudinally cut tubers and extracted by 0·6M metaphosphoric acid at 2°C. This extract was shaken with diethyl ether to remove lactic, malic, and isocitric acids for enzymatic estimation. The ether extract was evaporated and the residue dissolved in water. Lactic acid was estimated by the method described in a Biochemica Boehringer pamphlet (determination of the L-(+)-lactate concentration in blood or serum, u.v. test with NADH). Malic acid was determined with malate dehydrogenase by the technique of Solomos (1963). Isocitric acid was determined according to Ochoa (1948) and Pritchard (1959).

Citric and acetic acids and acetaldehyde were estimated in the metaphosphoric acid extract. Citric acid was estimated colorimetrically according to the method proposed by Taylor (1953) and subsequently modified by Hughes (1960) and Solomos (1963). The microdiffusion method of Conway (1950) and Serlin and Colzias (1955) was adopted for the estimation of acetic acid. Acetaldehyde was estimated by the technique of Clausen (1922).

The respiratory CO_2 was absorbed in a solution of NaOH in a Pettenkoffer tube. The amount of CO_2 was estimated by titration of the NaOH solution with standard HCl after precipitation of the carbonate present with saturated $BaCl_2$. The CO_2 production of different samples under the same experimental conditions was similar, and therefore mean values are shown in the figures. The time when samples were transferred to nitrogen is shown as 0 hr in the figures. Arrows mark the transition from air to nitrogen and from nitrogen to air.

III. Results and Discussion

(*a*) *Changes in CO_2 Output and Lactate Concentration during and after Anaerobiosis*

In experiment 1, the CO_2 output in nitrogen [Fig. 1(*a*)] fell to about one-third that of the air sample. Restoration to air resulted in a rapid increase in the CO_2 output. There was little change in the CO_2 output of samples kept in air. Similar changes in CO_2 output were observed in experiments 2 and 3 [Figs. 1(*b*) and 1(*c*)].

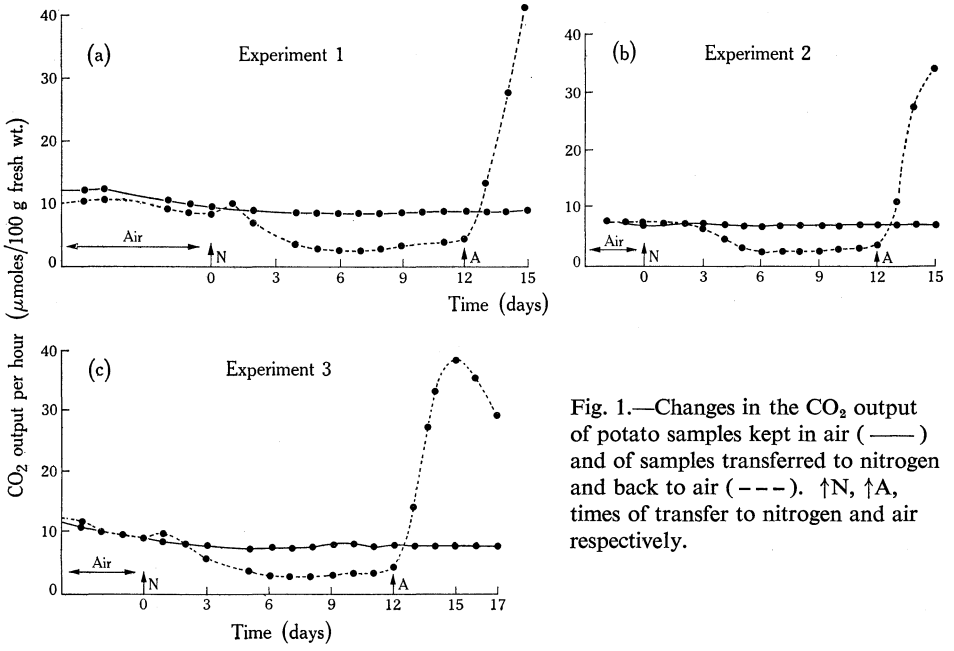

Fig. 1.—Changes in the CO_2 output of potato samples kept in air (——) and of samples transferred to nitrogen and back to air (– – –). ↑N, ↑A, times of transfer to nitrogen and air respectively.

