

The Effect of Light on the Tricarboxylic Acid Cycle in Green Leaves

II. INTERMEDIARY METABOLISM AND THE LOCATION OF CONTROL POINTS

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E. A. CHAPMAN AND D. GRAHAM

Plant Physiology Unit, Commonwealth Scientific and Industrial Research Organization, Division of Food Research, and School of Biological Sciences, Macquarie University, North Ryde 2113, Sydney, Australia

ABSTRACT

Long term feeding of acetate-2-¹⁴C, ¹⁴CO₂, citrate-1,5-¹⁴C, fumarate-2,3-¹⁴C, and succinate-2,3-¹⁴C to mung bean (*Phaseolus aureus* L. var. Mungo) leaves in the dark gave labeling predominantly in tricarboxylic acid cycle intermediates. Kinetics of the intermediates during dark/light/dark transitions showed a light-induced interchange of ¹⁴C between malate and aspartate, usually resulting in an accumulation of ¹⁴C in malate and a decrease of it in aspartate. ¹⁴C-Phosphoenolpyruvate also showed a marked decrease during illumination. Changes in other intermediates of the tricarboxylic acid cycle were relatively minor. The kinetic data have been analyzed using the Chance crossover theorem to locate control points during the dark/light/dark transitions. The major apparent control points are located at malate and isocitrate dehydrogenases, and less frequently at citrate synthase and fumarase. These findings are explained in terms of the light-induced changes in adenine nucleotides and nicotinamide adenine dinucleotides.

In the previous paper (9) evidence was presented which indicated that a relatively high light intensity had only a temporary effect in depressing the rate of the tricarboxylic acid cycle in detached green leaves of mung bean (*Phaseolus aureus* L. var. Mungo). The tricarboxylic acid cycle appeared to adjust quickly to illumination, and the subsequent rate of oxidation was at least as high in the light as in the dark, judged by the rate of accumulation of ¹⁴C in either succinate in the presence of malonate or in citrate in the presence of fluoroacetate.

Earlier studies on intermediary respiratory metabolism of mung bean leaves under illumination showed that several of the intermediates of the tricarboxylic acid cycle were interconverted to all the other detectable intermediates of the cycle both in the light and the dark (14). Rapid changes in the relative concentrations of ¹⁴C-labeled tricarboxylic acid cycle intermediates were shown to occur during the dark to light transition (13). These changes were postulated to be related to the observed changes in nicotinamide adenine dinucleotides on illumination (13). This work is confirmed and extended by the present study, and the results are analyzed by the Chance crossover theorem (6) to determine control points in the tricarboxylic acid cycle.

The crossover theorem was derived by Chance and used in

the elucidation of the sites of phosphorylation in the oxidative electron transport chain (7, 8). This theorem (5) may be stated as follows: in a linear sequence of reactions, if the steady state flux of carbon is decreased with consistent increases (+) and decreases (–) in the steady state levels of the intermediates, a (+, –) pair in the sequence of changes is called a crossover point and can only occur at a site of interaction or control point. A (–, +) pair in the sequence is called a reverse crossover and can occur anywhere between control points. Such a reverse crossover does not indicate a control point. Chance points out (5) that even though only one crossover point is identified, more than one may exist since a site of interaction does not always give rise to a crossover point.

The derivation of a mathematical formula (12) extended the application of the crossover theorem to the determination of control points in the glycolytic sequence in yeast (12) and pea seeds (Rowan's analysis [33] of the results of Barker [1]) and in the tricarboxylic acid cycle in bananas (28).

The crossover theorem has been applied in the present study to the tricarboxylic acid cycle intermediates during dark/light/dark transitions which are likely to result in changes in carbon flux. The main conclusions drawn from the analysis are that the tricarboxylic acid cycle in mung bean leaves undergoes a series of changes in control points at citrate synthase, fumarase, isocitrate and malate dehydrogenases during the transition from dark to light. The changes can be correlated with known light-induced changes of the adenine nucleotides (34) and of the nicotinamide adenine dinucleotides (13, 16, 19, 29, 30). After the major initial changes in the light, the ¹⁴C-labeled intermediates of the tricarboxylic acid cycle approximate to a new steady state in which it appears probable that the ratio of oxidized-reduced NAD continues to play an important role in the regulation of the cycle. On transition from light to dark the major apparent control point is at isocitrate dehydrogenase.

