

EFFECTS OF OXYGEN AND CARBON DIOXIDE CONCENTRATION ON THE
PHOTOSYNTHETIC CARBON METABOLISM OF MAIZE AND TOBACCO LEAVES.

by

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ABSTRACT

An infra-red gas analyzer was used to measure rates of photosynthesis by maize and tobacco leaves in atmospheres containing various partial pressures of O_2 and CO_2 . With atmospheres containing 100-300 ppm of CO_2 photosynthesis by maize was fastest in the presence of 21 to 40% oxygen but with more than 300 ppm CO_2 the maximum rate was obtained in the absence of oxygen. Whatever the concentration of CO_2 in the atmosphere, photosynthesis by tobacco leaves was fastest in the absence of oxygen. Explanations for these effects were sought by studying the metabolism by leaves of ^{14}C -labelled CO_2 , glycine, alanine and malate. Production of glycine from CO_2 was increased in both maize and tobacco when the concentration of oxygen was increased. In light and darkness serine was a major product of metabolism by maize of $[2 - ^{14}C]$ glycine; sucrose synthesis from glycine was light-dependent and required oxygen. Therefore, the C_2 pathway is active in maize as well as in C_3 -plants. Increased concentrations of oxygen above 40% decreased photosynthesis by maize and caused C_4 acids to accumulate. The ability of the Calvin cycle to accept carbon from the C_4 -cycle was presumably impaired by stimulation of the C_2 pathway and inhibition of RuDP carboxylase by oxygen.

In the presence of lower concentrations of CO_2 , when decreasing oxygen below about 40% caused a decrease in photosynthesis, there was increased accumulation of newly assimilated carbon in aspartate. With low concentrations of CO_2 , and low oxygen, reductive amination and therefore availability of amino donors may be increased. Accumulated aspartate may inhibit reactions in the C_4 -cycle. Experiments in which ammonium chloride was supplied to leaves to uncouple photophosphorylation gave inconclusive results. Under anaerobic conditions photosynthesis by maize in $[^{14}C] CO_2$

lead to accumulation of ^{14}C in alanine. In the light, but not in the dark, maize converted both alanine and malate to sucrose by a mechanism that was stimulated by oxygen.

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ABBREVIATIONS

DCMU - 3- (3, 4-dichlorophenyl) -1, 1- dimethyl urea

HEPES - N-2-hydroxyethylpiperazine-N-2-ethane sulphonic acid

IRGA - infra-red gas analyzer

PEP - Phosphoenolpyruvate

PGA - Phosphoglycerate

RuDP - ribulose 1, 5 diphosphate

UDPG - uridine diphosphate glucose

INTRODUCTION

1. Effect of Oxygen on the Rate of Photosynthesis
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Higher plants are frequently divided into three groups because differences have been recognized in their photosynthetic metabolism. Maize (Zea mays) and tobacco (Nicotiana tabacum) were used as examples from two of these groups. The third group, consisting of succulent plants adapted to growing in very dry situations, were not considered. Maize is regarded as typical of C_4 -plants, in which C_4 acids are important initial products of photosynthesis. Tobacco, on the other hand, is from the group of plants, often called C_3 -plants, in which PGA is the earliest product of photosynthesis that has been detected. There are also well recognised differences in the leaf anatomy between C_3 - and C_4 -plants. The concentration of oxygen and carbon dioxide in the atmosphere affect the rate of photosynthesis by maize and tobacco in different ways and the intention was to define these differences and investigate their cause.

1. Effect of O_2 on the Rate of Photosynthesis

Warburg (1920) first demonstrated that the unicellular alga, Chlorella pyrenoidosa, in the presence of high light intensity and high CO_2 concentration produced oxygen at a considerably greater rate in 2% than in 100% O_2 . The apparent rate of O_2 evolution (photosynthesis) was increased in passing from high to low O_2 . This inhibitory effect of high O_2 on the rate of photosynthesis was subsequently termed "the Warburg effect". This phenomenon was thought not to be due to an acceleration of normal dark respiration (Turner, Todd and Brittain, 1956), as no change was detected in the rate of respiration in the dark when the O_2 concentration was increased from 2% to 100%. Many of Warburg's observations were subsequently confirmed by a number of workers including Wassink et al (1938). The inhibitory effect of high O_2 concentration on the rate of photosynthesis was shown to be reversible by Tamiya and Huzisige (1949).

The rate of photosynthesis in the above experiments was measured as O_2 evolution but McAlister and Myers (1940) also demonstrated the Warburg effect, using young wheat leaves, by measurement of the inhibition of CO_2 uptake. Subsequently the effect was found to be widespread throughout the plant kingdom (Turner, Todd and Brittain, 1956). The early work concerned with the inhibitory effect of O_2 on the rate of photosynthesis was thoroughly reviewed by Turner and Brittain (1962).

Björkman (1965) found that the rate of photosynthetic CO_2 uptake was greater in 2% O_2 than in air, hence the presence of O_2 even at 21% inhibited photosynthesis. The increase in the rate of photosynthesis when the O_2 concentration was decreased from 21% to 2% was rapid and reversible, and the rate of dark respiration was little affected by such changes in O_2 concentration. These effects were exhibited by all the higher plant species Björkman studied. Forrester, Krotkov and Nelson (1966a) investigated the effect of O_2 concentration on the rate of photosynthesis by leaves of soybean. They confirmed the presence of the Warburg effect and demonstrated its reversibility. Forrester et al (1966b) found that the rate of photosynthesis by leaves of maize was also inhibited by increased O_2 concentration, though to a lesser extent than observed in soybean and this inhibition caused by high O_2 concentrations was found to be only partially reversible. Subsequently the rate of photosynthesis by a number of species of higher plants, including tobacco, was shown to be increased if the O_2 concentration in the atmosphere was decreased below that in air (Tregunna, Krotkov and Nelson, 1966; Hesketh, 1967; Poskuta, 1968). Björkman (1967) demonstrated that the overall growth rate of Mimulus cardinalis and Marchantia polymorpha was enhanced in the presence of a low (4%) O_2 concentration compared to 21% O_2 ; but the overall growth rate of maize was not increased in the presence of 4% O_2 compared to 21%. Downes and Hesketh (1968) confirmed that maize and other tropical grasses did not show a

significant enhancement of photosynthesis when the concentration of O_2 in the atmosphere was decreased below 21%, but the rate of photosynthesis by temperate grasses was increased 23% to 60% if the concentration of O_2 in the atmosphere was decreased from 21% to near 0%.

2. The Interaction between the Effects of Carbon Dioxide and Oxygen Concentration on the Rate of Photosynthesis

The magnitude of the Warburg effect was assessed from the rate of photosynthesis by Chlorella species in media equilibrated with 100% O_2 or 21% O_2 compared with rates in media equilibrated with N_2 (Wassink et al., 1938; Tamiya and Huzisige, 1949; Briggs and Whittingham, 1952). In the presence of relatively high CO_2 concentrations there was a marked inhibition of photosynthesis in 100% O_2 compared to 0% O_2 but there was no inhibitory effect in 21% O_2 compared to 0% O_2 . McAlister and Myers (1940) showed that the rate of CO_2 assimilation by wheat leaves in high light and 0.03% CO_2 was reduced by 30 to 50% in the presence of 20% O_2 compared to 0.5% O_2 . It was found that the Warburg effect was greater when CO_2 was limiting (Tamiya and Huzisige, 1949). Björkman (1967) demonstrated also that the increase in growth rate of plants grown at 4% O_2 compared with 21% O_2 was greater at low CO_2 concentration (0.011%) compared to high CO_2 concentration (0.064%) and Jolliffe and Tregunna (1968) concluded from their work using wheat leaves that the inhibition of photosynthesis by O_2 was greater in the presence of low CO_2 concentrations. The rate of photosynthesis by Conocephalum conicum was measured over a range of CO_2 concentrations from 0 to 600 ppm at low (2%) and high (50%) concentrations of O_2 by Fock, Krotkov and Calvin (1969). The rate of photosynthesis was directly proportional to the CO_2 concentration at low CO_2 concentrations in 21% O_2 , whereas this was not so when the O_2 concentration was 50%. Photosynthesis in 2% O_2 became saturated at lower CO_2 concentrations than

was the case of photosynthesis in 50% O_2 . The rate of photosynthesis was considerably decreased in 50% O_2 compared with 21% O_2 at all CO_2 concentrations measured, the inhibition by O_2 was found to be reversible.

Arnon, Allen and Whatley (1954) demonstrated that CO_2 fixation by isolated chloroplasts was inhibited by O_2 and Gibbs, Ellyard and Latzko (1968), using whole spinach chloroplasts, have since found that the degree of inhibition by O_2 was dependent upon CO_2 concentration. The Warburg effect exhibited by isolated chloroplasts was found to be reversible.

In conclusion, the rate of photosynthesis, whether measured by O_2 evolution or CO_2 uptake, is inhibited by increased concentrations of O_2 . The inhibition is particularly apparent when the concentration of CO_2 in the atmosphere is low. The rate of photosynthesis by many temperate species, e.g. tobacco, is maximal in atmospheres without O_2 ; the rate of photosynthesis by tropical species, such as maize, is less affected by the concentration of O_2 in the atmosphere. Investigations of the effect of O_2 , and the interaction between O_2 and CO_2 , on the rate of photosynthesis was continued by studies of respiration in the light, photorespiration, and on the other hand the effect of the gaseous environment, with regard to O_2 and CO_2 concentration, on photosynthetic carbon metabolism was studied. The evidence concerning photorespiration from studies of gaseous exchange, will be considered initially.

3. Photorespiration

The term photorespiration is used to describe a process or combination of processes which involve the evolution of CO_2 and the consumption of O_2 by a green plant during illumination. The investigation of such a process in the presence of CO_2 assimilation and O_2 evolution by photosynthesis is difficult.

O_2 consumption by illuminated plants has been investigated using the ^{18}O isotope and mass spectrometry; Brown and co-workers were responsible for much of the early work using this technique. Brown (1953) found no evidence for a light stimulated O_2 uptake during photosynthesis by either Chlorella or leaves of tobacco. He concluded that there was no effect of light on the rate of respiration. Govindjee, Owens and Hoch (1963) reported that the uptake of $[^{18}O]O_2$ by Chlorella in darkness was inhibited by low intensity light. However this effect was not observed in Scenedesmus by Hoch, Owens and Kok (1963); and from the results of inhibitor experiments it was concluded that two mechanisms of O_2 uptake operated during illumination, one was the normal dark respiratory process which was inhibited by light and the other a process of O_2 uptake enhanced by increased light intensity.

Ozbun, Volk and Jackson (1964) reported that the rate of O_2 consumption by leaves of bean was greater at 300 ft candles illumination than in darkness and that increasing the light intensity to 1500 ft candles did not significantly alter the rate of consumption. Further work by Ozbun et al (1965a; 1965b) confirmed this observation. They noted that there was evolution and uptake of O_2 at the CO_2 compensation point. The rate of O_2 uptake by leaves of soybean in the light was inversely related to the CO_2 concentration in the atmosphere and was slower than the rate of O_2 evolution (Mulchi, Volk and Jackson, 1968). Illuminated (1200 ft candles) maize leaves consumed O_2 at rates equal to or exceeding the rate of darkness, even at the CO_2 compensation point (Jackson and Volk, 1969).

The measurement of $^{18}O_2$ uptake by plants has been restricted to the study of O_2 exchange in the presence of low concentrations of O_2 . The data discussed pertains therefore to the consumption of O_2 in the light and darkness by processes having a high affinity for O_2 .

CO_2 evolution from the leaves of higher plants during illumination has been studied by a number of techniques in attempts to distinguish between it and the CO_2 consumed by photosynthesis. Investigators have distinguished between these two processes by studying the CO_2 exchange immediately upon darkening, the CO_2 compensation point or CO_2 evolution into a CO_2 -free atmosphere. Each of these methods will be considered.

It was demonstrated, by Decker (1955) and subsequently by Tregunna, Krotkov and Nelson (1961), that when an illuminated leaf of tobacco was darkened in air there was an immediate burst of CO_2 evolution, followed by a second burst prior to the attainment of a steady lower rate of CO_2 evolution due to normal dark respiration. The magnitude of the first burst of CO_2 was increased with increased light intensity during the period of illumination. A similar response by leaves of soybean and peperonia was observed but the effect was absent when non-chlorophyllous leaves were used (Tregunna et al, 1964). When leaves of tobacco were supplied with $[^{14}\text{C}] \text{CO}_2$ during the light period, large amounts of $^{14}\text{CO}_2$ were evolved during the first 75 sec of darkness; the $^{14}\text{CO}_2$ evolved having a similar specific activity to the $^{14}\text{CO}_2$ assimilated in the light (Tregunna et al, 1964). It was concluded that the CO_2 evolved immediately upon darkening was derived from recent products of photosynthesis. There was no burst of CO_2 evolution when illuminated maize leaves were darkened.

The effect of O_2 and CO_2 concentration on the post-illumination CO_2 burst from leaves was investigated. Tregunna et al (1966) reported that the burst from leaves of tobacco was absent in the presence of an atmosphere containing a low (2%) concentration of O_2 . Egle and Fock (1967), using Conocephalum conicum, reported that the post-illumination burst was increased with increasing O_2 partial pressure in an atmosphere containing 300 ppm CO_2 , however in the presence of 1200 ppm CO_2 the outburst of CO_2

during the first minute of darkness was absent. The effect of CO_2 concentration has been further studied using concentrations of CO_2 from 100 ppm to 600 ppm by Fock, Krotkov and Calvin (1969). They showed that the magnitude of the post-illumination CO_2 burst was increased when the concentration of CO_2 present was increased in the range 0 to 300 ppm during the period of illumination, but with further increases in the concentration of CO_2 the post-illumination burst was decreased.

It has been suggested that the post-illumination burst of CO_2 is due to a photorespiratory process; photosynthesis ceases on darkening but photorespiration continues briefly until its substrates, produced during illumination are consumed (Decker, 1957; Tregunna et al, 1961; Ludwig, Krotkov and Calvin, 1969). Forrester, Krotkov and Nelson (1966b) found that there was no post-illumination burst of CO_2 from leaves of maize even in the presence of high concentrations of O_2 . Recent work by Björkman, Gauhl and Nobs (1970) and Downton (1970) has shown that plants such as Amaranthus edulis and Atriplex rosea, which are believed to have a photosynthetic carbon metabolism similar to that of maize, do show a post-illumination burst of CO_2 but that it is insensitive to O_2 partial pressure.

Green plants, maintained at constant light intensity and temperature, in a closed vessel will deplete the CO_2 of the enclosed atmosphere only to a certain limiting concentration of CO_2 ; this concentration of CO_2 is termed the CO_2 compensation point. Moss (1962) demonstrated that leaves of maize and sugarcane had compensation points of less than 10 ppm CO_2 , whereas under the same experimental conditions leaves of tobacco, geranium and orchard grass had compensation points of 60 ppm CO_2 or greater. Subsequent work has indicated that higher plants can be separated into two groups depending upon whether they exhibit high or low compensation point concentrations of CO_2 (El-Sharkawy and Hesketh, 1965; Forrester et al, 1966b; Tregunna and Downton, 1967; Downton and Tregunna, 1968a; Osmond,

Troughton and Goodchild,, 1969). The CO_2 compensation points, of plants having high CO_2 compensation point values, were found to be sensitive to the concentration of O_2 present and were increased with increased concentrations of O_2 from 0% to 100% by volume (Forrester et al, 1966a; Tregunna and Downton, 1967; Fock, Krotkov and Canvin, 1969). Forrester et al (1966b) found that the low CO_2 compensation point of leaves of maize was insensitive to the presence of increased concentrations of O_2 , even up to 100% O_2 . This was confirmed by Poskuta (1969). Heichel and Musgrave (1969) reported significant differences in CO_2 compensation points when they compared different maize genotypes. However Moss, Willmer and Crookston (1971) determined the CO_2 compensation point values of a large number of maize genotypes, including those studied by Heichel and Musgrave, and they found that the CO_2 compensation point values were not significantly different and were all very low. The CO_2 compensation points are usually assumed to result from the production of CO_2 by respiratory processes at a rate which is equal to the rate of photosynthesis at that particular CO_2 concentration.

A CO_2 evolution into a CO_2 -free atmosphere from green leaves of a number of higher plant species, during illumination, has been demonstrated by El-Sharkawy and Hesketh (1965), Moss (1966a), El-Sharkawy, Loomis and Williams (1967), Zelitch and Day (1968) and Hew, Krotkov and Canvin (1969). The species studied included sunflower, cotton, sugarbeet, oat, tomato and tobacco. Leaves of maize, sorghum and Amaranthus edulis, however, did not evolve measureable amounts of CO_2 into a CO_2 -free atmosphere in the light (El-Sharkawy and Hesketh, 1965; El-Sharkawy et al, 1967; Yemm and Bidwell, 1969; Hew et al, 1969). Troughton (1971), using several techniques, could not detect any significant CO_2 evolution in the light from attached leaves of maize.

Yemm (1969) studied the effect of pretreatment in atmospheres containing different concentrations of CO_2 on the subsequent CO_2 evolution into CO_2 -free air from leaves of barley in light and darkness. He showed that as the CO_2 concentration during pretreatment was decreased the rate of CO_2 evolution in the light was increased but that the rate of CO_2 evolution in darkness was not appreciably altered even after pretreatment in the presence of very low CO_2 concentrations. Ludwig et al (1969), using leaves of sunflower, showed that during photosynthesis from 355 ppm CO_2 all the CO_2 evolved was derived from the immediate products of fixation.

The presence of high O_2 concentrations in atmospheres devoid of CO_2 was shown to increase the CO_2 evolution in the light from leaves of plant species, including tobacco, (Poskuta, Nelson and Krotkov, 1966; Zelitch, 1968; Zelitch and Day, 1968); conversely the presence of low O_2 concentrations in a CO_2 -free atmosphere was shown to inhibit CO_2 evolution in the light (Ludwig, Krotkov and Calvin, 1969; Ludwig and Calvin, 1971). Leaves of maize, however, did not evolve detectable amounts of CO_2 into atmospheres devoid of CO_2 during illumination, even in the presence of high O_2 concentration (Zelitch, 1968; Troughton, 1971).

El-Sharkawy, Loomis and Williams (1967) found that leaves of maize and Amaranthus edulis did evolve CO_2 into a CO_2 -free atmosphere in the light, after supplying a photosynthetic inhibitor, DCMU, to the plants but the rate of CO_2 evolution in darkness was not affected by the inhibitor. They suggested, therefore, that these plants do produce CO_2 during illumination but that it is re-fixed photosynthetically and not evolved from the leaf. Irvine (1970) demonstrated that when a sugarcane or sorghum plant, which had previously assimilated $[^{14}\text{C}] \text{CO}_2$, was placed with an unlabelled sorghum plant in a closed illuminated chamber, the unlabelled plant became ^{14}C labelled. He suggested this was evidence for a photo-respiratory evolution of CO_2 .

Photorespiration has been shown to be a process different from dark respiration by a number of workers. Unlike photorespiration, CO_2 evolution in darkness due to normal mitochondrial respiration was found to be insensitive to concentrations of O_2 above 2% by volume (Warburg, 1920; Poskuta, Nelson and Krotkov, 1966; Tregunna, Krotkov and Nelson, 1966; Forrester, Krotkov and Nelson, 1966a; 1966b; Tregunna and Downton, 1967; Hew and Krotkov, 1968; Poskuta, 1968; Yemm, 1969). Photorespiration has also been distinguished from dark respiration by its different response to the inhibitors, KCN and DCMU, (Poskuta, Nelson and Krotkov, 1966; 1967) and to temperature (Hew and Krotkov, 1967). Yemm (1969) showed that after leaves of barley had photosynthesised in the presence of $[\text{}^{14}\text{C}] \text{CO}_2$, the $^{14}\text{CO}_2$ released from the leaves into an atmosphere of CO_2 -free air had a specific activity similar to that of the $[\text{}^{14}\text{C}] \text{CO}_2$ supplied but when the leaves were darkened the specific activity of the $^{14}\text{CO}_2$ evolved into CO_2 -free air was decreased but increased again on re-illumination. These results indicated that CO_2 evolved in the light was produced from recent products of photosynthesis whilst the substrate of CO_2 evolution in darkness was different. The work of Ludwig and Calvin (1971) has indicated that the rate of photorespiration during photosynthesis in the presence of 21% O_2 (3500 ft candles, 25°C) is over three times the steady state rate of dark respiration by leaves of sunflower.

Higher plants can be separated into two groups on the basis of the various measurements described of gaseous exchange between photosynthesising leaves and the atmosphere. Photosynthesis by temperate species, such as tobacco, was increased by the lowering of the concentration of O_2 in the atmosphere below 21% by volume; whereas photosynthesis by tropical species, e.g. maize, was not significantly greater in the absence of O_2 (Hesketh;

1967; Forrester et al, 1966b; Downes and Hesketh, 1968). Plant species like tobacco had other physiological characteristics in common. Photorespiration was easily measured by CO_2 evolution in light, they had high CO_2 compensation points and both of these phenomena were sensitive to O_2 concentration. On the other hand, with plants like maize and other tropical species evolution of CO_2 in the light was not easily detected, the CO_2 compensation points were very low and were insensitive to O_2 concentration. The magnitude of the CO_2 compensation point has been used therefore as the diagnostic character for distinguishing those plants which behave physiologically in a similar way to tobacco and those of the other group which behave like maize (Tregunna and Downton, 1967; Downton and Tregunna, 1968a; Tregunna, Downton and Jolliffe, 1969).

4. The Dicarboxylic Acid Pathway of Photosynthesis.

Species of plants with low CO_2 compensation points differ from plants with high CO_2 compensation points not only in certain physiological characteristics but also in their photosynthetic carbon metabolism. The assimilation of CO_2 into compounds with three carbon atoms mediated by ribulose 1;5 diphosphate carboxylase was described by Calvin and co-workers (Bassham and Calvin, 1957). A survey by Norris, Norris and Calvin (1955) showed the carbon reduction cycle, with RuDP carboxylase as the primary carboxylating enzyme, to be functional in species from nine phyla of plants. Recent investigations of the photosynthetic carbon metabolism of certain tropical species, including maize and sugarcane, have demonstrated that these plants differ from those studied by Calvin et al.