After 12 days in nitrogen, the lactate content of samples in experiment 1 increased to 1000 μmoles/100 g from the control value of 10 μmoles/100 g [Fig. 2(*a*)]. On

return to air lactate concentration fell rapidly, the loss being about 20, 45, and 17% in the first, second, and third day respectively. The changes in lactate content in experiments 2 and 3 [Figs. 2(*b*) and 2(*c*)] were similar.

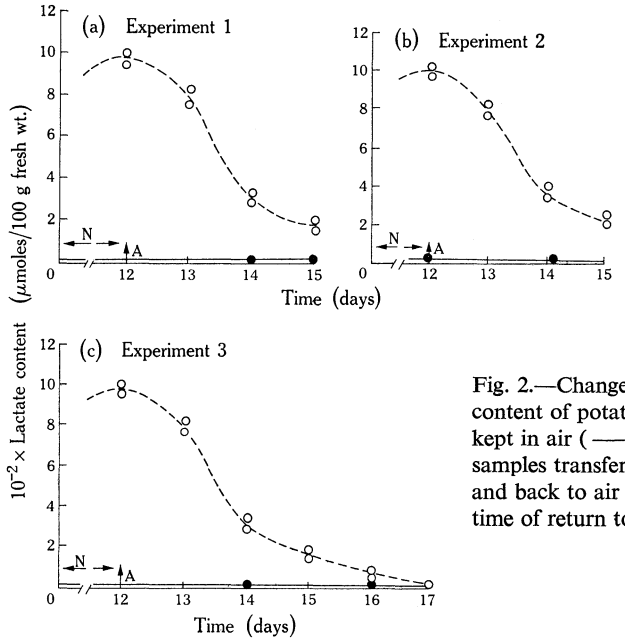

Fig. 2.—Changes in lactate content of potato samples kept in air (——) and of samples transferred to nitrogen and back to air (– – –). ↑A, time of return to air.

(*b*) *Changes in Citrate, Malate, and Isocitrate Concentrations*

Figures 3(*a*), 3(*b*), and 3(*c*) show the changes in citrate content recorded during experiments 1, 2, and 3 respectively. In the first experiment citrate content increased slightly in samples kept in air and did not change significantly either in nitrogen or in air after nitrogen, but in the other two experiments there was a loss of citrate during 12 days in nitrogen and on return to air citrate increased slightly. Both of these changes were statistically significant in the third experiment. The citrate content in air after nitrogen was lower than the corresponding value for control samples kept in air.

There was a small decrease in malate content in samples in nitrogen [Fig. 3(*d*)] and an increase in air after nitrogen. The difference between the mean values in air and nitrogen was statistically significant, as was the increase in air after nitrogen.

Table 1 shows the amounts of lactate lost in the three experiments during the first and second days in air after nitrogen which was not accounted for as CO_2. If the additional lactate accumulated as citrate or malate, only small relative increases in these acids would occur since the total concentrations of citrate were as large as 2000–2600 μmoles and of malate 627–656 μmoles per 100 g of potatoes. Table 1 also shows that in the first day after return to air, the increase in citrate and malate contents together in the second experiment, and in citrate alone in the third experiment, was greater than the unaccounted for lactate. Thus in the first day in air after nitrogen a part of the gain in citrate and malate might be derived from glycolysis. During

the second day in air after nitrogen the increase in citrate and malate was much less than the unaccounted for lactate. Thus at this stage lactate must be converted in part to substances other than CO_2, citrate, and malate.