MATERIALS AND METHODS

The growth of mung bean seedlings (*Phaseolus aureus* L. var. Mungo) and the experimental and analytical procedures have been described in the previous paper (9).

RESULTS

Figure 1, a through d, and Figure 2 show the effects of light on the percentage distribution of ¹⁴C in aspartate, malate, PEP¹,

¹ Abbreviation: PEP: phosphoenolpyruvate.

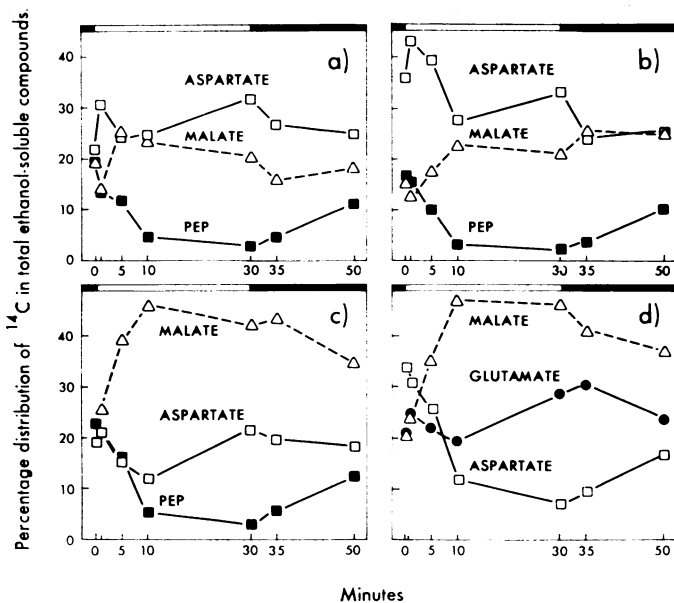


FIG. 1. Transient changes in some ^{14}C -labeled intermediates during dark/light/dark transitions. Each sample of five leaves was fed acetate-2- ^{14}C (a), citrate-1,5- ^{14}C (b), fumarate-2,3- ^{14}C (c), or succinate-2,3- ^{14}C (d) in the dark for 2 hr then transferred to water in an air draught for 15 min before beginning the experiments. (For details see "Materials and Methods" in ref. 9.) Solid bar represents darkness and open bar represents illumination.

and glutamate in excised leaves to which acetate-2- ^{14}C , citrate-1,5- ^{14}C , fumarate-2,3- ^{14}C , and succinate-2,3- ^{14}C (Fig. 1, a through d, respectively) and $^{14}\text{CO}_2$ (Fig. 2) were fed in the dark, and then were replaced by distilled water. In the experiments in which fumarate-2,3- ^{14}C , succinate-2,3- ^{14}C , and $^{14}\text{CO}_2$ were fed, illumination resulted in a large increase in the proportion of ^{14}C in malate and an apparently reciprocal decrease of ^{14}C in aspartate (Fig. 1, c and d, and Fig. 2). Marked reciprocal changes in ^{14}C -malate and ^{14}C -aspartate were also found on illumination in the experiments in which acetate-2- ^{14}C and citrate-1,5- ^{14}C were fed (Fig. 1, a and b), although the total changes were less than those described when the other three substrates were fed. For all five experiments the changes were

strictly light-dependent, because in control experiments during a 50-min time course in the dark only insignificant changes were found in the proportions of ^{14}C in the various intermediates. Return of the leaves to darkness resulted in further reciprocal changes of ^{14}C between malate and aspartate in the case of the citrate-1,5- ^{14}C and succinate-2,3- ^{14}C feeding experiments, but such reciprocity was not so clear in the experiments in which acetate-2- ^{14}C , fumarate-2,3- ^{14}C , or $^{14}\text{CO}_2$ were used to label the leaves.

In the experiments in which acetate-2- ^{14}C , citrate-1,5- ^{14}C , and fumarate-2,3- ^{14}C were fed there was also a rapid decrease in the proportion of ^{14}C in PEP on illumination. Return to darkness resulted in a slow reversal of this change. It is noteworthy that ^{14}C -glutamate was a significant proportion of the total ^{14}C in ethanol-soluble compounds only in the experiments in which $^{14}\text{CO}_2$ or succinate-2,3- ^{14}C were fed.