Kortschak, Hartt and Burr (1965) found that the major ^{14}C labelled photosynthetic products in sugarcane leaves after a brief exposure to ^{14}C CO_2 were dicarboxylic acids with four carbon atoms, namely malate and aspartate. Previously Calvin and co-workers had found that malate was an

early product of $[^{14}\text{C}]\text{CO}_2$ photosynthesis by Scenedesmus but Bassham et al (1950) demonstrated that, although pretreatment of Scenedesmus with malonate inhibited the formation of $[^{14}\text{C}]\text{malate}$, it did not affect the labelling of the α and β -C atoms of phosphoglyceric acid. Kortschak et al, however, showed that, in sugarcane leaves, malonate not only inhibited malic acid synthesis but decreased the rate of photosynthesis and the synthesis of phosphoglyceric acid. These results suggested that malic acid was an essential precursor of phosphoglyceric acid in photosynthesis by sugarcane. Similar conclusions were reached by Hatch and Slack (1966). Using samples of sugarcane and maize leaves, they found that malate and aspartate were major primary products of $[^{14}\text{C}]\text{CO}_2$ fixation after brief periods of photosynthesis and that the ^{14}C was located in the carbon 4 position in these acids. After longer periods of photosynthesis in $[^{14}\text{C}]\text{CO}_2$ a larger proportion of the radioactivity appeared in 3-PGA, hexose monophosphates and sucrose. Hatch and Slack proposed a scheme to account for their observations in which a three carbon acceptor compound was carboxylated to produce a four-carbon dicarboxylic acid. The fourth carbon atom of the dicarboxylic acid was the source of carbon 1 of 3-phosphoglycerate and they therefore postulated a trans-carboxylation reaction with a two-carbon acceptor compound. After trans-carboxylation the remaining three carbon compound was used for resynthesis of the CO_2 acceptor. The four-carbon dicarboxylic acid, oxaloacetic acid, was formed before malate and aspartate in sugarcane, maize and sorghum, and therefore carboxylation of phosphoenolpyruvate was favoured as the reaction responsible for primary CO_2 fixation in these species (Slack and Hatch, 1967). After photosynthesis from $[^{14}\text{C}]\text{CO}_2$ by leaves of maize, Hofstra and Nelson (1969) studied the distribution of ^{14}C in the products of photosynthesis during a subsequent period of fixation in the presence of unlabelled CO_2 . They found that sucrose was rapidly labelled with a loss of ^{14}C from aspartate, malate, glycine and alanine.

These investigations showed that higher plants could be distinguished into two groups: those whose primary carboxylating enzyme was RuDP carboxylase where the first product was a 3-carbon compound (3-PGA) and those whose first product appeared to be a 4-carbon acid (oxaloacetic acid). The former group of plants were termed C_3 -species and the latter C_4 -species. There is considerable evidence that maize, sugarcane and other C_4 -plants contain much higher activities of PEP carboxylase than the C_3 -plants (Slack and Hatch, 1967; Johnson and Hatch, 1968; Osmond, Troughton and Goodchild, 1969; Whelan, Benedict and Sackett, 1970; Chen, Brown and Black, 1971; Waygood, Arya and Pan, 1971). Although the activity of malic dehydrogenase (malic enzyme) was found to be considerably higher in C_4 -plants than in C_3 -plants it was thought this enzyme probably did not contribute significantly to the primary fixation of CO_2 (Slack and Hatch, 1967; Slack, Hatch and Goodchild, 1969). Malate and aspartate were rapidly formed from oxaloacetate; the conversion to malate being mediated by an NADP-specific malate dehydrogenase (Hatch and Slack, 1969a). Johnson and Hatch (1969) supported the suggestion that PEP carboxylase was the enzyme responsible for the primary carboxylation reaction and showed that the path of carbon from the four-carbon dicarboxylic acids was via PGA and intermediates of the reductive pentose phosphate cycle. They found no evidence for a two carbon acceptor compound. Berry, Downton and Tregunna (1970) proposed a mechanism to explain the carboxyl transfer from the C_4 -acids to 3-PGA. They suggested that CO_2 was released from malate by malic enzyme and re-fixed by Ru, 1, 5 DP carboxylase, without loss of CO_2 to the atmosphere.

A photosynthetic process for carbon assimilation, such as the C_4 -pathway, depends on regeneration of the carbon acceptor. There has been considerable investigation of this aspect of C_4 -metabolism and the enzymes responsible have been purified and their location in leaf cells studied.

An enzyme from Escherichia coli, phosphoenolpyruvate synthetase, was described by Cooper and Kornberg (1965) which catalyzed the conversion of pyruvate and ATP to phosphoenolpyruvate, AMP and PP_i . A similar enzyme was isolated from leaves of maize, sugarcane and sorghum by Hatch and Slack (1967); no activity was found in extracts of leaves of silverbeet, wheat and oat (C_3 -plants). Further work by Hatch and Slack (1968) and Johnson and Hatch (1968) showed that the enzyme was active in the leaves of a number of C_4 -plants and its activity was enhanced during illumination (Slack, 1968; Hatch and Slack, 1969b). The enzyme catalyzed the reversible reaction: pyruvate + ATP + orthophosphate $\rightarrow$ phosphopyruvate + AMP + pyrophosphate, and was re-named pyruvate phosphate dikinase (Andrews and Hatch, 1969). Two other enzymes required to complete a system for regenerating PEP, adenylate kinase and pyrophosphatase, were also detected and these together with pyruvate phosphate dikinase were located mainly in the mesophyll cells of C_4 -plants (Slack, Hatch and Goodchild, 1969; Hatch, Slack and Bull, 1969; Slack, 1969; Osmond, Troughton and Goodchild, 1969; Chen, Brown and Black, 1971). Chen et al (1971) showed that pyruvate phosphate dikinase and adenylate kinase were present to a greater extent in C_4 -plants than in C_3 -plants.

The distribution of ^{14}C among the products was not significantly altered during short-term photosynthesis in $[^{14}C]CO_2$ by increasing the concentration of carbon dioxide in the atmosphere (0.03% CO_2 to 3.87% CO_2) or by altering the light intensity (1470 to 9100 ft candles) (Hatch, Slack and Johnson, 1967). The presence of the C_4 -dicarboxylic acid pathway has been established in other species of tropical plants by Hatch, Slack and Johnson (1967), Osmond (1967), Johnson and Hatch (1968), Chen, Brown and Black (1971), Usuda, Kanai and Takeuchi (1971), Downton (1971) and Schüch (1971).

The assimilation of carbon into C_4 -dicarboxylic acids in the dark is a feature of Crassulacean acid metabolism (Walker, 1962) but Kortschak, Hartt and Burr (1965) and Hatch and Slack (1966) found that the dark rate of $[^{14}C]CO_2$ fixation by sugarcane leaves was very slow, with no incorporation of ^{14}C into phosphorylated compounds. Usuda, Kanai and Takeuchi (1971) studied $[^{14}C]CO_2$ assimilation by Amaranthus retroflexus, a C_4 -plant, and found that in this species $[^{14}C]CO_2$ fixation in the dark was also slow.

All the leaves of C_4 -plants, which have been studied, possess a well developed parenchymatous bundle sheath, surrounding the vascular tissue. The closely packed cells of the bundle sheath were large and contained a dense concentration of chloroplasts (Downton and Tregunna, 1968a; Bisalputra, Downton and Tregunna, 1969). The arrangement of cells in the bundle sheaths has not been observed in the leaves of plants with high compensation points (C_3). Even within the same genus the species with low CO_2 compensation points, e.g. Atriplex rosea, possessed a distinct bundle sheath surrounding the vascular tissue whereas species with high CO_2 compensation points e.g. Atriplex hastata, lacked a distinct bundle sheath (Downton, Bisalputra and Tregunna, 1969).

Maize, sugarcane and a number of other C_4 -plants exhibit a distinct dimorphism in their chloroplast structure (Laetsch, Stetler & Vlitos, 1966; Laetsch 1968; Bachmann, Robertson and Bowen, 1969; Bisalputra et al, 1969). The larger chloroplasts of the bundle sheath cells were found to possess parallel separate lamellae extending from one end of the plastid to the other, with little or no appression between adjacent lamellae; thus grana were absent or present in only very rudimentary form. The chloroplasts of the mesophyll cells possess the usual arrangement of thylakoids in grana observed generally in the chloroplasts of higher plants. An accumulation

of starch in the chloroplasts of the bundle sheath of maize and sorghum has been reported (Laetsch, et al, 1966; Laetsch, 1968; Bachmann et al, 1969; Bisalputra et al, 1969; Black and Mollenhauer, 1971). The bundle sheath was found to be surrounded by the larger parenchymatous cells of the mesophyll. The chloroplasts of these cells were smaller than those of the bundle sheath and generally starch grains were few and very small (Downton and Tregunna, 1968a; Bisalputra et al, 1969). However, this feature was not common to all plants with bundle sheaths, Downton and Tregunna (1968a) and Usuda et al (1971) observed large starch grains in the chloroplasts of cells of both the mesophyll and bundle sheath cells of Amaranthus retroflexus L.

Downton (1970) suggested that C_4 -plants can be divided into two groups by determining whether malate or aspartate was the major product of short-term photosynthesis. He suggested that the predominant production of malate e.g. in maize and sugarcane, was correlated with the presence of only rudimentary grana in the chloroplasts of the bundle sheath and an absence of a post-illumination CO_2 burst. Amaranthus edulis, Amaranthus retroflexus, Atriplex rosea and Atriplex retroflexus were cited as examples of the second group characterised by the synthesis of aspartate as the major product of short-term photosynthesis. The plants were further distinguished by the presence of grana in the chloroplasts of the bundle sheath cells and the presence of a post-illumination CO_2 burst that was insensitive to O_2 .

Hew and Gibbs (1970) found no differences between the relative activities of the photosynthetic electron transport systems in chloroplasts from maize, sorghum and sugarcane (C_4 -plants) and the relative activities of the photosynthetic electron transport systems in chloroplasts from spinach (C_3 -plant). The ferridoxin of maize has been shown to be similar

to that of C_3 -plants in amino acid composition, iron content, molecular weight and biological activity (Crawford and Jensen, 1971). There is, however, evidence that in other respects the photosynthetic electron transport system of chloroplasts, without grana, in the bundle sheath cells of C_4 -plants is modified.

The relative activity of photosystem I and photosystem II has been correlated with the organization of lamellae in the chloroplasts. Homann and Schmid (1967) and Homann, Schmid and Gaffron (1967), using chloroplasts isolated from normal and mutant tobacco plants, suggested that, although photosystem I was fully functional in separate lamellae in chloroplasts, without grana, the complete photosynthetic electron transport system including O_2 evolution by photosystem II required the close contact of at least two lamellae as found in grana. Woo et al (1970) suggested that the chloroplasts of the bundle sheath cells of Sorghum bicolor and Zea mays, which do not have grana, were either deficient in photosystem II or it was inactive. The chloroplasts of the mesophyll cells had active photosystems I and II, as had the chloroplasts from both the bundle sheath and mesophyll cells of Atriplex spongiosa, which has chloroplasts with grana in both types of cell. Anderson, Woo and Boardman (1971) showed that the chloroplasts (without grana), from the bundle sheath cells of Sorghum bicolor, were similar in composition to fragments of chloroplasts from spinach which were active only in photosystem I. They concluded that these chloroplasts from S. bicolor were deficient in photosystem II but that chloroplasts of the cells of the mesophyll had active photosystems I and II. Anderson et al suggested that the activity of photosystem II was greater in chloroplasts from the bundle sheath cells of maize compared with those from the bundle sheath of S. bicolor. Bazzaz and Govindjee (1971) reported that the chloroplasts from the bundle sheath cells of maize contained up to 50%

photosystem II activity compared to chloroplasts from the mesophyll cells. They confirmed that the chloroplasts from the bundle sheath cells were relatively more active in photosystem I than in photosystem II. The two photosystems were probably linked in these chloroplasts. Further work by Anderson, Boardman and Spencer (1971) showed that the rate of non-cyclic phosphorylation by intact isolated chloroplasts, from the bundle sheath cells of maize, was only 11 to 19% of the rate attained by intact chloroplasts from the mesophyll cells, though both types of chloroplasts were capable of high rates of cyclic phosphorylation. Chang and Troughton (1972) found that the ratio of the amount of chlorophyll a to the amount of chlorophyll b was greater in C_4 -plants, e.g. Atriplex spongiosa and Zea mays, than in C_3 -plants e.g. Trifolium repens, Vicia faba and Spinacia oleracea. This can be interpreted as evidence for relatively more photosystem I than photosystem II in C_4 -plants, since the light-trap for photosystem I contains a higher concentration of chlorophyll a than the light-trap for photosystem II.

The quantitative and qualitative relationship between the photosystems of the bundle sheath chloroplasts, without grana, and the chloroplasts of the mesophyll cells, with grana, remains, however, a controversial problem. Polya and Osmond (1972) reported a major deficiency in photosystem II-dependent electron transport in the chloroplasts of the bundle sheath cells of Sorghum bicolor and Zea mays. Recent investigations by Andersen et al (1972) indicated that the chloroplasts of the bundle sheath cells of maize possessed photosystem II, but these chloroplast preparations had little capacity to photoreduce NADP to water. Andersen et al, therefore, suggested that photosystems I and II in these chloroplasts were not linked for electron flow. On the other hand Smillie et al (1972) studied this problem and concluded that chloroplasts from the bundle sheath cells of maize were capable of NADP reduction and that electron transport through both photosystems was functional.

Comparative studies of various enzymes involved in photosynthetic carbon metabolism have sought to show differences between the activities and location of enzymes extracted from C_4 -plants compared to those from C_3 -plants. It was reported by Slack and Hatch (1967) and Johnson and Hatch (1968) that the activity of ribulose 1, 5 diphosphate carboxylase in maize, sugarcane and other plants possessing the C_4 -dicarboxylic acid pathway, was considerably less than that found in C_3 -plants. Further work by Björkman and Gauhl (1969) indicated that there was no marked difference in the level of RuDP carboxylase between the two types of plant. The activity of RuDP carboxylase was apparently restricted to the chloroplasts of the bundle sheath cells of C_4 -plants (Björkman and Gauhl, 1969; Slack, Hatch and Goodchild, 1969; Edwards, Lee, Chen and Black, 1970). This conclusion is, however, controversial. Baldry, Bucke and Coombs (1971) and Bucke and Long (1971) showed that the mesophyll cells, containing chlorophyll, of sugarcane and maize did contain RuDP carboxylase. They suggested that it had been difficult to detect in previous investigations because of inactivation by phenolic compounds which were also extracted from the leaves.

The location of PEP carboxylase in the leaves of C_4 -plants has been studied. Slack, Hatch and Goodchild (1969), Edwards et al (1970) and Berry et al (1970) reported that the activity of PEP carboxylase was largely confined to the mesophyll cells. Some PEP carboxylase was extracted from chloroplasts from spinach leaves by Rosenberg et al (1958). Baldry, Bucke and Coombs (1969) demonstrated that isolated sugarcane chloroplasts were capable of CO_2 assimilation in the presence of light and PEP. Slack and Hatch (1967) reported the presence of PEP carboxylase in chloroplasts from maize, sugarcane and sorghum, and further work by Slack, Hatch and Goodchild (1969) suggested that the enzyme may be associated with the

bounding membrane of the chloroplast. However Baldry, Bucke and Coombs (1971) and Bucke and Long (1971) recently suggested that in maize and sugar-cane PEP carboxylase was in the cytoplasm. Waygood et al (1971) found PEP carboxylase both in the chloroplasts and supernatant liquid from maize. The enzyme may therefore be in the cytoplasm.

Ting (1968) showed that PEP carboxylase from maize roots was inhibited by L-malate, oxaloacetate and aspartate. Aspartate also inhibited PEP carboxylase from Salmonella (Maeba and Sanwal, 1969). Kluge and Osmond (1972) have recently reported inhibition of PEP carboxylase from Bryophyllum tubiflorum and Sedum praeacanthum by L-malate and pyruvate. The inhibitory effect of these compounds on the activity of PEP carboxylase may be of importance in the control of C_4 -metabolism.

Carbonic anhydrase may fulfill an important role in photosynthesis by mediating the release of CO_2 at the primary carboxylation site. In low concentrations of CO_2 , Chlorella grown in high concentrations of CO_2 (5%) initially photosynthesized more slowly than Chlorella grown in low concentrations of CO_2 (Graham and Whittingham, 1968). This observation correlated with the amount of carbonic anhydrase in the cells (Reed and Graham, 1968). As Chlorella adapted to conditions with a low concentration of CO_2 , carbonic anhydrase increased markedly, so the carbonic anhydrase activity appeared to control the rate of photosynthesis. Everson (1969) reported that inhibition of carbonic anhydrase in chloroplast extracts also caused a significant inhibition of CO_2 fixation. Carbonic anhydrase activity may enhance CO_2 concentration in the locality of RuDP carboxylase, thus facilitating a greater rate of carboxylation. Failure to add carbonic anhydrase may account for the discrepancies between the rate of carboxylation of RuDP measured in vitro and the rate of photosynthesis in vivo. Everson and Slack (1968) found that there was less carbonic anhydrase

in C_4 -plants than in C_3 -plants. Chen, Brown and Black (1970) found that the possession of a low CO_2 compensation point (characteristic of C_4 -plants) was correlated with a low carbonic anhydrase activity; conversely a high CO_2 compensation point was correlated with high carbonic anhydrase activity. However, Osmond et al (1969) found little difference in the amounts of carbonic anhydrase in leaves of Atriplex hastata (C_3 -plant) and A.spongiosa (C_4 -plant).

The activities of certain enzymes have been shown to be greater in the leaves of C_4 -plants than in C_3 -plants. Relative activities of various enzymes are different also between mesophyll and bundle sheath cells of C_4 -plants. It is particularly difficult, however, to extract organelles and functional enzymes from C_4 -plants, especially the tropical grasses; and Bassham's statement in his review of photosynthesis (1964) may be very pertinent:

"The failure of attempts to isolate any or sufficient enzyme activity for a given proposed reaction is not an overwhelming argument against the occurrence of that reaction in an in vivo metabolic pathway".

The cellular locus of CO_2 fixation by C_4 -plants has been studied by the use of autoradiography. Moss and Rasmussen (1969) found that after 2 min photosynthesis from $[^{14}C]CO_2$ by leaves of maize most of the ^{14}C assimilated was present in the bundle sheath cells and little in the mesophyll cells. Berry, Downton and Tregunna (1970) found that the distribution of ^{14}C , after 6 sec photosynthesis from $[^{14}C]CO_2$ by leaves of Gomphrena globosa (a C_4 -plant), corresponded to the distribution of chlorophyll i.e. CO_2 fixation was restricted to cells of the mesophyll and bundle sheath, which surround the vascular bundle. In leaves of maize, they found that ^{14}C was mostly restricted to the cells of the bundle sheath. A more detailed study by Bednarz and Rasmussen (1972) showed that,

after only 10 sec photosynthesis from $[^{14}\text{C}] \text{CO}_2$ by leaves of maize, there was more ^{14}C present in the cells of the bundle sheath than in the cells of the mesophyll. These investigations indicated that either CO_2 fixation by leaves of maize was largely confined to the cells of the bundle sheath or that rapid translocation of recent photosynthate occurred from the cells of the mesophyll to those of the bundle sheath.

5. Effects of CO_2 and O_2 Concentration on the Products of Photosynthetic Carbon Metabolism.

Two effects of light on the carbon metabolism of both algae and higher plants were recognized by Benson and Calvin (1950). Light not only initiated the reactions of photosynthetic CO_2 fixation but also inhibited the entry of photosynthetic products into the tricarboxylic acid cycle. Glycine and glycollate were rapidly labelled in barley leaves during photosynthesis from $[^{14}\text{C}] \text{CO}_2$ and a significant amount of ^{14}C was found in these compounds after only 5 sec photosynthesis in $[^{14}\text{C}] \text{CO}_2$. On darkening ^{14}C in these compounds rapidly disappeared. From subsequent investigations using algae Calvin and co-workers found that glycollic acid and glycine were products of photosynthesis formed later than the sugar phosphates (Buchanan *et al*, 1952). Bassham *et al* (1954) observed that $[^{14}\text{C}]$ glycollate synthesised in *Scenedesmus* was increased considerably when the CO_2 concentration was decreased from 1% to 0.003% in the presence of 21% O_2 .

The effect of a change in CO_2 concentration on carbon metabolism was studied in more detail by Wilson and Calvin (1955). The overall rate of photosynthesis by algae was decreased when the CO_2 concentration was lowered from 1% to 0.03% but the decrease in rate was not immediate and was not significant until the concentration of ribulose 1,5 diphosphate had fallen. Less sucrose was synthesised in 0.03% CO_2 and there was an increase in the concentration of glycollic acid. When the CO_2 concentration

was increased again to 1% the concentration of glycollate fell. The effect of CO_2 concentration on the ^{14}C products of photosynthesis in Chlorella was studied by Whittingham and Pritchard (1963). They observed increased incorporation of ^{14}C into sugar monophosphates, PGA and sucrose when the CO_2 concentration was increased from 0.1% to 4.0% by volume. The incorporation into serine, alanine, malate and aspartate was not markedly affected but incorporation into the two-carbon compounds, glycollate and glycine, was considerably decreased when the CO_2 concentration was increased. Graham and Whittingham (1968) found that after 6 min photosynthesis by Chlorella in $[^{14}\text{C}] \text{CO}_2$ the % ^{14}C incorporated into glycine, serine and alanine was considerably greater in the presence of 0.03% CO_2 than with 5% CO_2 .

Investigations of the effect of CO_2 concentration on the products of photosynthesis by leaves of higher plants showed that the responses were similar to those observed in the unicellular algae. Tolbert (1958) reported that during photosynthesis from $[^{14}\text{C}] \text{CO}_2$ by leaves of Sedum alboroseum the % ^{14}C incorporated into glycollate, glycine and serine was much less in the presence of 4% CO_2 compared to 0.03% CO_2 . Mortimer (1959) found that in several higher plants there was an increased rate of photosynthesis when the CO_2 concentration was raised from 0.03% to 2.0% and that the relative amounts of products were changed. At low CO_2 concentrations significant amounts of ^{14}C were incorporated into glycine, serine and glyceric acid whereas at high CO_2 concentrations ^{14}C was incorporated predominantly into sucrose and alanine.