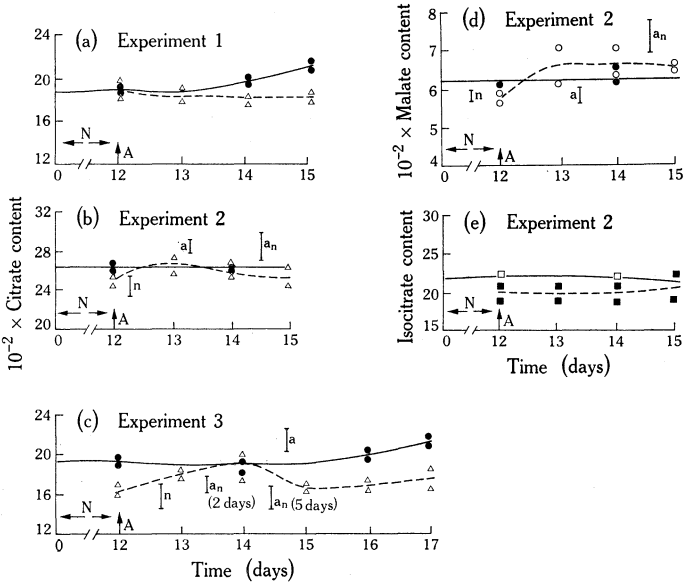

Fig. 3.—Changes in citrate (*a–c*), malate (*d*), and isocitrate (*e*) contents of potato samples kept in air (——) and of samples subjected to anaerobiosis then returned to air (– – –). All concentrations are expressed as μmoles/100 g fresh weight. ↑A, time of restoration to air. The magnitude of the standard errors are shown on Figures 3(*b*)–3(*d*) for samples in nitrogen (n), air after nitrogen (a_n), and air throughout (a).

TABLE 1

CHANGES IN LACTATE COMPARED WITH CO_2 OUTPUT AND IN CITRATE AND MALATE DURING RECOVERY FROM ANAEROBIOSIS

Values are given as μmoles per 100 g of potatoes per day

Experiment	Time in air after nitrogen (days)	Lost lactate not accounted for as extra CO_2*	Increase in citrate†	Increase in malate†
1	1	155	Nil	—
	2	311	Nil	—
2	1	163	159	47
	2	271	80	47
3	1	119	160	—
	2	273	170	—

* Extra CO_2 is obtained by subtracting CO_2 output of samples kept in air from CO_2 output of samples in air after nitrogen.

† Above level recorded while samples were kept in nitrogen.

Since citrate increased in air after nitrogen in experiments 2 and 3 but not in experiment 1, no firm conclusion can be drawn from these results as to whether this acid increases on return of potatoes to air. However, the results of experiment 1 do not preclude the hypothesis that the turnover rate in the TCAC was increased. The fact that citrate did not increase on transfer of the samples to air after nitrogen

in the first experiment might have been due to an increase in the rate of utilization of citrate at the mitochondrial level which compensated for the faster rate of production. Moreover, there are various examples in the literature where no change in the concentration of a certain acid occurred although the turnover rate in the TCAC appeared to be increased. In strawberry leaves treated with iodoacetate, the increase in respiration was followed by an increase in pyruvate and oxaloacetate but not in citrate (Barker and Younis 1965) and during the ripening of banana, ketoacids and malate increased following the increase in respiration but citrate content was unchanged (Solomos 1963).

The loss of citrate and malate during anaerobiosis [Figs. 3(*a*)–3(*d*)] may be considered indicative of utilization of these acids through the TCAC. Wager (1961) observed in peas kept under anaerobic conditions a decrease in citrate and malate and an increase in succinate contents, and suggested that the changes represented movement round the TCAC.

The concentration of isocitrate [Fig. 3(*e*)] ranged from 21·1 to 22·6 μmoles/100 g in samples kept in air in experiment 2. There was no significant change in nitrogen and in air after nitrogen.

The behaviour of isocitrate has been found to differ from that of malate and citrate by Stutz and Burris (1951), Varner and Burrell (1950), and Vickery (1952). The equilibrium established by aconitate hydratase between citrate and isocitrate favours citrate and this may be one of the reasons for the absence of any detectable changes in isocitrate content in air after nitrogen.

(*c*) *Changes in Acetaldehyde and Acetate Contents following Anaerobiosis*

On return to air after nitrogen in experiment 3 acetate increased, reaching a maximum in the first and second day, then fell slowly [Fig. 4(*a*)]. The acetate content of samples kept in air ranged from 43 to 67 μmoles/100 g of potatoes, whilst the acetaldehyde content of these samples was 0·76–0·97 μmoles/100 g. Acetaldehyde content increased in nitrogen, and increased further in air after nitrogen, reaching a maximum in the first day [Fig. 4(*b*)].

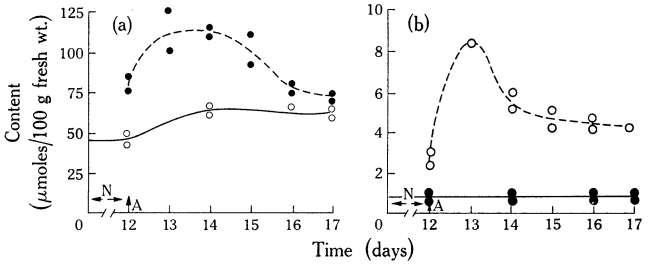

Fig. 4.—Changes in acetate (*a*) and acetaldehyde contents (*b*) of potato samples kept in air (——) or subjected to anaerobiosis then returned to air (– – –). ↑A, time of restoration to air.