Table I shows the percentage distribution of ^{14}C among other ethanol-soluble compounds in the dark and after illumination for 10 min. In general the light-induced changes in ^{14}C in these compounds were small relative to the changes observed in

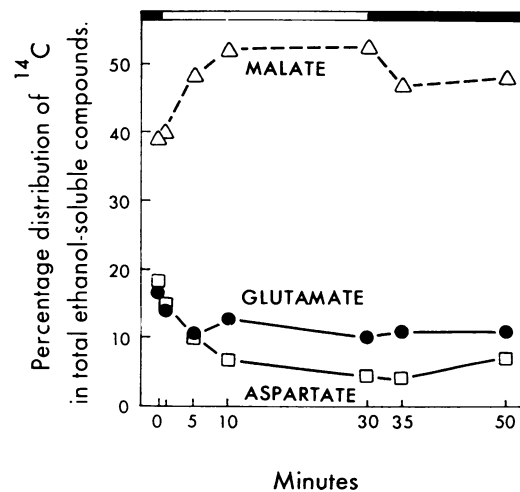


FIG. 2. Transient changes in some ^{14}C -labeled intermediates during dark/light/dark transitions. Leaves were fed $^{14}\text{CO}_2$ in the dark for 2 hr and treated as described for Figure 1. Solid bar represents darkness and open bar represents illumination.

Table I. Percentage Distribution of ^{14}C in Some Ethanol-soluble Compounds in Dark and Light

Excised leaves were labeled in the dark then placed in water in an air draught. Experimental treatments were then carried out as described in "Materials and Methods." The results shown are for the same experiments given in Figures 1 and 2, and list those compounds not shown therein.

	Acetate-2- ^{14}C		$^{14}\text{CO}_2$		Citrate-1,5- ^{14}C		Fumarate-2,3- ^{14}C		Succinate-2,3- ^{14}C	
	0 dark	10 min light	0 dark	10 min light	0 dark	10 min light	0 dark	10 min light	0 dark	10 min light
Alanine	2.1	ND ¹	ND	ND	ND	ND	9.2	4.0	ND	1.3
Arginine	ND	ND	5.5	5.2	ND	ND	ND	ND	ND	ND
Asparagine	2.6	6.7	ND	ND	2.5	7.7	7.0	11.9	ND	ND
Citrate	7.3	4.6	14.6	12.7	— ²	— ²	5.0	6.0	9.5	7.5
Fumarate	11.2	9.9	trace	trace	9.0	9.2	— ²	— ²	trace	trace
Glutamate	1.4	1.0	16.8	12.9	0.5	0.6	1.6	1.7	20.8	19.3
Glutamine	ND	ND	trace	2.6	ND	ND	ND	ND	10.5	10.9
Phosphoenolpyruvate	19.9	2.8	ND	ND	17.0	2.4	23.1	3.0	ND	ND
Succinate	trace	2.6	2.0	1.6	1.5	3.7	1.5	0.7	— ²	— ²
Sucrose	ND	ND	1.2	3.8	ND	ND	ND	ND	ND	ND

¹ Not detected.

² ^{14}C -substrate fed to leaves and not included in total ethanol-soluble compounds.

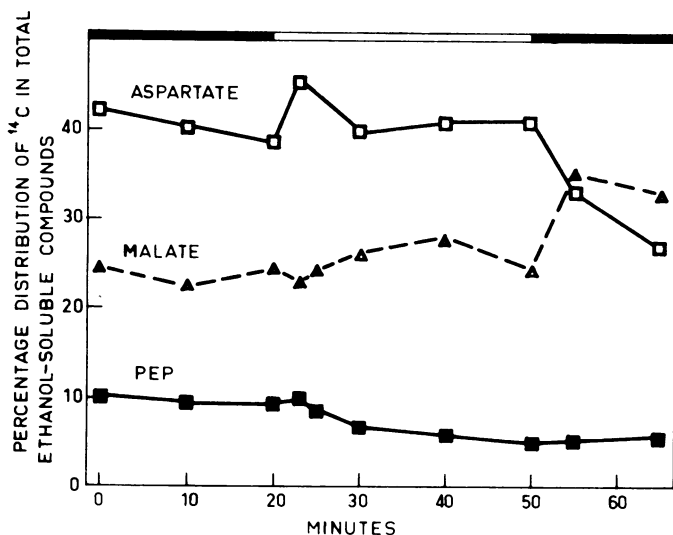


FIG. 3. Transient changes in some ^{14}C -labeled intermediates during dark/light/dark transitions in the presence of 0.1 mM DCMU. Leaves were fed citrate-1,5- ^{14}C in the dark for 2 hr and treated as described for Figure 1. Solid bar represents darkness and open bar represents illumination.