Further investigations into the effect of CO_2 concentration on photosynthetic carbon metabolism were concerned with the interaction between CO_2 and O_2 concentration. Tolbert and Zill (1956) demonstrated that during photosynthesis from $[^{14}\text{C}] \text{CO}_2$ by Chlorella some 3 to 10% of

the total ^{14}C assimilated was excreted into the suspending medium as glycollate. The magnitude of the glycollate excretion was dependent upon aerobic conditions and the concentration of HCO_3^- ions in the medium. Bassham and Kirk (1962) studied the distribution of ^{14}C between the products of photosynthesis in $[^{14}\text{C}]\text{CO}_2$ by Chlorella in the presence of either 100% O_2 or N_2 . They found that the ^{14}C incorporated into glycollate and glycine was markedly increased in the presence of O_2 compared to N_2 whereas the ^{14}C incorporated into ribulose diphosphate, PGA and alanine was less in O_2 than in N_2 . Whittingham, Coombs and Marker (1967) investigated the effect of O_2 concentration on the distribution of ^{14}C between the products of photosynthesis by Chlorella in 0.03% $[^{14}\text{C}]\text{CO}_2$ in two ways. Firstly, during steady state photosynthesis, they found that the synthesis of $[^{14}\text{C}]$ glycollate was increased with increased O_2 concentration and this was associated with a decrease in the size of the sugar phosphate pools and synthesis of less sucrose. Secondly, during the transition from 21% O_2 to 99.7% O_2 , there was increased formation of glycollate which was again accompanied by a decrease in amounts of sugar phosphates present.

The effect of 100% O_2 compared to 21% O_2 on ^{14}C in products of photosynthesis from $[^{14}\text{C}]\text{CO}_2$ by twigs of Balsam fir was investigated by Maleszewski and Nelson (1968). They found that the presence of high O_2 concentration was associated with decreased ^{14}C in sugars and increased ^{14}C in amino acids; this effect was increased at low CO_2 concentrations. The ^{14}C in glycine and serine was greater at high O_2 concentration but there was less ^{14}C in alanine. Similar studies by Fock, Egle, Schaub and Hilgenberg (1969) of photosynthesis from $[^{14}\text{C}]\text{CO}_2$ by sunflower leaves showed a major shift in the distribution of ^{14}C from carbohydrates to amino acids with increasing O_2 concentration. Leonard and Bidwell (1969) reported that, whether the concentration of CO_2 was high or low, the

absence of O_2 resulted in an increase in PGA and alanine but little change in Calvin cycle intermediates during photosynthesis by leaves of barley. They suggested that O_2 has an inhibitory effect on the carboxylation of ribulose diphosphate. Voskresenskaya, Wiil, Grishina and Pärnik (1970) found that, during photosynthesis by leaves of bean in $0.03\% [^{14}C] CO_2$, less ^{14}C was incorporated into glycollate and glycine but more into alanine and carbohydrates in the presence of $0.5\% O_2$ than was the case in $21\% O_2$. Lewanty, Maleszewski and Poskuta (1971) found that less ^{14}C was incorporated into PGA, alanine, glycine, serine and sugars during photosynthesis by leaves of maize from $[^{14}C] CO_2$ in an atmosphere of $100\% O_2$ compared to $21\% O_2$. The effects of O_2 and CO_2 concentration on the products of photosynthetic carbon metabolism by maize, or other C_4 -plants, had been little studied and it was not possible to make any rigorous comparison with the studies discussed using C_3 -plants.

Photosynthesis by isolated chloroplasts was also influenced by the concentration of O_2 and CO_2 . Glycollic acid was a major product of photosynthesis by isolated chloroplasts (Kearney and Tolbert, 1962). Chloroplasts fixing CO_2 in the presence of O_2 contained less Ru, 1,5, DP and triose phosphates but more pentose monophosphates and glycollate than chloroplasts fixing CO_2 in N_2 (Ellyard and Gibbs, 1967). Incorporation of ^{14}C into glycollate was also dependent upon $[^{14}C] HCO_3^-$ concentration. The maximum $[^{14}C]$ glycollate production was achieved under high O_2 and very low HCO_3^- concentration. Vandor and Tolbert (1968) confirmed that glycollate formation by intact isolated spinach chloroplasts was dependent on the presence of O_2 . The concentration of CO_2 and O_2 , therefore, affects the overall rate of photosynthesis and also the distribution of recently assimilated carbon among the products of photosynthesis. Carbon incorporated into the two-carbon compounds, glycollic acid and glycine, was increased when the concentration of O_2 in the atmosphere was increased or the concentration of CO_2 decreased.

The most marked effect of increased O_2 concentration on carbon metabolism during photosynthesis was, therefore, the production of the two-carbon compounds, glycollate and glycine. The metabolism of these compounds has received considerable attention and the studies concerned with this aspect of photosynthetic carbon metabolism will be considered.

6. The C2 Pathway

It was suggested by Wilson and Calvin (1955) that glycollate was derived by oxidation of the two-carbon compound which is an intermediate in the reactions of sugar phosphates catalyzed by transketolase in the photosynthetic carbon reduction cycle. Calvin et al (1951) showed that after 15 sec photosynthesis by barley leaves in $[^{14}C]CO_2$ the glycollate was equally labelled in both carbon atoms. A possible precursor of glycollate was phosphoglycollate which could be derived from the sugar phosphates of the Calvin cycle. Phosphoglycollate was shown to be a product of photosynthesis by Buchanan et al (1952) and Kearney and Tolbert (1962). The presence of a specific phosphoglycollate phosphatase in leaves has been established by Richardson and Tolbert (1961) and Yu, Tolbert and Orth (1964).

Zelitch (1965) proposed that glycollate arose from a primary carboxylation because the specific activity of the $[^{14}C]$ glycollate was higher than that of the carboxyl group of PGA after photosynthesis by tobacco leaves in $[^{14}C]CO_2$. This result was probably an artifact since Hess and Tolbert (1966) subsequently reported that carboxyl labelled PGA was the primary product of photosynthesis in tobacco leaves and that glycollate, glycine and serine were secondary products.

The presence of an enzyme (glycollic acid oxidase) in green leaves capable of oxidising α -hydroxy acids with the concomitant consumption of

O_2 was reported by Clagett, Tolbert and Burris (1949). Tolbert and Burris (1950) suggested that glycollic acid oxidase was associated with photosynthesis as they demonstrated that it was activated, or formed, following exposure of plants to light and it was absent in albino plants. Kenten and Mann (1952) demonstrated that hydrogen peroxide was produced during the oxidation of glycollic acid or lactic acid. The enzyme was isolated and purified by Zelitch and Ochoa (1953). Glycollic acid oxidase catalyzed the oxidation of glycollic acid to glyoxylic, and L-lactic acid to pyruvic acid with the production of hydrogen peroxide. Various α -hydroxysulphonates, shown by Zelitch (1957) to be inhibitors of glycollic acid oxidase, caused an accumulation in the light of glycollic acid in leaves of spinach, bean, tomato and maize (Zelitch, 1958; 1959).

The metabolism of $[^{14}C]$ glycollic acid and related compounds by detached leaves or leaf disks was studied by Tolbert and Cohan (1953). The major products of the metabolism of $[^{14}C]$ glycollate were glycine, serine and an unknown compound. Evidence was presented by Wang and Hao (1960) that $[2-^{14}C]$ glycine supplied to leaves of wheat was metabolised to ^{14}C sugars. Pritchard, Whittingham and Griffin (1961) studied the distribution of ^{14}C between the products of photosynthesis by Chlorella in $[^{14}C]CO_2$ in the presence of an inhibitor, isonicotinyl hydrazide. There was an increase in the amounts of ^{14}C in glycollate and glycine but incorporation of ^{14}C into serine and sugar phosphates was inhibited.

A pathway from glycollate to hexose sugars was proposed by Wang and Waygood (1962) based on their isotopic competition experiments using wheat leaves. They found that unlabelled glycollate supplied with $[^{14}C]$ glycine or $[^{14}C]$ serine did not decrease the ^{14}C incorporated into sugars whereas the synthesis of $[^{14}C]$ sugars from $[^{14}C]$ glycollate was decreased by added

Study of the metabolism of specifically labelled $[^{14}\text{C}]$ glycollate, glycine and serine in wheat leaves by Wang and Burris (1963) supported this pathway. Glyceric acid was the major organic acid formed by the metabolism of glycine and $[^{14}\text{C}]$ phosphoserine was formed from $[2-^{14}\text{C}]$ glycine, $[1-^{14}\text{C}]$ serine and $[3-^{14}\text{C}]$ serine. The metabolism of $[^{14}\text{C}]$ glycine in non-green plant tissue was investigated by Sinha and Cossins (1964). The major products were found to be serine, glyoxylate and CO_2 . Using various plant tissues they found that the metabolism of glyoxylate resulted in all cases in the synthesis of glycine, serine and CO_2 . A transaminase capable of converting glyoxylate to glycine was found by Sinha and Cossins (1965). Cossins and Sinha (1966) showed that, in the presence of a soluble protein fraction from wheat leaves, two molecules of glycine were converted to one molecule each of serine and CO_2 . The conversion had an absolute requirement for tetrahydrofolate.

The work of Asada et al (1965) demonstrated that glycine and serine were synthesised via glycollate and glyoxylate during photosynthesis by leaves of tobacco and spinach. Ongun and Stocking (1966) confirmed the presence in tobacco leaves of a pathway synthesising carbohydrates from glycollate. Mifflin, Marker and Whittingham (1966) found that $[^{14}\text{C}]$ glycollate and $[^{14}\text{C}]$ glycine supplied exogenously to excised pea leaves were both metabolised via serine to sucrose. The metabolism of serine to sucrose but not glycine to serine was light-dependent. During a period of photosynthesis in 0.03% CO_2 , after photosynthesis in $[^{14}\text{C}] \text{CO}_2$, ^{14}C in sucrose increased while ^{14}C in glycine and serine decreased (Mifflin et al, 1966). On the other hand, when photosynthesis in $[^{14}\text{C}] \text{CO}_2$ was followed by photosynthesis in 4% $^{12}\text{CO}_2$ there was a more rapid loss of ^{14}C from sugar phosphates but ^{14}C in glycine, serine and sucrose did not change while ^{14}C in other amino acids and in the alcohol-insoluble fraction increased.

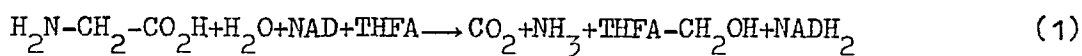
Two pathways for serine biosynthesis in animal tissues were demonstrated by Walsh and Sallach (1966) one, 'the phosphorylated pathway' involved reduction of 3-PGA to phosphohydroxypyruvate and its subsequent transamination from L-glutamate to form phosphoserine; the other, the 'nonphosphorylated pathway', was by the oxidation of D-glycerate to hydroxypyruvate and subsequent transamination from L-alanine to form serine. Cheung, Rosenblum and Sallach (1968) investigated the amounts of these transaminases, 3-phosphoglycerate dehydrogenase and D-glycerate dehydrogenase in a variety of plant tissues and suggested that the phosphorylated pathway was of major importance for serine biosynthesis in tissues, such as the apical meristem, where there was rapid cell proliferation whilst the 'non-phosphorylated pathway' operated in green leaves.

From their studies using isolated chloroplasts, Chang and Tolbert (1965) suggested the possibility that serine was synthesised from 3-PGA in chloroplasts whilst in the cytoplasm it was by the glycollate pathway. Previous work using isolated chloroplasts showed that glycollate was possibly excreted by chloroplasts, and that, although added [^{14}C] glycollate could not re-enter chloroplasts [^{14}C] glyoxylate could readily do so (Kearney and Tolbert, 1962).

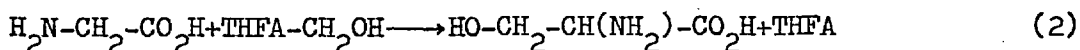
Glycollic acid oxidase was thought by Tolbert and Cohan (1953) and Delavan and Benson (1958) to be located in the chloroplast. Thompson and Whittingham (1968), however, showed that glycollic acid oxidase was in the cytoplasm. Tolbert and co-workers (1971) have since established that several enzymes of the C2 pathway, namely glycollate oxidase, hydroxypyruvate reductase, glutamate-glyoxylate aminotransferase, serine-glyoxylate transferase and catalase are present in organelles called peroxisomes. The metabolism of glycollate to glycine is, therefore, probably located in the peroxisomes. Frederick and Newcomb (1971) studied the distribution of

microbodies (peroxisomes) in the leaves of grasses. They found that C_3 -species possessed a greater number of peroxisomes than C_4 -species. In C_4 -species, including maize, electron microscopic investigations have revealed the presence of a greater number of mitochondria and peroxisomes in the cells of the bundle sheath than in those of the mesophyll (Laetsch, 1968; Downton, Bisalputra and Tregunna, 1969; Osmond, Troughton and Goodchild, 1969; Frederick and Newcomb, 1971; Black and Mollenhauer, 1971; Usuda et al, 1971).

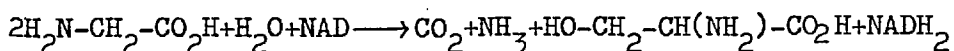
In animals an enzyme system responsible for the conversion of glycine to serine was found to be located in the mitochondria (Sanadi and Bennett, 1960). Kisiaki, Yoshida and Imai (1971b) found that glycine decarboxylase activity, associated with serine synthesis, was present in mitochondrial preparations from spinach leaves, and was thought to be entirely localized in the mitochondria (Kisiaki, Imai and Tolbert, 1971a). Glycine decarboxylase mediated the cleavage of glycine producing CO_2 and NH_3 . The system was stimulated by pyridoxal phosphate and NAD, and required tetrahydrofolate (THFA). It was suggested that decarboxylation of glycine was followed by the synthesis of serine by hydroxymethylation of a second molecule of glycine catalyzed by serine hydroxymethyltransferase. This enzyme was predominantly located in the mitochondria but was present also in the chloroplasts (Kisiaki et al, 1971a). Kisiaki et al (1971b) suggested a close relationship between the production of CO_2 , decarboxylation of glycine and the synthesis of serine catalyzed by their mitochondrial preparations from spinach. Conditions, for example lack of O_2 , which increased CO_2 evolution also increased the production of NH_3 and the synthesis of serine. They summarised the reactions as follows:



(glycine decarboxylase)



Overall reaction:



Kisaki et al (1971b) suggested that reduction of NAD may be of importance as a regulatory mechanism in the mitochondrion. Further work concerned with the synthesis of serine has recently been reported by Bird, Cornelius, Keys and Whittingham (1972a) who investigated a similar biochemical system to that of Kisaki et al (1971a; 1971b), isolated from leaves of tobacco. Here, however, the synthesis of serine from glycine was O_2 -dependent and 3% by volume gave the maximum rate of serine synthesis. Effects of inhibitors of mitochondrial electron transport on this system showed that the reaction required the cytochrome system of the mitochondrion. Bird et al found that NAD was not required as a cofactor but ADP was, and was phosphorylated to give ATP. The free energy change was such that theoretically 3 molecules of ATP might be formed for each molecule of serine synthesised. Their work suggests that a function of the C2 pathway is to effect light-dependent phosphorylation in the mitochondrion. Further investigations of this reaction by Bird et al (1972b) demonstrated that 2 molecules of ATP were synthesised with the production of each molecule of serine. Tolbert (1971) proposed that the C2 pathway involved the flow of carbon from the chloroplast to the peroxisome and then to the mitochondrion.

The site of synthesis of sucrose, the terminal product of the C2 pathway, has been investigated by a number of workers. Non-aqueous techniques for chloroplast separation produced conflicting results. Heber and Willenbrink (1964) found that UDPG and sucrose were synthesised cytoplasmically while Stocking et al (1963) found sucrose was formed first

in the chloroplasts. Bird, Porter and Stocking (1965) studied the intracellular localisation of the enzymes associated with sucrose synthesis in tobacco leaves. Enzymes needed for the synthesis of UDPG, and the formation of sucrose and sucrose phosphate from it, were located mainly within the chloroplast.

The effect of O_2 and CO_2 concentration on photorespiration and on the entry and metabolism of carbon via the C2 pathway indicates that the C2 pathway may be the biochemical process responsible for photorespiration. The synthesis of glycollate is probably O_2 -dependent and there are at least two O_2 -dependent reactions in the C2 pathway: the oxidation of glycollic acid, which is mediated by glycollic acid oxidase and has a low affinity for O_2 (Frigerio and Harbury, 1958) and the synthesis of serine from glycine by a catalytic system which has a high affinity for O_2 (Bird *et al.*, 1972a). The reactions catalyzed by glycollate oxidase and the conversion of glycine to serine may also be the reactions of the C2 pathway which account for the CO_2 evolved during photorespiration. Zelitch and Ochoa (1953) showed that hydrogen peroxide, formed with glyoxylate when glycollate was oxidized by O_2 in the presence of glycollic acid oxidase, can react non-enzymatically with glyoxylate to yield formate, CO_2 and water. However Tolbert (1971) has established that glycollic acid oxidase and catalase are both located in the peroxisomes. Oxidation of glycollic acid to glyoxylate can occur, therefore, in these organelles but because sufficient catalase is also present hydrogen peroxide produced in the reaction would be rapidly decomposed and further non-enzymatic oxidation of glyoxylate to formate and CO_2 is unlikely to take place. Kisiaki and Tolbert (1970) found that when $[1-^{14}C]$ glycollate or $[1, 2-^{14}C]$ glyoxylate were supplied to peroxisomes $[^{14}C]$ glycine was produced but there was no oxidation of either compound to CO_2 . They found that their preparations of chloroplasts were able to oxidize

glyoxylate, but not glycollate, slowly to form CO_2 . Halliwell, Leek and Butt (1972) reported decarboxylation of $[^{14}\text{C}]$ glycollate and $[^{14}\text{C}]$ glyoxylate with the evolution of $^{14}\text{CO}_2$ by peroxisome preparations from leaves of spinach beet. The decarboxylations were inhibited in the presence of N_2 but were considered to be insufficient to account for the CO_2 evolution due to photorespiration. Zelitch (1972) has recently found that $[1-^{14}\text{C}]$ glyoxylate can be rapidly decarboxylated by envelope-free chloroplasts from spinach in the presence of light and O_2 . He found that $[1-^{14}\text{C}]$ glycine was decarboxylated at only 1% of the rate of decarboxylation of $[1-^{14}\text{C}]$ glyoxylate by these chloroplast preparations. The metabolism of glycine to serine is the other reaction of the C2 pathway which results in the evolution of CO_2 (Cossins and Sinha, 1966; Kisaki, Yoshida and Imai, 1971b; Bird et al. 1972a).

The evolution of CO_2 by photorespiration has been studied from leaves which have been exogenously supplied with ^{14}C labelled or unlabelled substrates. Sinha and Cossins (1965) found that $^{14}\text{CO}_2$ was produced when leaves of pea were supplied with $[^{14}\text{C}]$ glyoxylate, with the incorporation of ^{14}C into glycine and serine. Moss (1966b) found that potassium glycollate supplied to detached leaves of tobacco increased the CO_2 evolution into CO_2 -free air. Both tobacco and maize leaves evolved $^{14}\text{CO}_2$ when supplied with either $[1-^{14}\text{C}]$ glycollate or $[2-^{14}\text{C}]$ glycollate (Zelitch, 1966) and with both species more ^{14}C was released from the $[1-^{14}\text{C}]$ than from the $[2-^{14}\text{C}]$ substrate. Moss (1967) reported that the metabolism of glycollate supplied exogenously to the leaves was very much more rapid in tobacco than maize. The evolution of CO_2 in the light from leaves of wheat and oat supplied with glycollate was approximately doubled compared with untreated leaves, but the supply of glycollate to leaves of maize only increased the evolution of CO_2 by 45% compared to untreated leaves (Downton and Tregunna, 1968b). The

increased evolution of CO_2 from leaves supplied with glycollate was not observed in the presence of low O_2 concentration (2%) (Downton and Tregunna, 1968b). When $[1-^{14}\text{C}]$ glycine or glycollate were supplied exogenously to tobacco leaves, $^{14}\text{CO}_2$ was evolved more rapidly in the dark than in the light (Kisaki and Tolbert, 1970). This was because re-fixation of the $^{14}\text{CO}_2$ by photosynthesis was prevented in the dark while the steps in the C2 pathway between glycollate and serine were not light-dependent. In darkness $[^{14}\text{C}]$ glycollate, $[^{14}\text{C}]$ glyoxylate and $[^{14}\text{C}]$ serine were decarboxylated more slowly than $[1-^{14}\text{C}]$ glycine. Kisaki and Tolbert (1970) found that in the light $[1-^{14}\text{C}]$ glycine was equally good or better as substrate for $^{14}\text{CO}_2$ evolution from disks of tobacco leaf than $[1-^{14}\text{C}]$ glycollate. $^{14}\text{CO}_2$ was evolved from segments of maize leaf when $[1-^{14}\text{C}]$ glycine or $[1-^{14}\text{C}]$ glycollate was supplied as substrate and there was a greater evolution of CO_2 from $[1-^{14}\text{C}]$ glycine than $[1-^{14}\text{C}]$ glycollate. Intermediates of the C2 pathway (glycollate, glyoxylate and glycine) have been shown, therefore, to be substrates for CO_2 evolution when supplied exogenously to samples of leaves from both C_3 - and C_4 -plants.