These large increases in acetate and acetaldehyde during recovery from anaerobiosis might have arisen from decarboxylation and oxidation of pyruvate. Pyruvate has been reported to increase in potatoes kept in air after nitrogen (Barker

and Mapson 1953, 1963). Similarly, in air after nitrogen Biale and Shepherd (1939) observed a large production of acetaldehyde in oranges and lemons and suggested that acetaldehyde and extra CO_2 might have come from pyruvate, and Wager (1961) observed an increase in acetate in peas after anaerobiosis and suggested that it might have come from pyruvate by oxidation.

IV. Acknowledgment

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V. References

ap Rees, T., and Beevers, H. (1960).—Pentose phosphate pathway as a major component of induced respiration of carrot and potato slices. *Pl. Physiol., Lancaster* **35,** 839–47.

Barker, J. (1963).—Studies in the respiratory and carbohydrate metabolism of plant tissues. XIII. The influence of oxygen at high pressure in increasing and decreasing the respiration of potatoes at 15°C. *Proc. R. Soc.* B **158,** 143–55.

Barker, J., and Mapson, L. W. (1953).—Studies in the respiratory and carbohydrate metabolism of plant tissues. V. Experimental studies of the formation of carbon dioxide and of the changes in lactic acid, sucrose and in certain fractions of ketoacids in potato tubers in air following anaerobic conditions. *Proc. R. Soc.* B **141,** 321–62.

Barker, J., and Mapson, L. W. (1955).—Studies in the respiratory and carbohydrate metabolism of plant tissues. VII. Experimental studies with potato tubers of an inhibition of the respiration and of a "block" in the tricarboxylic acid cycle induced by "oxygen poisoning". *Proc. R. Soc.* B **143,** 523–49.

Barker, J., and Mapson, L. W. (1963).—Studies in the respiratory and carbohydrate metabolism of plant tissues. XII. Further studies of the formation of CO_2 and the changes in lactate, alcohol, sucrose, pyruvate and α-ketoglutarate in potato tubers in nitrogen and in air following anaerobic conditions. *Proc. R. Soc.* B **157,** 383–402.

Barker, J., and Younis, M. E. (1965).—Studies in the respiratory and carbohydrate metabolism of plant tissues. XVI. Further studies of the influence of iodoacetate as a stimulant of respiration in strawberry leaves. *J. exp. Bot.* **16,** 59–73.

Biale, J. B., and Shepherd, A. D. (1939).—*Proc. Am. Soc. hort. Sci.* **37,** 543.

Clausen, S. W. (1922).—A method for the determination of small amounts of lactic acid. *J. biol. Chem.* **52,** 263–80.

Conway, E. J. (1950).—"Microdiffusion and Analysis and Volumetric Error." 3rd Edn. (Crossley Lockweed and Sons: London.)

Hughes, C. S. (1960).—Ph.D. Thesis, University of Cambridge.

Ochoa, S. (1948).—Biosynthesis of tricarboxylic acids by carbon dioxide fixation. *J. biol. Chem.* **174,** 133–57.

Pritchard, G. G. (1959).—Ph.D. Thesis, University of Cambridge.

Serlin, I., and Colzias, G. C. (1955).—Microdiffusion of acetic acid as an assay for acetylcholinesterase. *J. biol. Chem.* **215,** 263.

Solomos, T. (1963).—Ph.D. Thesis, University of Cambridge.

Stutz, R. E., and Burris, R. H. (1951).—Photosynthesis and metabolism of organic acids in higher plants. *Pl. Physiol., Lancaster* **26,** 226–43.

Taylor, T. G. (1953).—A modified procedure for the microdetermination of citric acid. *Biochem. J.* **54,** 48–9.

Varner, J. E., and Burrell, R. C. (1950).—Use of C^{14} in the study of the acid metabolism of *Bryophyllum calycinum. Archs Biochem.* **25,** 280–7.

Vickery, H. B. (1952).—The behaviour of isocitric acid in excised leaves of *Bryophyllum calycinum* during culture in alternating light and darkness. *Pl. Physiol., Lancaster* **27,** 9–17.

Wager, H. G. (1961).—The effect of anaerobiosis on acids of the tricarboxylic acid cycle in peas. *J. exp. Bot.* **12,** 34–46.