^{14}C -malate, ^{14}C -aspartate, and ^{14}C -PEP. It is clear that the method of feeding ^{14}C -labeled intermediates of the tricarboxylic acid cycle or $^{14}\text{CO}_2$ to leaves in the dark resulted in the major proportion of the ethanol-soluble ^{14}C being present in intermediates of tricarboxylic acid cycle or compounds closely related to them (e.g., aspartate and glutamate).

Effect of DCMU. In order to determine the influence of photosynthesis on the light-induced changes in tricarboxylic acid cycle intermediates, leaves previously fed citrate-1,5- ^{14}C in the dark were treated with 0.1 mM DCMU. The results in Figure 3 show that the light-induced changes in ^{14}C -malate, ^{14}C -aspartate, and ^{14}C -PEP were greatly diminished in the presence of the inhibitor, compared with the control (Fig. 1b).

Determination of Control Points in the Tricarboxylic Acid Cycle. The results shown in Figures 1 to 3 and in Table I have been used to determine the control points in the tricarboxylic acid cycle during dark/light/dark transitions by the application of the Chance crossover theorem (5). Crossover points were derived as follows. The tricarboxylic acid cycle intermediates were expressed in a linear series. ^{14}C -Aspartate and ^{14}C -glutamate have been used in place of their respective ketoacids because it is impracticable to isolate and measure the ketoacids in the same leaf samples used for the other intermediates. It is reasonable to assume that these two amino acids are in equilibrium with their ketoacids because of the presence of adequate levels of transaminases and because of the higher activity and greater affinity for substrates of these compared with glutamic dehydrogenase. (For examples see references 4, 10, 21, 26, 37, 43.) The relative percentage changes in each of the intermediates during certain time intervals was calculated according to the formula (ref. 6)

$$\frac{C_{t_2} - C_{t_1}}{\frac{C_{t_1} + C_{t_2}}{2}} \times 100$$

The time intervals chosen were: dark (0 min) to 1 min light; 1 min light to 5 min light; 5 min light to 30 min light and 30 min light to 5 min dark. Examples of plots of crossover points are shown in Figure 4, a through c, for the experiment in

which acetate-2- ^{14}C was previously fed to the leaves in the dark. Control points were determined by considering the relative fluxes of carbon in the cycle during a particular time interval in relation to the observed crossovers in Figure 4. Evidence was presented in the previous paper (9) that the carbon flux in the tricarboxylic acid cycle decreased during the first 5 min of illumination compared with that in the dark. There followed an increase in carbon flux to a value as great as or greater than that in the preceding dark period. Return