The effect of inhibitors on photorespiratory CO_2 evolution and the C2 pathway has been studied. Zelitch (1958 and 1959) demonstrated that when glycollic acid oxidase was inhibited by α -hydroxysulphonates glycollic acid accumulated in the leaves of both tobacco and maize in the light. He found that the rate of $[^{14}\text{C}] \text{CO}_2$ uptake by leaves of tobacco was increased in the presence of α -hydroxy-2-pyridinemethanesulphonic acid at 30°C but there was no increase in the rate of assimilation by leaves of maize in the presence of the inhibitor (Zelitch, 1966). Tolbert and Hess (1966) found that the ^{14}C incorporated into amino acids was decreased in Chlamydomonas or Chlorella during photosynthesis from $[^{14}\text{C}] \text{CO}_2$ in the presence of α -hydroxysulphonate compounds. The light-dependent CO_2

evolution from leaves of tobacco into CO_2 -free air was suppressed when the leaves were supplied with α -hydroxysulphonate (Moss, 1968; Zelitch, 1968).

Kisaki and Tolbert (1970) showed that in light and darkness the CO_2 evolution from disks of tobacco leaf supplied with $[1-^{14}\text{C}]$ glycollate was decreased in the presence of α -hydroxy-2-pyridinemethanesulphonate. When $[1-^{14}\text{C}]$ glycine was supplied in darkness the CO_2 evolution was unaffected by the presence of the inhibitor but in the light the presence of α -hydroxy-sulphonate increased the $^{14}\text{CO}_2$ evolved from $[1-^{14}\text{C}]$ glycine. Kisaki and Tolbert suggested that the increased $^{14}\text{CO}_2$ evolution in the light was due to the formation of unlabelled glycine and inhibition of the re-fixation of $^{14}\text{CO}_2$ by the presence of the inhibitor.

Pritchard, Whittingham and Griffin (1961) found that the presence of isonicotinylhydrazide (INH) inhibited the conversion of glycine to serine by Chlorella. During photosynthesis from $[^{14}\text{C}]\text{CO}_2$ by Chlorella in the presence of INH more $[^{14}\text{C}]$ glycollate and $[^{14}\text{C}]$ glycine were synthesised in the presence of air or 100% O_2 than were synthesised in the absence of the inhibitor (Whittingham, Coombs and Marker, 1967). After $[^{14}\text{C}]\text{CO}_2$ assimilation by tobacco leaves in the light; Goldsworthy (1966) found that the specific activity of $^{14}\text{CO}_2$ released into a CO_2 -free atmosphere was decreased when the leaves had been previously treated with solutions of either α -hydroxy-2-pyridinemethanesulphonate or INH. Kisaki and Tolbert (1972) found that when $[1-^{14}\text{C}]$ glycine or $[1-^{14}\text{C}]$ glycollate was supplied exogenously to disks of leaves of tobacco in the presence of INH, the $^{14}\text{CO}_2$ evolution in light or darkness from either of these substrates was inhibited compared to that from disks supplied with the ^{14}C substrates in the absence of the inhibitor. If the immediate substrate of photorespiration was either glycollate or glyoxylate INH would not have inhibited $^{14}\text{CO}_2$ evolution from $[^{14}\text{C}]$ glycollate. Inhibitors of photorespiratory CO_2 evolution

in the light, therefore, also inhibit the flow of carbon through intermediates of the C2 pathway.

The investigations of photorespiration have shown that the substrates of this process became rapidly labelled with ^{14}C during photosynthesis from $[^{14}\text{C}]\text{CO}_2$ by leaves and that these substrates were soon depleted when the leaf was darkened (Tregunna et al, 1964; Ludwig et al, 1969; Ludwig and Calvin, 1971). Glycollate and glycine were found to be rapidly labelled during photosynthesis from $[^{14}\text{C}]\text{CO}_2$ and to rapidly lose ^{14}C labelling on darkening (Benson and Calvin, 1950). The CO_2 evolution from leaves of sunflower was saturated with ^{14}C after 10 min steady state photosynthesis in the presence of 300 ppm $[^{14}\text{C}]\text{CO}_2$ in air, and after a subsequent period (10 min) of photosynthesis in $^{12}\text{CO}_2$ the CO_2 evolved by photorespiration was almost unlabelled (Atkins, Calvin and Fock, 1971). Atkins et al suggested that the kinetics of ^{14}C labelling of the substrate of photorespiration should be similar. They found that those compounds which reached isotopic equilibrium after 10 mins of photosynthesis from $[^{14}\text{C}]\text{CO}_2$ and which rapidly lost ^{14}C labelling in the presence of unlabelled CO_2 were intermediates of the Calvin cycle, glycine and serine. The flow of carbon through serine accounted for a considerable proportion of the total carbon assimilated photosynthetically and it was of a magnitude consistent with serine being an intermediate of a pathway for photorespiration. If this were the case, and most glycine and serine were produced via the C2 pathway from glycollate, it would not be possible for a significant amount of CO_2 to be produced prior to the synthesis of serine by a non-enzyme oxidation of glycollate as proposed by Zelitch (1966).

The C2 pathway therefore has the following characteristics in common with photorespiration: it is a light-dependent pathway in that it is

closely associated with photosynthetic carbon metabolism, it is O_2 -dependent and the flow of recently assimilated carbon into intermediates of the C2 pathway is increased in the presence of increased O_2 concentrations and decreased CO_2 concentrations. Intermediates of the C2 pathway supplied exogenously to leaves of both C_3 - and C_4 -plants are used as substrates for CO_2 evolution and certain reactions of the pathway, notably the synthesis of serine from glycine, involve decarboxylation.

For C_3 -plants, including tobacco, there is considerable evidence for the presence of an active C2 pathway metabolism and hence photorespiration. On the other hand, the decarboxylation of intermediates of the C2 pathway supplied exogenously to leaves of maize indicated that this pathway may be functional in maize and, in addition, enzyme studies indicated that the enzymes mediating certain reactions of the C2 pathway were present in leaves of maize (Tolbert *et al.*, 1969; Rehfeld, Randall & Tolbert, 1970). However the effects of O_2 and CO_2 concentration on the products of photosynthesis by maize, or other C_4 -plants, had not been studied when the present work was initiated.

7. The Interaction of O_2 with Photoelectron Transport.

The effect of O_2 on photosynthesis involves not only the 'dark' reactions of carbon metabolism but also the light-dependent reactions which result in the production of energy (ATP) and reducing power (NADPH). The phosphorylation of ADP, the reduction of NADP and the oxidation of water to molecular O_2 is effected by a light stimulated electron flow.

Mehler (1951a; 1951b) found that, in the absence of other oxidants or Hill reagents, isolated chloroplasts consumed O_2 . He concluded that O_2 was acting as a Hill reagent, accepting electrons from the reducing end of the electron transport system and being converted to H_2O_2 . Mehler suggested this reaction might occur in vivo during photosynthesis under

conditions when CO_2 was limiting and at high O_2 concentrations. Mehler and Brown (1952) used O_2 isotopes to confirm the occurrence of the Mehler reaction in chloroplasts. Heber and French (1968) found that, when catalase was poisoned, O_2 was consumed by both intact and broken chloroplasts and this consumption of O_2 was inhibited by DCMU. They suggested that this was evidence for a functional Mehler reaction in chloroplasts. The results indicated that O_2 reacted with the primary reductant of photosystem I and not significantly with the reductant produced by photosystem II. The rate of reduction of PGA and nitrate by isolated chloroplasts was decreased in the presence of N_2 compared to air, indicating an O_2 requirement by isolated intact chloroplasts (Heber and French, 1968). O_2 may react with the electron transport chain at a site near photosystem I and before the site ferredoxin (Mathieu, 1969). Whitehouse, Ludwig and Walker (1971), using preparations of isolated chloroplasts, suggested that the rate of the Mehler reaction was principally limited by the rate of transfer of electrons to molecular O_2 . The addition of NH_4Cl increased the rate of the Mehler reaction provided that a suitable artificial electron carrier was present to facilitate electron transport to O_2 .

Arnon, Allen and Whatley (1954) found that photophosphorylation by isolated chloroplasts was inhibited under anaerobic conditions. Subsequently Forti and Jagendorf (1961) showed that endogenous photophosphorylation in the absence of redox dyes was O_2 -dependent. Arnon et al (1961) recognized three types of photophosphorylation in isolated chloroplasts; cyclic, non-cyclic and pseudocyclic phosphorylation. The formation of ATP by cyclic phosphorylation was effected by a cyclic flow of electrons without a net change in any exogenous electron donor or acceptor. Non-cyclic photophosphorylation was the production of ATP with the reduction of a

Hill reagent or NADP (as in vivo) and the evolution of O_2 . Pseudocyclic photophosphorylation was considered to be a form of cyclic phosphorylation except that it was dependent upon the presence of O_2 . Arnon et al concluded, however, that "the intervention of oxygen in non-cyclic photophosphorylation resulted in a loss of biochemically useful energy which would have normally been used for forming a strong reductant i.e. TPNH". There is, therefore, evidence that under certain conditions in vitro O_2 can interact with the electron transport system. Heber (1969) presented evidence that O_2 can interact in vivo with photoelectron transport in the leaves of a number of plants including spinach. On the other hand, Raven (1970) using Hydrodictyon africanum, found no evidence that pseudocyclic phosphorylation was required in vivo for steady-state photosynthesis.

8. The Effects of NH_4Cl and $NaCl$ on Photosynthesis.

The energy and reducing power needed for the incorporation of one molecule of CO_2 into carbohydrate was thought by Chen, Brown and Black (1969) to be 3 molecules of ATP and 2 molecules of NADPH for C_3 -plants but 5 molecules of ATP and 2 of NADPH in C_4 -plants. The conclusion for C_4 -plants was based on the theory that carboxylation of PEP and RuDP occur in sequence in these plants. Chen et al proposed that the additional energy required was supplied by increased cyclic photophosphorylation.

Schlürmann, Buchanan and Arnon (1971) demonstrated the importance of the ratio of ATP:NADPH in determining the relative amounts of ^{14}C in products of photosynthesis by isolated intact chloroplasts from $[^{14}C]CO_2$. Sugar phosphates synthesis was increased and phosphoglycerate synthesis decreased when non-cyclic phosphorylation was inhibited. The inhibition of cyclic phosphorylation resulted in less synthesis of sugar phosphates and more synthesis of phosphoglycerate. A high ATP:NADPH ratio probably favoured the conversion of phosphoglycerate to carbohydrate.

Environmental factors and inhibitors, which may affect the ratio of ATP:NADPH, might therefore, be expected to affect photosynthetic carbon metabolism in vivo.

a) Effect of NH_4^+ on Photoelectron Transport and Photophosphorylation

Kroghmann, Jagendorf and Avron (1959) suggested that ammonium ions acted as very effective uncouplers of photophosphorylation and that the effect was reversible. Good (1960) reported that ammonia and a number of mono-functional amines increased the rate at which ferricyanide and other oxidants were reduced by illuminated chloroplasts. He suggested that the activation was caused by the unionized amine. These observations were confirmed and extended to other nitrogenous bases by Hind and Whittingham (1963). Hind and Jagendorf (1965) suggested that uncouplers such as ammonia increased the rate of breakdown of the high energy state, an intermediate in photophosphorylation.

b) Cl^- ions in Photoelectron Transport

Chloride ions were considered to be essential to the O_2 -evolving system (Warburg and Lüttgens, 1946). Subsequently Bové, Bové, Whatley and Arnon (1963) found that chloride ions were required for photochemical reactions involving O_2 evolution. Hind, Nakatani and Izawa (1969) found that non-cyclic electron flow by broken chloroplasts, was dependent upon chloride ions and that the effect was greater after uncoupling by ammonia. They suggested that the chloride effect was associated closely with photosystem II. Heath and Hind (1969) from their studies of the effect of chloride ions on the fluorescence of isolated chloroplasts suggested that the site of chloride involvement was on the oxidative or water splitting side of photosystem II. Treatment of isolated chloroplasts with NaCl can result in uncoupling (Kroghmann et al, 1959). A more detailed study of the uncoupling effect of anions by Good (1962) indicated that Cl^- ions were

feeble uncouplers but that their effect could be of greater magnitude under circumstances where there was an interaction with other anions.

c) Effects of Ammonia on Carbon Metabolism

Holm-Hansen, Nishida, Moses and Calvin (1959) found that the addition of NH_4Cl changed the distribution of ^{14}C between products of short-term photosynthesis by Chlorella in the presence of $[^{14}\text{C}]\text{CO}_2$. Less ^{14}C was incorporated into sugar phosphates and more into amino acids. Hiller and Whittingham (1964) found that in the presence of NH_4Cl less $[^{14}\text{C}]$ malate was formed during photosynthesis by Chlorella from $[^{14}\text{C}]\text{CO}_2$ and the ^{14}C incorporated into glycollic acid was decreased. The results were consistent with the expected decrease in ATP synthesis caused by the presence of NH_4Cl . Hiller (1970) showed that, during photosynthesis from 2% $[^{14}\text{C}]\text{CO}_2$ by Chlorella, the presence of 10^{-2}M NH_4Cl decreased synthesis of sucrose, polysaccharides, PGA, fructose and sedoheptulose monophosphates. Synthesis of glucose monophosphate and fructose and ribulose diphosphates was unaffected. There was a rapid increase in synthesis of amino acids and tricarboxylic cycle acids, partly accounted for by the decreased synthesis of the sugar phosphates, but mostly by decreased synthesis of sucrose and polysaccharides. Hiller suggested that NH_4Cl stimulated the conversion of PGA to phosphoenolpyruvate and pyruvic acid, and that it may have a direct effect on phosphoglyceromutase, enolase or pyruvate kinase in vivo.

Kanazawa, Kirk and Bassham (1970) studied the effects of NH_4Cl on photosynthetic carbon metabolism by Chlorella in 1.6% $[^{14}\text{C}]\text{CO}_2$. In the presence of NH_4Cl the synthesis of $[^{14}\text{C}]$ PEP was increased compared to $[^{14}\text{C}]$ 3-PGA, but the synthesis of these compounds was reduced by NH_4Cl compared to untreated cells. The ^{14}C incorporated into pyruvic acid and alanine was increased in the presence of NH_4Cl whilst the incorporation of ^{14}C

into serine was unaffected. There was an increased rate of metabolism of ^{14}C by the tricarboxylic acid cycle with an increase of $[\text{}^{14}\text{C}]$ malate, citrate, glutamate, glutamine and aspartate, and an increased rate of ^{14}C incorporation into lipids. The increased synthesis of $[\text{}^{14}\text{C}]$ amino acids was mostly at the expense of sucrose synthesis. Starch synthesis was decreased only slightly. The decrease in $[\text{}^{14}\text{C}]$ sucrose synthesis was apparently due to inhibition of the reaction between UDP-glucose and fructose-6-phosphate. The rate of conversion of fructose 1, 6 diphosphate was also decreased by ammonia. The data supported the suggestion that ammonia stimulated pyruvate kinase. Miller and Evans (1957) found that univalent cations, among them NH_4^+ , stimulated phosphorylation of ADP by PEP catalyzed by pyruvate kinase in vitro.

The effects of ammonia toxicity in higher plants has been studied by a number of workers. Vines and Wedding (1960) suggested that ammonia toxicity to plants was the result of an interaction of NH_4^+ with the electron transport system of the mitochondrion and especially its oxidation of NADH. Matsumoto, Wakiuchi and Takahashi (1968) studied the concentration of sugars in cucumber leaves during ammonium toxicity and concluded that the translocation of glucose was inhibited by NH_4^+ . Wakiuchi et al (1971) found that the activity of pyruvate kinase was increased in the leaves of cucumber plants treated with NH_4Cl . A further study of Wakiuchi et al (1972) showed that upon addition of NH_4Cl to cucumber leaves there was an immediate increase in the rate of respiration followed by a slow return to normal rates.

d) Effect of NaCl on Carbon Metabolism.

The requirement for univalent cations for the reaction mediated by pyruvate kinase was found to be satisfied by NaCl, though the Na^+ salt was less effective than the K^+ salt (Miller and Evans, 1957). NaCl

increased the activity of malic dehydrogenase from spinach leaves (Hiatt and Evans, 1960). Joshi, Dolan, Gee and Saltman (1962) showed that the distribution of ^{14}C among the products formed by homogenates of spinach leaves from $[^{14}\text{C}]\text{CO}_2$ in the dark was similar to that in intact leaves. The addition of NaCl, however resulted in an increased synthesis of $[^{14}\text{C}]$ amino acids. Hartt (1967) used NaCl to induce water-stress in sugarcane plants. She found that translocation of photosynthate was decreased but the photosynthetic metabolism of $[^{14}\text{C}]\text{CO}_2$ was not markedly affected. Harvey and Gibbs (1970) found that there was a greater retention of products within isolated chloroplasts that were fixing CO_2 in the light when NaCl was included in the medium. Osmond and Greenway (1972) showed that NaCl inhibited PEP carboxylase from C_4 -plants. PEP carboxylase from C_3 -plants was not as sensitive to NaCl as the enzyme from C_4 -plants and RuDP carboxylase was much less sensitive to NaCl than PEP carboxylase. The effect of KCl on PEP carboxylase activity was similar to that of NaCl. The ^{14}C distribution among the products formed by photosynthesis from $[^{14}\text{C}]\text{CO}_2$ by C_4 -plants was not affected by NaCl; there was no decrease in the amount of ^{14}C incorporated into the four-carbon dicarboxylic acids. Winter and Von Willert (1972) found that Mesembryanthemum crystallinum, when grown hydroponically in a saline medium, exhibited CO_2 uptake in darkness and at the same time malate was synthesised in the leaves. This metabolism similar to that of Crassulacean plants, was absent in untreated plants.

9. Justification for the Experiments Described in this Thesis.

Light-dependent CO_2 evolution from leaves of tobacco is easy to measure and its rate is increased by increased concentrations of O_2 in the atmosphere. The rate of apparent photosynthesis is decreased with increased concentration of O_2 so that the maximum rate of photosynthesis is observed in atmospheres devoid of O_2 . The apparent decrease in the assimilation of

CO_2 in the presence of O_2 is therefore largely accounted for by the simultaneous loss of CO_2 through photorespiration. The evolution of CO_2 in the light from leaves of maize is difficult to measure even in the presence of high concentrations of O_2 . The results of Forrester et al (1966b) however, show that the presence of increased concentrations of O_2 did decrease the rate of photosynthesis by leaves of maize. The experiments described aimed to investigate the effects of O_2 on the rates of photosynthesis by maize, a C_4 -plant, and tobacco, a C_3 -plant, and then, having established the differences in the effects of O_2 concentration on the rate of assimilation of CO_2 , to study the differences in biochemistry of photosynthesis that were responsible.

The effect of high and low CO_2 concentrations, in atmospheres containing different concentrations of O_2 , on the photosynthetic carbon metabolism by leaves of maize was studied in greater detail. The metabolism of certain photosynthetic intermediates was investigated, in light and darkness, by supplying these compounds exogenously to leaves of maize in the presence of atmospheres containing different concentrations of O_2 and CO_2 .

The effect of O_2 on photosynthesis may not be due only to direct effects on carbon metabolism since there is also evidence that it can interact with photoelectron transport. This evidence shows that the concentration of O_2 present may influence carbon metabolism by an effect on photoelectron transport that alters the ratio of ATP to NADPH synthesis. There is also the theoretical possibility that the energy requirement for photosynthesis may be greater for C_4 -plants than C_3 -plants (Chen et al, 1969). The effect of O_2 on the rate of photosynthesis by leaves of maize and tobacco was investigated therefore, in the presence of NH_4Cl , an uncoupler of photophosphorylation. It was considered possible that Cl^- ions may have an effect in addition to the effect of the NH_4^+ ions and similar experiments were therefore conducted in the presence of NaCl .

MATERIALS AND METHODS

1. Plant Material

2. Measurements of Rates of Photosynthesis in Atmospheres Containing Different Proportions of Oxygen and Carbon dioxide.
 - a. Preparation of gas mixtures
 - b. Apparatus
 - c. Leaf tissue
 - d. Procedure
 - e. Calculation of Results.

3. Supply of $[^{14}\text{C}]\text{CO}_2$ at 100 ppm in Atmospheres consisting of either O_2 -free N_2 , 21% O_2 or 80% O_2 .
 - a. Apparatus
 - b. Preparation of gas mixtures
 - c. Leaf chamber and leaf tissue
 - d. Procedure
 - e. Extraction of leaf tissue
 - f. Measurement of radioactivity
 - g. Measurement of radioactivity of the insoluble residue.

4. Supply of $[^{14}\text{C}]\text{CO}_2$ at 5000 ppm in Atmospheres consisting of either O_2 -free N_2 , 21% O_2 or 80% O_2 .
 - a. Apparatus
 - b. Leaf chamber and leaf tissue
 - c. Preparation of gas mixtures and Procedure
 - d. Extraction of leaf tissue
 - e. Measurement of radioactivity

5. Supply of $[^{14}\text{C}] \text{CO}_2$ at 300 ppm in Atmospheres Containing Different Proportions of O_2 to Samples of Maize and Tobacco Leaves Treated with NH_4Cl and NaCl Respectively.
 - a. Apparatus
 - b. Preparation of gas mixtures
 - c. Leaf chamber and leaf tissue
 - d. Procedure
6. Supply of $[2 - ^{14}\text{C}]$ glycine, $[\text{L} - \text{U} - ^{14}\text{C}]$ malic acid and $[\text{L} - \text{U} - ^{14}\text{C}]$ alanine.
 - a. Preparation of gas mixtures
 - b. Solutions of ^{14}C substrates
 - c. Leaf samples
 - d. Leaf chambers
 - e. Procedure
 - f. Extraction of leaf tissue
 - g. Measurement of radioactivity
7. Supply of $[^{14}\text{C}] \text{CO}_2$ at 100 ppm in O_2 -free N_2 or CO_2 -free air to Samples of Maize Leaf in the Light for Periods of 20, 40, 60, 120, 300 and 600 seconds.
 - a. Preparation of gas mixtures
 - b. Leaf chambers and leaf tissue
 - c. Procedure
 - d. Extraction of leaf tissue
 - e. Measurement of radioactivity

8. Analysis of ^{14}C Products

- a. Two-dimensional paper chromatography
- b. Autoradiography
- c. Elution of paper chromatograms
- d. Measurement of radioactivity
- e. Identification of ^{14}C compounds by thin layer chromatography
- f. Separation of glycine and serine
- g. Determination of the total ^{14}C content of ethanol/water insoluble residues by combustion.