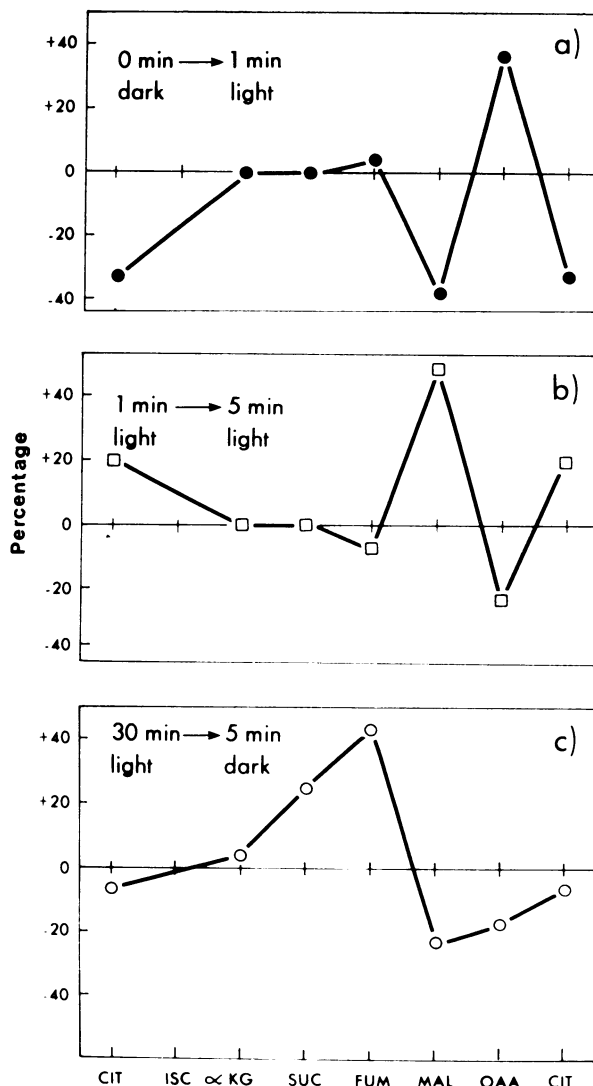


FIG. 4. Crossover points in the tricarboxylic acid cycle. Crossover points were obtained by application of the formula

$$\frac{C_{t_2} - C_{t_1}}{\frac{C_{t_1} + C_{t_2}}{2}} \times 100$$

to data shown in Figure 1a (acetate-2- ^{14}C fed). C_{t_1} and C_{t_2} are amounts of ^{14}C in an intermediate (expressed as percentages of the total ethanol-soluble compounds) at times t_1 and t_2 minutes, respectively. Intermediates of the tricarboxylic acid cycle are abbreviated as follows: CIT: citrate; ISC: isocitrate; αKG : α -ketoglutarate; SUC: succinate; FUM: fumarate; MAL: malate; OAA: oxaloacetate. In the experiments, ^{14}C -aspartate and ^{14}C -glutamate were measured and assumed to be in rapid equilibrium with oxaloacetate and α -ketoglutarate (see text). Accordingly the latter intermediates of the tricarboxylic acid cycle are shown in the figure.

to darkness after illumination is assumed to result in an increase in respiratory carbon flux due to the utilization of photosynthetic intermediates (2). According to the crossover theorem (6), a control point is found wherever a crossover occurs which is similar in direction to the relative carbon flux. For example, in Figure 4 citrate synthase and fumarase are control points for the time interval dark to 1 min light (Fig. 4a), malate dehydrogenase and probably isocitrate dehydrogenase for the interval 1 min light to 5 min light (Fig. 4b), and isocitrate dehydrogenase for the interval 30 min light to 5 min dark (Fig. 4c). Table II summarizes the crossover points derived in a similar manner for the other ^{14}C -substrates fed, including the experiment in which DCMU was present. The control points which are less definitive, such as at isocitrate dehydrogenase for the interval 1 min to 5 min light in the above example, have been listed in brackets in Table II. For the majority of ^{14}C -labeled substrates fed, the results show that the major control points occur at isocitrate dehydrogenase in the 1st min of illumination, malate dehydrogenase in the 1 to 5 min interval in the light, and again at isocitrate dehydrogenase on transfer from the light to the dark. During the interval 5 min light to 30 min light, the amounts of ^{14}C -labeled tricarboxylic acid cycle intermediates seem to approximate to a steady state (Figs. 1-3), and consequently the frequency of control points is diminished.

Effect of DCMU on Control Points. Table II also shows the

control points obtained when leaves previously fed citrate-1,5- ^{14}C in the dark were illuminated in the presence of 0.1 mM DCMU. It should be noted that some of the time intervals were slightly different in the experiment with DCMU. During the initial period of illumination, citrate synthase is the major control point with a less definite control point at fumarase. These are followed by control points at isocitrate dehydrogenase and malate dehydrogenase. Return to darkness again gives citrate synthase and fumarase as control points.

DISCUSSION

It was concluded in the previous paper (9) that the tricarboxylic acid cycle was operative both in the dark and in the light in mung bean leaves. In this paper we have examined the kinetics of the tricarboxylic acid cycle intermediates during dark/light/dark transitions. The technique of feeding ^{14}C -labeled compounds to mung bean leaves for 2 hr in the dark resulted in ^{14}C being present predominantly in intermediates of the tricarboxylic acid cycle and compounds related to them, such as aspartate, glutamate and PEP (Figs. 1 and 2, and Table I).

On illumination of the ^{14}C -labeled leaves there was a light-dependent, reciprocal interchange of ^{14}C between aspartate and malate (Figs. 1 and 2), giving a substantial increase in the proportion of ^{14}C in malate in the experiments in which ^{14}C -fu-

Table II. Summary of Control Points in the Tricarboxylic Acid Cycle During Dark-Light-Dark Transitions

The Chance Crossover Theorem was applied to data obtained from the experiments shown in Figures 1, 2, and 3. Crossover diagrams such as those shown in Figure 4 were constructed and from knowledge of the carbon flux (see text), control points were determined. + indicates a control point; [+] indicates a less definitive control point.

Time Interval	Relative Carbon Flow	Enzyme	^{14}C -substrate Fed					
			Acetate- 2- ^{14}C	$^{14}\text{CO}_2$	Fumarate- 2,3- ^{14}C	Succinate- 2,3- ^{14}C	Citrate- 1,5- ^{14}C	Citrate- 1,5- ^{14}C (+ DCMU) ¹
Dark (0 min), light (1 min)	Decreasing	Citrate synthase	+					+
		Isocitrate dehydrogenase		+	+		+	
		α -Ketoglutarate oxidase						
		Succinate oxidase						
		Fumarase	+					[+]
Light (1 min), light (5 min)	Decreasing	Malate dehydrogenase		+		+		
		Citrate synthase		+				
		Isocitrate dehydrogenase	[+]					+
		α -Ketoglutarate oxidase						
		Succinate oxidase						
Light (5 min), light (30 min)	Increasing	Fumarase	+		+	+	+	+
		Malate dehydrogenase						
		Citrate synthase					+	
		Isocitrate dehydrogenase		+		+		
		α -Ketoglutarate oxidase						
Light (30 min), dark (5 min)	Increasing	Succinate oxidase						
		Fumarase	+					
		Malate dehydrogenase		+	+	+	+	+
		Isocitrate dehydrogenase	+					
		Citrate synthase						

¹ Time intervals in this experiment were: dark (0 min) to light (3 min); 3 min light to 5 min light; the other intervals were as shown.

marate, ^{14}C -succinate, or $^{14}\text{CO}_2$ were fed (Fig. 1, c and d, and Fig. 2). This light-dependent interchange of ^{14}C between aspartate and malate which was described earlier (13) appears to be a mitochondrial process, because it was inhibited by malonate or fluoroacetate (9) which are well documented inhibitors of mitochondrial enzymes (41, 42). Illumination also resulted in a sharp decrease in ^{14}C in PEP in those experiments in which this compound was detected (Fig. 1, a-c). It seems likely that PEP is carboxylated to give oxaloacetate, which is reduced to ^{14}C -malate in the light.

In the previous paper it was shown that, under the same conditions as those in the present experiments, 0.1 mM DCMU inhibited photosynthesis, measured as $^{14}\text{CO}_2$ fixation, about 82%. The finding that the light-induced changes in ^{14}C -malate, ^{14}C -aspartate, and ^{14}C -PEP were greatly diminished in the presence of DCMU indicates that the products of photosynthetic electron transport, ATP and NADPH, are necessary for the light-induced changes in tricarboxylic acid intermediates and related compounds.

In interpreting the changes discussed above, it is necessary to consider the possibility of multiple pools of the various tricarboxylic acid cycle intermediates. The vast majority of the leaf cells are of two types, the palisade and the spongy mesophyll cells. Microscopic observations showed that the former contained a higher proportion of chloroplasts than the latter but it is likely that both show generally similar responses to light. The contribution of epidermal and vascular tissue to the metabolic changes is likely to be small.

The problem of intracellular compartmentation has been extensively studied in corn roots (15, 23, 24, 26, 38), in the green alga *Scenedesmus* (27), and in beet discs (31). It has been established that plant cells contain at least two pools of each organic acid. The probable sites of these pools are the vacuole, mitochondrion, cytoplasm, and possibly chloroplast and peroxisome. Of these, the vacuolar pool is probably the largest but also the least metabolically active (26). This multiplicity of pools is exemplified by malate. After pulse-labeling in corn roots and beet discs, it was found that ^{14}C -malate produced by dark $^{14}\text{CO}_2$ fixation or supplied exogenously, was in an extramitochondrial pool (23, 24, 31). Short term experiments, however, in which substrates such as ^{14}C -acetate, which are metabolized by the mitochondrion, were fed to corn roots yielded ^{14}C -malate in a mitochondrial pool (15, 38). In the light in *Scenedesmus* (27), ^{14}C -malate produced by carboxylation of pyruvate was not in equilibrium with mitochondrial malate during a short term experiment.