1. Plant Material

Seeds of Zea mays var. Golden Bantam (Carters Seeds Ltd) were germinated individually in 3" pots and the plants transferred to 4½" pots after 7 days. The seed was germinated and the plants were grown in a Saxcil growth chamber providing automatic watering, high humidity, a 16 hr. light period (1900 ft candles) at 28°C; and an 8 hr. dark period at 25°C. After 19 days the plants were transferred from the growth cabinet to a greenhouse where automatic watering was provided and daylight was supplemented with fluorescent lighting (16 hr. light period). The minimum temperature was 25°C and the minimum light intensity 1200 ft candles.

All samples were cut from the third and fourth leaves of the plants during the period 19 to 23 days from sowing. Nicotiana tabacum var. White Burley was grown in a greenhouse in daylight supplemented with fluorescent lighting, the plants being repotted at intervals; they were in 10" pots when used. The plants were supplied twice a week with a commercial nutrient solution (Fison's liquinure).

All experiments were commenced four hours after the beginning of the light period in an attempt to standardise the leaf material with regard to the diurnal fluctuations in the physiology and biochemistry of the plant.

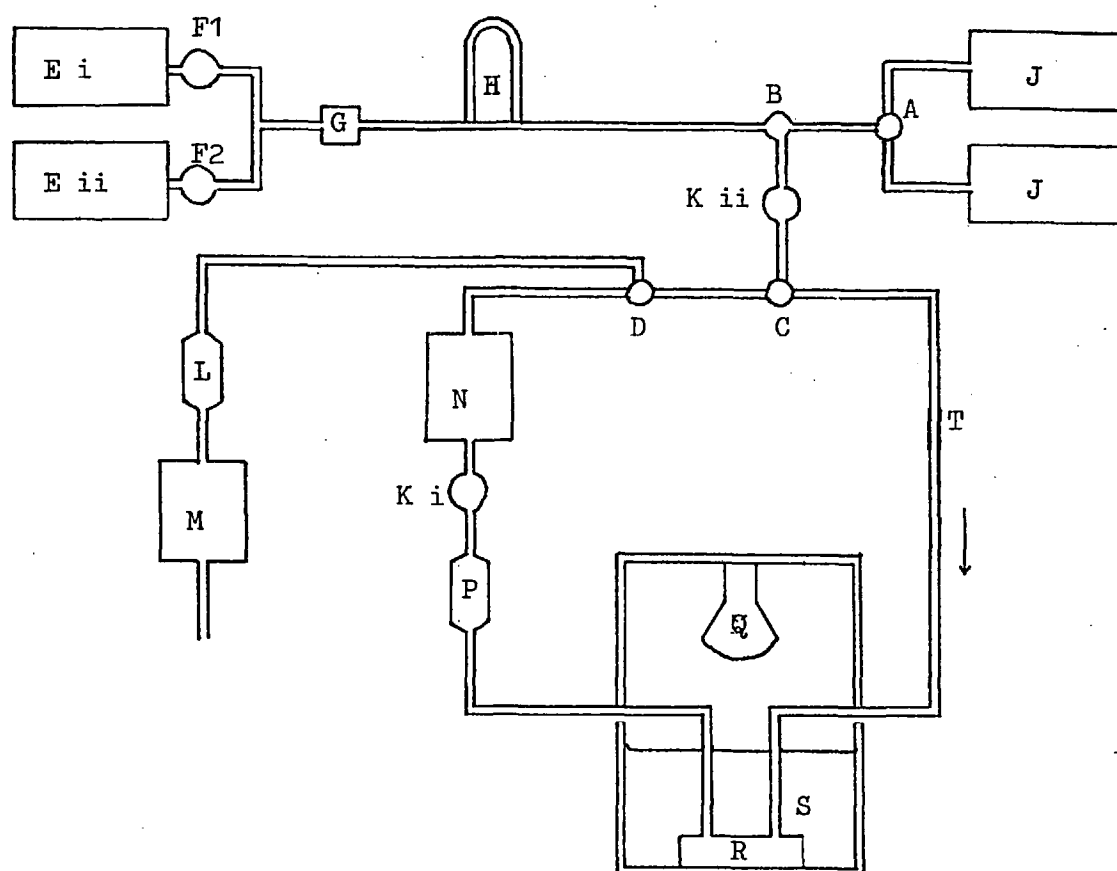
2. Measurements of Rates of Photosynthesis in Atmospheres Containing Different Proportions of Oxygen and Carbon dioxide.

(a) Preparation of gas mixtures

For measurements at zero and 100% oxygen, oxygen-free nitrogen and pure oxygen were used directly from pressurised cylinders supplied by British Oxygen Co Ltd. Carbon dioxide-free air was obtained by pumping air through a drying tower containing soda-lime (Carbasorb - B.D.H.).

FIGURE:1

Apparatus used to measure rates of photosynthesis by samples of maize and tobacco leaves in atmospheres containing different concentrations of O_2 and CO_2 .



- A, B, C, & D, 3-way glass taps;
 E, Gas-cylinders containing (i) O_2 (ii) O_2 -free N_2 under pressure;
 F (1 & 2), Pressure-reducing valves;
 G, Flostat;
 H, Manometer;
 J, Double-walled polythene bags;
 K, (i & ii), Diaphragm pumps;
 L, Drying-tube containing silica gel;
 M, Oxygen analyzer;
 N, Infra-red gas analyzer;
 P, Drying-tube containing magnesium perchlorate;
 Q, Spot lamp;
 R, Leaf chamber;
 S, Constant-temperature water bath;
 T, Section of rubber tubing.

All other atmospheres used were obtained by mixing oxygen-free nitrogen and oxygen in polythene bags. The oxygen-free nitrogen and oxygen cylinders were each connected through a pressure reducing valve set at 10 lbs/sq in to a flostat (Flowstat minor supplied by G.F. Platon Ltd, Wella Road, Basingstoke, Hants.) to provide a constant gas flow. The output from the flostat was measured by means of a flow-meter and set to 4 l/min. The nitrogen: oxygen mixtures were prepared in large polythene bags, with double walls each 500 gauge, made from lay-flat polythene tubing, 4 feet diameter, supplied by Transatlantic Plastics Ltd. Two of these bags were connected in the filling system by two 3-way glass vacuum taps (A and B, Fig. 1). Each bag was evacuated and oxygen-free nitrogen passed in at 4 l/min for an appropriate time followed by oxygen at 4 l/min until the required relative proportion of the gas was in the bag. An 80% oxygen: 20% nitrogen mixture was prepared, for example, by passing oxygen at 4 l/min into the bag for 8 mins followed by oxygen-free nitrogen at 4 l/min for 2 min. The gases inside the bag were mixed by gentle manipulation of the walls of the bag. The composition of the prepared gas mixture did not alter significantly during the time required to complete each experiment (2 hr). Mixtures of argon and oxygen were prepared in a similar manner. The oxygen concentration of the gas mixtures was determined by passing samples through a drying tube (silica gel) and into a paramagnetic oxygen analyzer (Type OA 150 supplied by Servomex Controls Ltd. Crowborough, Sussex).

b. Apparatus

The apparatus is shown in the diagram (Fig 1). The leaf chamber was a clear perspex box, external dimensions 20.5 x 10 x 3.5 cm, with an internal volume of 216 ml. The gas stream was passed into the chamber and over the leaf surface through inlet and outlet tubes at opposite ends of the lid. The chamber was maintained at 28°C by submersion in a constant

temperature water bath, equipped with both heating and refrigeration units, and was illuminated at 1950 ft candles by a 250 w reflector spotlight (Mazda).

The gas, circulated at 4 l/min by a diaphragm pump (i), was dried before entering the infra-red gas analyzer by passage through a drying tube containing magnesium perchlorate. The carbon dioxide concentration of the gas stream was measured using a 'null balance' infra-red gas analyzer, made by Hilger-IRD, Ltd. The instrument was designed to measure carbon dioxide concentration in three scale ranges - zero to 100 ppm, zero to 1000 ppm and zero to 10,000 ppm. It was not possible during the course of experiments using a closed circuit to change to a more sensitive range without introducing a volume of carbon dioxide-free air and therefore changing the composition of the gas mixture (oxygen concentration). The investigation of the effect on photosynthetic rate of carbon dioxide concentrations from 6000 ppm to the leaf compensation point was therefore conducted using separate leaf samples for each of the scale ranges. A linear Toa electronic polyrecorder (T.E.M. Sales Ltd, Crawley, Sussex) connected to the gas analyzer recorded continuously the change of carbon dioxide concentration. The diaphragm pumps (i and ii) used were the 'Dymax' type supplied by Charles Austin Ltd. Glass tubing was used wherever possible to minimise diffusion of carbon dioxide; connections were made, with $\frac{1}{4}$ " diameter PVC tubing and Quickfit all-glass joints except for a section 1" long of rubber tubing (see Fig. 1) into which injections of gas were made. All vacuum taps and ground glass joints were made gas-tight using silicone high vacuum grease.

The closed system was tested for leaks by circulating in it carbon dioxide-free air; it was judged free of leaks if no diffusion of CO₂ in from the atmosphere could be detected in 30 min. As a further test, the

system was partly evacuated and the retention of the vacuum measured using a simple mercury manometer. The total volume of the closed system including the leaf chamber was approximately 600 - 800 ml, the exact volume was determined by measuring the dilution of a known concentration of CO_2 in a known volume of N_2 which was injected into the closed system.

c. Leaf tissue

Samples of maize leaf consisted of three segments, two cut from the third and one from the fourth leaf of a 19 - 23 day old plant. The three segments (total area approximately 100 cm^2 , fresh weight approximately 1g) were strips of lamina cut from either side of the midrib. For experiments using tobacco leaves, pieces of lamina having an area of 100 cm^2 were used.

d. Procedure

The leaf tissue was floated with its adaxial or dorsal surface in contact with 20 ml distilled water contained in the leaf chamber and allowed to equilibrate for one hour with air flowing through at 4 l/min. The required carbon dioxide-free oxygen: nitrogen mixture was then passed into the system (4 l/min pump ii) for one minute through the 3-way taps C and D (Fig.1) to fill the entire system with the mixture. The system was closed and pure CO_2 was injected through the rubber tubing using a syringe and hypodermic needle to give the required CO_2 concentration in the system. The gas was circulated at 4 l/min by pump (i), (Fig.1) and the rate of depletion of carbon dioxide measured. The procedure was then repeated for various oxygen concentrations. The magnesium perchlorate and the 1" length of rubber tubing in the circuit were replaced prior to the introduction of each gas mixture.

In further experiments the same procedure was used except that during the initial one hour equilibration period the leaf sample was floated not on distilled water but on aqueous solutions of ammonium chloride buffered with either phosphate buffer or HEPES, sodium chloride or phosphate buffer, as shown in Table 1.

TABLE:1

Solutions Supplied to Samples of Maize and Tobacco Leaves During the First Hour After Excision

Sample of Leaf	Solution on which the sample of leaf was floated during the first hour after excision
maize	2.56 m M NH_4Cl in 0.2 M phosphate buffer, pH 7.6 5.12 m M NH_4Cl in 0.2 M phosphate buffer, pH 7.6 0.2 M phosphate buffer pH 7.6
maize and tobacco	5.12 m M NH_4Cl in 0.01 M HEPES pH 7.4 5.12 m M NH_4Cl in 0.01 M HEPES buffer pH 7.4.
maize	10.24 m M NaCl 5.12 m M NaCl
tobacco	5.12 m M NaCl

This treatment was followed by a further 40 mins during which the leaf tissue was floated on 20 ml distilled water in the stream of air flowing at 4 l/min.

e. Calculation of Results

The Toa electronic polyrecorder measured the decrease with time in the CO_2 concentration in the closed system. The rate of change of CO_2 concentration at a particular CO_2 concentration was obtained by drawing a tangent to the line plotted by the recorder at that CO_2 concentration. The rate of apparent photosynthesis ($\mu\text{l dm}^{-2}\text{hr}^{-1}$) at that CO_2 concentration was calculated from the rate of decrease in CO_2 by making appropriate allowances for area of the sample of leaf used and for total volume of the closed system.

3. Supply of ^{14}C CO_2 at 100 ppm in Atmospheres consisting of either O_2 -free N_2 , 21% O_2 or 80% O_2

(a) Apparatus

The apparatus is shown in the diagram, Fig. 2.

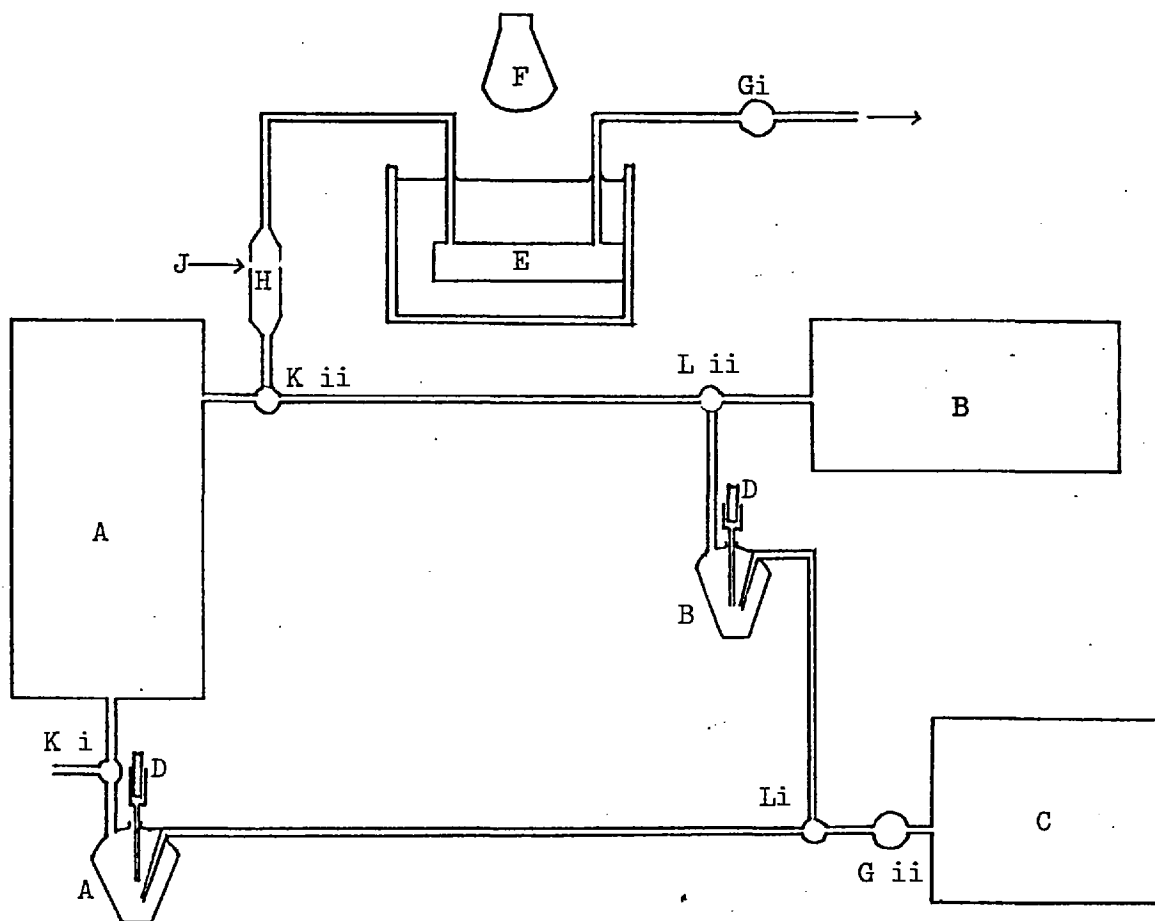
(b) Preparation of gas mixtures

The three double-walled polythene bags A, B and C (Fig 2) were similar to those described previously (see 2(a)). Bag C was filled with either O_2 -free N_2 or 80% O_2 : 20% O_2 -free N_2 by the technique described (see section 2a) and the oxygen concentration measured. The gas stored in this bag was used to fill bags A and B. While bags A and B were being filled either ^{14}C CO_2 to give 100 ppm (bag A) or unlabelled CO_2 to give 300 ppm (bag B) was evolved into the gas stream. Air mixtures containing either 100 ppm ^{14}C CO_2 (bag A) or 300 pm $^{12}\text{CO}_2$ (bag B) were prepared in a similar manner except that the CO_2 -free air was drawn in by pump ii, through two towers containing soda-lime arranged in parallel, without storage in bag C.

Before filling bags A and B, the CO_2 -free gas to be used for a particular leaf sample was passed at 4 l/min for 4 min through flask A and

FIGURE:2

Apparatus used to supply (1) samples of maize leaves with 100 ppm $[^{14}\text{C}] \text{CO}_2$ in either O_2 -free N_2 , 21% O_2 , or 80% O_2 , and (2) samples of maize and tobacco leaves with 300 ppm $[^{14}\text{C}] \text{CO}_2$ in atmospheres containing different concentrations of O_2 .



Flask A, Flask containing $\text{Na}_2 [^{14}\text{C}] \text{CO}_3$;

Flask B, Flask containing Na_2CO_3 ;

Polythene bag A, Containing either 100 or 300 ppm $[^{14}\text{C}] \text{CO}_2$ in the required mixture of O_2 & N_2 ;

B, Containing 300 ppm $^{12}\text{CO}_2$ in required mixture of O_2 & N_2 ;

C, Containing CO_2 -free required mixture of O_2 & N_2 ;

D, Syringes containing 50% (v/v) lactic acid;

E, Leaf chamber (see Fig. 3);

F, Spot lamp;

G, (i & ii), Diaphragm pumps;

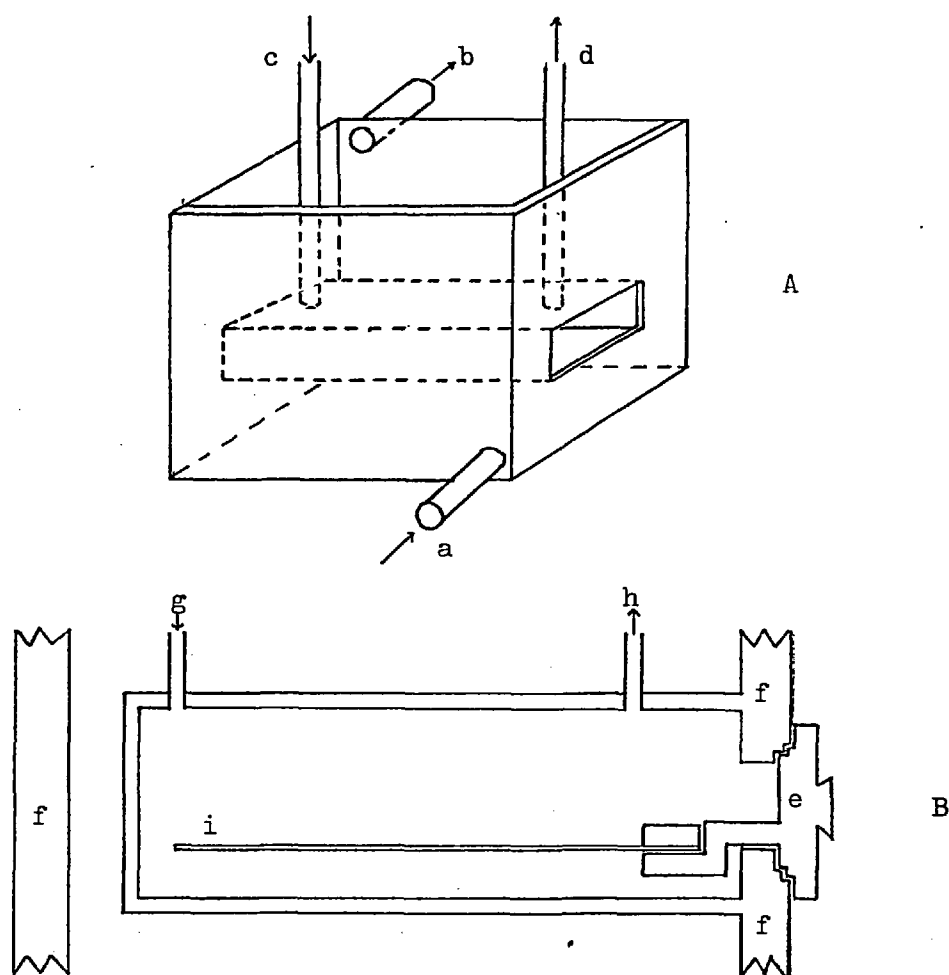
H, Drying-tube containing magnesium perchlorate;

J, Entry for air supply;

K, (i & ii), 3-way glass taps;

L, (i & ii), 2-way glass taps;

FIGURE:3.



A. Three-dimensional view of chamber with door removed.

a, entry for water;
c, entry for gas;

b, overflow for water;
d, exit for gas.

B. Transverse section of leaf chamber with part of the water bath.

e, door of leaf chamber with handle;
f, walls of water bath;
g, entry for gas;
h, exit for gas;
i, leaf tissue.

for 2 min at 4 l/min through flask B; bags A and B were evacuated to remove air. Gas from bag C or CO_2 free air (24 l) was passed 4 l/min through either flask A into bag A or flask B into bag B while CO_2 was generated by the injection of aqueous Na_2CO_3 solution into 0.3 ml 50% lactic acid in the appropriate flask. The evolution of carbon dioxide was facilitated by warming the flask. $[^{14}\text{C}]\text{Na}_2\text{CO}_3$ supplied by the Radiochemical Centre, Amersham (specific activity 55.5 mCi/m mol) was suitably diluted with analar $\text{Na}_2^{12}\text{CO}_3$ before use.

(c) Leaf chamber and leaf tissue

The leaf chamber was a clear perspex box : external dimensions 20 x 10 x 3 cm, with an internal volume of 375 ml. The box was submerged in a clear perspex water bath 21 x 21 x 21 cm; so that access to the leaf chamber was through a door in the wall of the water bath (see diagram, Fig. 3).