In the long term dark feeding experiments reported here, in which acetate, citrate, fumarate, or succinate were the labeled substrates, about 15 to 20% of the ^{14}C in the ethanol-soluble fraction was found in ^{14}C -malate in each case. Such equivalent proportionation indicates that it is likely the same pools of malate are being labeled with ^{14}C . During the initial part of the feeding period with ^{14}C -labeled respiratory substrates it is presumed that predominantly mitochondrial pool(s) will be labeled (15, 38), but after long term feeding ^{14}C will be distributed in more than one cellular compartment or metabolic pool.

After $^{14}\text{CO}_2$ labeling of mung bean leaves in the dark, the proportion of ^{14}C -malate is doubled (Fig. 2) compared with that after the feeding of other ^{14}C -labeled substrates (Fig. 1). The mitochondrial inhibitors, fluoroacetate and malonate, markedly reduce both the level of ^{14}C -malate in the dark and the increase in ^{14}C -malate in the light (9) after prior labeling of the leaves in the dark with $^{14}\text{CO}_2$. We conclude therefore that a large part of the ^{14}C -malate is metabolically active and is in a mitochondrial pool or in an extramitochondrial pool which is readily accessible to the mitochondrial pool. This met-

abolically active pool probably corresponds to the ^{14}C -malate derived from ^{14}C -labeled acetate, fumarate, citrate, and succinate. It is suggested that the remaining portion of the ^{14}C -malate derived from dark $^{14}\text{CO}_2$ fixation is in an additional, metabolically separate pool, because after long term treatment with fluoroacetate in the dark, the proportion of ^{14}C -malate cannot be reduced below 20% of the total ethanol-soluble ^{14}C (E. A. Chapman, unpublished results).

Specific activities were not determined because without knowledge of the individual pool sizes such data are of limited value. An attempt to measure individual pool sizes was made using a published nonaqueous technique to isolate cellular organelles (39). This technique proved unsatisfactory because control experiments revealed considerable cytoplasmic contamination of cellular organelles, particularly the mitochondrion. Using a differential labeling procedure, it was possible to correct for that cytoplasmic and vacuolar contamination of the organelles which occurred after cell breakage. Correction for contamination which occurred before cell breakage (for example, during lyophilization) was extremely difficult. Therefore, attempts to localize metabolic pools and the sites of changes in the labeled compounds associated with the tricarboxylic acid cycle were not pursued further.

The Chance theorem has been applied to the mitochondrial electron transport chain (8) and to glycolysis (12) to provide evidence of control sites in these sequences of reactions. In the case of the electron transport chain, unequivocal answers have been obtained and the results for the glycolytic system have been consistent with the known biochemical facts. However, as has been pointed out (36), the rigorous requirements for the application of the theorem cannot be readily obtained in most metabolic situations other than an electron transport chain. The principal criticisms of Scrutton and Utter (36), which are applicable to the results described here, refer to a lack of tissue homogeneity, a lack of single pools of metabolites, and a lack of knowledge of the concentration of such metabolites at the sites of action. Furthermore, it is probable that the various ^{14}C -substrates supplied to the leaves were not necessarily metabolized in the same way. For example, it is likely that acetate, fumarate, citrate, and succinate were primarily metabolized intramitochondrially but that the products of dark $^{14}\text{CO}_2$ fixation, mainly aspartate and malate, were metabolized in the cytosol. It cannot be denied that these criticisms are valid in considering the application of the Chance theorem to any metabolic sequence involving dissociable intermediates. Considerable care is therefore necessary in assessing the results of the application of the theorem to such data. Nevertheless, on application the theorem seems to provide results generally consistent with the known biochemical responses of leaf tissues to light.