Samples of maize leaf were similar to those employed for measurements of photosynthetic rate with the infra-red gas analyzer. One end of each of the three strips comprising the leaf sample was clamped between two perspex bars attached to the inside of the door of the leaf chamber (see Fig 3). A gas tight seal was made between the door and the leaf chamber with silicone grease. When inside the leaf chamber the adaxial surface of the leaf samples was in contact with 20 ml distilled water. The inlet and outlet tubes, used for circulation of gases through the leaf chamber, were at either end of the uppermost surface of the chamber (Fig. 3). The leaf chamber was maintained at a constant temperature, 28°C , by water circulating continuously through the water bath from the main water-bath which was equipped with heating and refrigeration units. The leaf sample was illuminated (1950 ft candles) from above by a reflector spotlight (Mazda 250 w). The walls of the water-bath were covered with black paper to exclude extraneous light.

(d) Procedure

The weighed leaf sample was equilibrated in the leaf chamber for one hour in the light with air passing through at 4 l/min. During this time the gas mixtures were prepared in bags A and B. After 1h the leaf chamber was connected through the drying tube to bag B (see Fig.2) and the gas containing 300 ppm CO₂ passed through the leaf chamber at 4 l/min for 1 min. The leaf chamber was then connected to bag A gas, containing 100 ppm [¹⁴C]CO₂, which was passed (4 l/min) through the chamber for 5 min. The leaf sample was withdrawn from the chamber and cut from the clamp with a razor blade so that it dropped into a 250 ml beaker containing 100 ml boiling 80% ethanol.

(e) Extraction of Leaf tissue

The leaf tissue was boiled for 2 min in 80% ethanol and the beaker was then transferred to ice. The tissue was cut into small pieces and homogenized using a Virtis "45" homogeniser run at full speed for 30 sec; after a pause of 30 sec, a further period of 30 sec at full speed was used to complete homogenization. The homogenate was centrifuged for 4 min at 1,200 x g. The supernatant liquid was decanted and stored in ice. The residue was re-extracted by heating in a water-bath with the following solutions: 25 ml boiling 80% ethanol for 2 min (twice), 25 ml boiling water for 3 min (twice) and 20 ml boiling 50% ethanol for 3 min. The suspension was centrifuged (1,200 x g) for 4 min between each extraction. The combined extracts, containing the ethanol/water soluble material from the leaf sample, were made up to a total volume of 250 ml with 80% ethanol. The total ¹⁴C content of this extract was measured (see Section 3f); the remaining ethanol/water soluble extract was concentrated to small volume in a rotary vacuum evaporator at below 35°C and made up to a total volume of 10 ml with absolute ethanol.

(f) Measurement of radioactivity

The total ^{14}C content of the ethanol/water soluble fraction was determined by taking an aliquot of the solution, and diluting it suitably. The diluted solution was dispensed (1 ml) onto planchets (Stock No. AL-12 Sigma Chemical Co.) and dried under an infra-red lamp (Osram 250w). The planchets were washed prior to use, with boiling 60% ethanol to remove traces of grease, and air dried. Each planchet was prepared, by spreading on it 0.3 ml 0.01% teepol in 50% ethanol which was dried under an i.r. lamp, before application of the radioactive sample.

The ^{14}C on each planchet was determined using a gas-flow proportional counter with an automatic sample changer (Nuclear Chicago Model No. D47). The carrier gas was 10% methane: 90% argon. The level of 'background' radioactivity was determined for each batch of samples using planchets spread with the teepol solution and dried. The ^{14}C content of a sample of a standard ^{14}C solution dried on a planchet was also determined with each group of samples to enable the radioactive content of all samples to be related to a common standard.

(g) Measurement of radioactivity of the insoluble residue.

The insoluble residue from the leaf samples was dried under vacuum in a desiccator. Portions of the dried residues were mixed with acid washed sand in thickened pyrex glass tubes, $5 \times \frac{5}{8}$ ", and ground with an electrically driven glass pestle to a fine powder. This was suspended in 10 ml absolute ethanol and the radioactivity of 1 ml samples of this suspension determined using the proportional gas-flow counter.

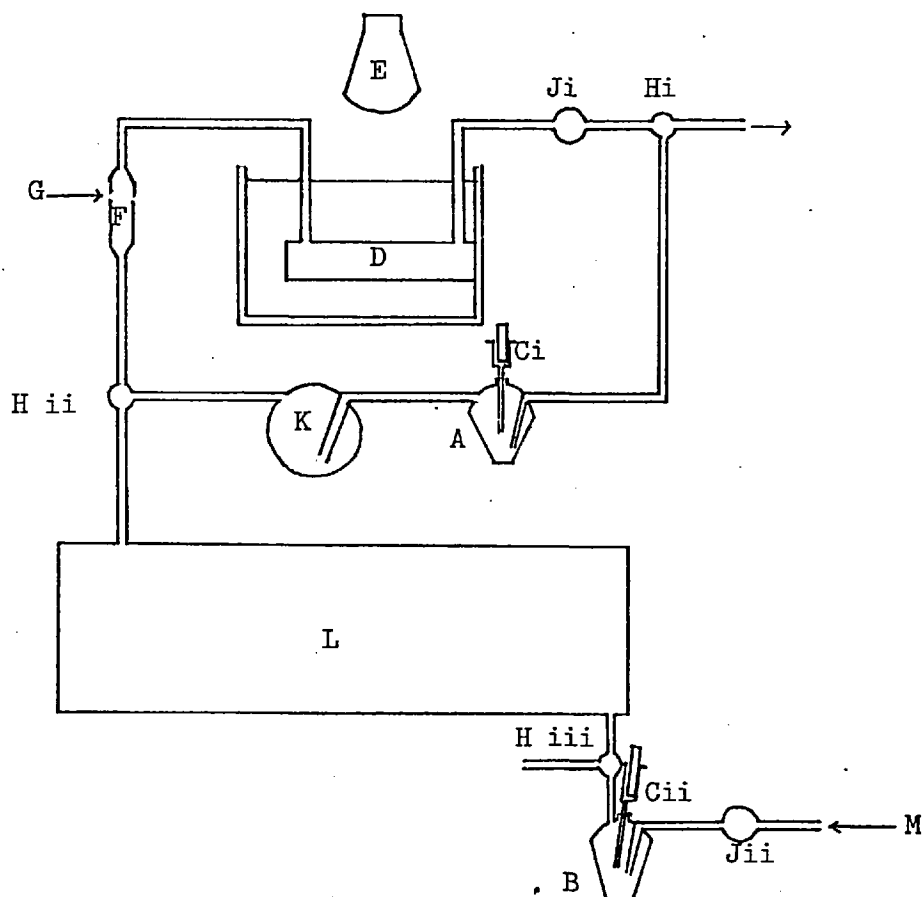
4. Supply of $[\text{}^{14}\text{C}]\text{CO}_2$ at 5000 ppm in atmospheres consisting of either
 O_2 -free N_2 , 21% O_2 or 80% O_2

(a) Apparatus

It was not possible to employ the open circuit technique, described for

FIGURE:4

Apparatus used to supply samples of maize leaves with 5000 ppm $[^{14}\text{C}]\text{CO}_2$ in either O_2 -free N_2 , 21% O_2 or 80% O_2 .



- A, Flask containing $\text{Na}_2[^{14}\text{C}]\text{CO}_3$;
- B, Flask containing $\text{Na}_2^{12}\text{CO}_3$;
- C, (i & ii), Syringes containing 50% v/v lactic acid;
- D, Leaf chamber (see Fig. 3);
- E, Spot lamp;
- F, Drying-tube containing magnesium perchlorate;
- G, Entry for air supply;
- H, (i, ii & iii), 3-way glass taps;
- J, (i & ii) diaphragm pumps;
- K, Flask (150 ml);
- L, Double-walled polythene bag to contain 300 ppm $^{12}\text{CO}_2$ in either O_2 -free N_2 , 21% O_2 or 80% O_2 ;
- M, Entry for CO_2 -free gas supply.

the supply of $[^{14}\text{C}]\text{CO}_2$ at 100 ppm, when the leaf sample was to be supplied with $[^{14}\text{C}]\text{CO}_2$ at 5000 ppm, because of the large quantities of radioactive carbon that would be required. A closed circuit method was therefore used. The closed system had an internal volume (1050 ml) such that during 5 min the photosynthetic activity of the leaf sample would not deplete the carbon dioxide available to a level which would significantly alter the rate of photosynthesis. The apparatus is shown in the diagram Fig. 4.

(b) Leaf chamber and leaf tissue.

The leaf chamber and leaf tissue samples used were similar to those used when supplying $[^{14}\text{C}]\text{CO}_2$ at 100 ppm (see section 3c).

(c) Preparation of gas mixtures and Procedure.

Before commencement of the experiment the section of the closed circuit between the two 3-way taps (i) and (ii) including flask A and the 150 ml flask was filled with the CO_2 -free gas to be used, by passing it through at 4l/min for 6 min (see Fig. 4). This section was then closed. The leaf sample was allowed to equilibrate in the leaf chamber for one hour (see Materials and Methods Section 3d). The double walled polythene bag was evacuated. The flask B was filled with the CO_2 -free gas by passing it through at 4l/min to waste through tap (iii) for 2 min. The gas was then allowed to pass into the double walled polythene bag for 6 min (at 4l/min). During this time CO_2 was generated into the gas stream by injecting into flask B, which contained 0.3 ml 50% lactic acid, sufficient $\text{Na}_2^{12}\text{CO}_3$ to make 300 ppm unlabelled CO_2 in 24l of gas in the bag.

After the one hour equilibration period, the leaf chamber was connected through the drying tube to the bag containing the gas with 300 ppm $^{12}\text{CO}_2$. This was passed through the leaf chamber, 4l/min for 1 min, to waste. During this time sufficient $[^{14}\text{C}]\text{Na}_2\text{CO}_3$ (specific activity $1.89 \mu\text{Ci}/\mu\text{mol}$) was injected into flask A which contained 0.3 ml 50% lactic acid, to make

the final concentration of CO_2 5000 ppm in 1050 ml. After 1 min, the ^{14}C labelled gas was allowed to pass through tap (ii) into the leaf chamber, the closed circuit was formed by turning tap (i) and the gas circulated at 4l/min for 5 min. The leaf sample was then dropped into boiling 80% ethanol as described above (Section 3d).

(d) Extraction of leaf tissue

The leaf tissue was extracted as described previously (see Section 3e).

(e) Measurement of radioactivity

The radioactive content of the ethanol/water soluble fraction was determined as described (see Section 3f). The total ^{14}C content of the insoluble residue was determined as described previously (see Section 3g).

5. Supply of ^{14}C CO_2 at 300 ppm in Atmospheres Containing Different Proportions of O_2 to Samples of Maize and Tobacco Leaves Treated with NH_4Cl and NaCl Respectively.

(a) Apparatus

The apparatus is shown in the diagram Fig. 2.

(b) Preparation of gas mixtures

The gas mixtures were prepared in a similar manner to that described previously for the supply of 100 ppm ^{14}C CO_2 in various atmospheres (see Section 3b), except that ^{14}C CO_2 was supplied at 300 ppm in the required gas mixture.

(c) Leaf chamber and leaf tissue

The leaf chamber was similar to that used before (see Section 3c and Fig.3). The maize sample comprised three strips of leaf tissue as used previously. The tobacco samples were similar to those used for measurements of rates of photosynthesis using the infra-red gas analyser (see Section 2c).

The rectangular tobacco leaf samples were held in the perspex clamp of the leaf chamber door by the margin of the tissue parallel to the shorter axis.

(d) Procedure

Samples of leaves were detached from the plants and allowed to equilibrate for 1 h, in the leaf chamber in the light, in an atmosphere of air flowing at 4 l/min. During this time their adaxial or dorsal surface was in contact with 20 ml of either a buffered solution of NH_4Cl or NaCl in water. After 1 h the solution was removed and the leaf samples allowed to equilibrate for a further 40 min in contact with 20 ml distilled water. The appropriate gas mixture containing 300 ppm $^{12}\text{CO}_2$ was then allowed to pass through the leaf chamber at 4 l/min for one min, followed by $[\text{}^{14}\text{C}]\text{CO}_2$ (specific activity $3.72 \mu\text{Ci}/\mu\text{mol}$) at 300 ppm in the same gas mixture (4 l/min). After 5 min the leaf sample was killed in boiling ethanol. The various treatments are summarised in Tables 2 and 3.

TABLE:2.

Solutions supplied to samples of maize leaves, supplied with $[\text{}^{14}\text{C}]\text{CO}_2$ at 300 ppm in atmospheres containing different proportions of O_2 for 5 min.

Solutions supplied to samples of leaf during the first hour after excision.

0.2 M sodium phosphate

5.12 mM ammonium chloride in

buffer, pH 7.6 (control)

0.2 M phosphate buffer pH 7.6

O_2 concentration of the atmosphere with 300 ppm $^{14}\text{C} \text{ CO}_2$. (% by volume)

0

0

38

42

76

78

TABLE:3

Solutions supplied to samples of tobacco leaves provided with $[^{14}\text{C}] \text{CO}_2$ at 300 ppm in atmospheres containing different proportions of O_2 .

Solutions supplied to samples of leaf during the first hour after excision

distilled water (control)

5.12 mM NaCl

O_2 concentration of the atmosphere with 300 ppm $^{14}\text{C} \text{ CO}_2$ (% by volume)

0

0

28

25

76

76

(e) Extraction of the leaf tissue

The leaf tissue was extracted in a similar manner to that described previously (see Section 3e).

(f) Measurement of radioactivity

The ^{14}C content of the ethanol/water soluble extract was determined using the proportional gas-flow counter as described (see Section 3f).

6. Supply of $[2 - ^{14}\text{C}]$ glycine, L - $[U - ^{14}\text{C}]$ malic acid and L - $[U - ^{14}\text{C}]$ alanine

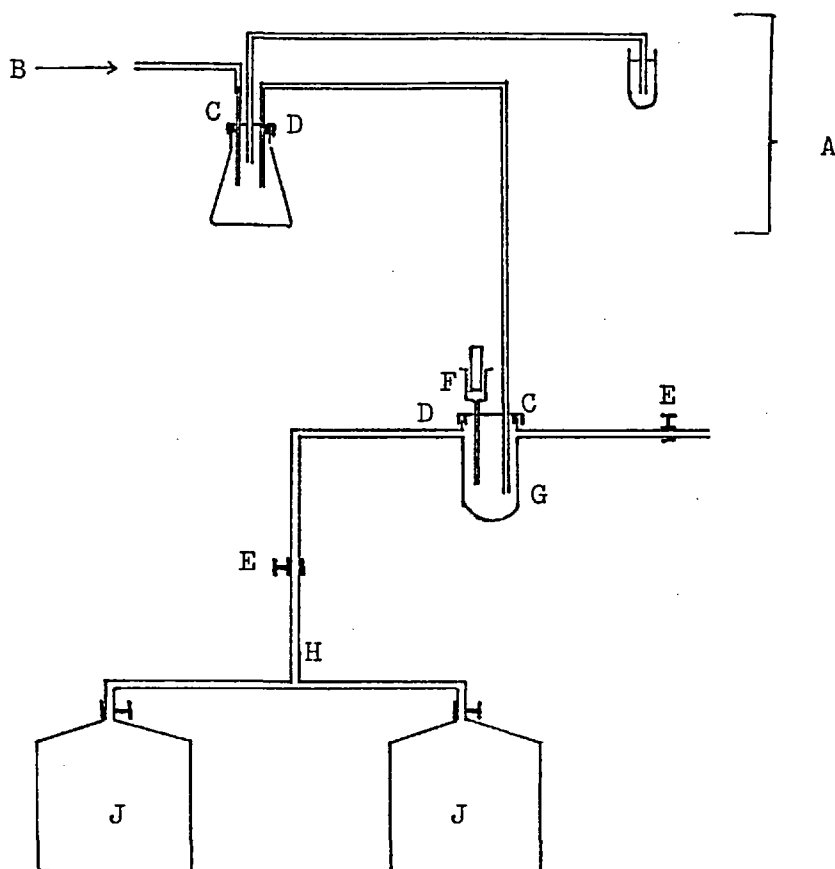
(a) Preparation of gas mixtures

The gas mixtures required were prepared and stored under pressure in metal cylinders, capacity 4.1 litres. The cylinders were cleaned prior to use by evacuation and refilling with air several times and by heating for periods in excess of 12 hours at 100°C in a vacuum oven (Townson & Mercer).

The apparatus used when filling the cylinders is shown in the diagram, Fig. 5. The cylinders and reaction vessel, containing an appropriate weight of Na_2CO_3 were evacuated using a vacuum pump (Model ISC 80, Edwards High

FIGURE:5

Apparatus used to fill storage cylinders with 100 or 5000 ppm $^{12}\text{CO}_2$, or with 100 ppm $[\text{}^{14}\text{C}]\text{CO}_2$, in O_2 -free N_2 or 21% O_2 .



- A, Apparatus to provide a continuous flow of gas at or slightly above atmospheric pressure;
- B, Supply of O_2 -free N_2 or CO_2 -free air under pressure;
- C, Hypodermic needles;
- D, Serum caps;
- E, Screw clips;
- F, Syringe containing 50% v/v lactic acid;
- G, Reaction vessel containing either an appropriate weight of Na_2CO_3 or aqueous $\text{Na}_2[\text{}^{14}\text{C}]\text{CO}_3$ suitably diluted with AR Na_2CO_3 injected after evacuation;
- H, pressure tubing;
- J, Metal gas-storage cylinders.

Vacuum Ltd) and the screwclips closed. Lactic acid (4 ml 50%) was injected through a hypodermic needle inserted into the serum cap of the reaction vessel onto the Na_2CO_3 . The reaction vessel was warmed to facilitate the evolution of carbon dioxide and the screwclip partially opened to allow the gas stream to enter the gas storage cylinders. Either O_2 -free N_2 or CO_2 -free air (special gas mixture supplied by B.O.C. Ltd) from pressurised cylinders was passed through a hypodermic needle inserted through a serum cap into the reaction vessel. When the pressure inside the cylinders was equal to that of the outside, the valves on the cylinders were closed. The cylinders were then filled to 27 lb in^{-2} from the pressurised B.O.C. cylinders through the standard B.O.C. pressure reducing valves. The following gas mixtures were prepared by this method: 0.01% CO_2 in oxygen-free nitrogen, 0.5% CO_2 in oxygen-free nitrogen, 0.01% CO_2 in carbon dioxide-free air and 0.5% CO_2 in carbon dioxide-free air.

(b) Solutions of the ^{14}C substrates.

$[2 - ^{14}\text{C}]$ glycine (specific activity 57 mCi/mM), L - $[U - ^{14}\text{C}]$ malic acid (specific activity 52 mCi/mM) and L - $[U - ^{14}\text{C}]$ alanine (specific activity 52 mCi / mM) were supplied by the Radiochemical Centre, Amersham. Each was made to a suitable volume in H_2O and diluted to $10 \mu\text{Ci}/\mu\text{mole}$ by addition of the required amount of unlabelled substrate. The pH of each solution was adjusted to 5.5 using a solution of NaOH or HCl.

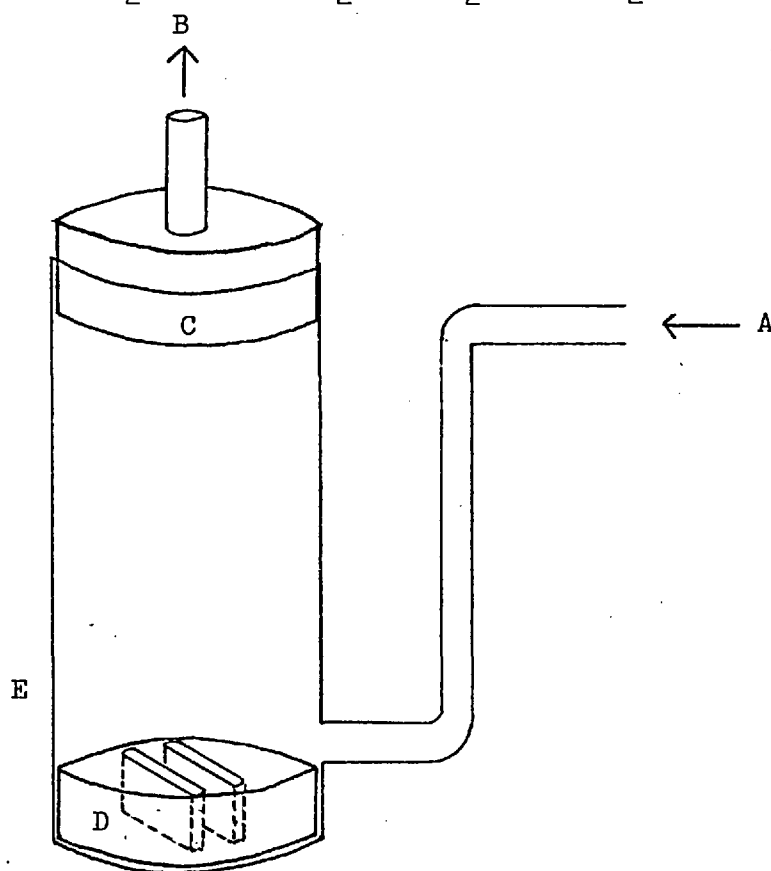
(c) Leaf Samples

Leaf segments, 2 x 1 cm, were cut from the third leaf of a 19 - 23 day old maize plant, using a metal template and a razor blade. The segment of leaf was cut with its longitudinal axis parallel to the midrib, from the middle region between the midrib and margin of the leaf where the total width of this region was 2 cm.

FIGURE:6

Leaf chamber used when samples of maize leaves were supplied with:

- (i) Solutions of ^{14}C substrates $[2-^{14}\text{C}]$ glycine, $[\text{U-}^{14}\text{C}]$ alanine or $[\text{U-}^{14}\text{C}]$ malate.
- (ii) 100 ppm $[\text{U-}^{14}\text{C}]$ CO_2 in either O_2 -free N_2 or 21% O_2 .



A, Entry for gas;

B, Exit for gas;

C, Rubber bung;

D, Perspex block with two slots (each of capacity 0.05ml);

E, Glass tube.