In Table II, it is shown that isocitrate dehydrogenase and malate dehydrogenase are major control points during the dark to light transition, and isocitrate dehydrogenase, a major control point during the transition from light to dark. Citrate synthase and fumarase appear less frequently as control points during either transition.

An explanation for control points during the dark/light/dark transitions at the enzymes citrate synthase, fumarase, isocitrate and malate dehydrogenases may be given in terms of the regulatory activity of the adenine nucleotides and nicotinamide adenine dinucleotides on these enzymes. The effect of light on these cofactors in green tissues is well documented. The ratio of ATP/ADP rises rapidly during the first minutes of illumination in both the chloroplastic and cytoplasmic plus mitochondrial fractions (34). The plant mitochondrial membrane is apparently freely permeable to ATP as shown by

measurements of isotopic exchange (20) and reverse electron flow (40). Those reactions of the tricarboxylic acid cycle which are controlled by the level of free ATP will be affected by the light-induced increase in ATP. Citrate synthase from a variety of sources is regulated in a complex manner by a number of modifiers including ATP (25) which is inhibitory (3). Fumarase from yeast (17) and pig heart mitochondria (32), and from saltbush (*Atriplex hastata*) and spinach (*Spinacia oleracea*) leaf mitochondria (E. A. Chapman, unpublished results) is inhibited by ATP. NAD⁺-dependent isocitrate dehydrogenase from yeast and *Neurospora* (35) is activated by ADP while the enzyme isolated from leaves of saltbush (*Atriplex hastata*) and spinach (*Spinacia oleracea*) is inhibited by ATP, and this inhibition can be overcome by addition of Mg²⁺ (E. A. Chapman, unpublished results). The peak in ATP level observed by Santarius and Heber (34) during the dark to light transition is of short duration, but from 1 to 3 min in the light a steady state level is achieved which is higher than the level in the dark. It is reasonable to assume that this higher level is maintained in the light and acts as a control for enzymes such as citrate synthase, fumarase, and isocitrate dehydrogenase.

Light-induced changes in the nicotinamide adenine dinucleotides in leaf cells also occur (13, 16, 19), resulting in only a slight change in the ratio of NADP/NADPH, but resulting in a large decrease in the ratio of NAD/NADH. These changes which occur primarily in the chloroplast could be transferred to the cytoplasm via a shuttle mechanism (18) and thence to the mitochondrion, which in plants is permeable to the nicotinamide adenine dinucleotides (22). The conversion of malate to oxaloacetate by malate dehydrogenase is particularly sensitive to the ratio of NAD/NADH (14). Any decrease in the ratio of NAD/NADH will favor the reduction of oxaloacetate to malate. The control point at malate dehydrogenase is therefore explicable on the basis of the known decrease in the ratio NAD/NADH in the light. Duggleby and Dennis (11) have found that mitochondrial isocitrate dehydrogenase isolated from peas is under allosteric control of NADH and is particularly sensitive to the mole fraction NADH/NAD + NADH. Both the increase in ATP and in the mole fraction of NADH/NAD + NADH could conceivably contribute to the appearance of isocitrate dehydrogenase as a control point during the dark/light transition.

During the transition from light to dark there is a decrease in both ATP and the reduced coenzymes. Enzymes under control of either of these cofactors are therefore likely to be activated. Citrate synthase, fumarase, malate and isocitrate dehydrogenases all appear as activated enzymes at this time although the major control is at isocitrate dehydrogenase.

In summary, the observation that carbon flow through the tricarboxylic acid cycle is reduced during the first minutes in the light is explicable in terms of rate limiting steps at isocitrate and malate dehydrogenases, and to a lesser extent at citrate synthase and fumarase. Subsequently, malate dehydrogenase becomes the major rate-limiting enzyme. The observations that, after a few minutes in the light, ¹⁴C ceases to accumulate in malate (Figs. 1 and 2), and carbon flow through the cycle resumes at a rate comparable to that in the dark (9) suggest that the accumulated malate can overcome the unfavorable coenzyme ratio by a mass action effect on malate dehydrogenase, allowing the oxidation of malate to proceed. On the establishment of the new approximate steady state in the light, control points are less apparent (Table II, 5 min light to 30 min light). On transfer to the dark there is an increase in carbon flow through the cycle (2) as isocitrate dehydrogenase and to a lesser extent malate dehydrogenase, citrate synthase, and fumarase are activated.

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