(d) Leaf chambers

Each leaf chamber (Fig. 6) was a 6 cm Pyrex glass tube, diameter 2.1 cm, with an internal volume of approximately 15 ml. Gas was passed into the chamber through a glass side tube near the base and flowed out through a glass tube in a rubber bung sealing the top of the chamber. The leaf sample stood with its basal cut end in one of two slots cut in a perspex block which rested on the bottom of the leaf chamber. Each slot held 0.05 ml of either distilled water or ^{14}C labelled metabolite. Four leaf chambers were fastened to the wall of a clear perspex water bath 24 x 14 x 16 cm and were illuminated laterally by two 150 w reflector spotlamps (Atlas) at 1950 ft candles. The water bath was maintained at constant temperature (28°) by continuous circulation of water from another constant temperature water bath equipped with heating and refrigeration units. The perspex blocks were orientated in the leaf chambers so that the slots were at right angles to the incident light.

During the equilibration period of the leaf tissue air was passed through the leaf chambers. The flow of air (200 ml/min) was regulated by controlling the escape of air through a side tube on the output of the pump with a screw clip, and was measured using a 600 ml flowmeter (Gapmeter supplied G.A. Platon).

(e) Procedure

The samples were cut from the leaf and placed in the leaf chambers with their basal cut ends in 0.05 ml distilled water in the slots furthest away from the incident light. The abaxial or ventral surfaces of the leaf were exposed to the incident light. The segments of leaf were equilibrated for one hour with air flowing through each chamber at 200 ml/min. Samples were then rapidly transferred to the second slot which contained 0.05 ml of substrate solution ($4\mu\text{Ci}$ $0.4\mu\text{mol}$) and at the same time the atmosphere in the leaf chamber was changed to a gas mixture from one of the small

storage cylinders (section 6a). The gas was passed through the leaf chamber at 200 ml/min. The flow rate was controlled by means of a flostat (Type MNBL supplied by G.A. Platon). After 40 min the leaf samples were removed from the leaf chambers and each killed in 10 ml boiling 80% ethanol.

(f) Extraction of leaf tissue

The samples were kept for 2 min in the boiling 80% ethanol after which each sample was cut into small pieces with scissors and ground using an electrically driven glass pestle. The suspension was transferred to a 15 ml pyrex glass tapered centrifuge tube and centrifuged for 4 min (1200 x g). The supernatant liquid was decanted, evaporated to reduce its volume, and then stored in ice. The residue was further extracted by heating it in a boiling water bath with the following solutions: 5 ml 80% ethanol (2 min), 1 ml distilled water (5 min) and 3 ml 50% ethanol (3 min). The suspension was centrifuged between each extraction and the supernatant liquids were combined with the first extract. After evaporation the total volume was adjusted to 5 ml.

The procedure was repeated so that leaf samples were supplied with each of the ^{14}C carbon labelled substrates, glycine, alanine and malic acid in the presence of each of the gas mixtures, 0.01% CO_2 in oxygen-free nitrogen, 0.01% CO_2 in carbon dioxide-free air, 0.5% CO_2 in oxygen-free nitrogen and 0.5% CO_2 in carbon dioxide-free air, in both light and darkness.

(g) Measurement of radioactivity

The ^{14}C content of the ethanol/water soluble fractions was determined as described previously (see Section 3f).

7. Supply of $[^{14}\text{C}] \text{CO}_2$ at 100 ppm in O_2 -free N_2 or CO_2 -free air to
Samples of Maize Leaf in the Light for periods of 20, 40, 60, 120,
300 and 600 seconds

(a) Preparation of gas mixtures

The gas mixtures containing $[^{14}\text{C}] \text{CO}_2$ ($19 \mu\text{Ci}/\mu\text{mole}$; 100 ppm) in O_2 -free N_2 or CO_2 -free air were prepared and stored under pressure in the small storage cylinders as described previously (see Section 6a). $[^{14}\text{C}] \text{CO}_2$ was generated by the action of 1 ml 50% lactic acid on $[^{14}\text{C}] \text{Na}_2\text{CO}_3$ suitably diluted with ANALAR $\text{Na}_2^{12}\text{CO}_3$ ($[^{14}\text{C}] \text{Na}_2\text{CO}_3$, 57 mCi/mM supplied by the Radiochemical Centre, Amersham).

(b) Leaf chambers and leaf tissue

Six leaf chambers similar to those utilized for the supply of ^{14}C labelled substrates were used (see Section 6d and Fig. 6). Pieces of maize leaf, 2 x 1 cm, were used similar to those described previously (see Section 6c).

(c) Procedure

The leaf samples were allowed to equilibrate for one hour with their basal cut ends in 0.05 ml distilled water with air passing 170 ml/min through the leaf chambers. Carbon dioxide-free nitrogen or air was then allowed to pass through the leaf chambers for one min (170 ml/min). The gas was supplied from pressurised (B.O.C. Ltd) cylinders containing oxygen-free nitrogen or carbon dioxide-free air, the flow was controlled by a pressure reducing valve and floatat. Gas mixtures containing the prepared $[^{14}\text{C}] \text{CO}_2$ (100 ppm) were passed through the leaf chambers at 170 ml/min. The leaf samples were removed from the leaf chambers after various times 20, 40, 60, 120, 300 or 600 sec. and killed in 10 ml boiling 80% ethanol.

(d) Extraction of the leaf tissue

The leaf tissue was extracted by the same procedure described in Section 6f.

(e) Measurement of radioactivity

The ^{14}C content of the ethanol/water soluble fractions was determined as described previously (see Section 3f) and the ^{14}C content of the insoluble residues was determined by total combustion in oxygen (see Section 8g).

8. Analysis of ^{14}C Products

Separation and identification of ^{14}C products in the ethanol/water soluble fractions.

(a) Two-dimensional paper chromatography

Aliquots of the ethanol/water soluble fractions were analyzed by two-dimensional descending paper chromatography. Each sample was applied as a 5 x 0.75 cm streak at a suitable distance from one corner of a 46 x 57 cm Whatman No.4 chromatography paper, and dried without heat in air. The first solvent was water-saturated phenol and the second a mixture of butan-1-ol, propionic acid and water prepared as described by Benson, Bassham, Calvin, Goodale, Hass and Stepka (1950). The solvents were not allowed to reach the edge of the paper. The paper was thoroughly dried in air without heat.

(b) Autoradiography

Separated radioactive compounds were located on the paper by autoradiography using 35.6 x 43.2 cm Kodak Crystallex X-ray film. The paper chromatograms were stapled to the film and kept in an envelope to exclude light. The time of exposure to the film depended on the quantity of radioactivity on the chromatogram.

(c) Elution of paper chromatograms

The radioactive areas of paper were cut out and the ^{14}C compounds eluted from the papers with distilled water (approx 3 ml collected for each piece).

(d) Measurement of radioactivity

Aliquots of the eluates were suitably diluted if necessary and the ^{14}C carbon content determined either using a proportional gas flow counter as previously described (see Section 3f) or using a gas flow counter (Nuclear Chicago Model No. 470) 98% argon: 2% propane as carrier gas.

The relative positions of the various compounds on the two-dimensional chromatograms, with water-saturated phenol as the first solvent and butan-1-ol: propionic acid: water as the second solvent, were similar to those reported by Bassham and Calvin (1957) using the same solvents. Therefore the identification of some compounds was based only on the position of the labelled compound on the two-dimensional chromatogram. In many cases, however, compounds isolated by two-dimensional paper chromatography were eluted from the paper chromatograms and identified by chromatography on thin layers of cellulose powder.

(e) Identification of ^{14}C compounds by thin layer chromatography.

The thin layer plates were prepared by spreading a 40 μ layer of cellulose (Whatman thin layer Chromedia CC41) on clean glass plates, either 10 x 20 cm or 20 x 20 cm supplied by Shandon Scientific Ltd using a Shandon spreader and dried in a stream of air.

Samples of eluates were evaporated to dryness over anhydrous CaCl_2 in a vacuum desiccator and the unknown compound dissolved in a small amount of water. This solution was applied to the prepared thin layer plate with suitable authentic marker compounds. The plate was developed using a

suitable solvent in a standard Shandon thin layer tank. The chromatogram was dried in air without heat, marked with radioactive ink and exposed to X-ray film. The image caused by the radioactive ink was used to align the autoradiogram and the thin layer chromatogram. The authentic marker compound was located by treatment of the chromatogram with the appropriate spray reagent, see Table 3. The unknown compounds identified, and the solvent systems in which they co-chromatographed, are shown in Table 4. The identity of sucrose was confirmed by hydrolysis of the sample with invertase and identification of the products, glucose and fructose, by co-chromatography.

(f) Separation of glycine and serine

The separation of glycine and serine was not always clearly defined on two-dimensional paper chromatograms. Glycine and serine, from such chromatograms, were eluted together and the eluate evaporated to dryness. The residue was dissolved in 0.1 ml distilled water and loaded onto 25.4 x 50.8 cm Whatman No.1 Chromatography paper with authentic glycine and serine samples as markers. This chromatogram was developed overnight by the descending method using as solvent a mixture of butan-1-ol, acetone, diethylamine and water (see Table 4). The radioactive compounds were located by autoradiography and the authentic compounds by ninhydrin spray (Table 3). Labelled glycine and serine samples were eluted and the radioactive content determined as described (see Section 3f).

(g) Determination of the total ^{14}C content of ethanol/water insoluble residues by combustion.

Schöniger oxygen combustion flasks, 500 ml (Quickfit and Quartz Ltd) were fitted with platinum baskets made from 25.4 mm squares of 0.025 mm platinum foil (B.D.H.) suspended from the glass hook inside the glass stoppers by platinum wire (S.W.G. 26, 0.46 mm diameter). A weighed sample of the dry residue (approximately 2 mg) was wrapped in a 2 cm square of

Greens no. 105 lens tissue together with a length of cotton thread (1.5 cm long), which had been soaked in a 20% aqueous solution of potassium nitrate and thoroughly dried. The wrapped sample was placed in the platinum basket. The combustion flask, containing 10 ml 0.2N caustic soda solution, was filled with pure oxygen. The cotton thread was ignited and the stopper, with the sample, placed quickly in the flask. The samples were completely combusted. The flasks were shaken for 30 min by an electrically driven shaker to ensure that all the $[^{14}\text{C}] \text{CO}_2$ formed was absorbed in the NaOH solution. The ^{14}C content of the solution was then determined using a Packard tri-carb liquid scintillation spectrometer. 1 ml aliquots were used with 10 ml toluene scintillation fluid with Triton X. 100 2:1 by volume. The scintillation fluid (Patterson and Greene, 1965) contained 0.4% w/v PPO (2, 5-diphenyloxazole) and 0.1% dimethyl-POPOP (1, 4 bis-2 - (4 - methyl - 5 - phenyloxazolyl) - benzene) in ANALAR toluene (Triton X 100, PPO and dimethyl - POPOP scintillation grade supplied by Packard).

The efficiency of combustion was determined by combusting known amounts of $[^{14}\text{C}]$ fructose adsorbed on 1 cm squares of Whatman No.1 paper. The recovery of ^{14}C was found to be 100%.

TABLE:3

Location Reagents for Paper and Thin-layer Chromatography

Compounds Detected	Location Reagent
Amino acids	Indane trione hydrate (ninhydrin) 0.1% w/v in absolute ethanol. After spraying, the chromatogram was heated (80 - 100°C) for a few minutes.
Organic acids	0.1% w/v bromocresol green in 99.5% ethanol. After addition of N NaOH until the colour of the bromocresol green solution was blue-green, the solution was diluted 1:4 with acetone (Smith, 1960).
Sugars	1% aniline and 1% diphenylamine in 10:1 v/v acetone: 85% phosphoric acid. After spraying, the chromatogram was heated (95 - 100°C) for a few minutes. (Smith, 1960).
Phosphate compounds	60% perchloric acid: N HCl: 4% ammonium molybdate: water, 5:10:25:60 v/v. The chromatogram was dried and exposed to ultra-violet light for 5 min (Dawson <u>et al</u> , 1959).

TABLE:4

Solvents used for the identification of the compounds shown below by thin-layer chromatography.

Solvent	Proportions mixed	Reference	Compound Identified
n-propyl acetate: 90% formic acid: water (twice)	11:5:3	Bieleski and Young (1963)	sucrose, sugar phosphates, malate, glycerate, 3-PGA, glycollate, citrate, phosphoenol pyruvate.
propan-1-ol: ammonia (s.g. 0.880) :water	6:3:1	Bieleski and Young (1963)	malate, glycerate, sugar phosphates
butan-1-ol: acetic acid: water	74:19:50	Benson <u>et al</u> (1950)	malate.
ethanol: 1M ammonium acetate, pH 7.5	7:5:3	Paladini and Leloir (1952)	3-PGA, citrate
butan-1-ol: acetone: diethylamine: water	10:10:2:5	Von Arx and Neher (1963)	aspartate, glycine, serine, threonine, alanine
propan-2-ol: 100% formic acid: water	40:2:10	Von Arx and Neher (1963)	glutamate, glycine, serine, alanine, aspartate.
water-saturated phenol (with 3% aqueous ammonia in equilibrium with the gas phase)	75:25	Von Arx and Neher (1963)	glycine, serine, threonine, aspartate glutamate

RESULTS

I. Rates of Photosynthesis by Samples of Maize and Tobacco Leaves in Gas Mixtures Containing Different Proportions of Oxygen and Carbon Dioxide

1. Preliminary Experiments
2. Measurements of Rates of Photosynthesis by Samples of Maize Leaf.
 - (a) Measurements from 10,000 ppm to the CO_2 compensation point.
 - (b) Measurements from 1000 ppm to the CO_2 compensation point.
 - (c) Measurements from 100 ppm to the CO_2 compensation point.
3. Measurements of Rates of Photosynthesis by Samples of Tobacco Leaves, (Measurements from 1000 ppm to CO_2 compensation point).

II. Rates of Photosynthesis by Samples of Maize and Tobacco Leaves Treated with Solutions of NH_4Cl or NaCl , in Gas Mixtures Containing Different Proportions of Oxygen and Carbon Dioxide. (Measurements from 1000 ppm to the CO_2 compensation point).

1. Preliminary Experiments.
2. Maize Leaves
 - (a) Leaf samples treated with solutions of NH_4Cl in phosphate buffer during the first hour after excision.
 - (b) Leaf samples treated with solutions of NH_4Cl in HEPES buffer during the first hour after excision.
3. Tobacco Leaves.

Samples of leaf treated with solutions of NH_4Cl in HEPES buffer.
4. Rates of Photosynthesis by Samples of Maize and Tobacco Leaves treated with NaCl During the First Hour after Excision.

(a) Maize Leaves

(b) Tobacco Leaves

III. Incorporation of ^{14}C into Ethanol/ H_2O -Soluble Products During 5 min Photosynthesis by Maize and Tobacco Leaves in Atmospheres Containing 300 ppm $[\text{}^{14}\text{C}]\text{CO}_2$

IV. Total Carbon Assimilated and the Distribution of ^{14}C between Products of 5 min Photosynthesis by Samples of Maize Leaves in 100 or 5000 ppm $[\text{}^{14}\text{C}]\text{CO}_2$ in Atmospheres of either O_2 -free N_2 , 21% O_2 or 80% O_2 .

1. Total Carbon Assimilated.

2. Distribution of ^{14}C Between Products Extracted from Maize Leaf After 5 min Photosynthesis in $[\text{}^{14}\text{C}]\text{CO}_2$.

V. ^{14}C in Various Compounds Formed by Samples of Maize Leaf During 5 min Photosynthesis in Atmospheres Containing $[\text{}^{14}\text{C}]\text{CO}_2$.

VI. Photosynthesis by Segments of Maize Leaf in O_2 -free N_2 or Air Containing 100 ppm $[\text{}^{14}\text{C}]\text{CO}_2$.

1. Total Carbon Assimilated.

2. Amounts of ^{14}C in Various Compounds Extracted with Ethanol and H_2O Following Photosynthesis in Atmospheres Containing 100 ppm $[\text{}^{14}\text{C}]\text{CO}_2$.

VII. Total Carbon Assimilated and Amounts of ^{14}C in Products of 5 min Photosynthesis by Maize Leaves treated with 5.12 mM Solutions of NH_4Cl in 0.2 M Phosphate Buffer or 0.2 M Phosphate Buffer.

VIII. Total Carbon Assimilated and Amounts of ^{14}C in Products of 5 min Photosynthesis by Tobacco Leaves treated with 5.12 mM Solutions of NaCl or Distilled Water.

IX. Metabolism of $[\text{U} - ^{14}\text{C}]$ Malate by Maize Leaf.

^{14}C Products formed in the Light.

^{14}C Products formed in the Dark.

Differences in Metabolism of $[\text{U} - ^{14}\text{C}]$ Malate in Light and Darkness.

X. Metabolism of $[\text{U} - ^{14}\text{C}]$ Alanine by Maize Leaf.

^{14}C Products Formed in the Light.

^{14}C Products Formed in the Dark.

Differences in $[\text{U} - ^{14}\text{C}]$ Alanine Metabolism in Light and Darkness.

XI. Metabolism of $[\text{2} - ^{14}\text{C}]$ Glycine by Maize Leaf.

^{14}C Products Formed in the Light.

^{14}C Products Formed in the Dark.

Differences in the Metabolism of $[\text{2} - ^{14}\text{C}]$ Glycine in Light and Darkness.

I. Rates of Photosynthesis by Samples of Maize and Tobacco Leaves in Gas Mixtures Containing Different Proportions of Oxygen and Carbon Dioxide

1. Preliminary Experiments.

Changes in the rate of photosynthesis were investigated after the leaves had been detached from the plant. Samples were kept in the leaf chamber for one hour in a stream of air. After this the rates of photosynthesis were measured (see Materials and Methods 2) continuously for 4 hours. Although the rates of photosynthesis with 600, 300 and 100 ppm CO_2 in 21% O_2 decreased with time, the decrease was not appreciable for the first two hours after equilibration in air. Subsequent measurements were made therefore between 1 and 3 hours after the leaves were detached from the plants.

Experiments were also conducted to determine whether the inhibition of photosynthesis by atmospheres containing 100% O_2 was reversible. Samples of maize leaf were equilibrated for 1 hour in air and the rates of photosynthesis with 600, 300 and 100 ppm CO_2 were measured in air. The measurements were repeated with CO_2 in an atmosphere of 100% O_2 , and then again with CO_2 in air. Rates of photosynthesis in 100% O_2 were always less than rates in air, and the rates of photosynthesis in air following measurements in 100% O_2 were lower than the rates in air before exposure of samples of leaf to 100% O_2 . Therefore the effect of high oxygen concentration on the rate of photosynthesis was not reversible and it was not possible to randomise the order in which atmospheres containing different concentrations of O_2 were supplied during an experiment. The gas mixtures were therefore used in a sequence in which the O_2 concentration was increased so that photosynthetic rate in 100% O_2 was the last measurement made. It was found, following such a sequence, that if measurements were repeated the rate of photosynthesis in air was always less than the rate measured before exposure to atmospheres of high O_2 concentration.

2. Measurements of Rates of Photosynthesis by Samples of Maize Leaf.

The CO_2 compensation point of samples of maize leaf was near zero and was independent of the ambient O_2 concentration.

(a) Measurements from 10,000 ppm to the CO_2 compensation point.

Rates of photosynthesis by samples of maize leaf were not significantly affected by CO_2 concentration in the range 1000 to 6000 ppm but, with this range of CO_2 concentration, rates were decreased by 35 to 40% if the O_2 concentration in the atmospheres was increased from 0 to 100% (Fig.7).

(b) Measurements from 1000 ppm to the CO_2 compensation point.

Rates of photosynthesis at 600, 300 and 100 ppm CO_2 in different gas mixtures are shown in Fig.8. Increased CO_2 concentration from 100 ppm to 600 ppm increased the rate of photosynthesis about 3 times. The actual rates for different leaf samples varied, presumably because of the inherent variability of the plant material. For any particular sample rates were higher in air than in an atmosphere of N_2 , but with increasing O_2 concentration above 50% the rate of photosynthesis decreased and in 100% O_2 was always less than in either air or N_2 . The increased rate of carbon fixation in air compared to N_2 was greater at low CO_2 concentrations (less than 300 ppm CO_2). Decreased photosynthesis in N_2 , compared with air, was not caused by a contaminant in the N_2 gas because similar results (Fig.9) were obtained when N_2 in the gas mixtures was replaced by argon.

(c) Measurements from 100 ppm CO_2 to the CO_2 compensation point.

Rates of photosynthesis at 60, 30 and 10 ppm CO_2 responded to increased O_2 concentration in the same manner as rates at 600, 300 and 100 ppm CO_2 . The rates of photosynthesis were less in pure N_2 and at high O_2 concentrations

than they were with 21 to 40% O_2 in the atmosphere. At very low CO_2 concentrations, below 30 ppm, the effect was small.

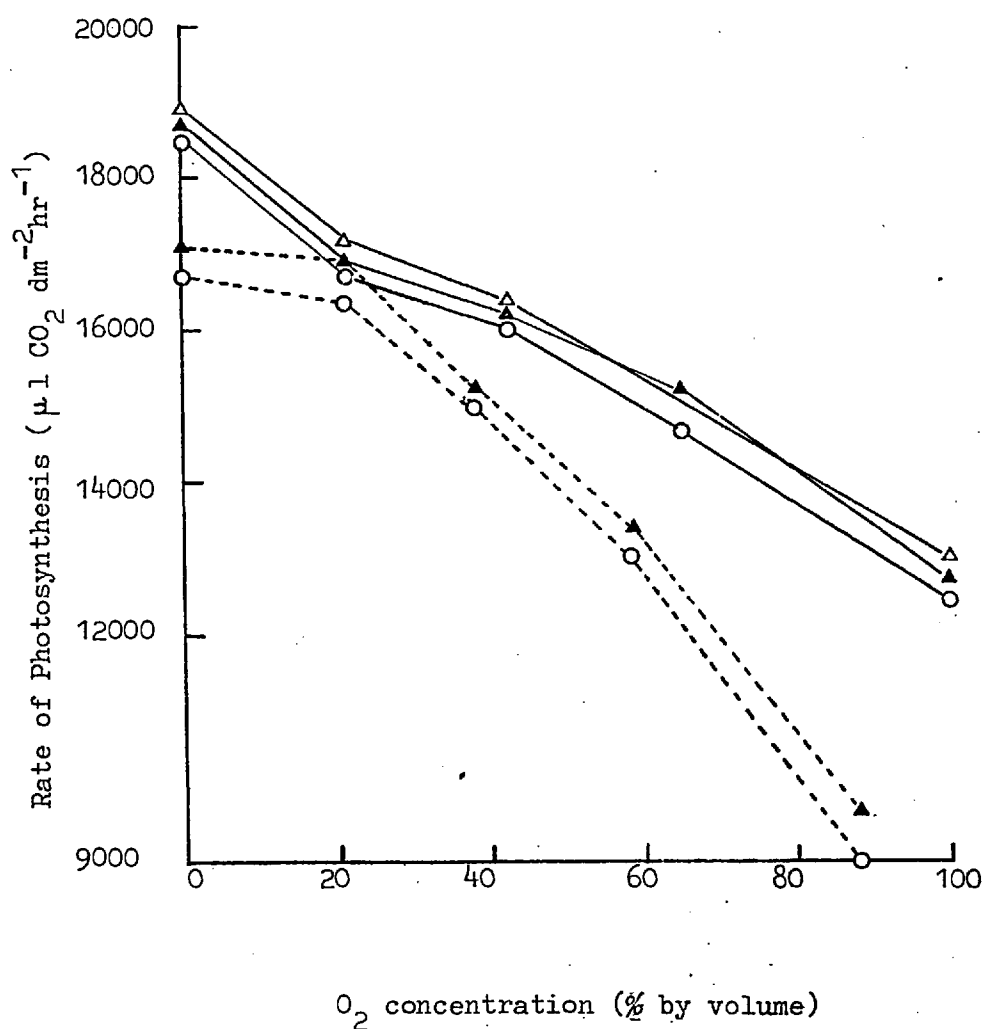
3. Measurements of Rates of Photosynthesis by Samples of Tobacco Leaves (Measurements from 1000 ppm to CO_2 compensation point).

The CO_2 compensation point of samples of tobacco leaf was 120 ppm at 21% O_2 and was increased when the atmosphere contained more O_2 (at 100% O_2 the CO_2 compensation point value was 380 ppm).

Photosynthesis by samples of tobacco leaves was increased when the concentration of CO_2 was increased in the range 300 to 700 ppm, at these concentrations of CO_2 the rate of photosynthesis was greatest when the atmosphere contained no oxygen (Fig.10).

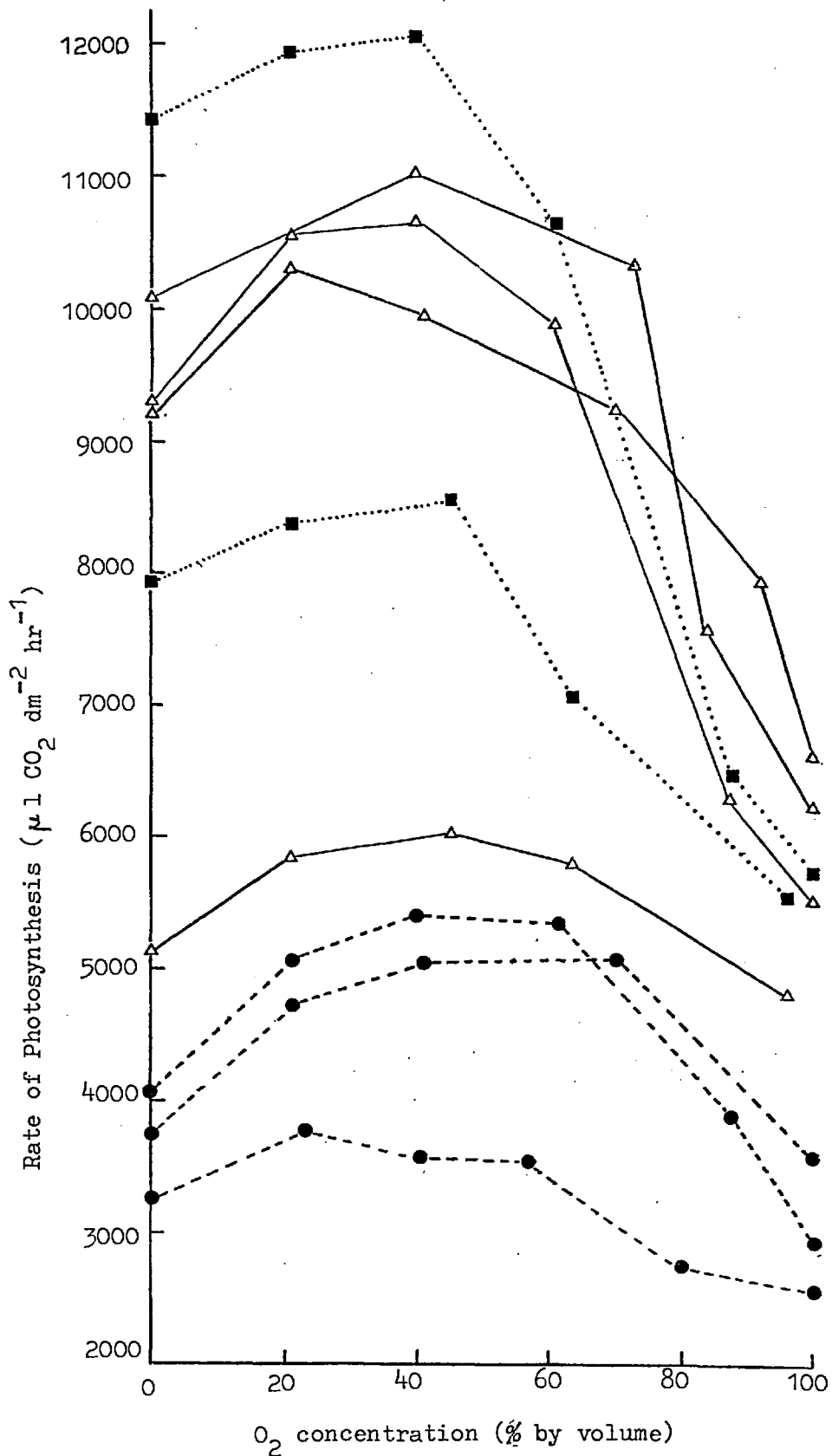
FIGURE:7

Rates of Photosynthesis by Maize Leaves at 6000 ppm, 3000 ppm and 1000 ppm CO_2 in Atmospheres Containing Different Concentrations of O_2 .



The rates of photosynthesis by samples of maize leaves, A— and B-----, were measured on different occasions at 6000 ppm (Δ), 3000 ppm ($\blacktriangle$) and 1000 ppm (o) CO_2 in the presence of different concentrations of O_2 using an I.R.G.A. The light intensity was 1950 ft candles and the temperature was 28°C .

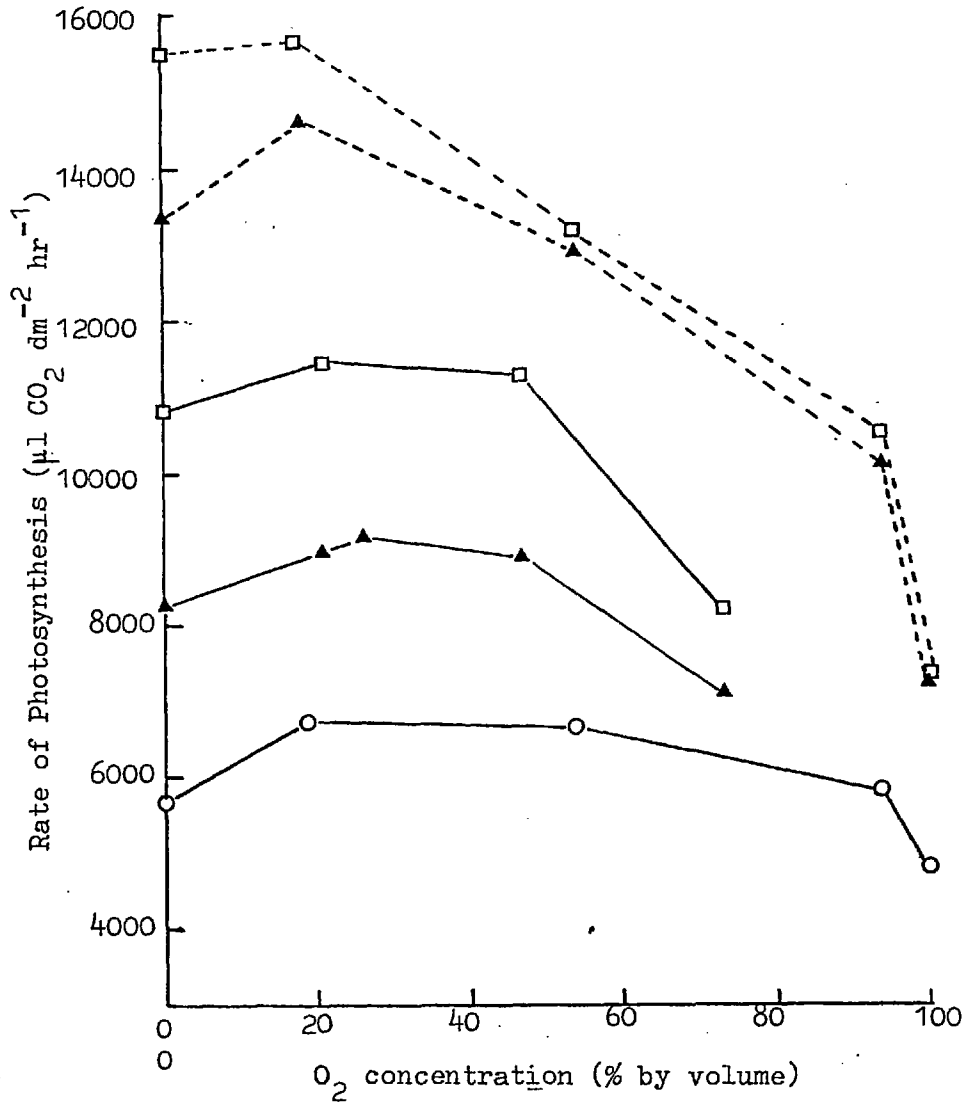
Rates of Photosynthesis by Maize Leaves at 600, 300 and 100 ppm CO_2 in Atmospheres Containing Different Concentrations of O_2 .



The rates of photosynthesis by samples of maize leaves were measured at 600 ppm (■.....■), 300 ppm (Δ——Δ) and 100 ppm (●-----●) in the presence of different concentrations of O_2 . The light intensity was 1950 ft candles and the temperature was 28°C .

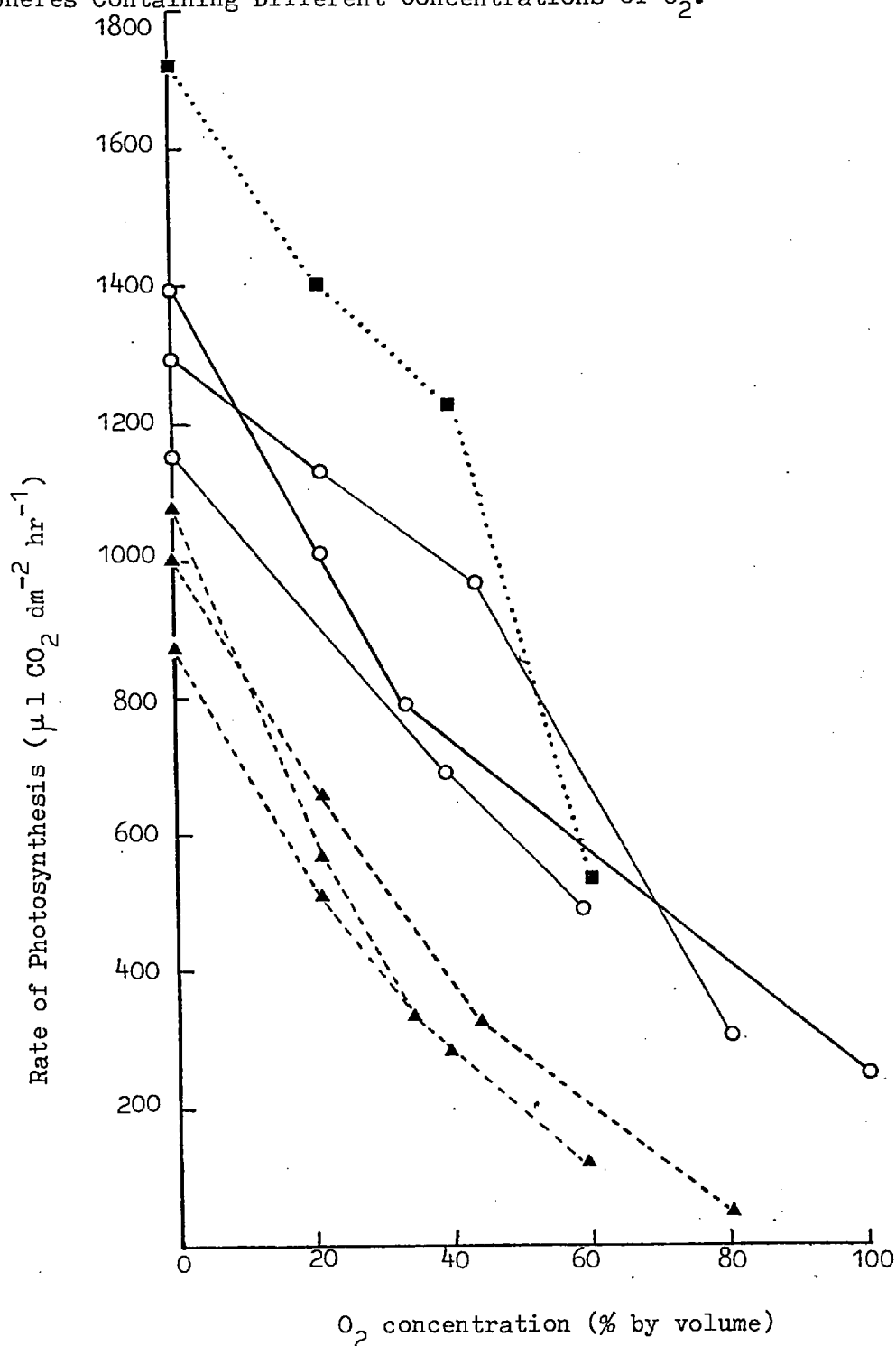
FIGURE:9

Rates of Photosynthesis by Maize Leaves at 600, 300 and 100 ppm CO_2 in Atmospheres Containing Different Concentrations of O_2 .



The rates of photosynthesis by samples of maize leaves, A---- and B—, were measured on different occasions at 600 ppm ($\square$), 300 ppm ($\blacktriangle$) and 100 ppm ($\circ$) CO_2 in the presence of different concentrations of O_2 in argon using an IRGA. The light intensity was 1950 ft candles and the temperature was 28°C .

Rates of Photosynthesis by Tobacco Leaves at 700, 600 and 300 ppm CO_2 in Atmospheres Containing Different Concentrations of O_2 .



The rate of photosynthesis by samples of tobacco leaves were measured at 700 ppm ($\blacksquare$), 600 ppm ($\circ$) and 300 ppm ($\blacktriangle$) CO_2 in the presence of different concentrations of O_2 using an IRGA. The light intensity was 1950 ft candles and the temperature was 28°C .

II. Rates of Photosynthesis by Samples of Maize and Tobacco Leaves Treated with Solutions of NH_4Cl or NaCl , in Gas Mixtures Containing Different Proportions of Oxygen and Carbon Dioxide (Measurements from 1000 ppm to the CO_2 compensation point).

1. Preliminary Experiments

Following treatment with NH_4Cl solution for 1 hr steady rates of photosynthesis, while the leaf sample was floated on distilled water, were not attained for 40 min. After this time rates of photosynthesis in air or N_2 were almost constant for 120 min. Similar conclusions were reached after treatment of samples with solutions of NH_4Cl in phosphate buffer. In the following experiments, therefore, the rates of photosynthesis were measured during the 2 hr subsequent to treatment of samples of leaf in the salt solutions and equilibration for 40 min floating on distilled water.

2. Maize Leaves.

(a) Leaf samples treated with solutions of NH_4Cl in phosphate buffer during the first hour after excision.

The effects of 2.56 or 5.12 mM NH_4Cl in 0.2 M phosphate buffer on rates of photosynthesis of samples of maize leaves are shown in Fig.11. Rates of photosynthesis after treatment of samples of leaf with phosphate buffer (control) responded as described previously to the concentration of CO_2 and O_2 in the atmosphere (see Fig.8), namely the rate of photosynthesis was less in N_2 or 80% O_2 than in 21% to 40% O_2 . Rates of photosynthesis of samples treated with 2.56 mM NH_4Cl in phosphate buffer responded in a similar manner to the control samples. Following treatments with 5.12 mM NH_4Cl in phosphate buffer the rate of photosynthesis in N_2 was not less than the rate in 21% to 40% oxygen.

- (b) Leaf Samples treated with solutions of NH_4Cl in HEPES buffer during the first hour after excision.

The rates of photosynthesis by samples of maize leaves treated with solutions of 0.01 M HEPES or 5.12 mM NH_4Cl in 0.01 M HEPES are shown in Fig.12. The rate of photosynthesis in O_2 -free N_2 relative to that in atmospheres containing 21 to 40% O_2 was greatest when the samples of leaf were treated in HEPES buffer containing 5.12 mM NH_4Cl .

3. Tobacco Leaves

Samples of leaf treated with solutions of NH_4Cl in HEPES buffer during the first hour after excision.

The rates of photosynthesis by samples of tobacco leaves treated with solutions of 5.12 mM NH_4Cl in HEPES buffer are shown in Fig.13. The rates decreased with increased O_2 concentration in the atmosphere in a similar manner to the response described previously (Fig.10) for untreated samples. Photosynthesis was most rapid in N_2 .

4. Rates of Photosynthesis by Samples of Maize and Tobacco Leaves Treated with NaCl During the First Hour after Excision.

The object of these experiments was to determine whether NaCl had the same effect on rates of photosynthesis as NH_4Cl .

(a) Maize Leaves.

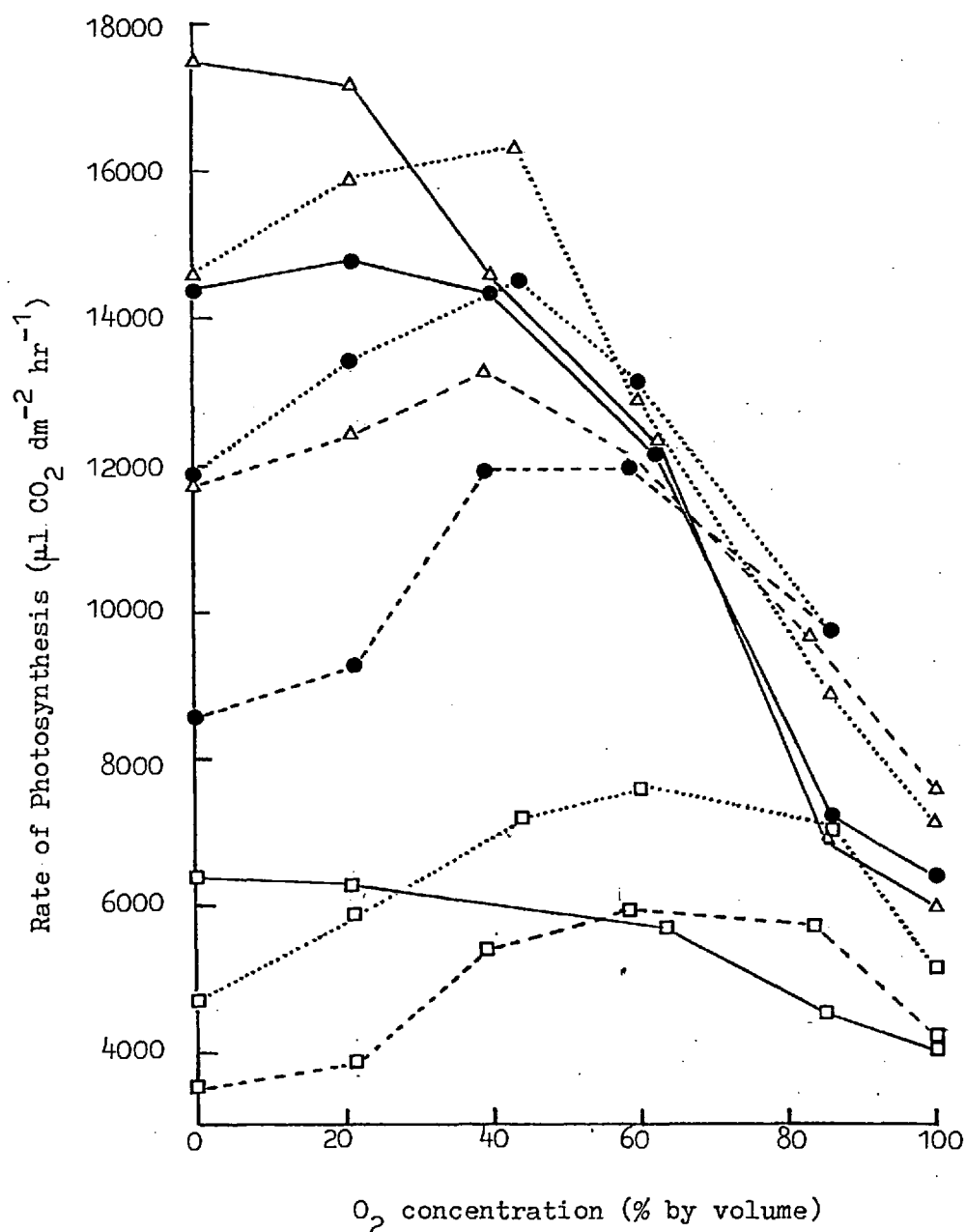
No significant effects were observed of NaCl on rates of photosynthesis by samples of maize leaf in atmospheres containing various concentrations of CO_2 and O_2 (Fig.14).

(b) Tobacco Leaves.

The effect of 5.12 mM NaCl on the rates of photosynthesis of samples of tobacco leaves was to make the response to O_2 in the atmosphere similar to that of untreated samples of leaf from maize. The rates were less in N_2 than in air and least in atmospheres containing 100% O_2 see Fig.14.

FIGURE:11

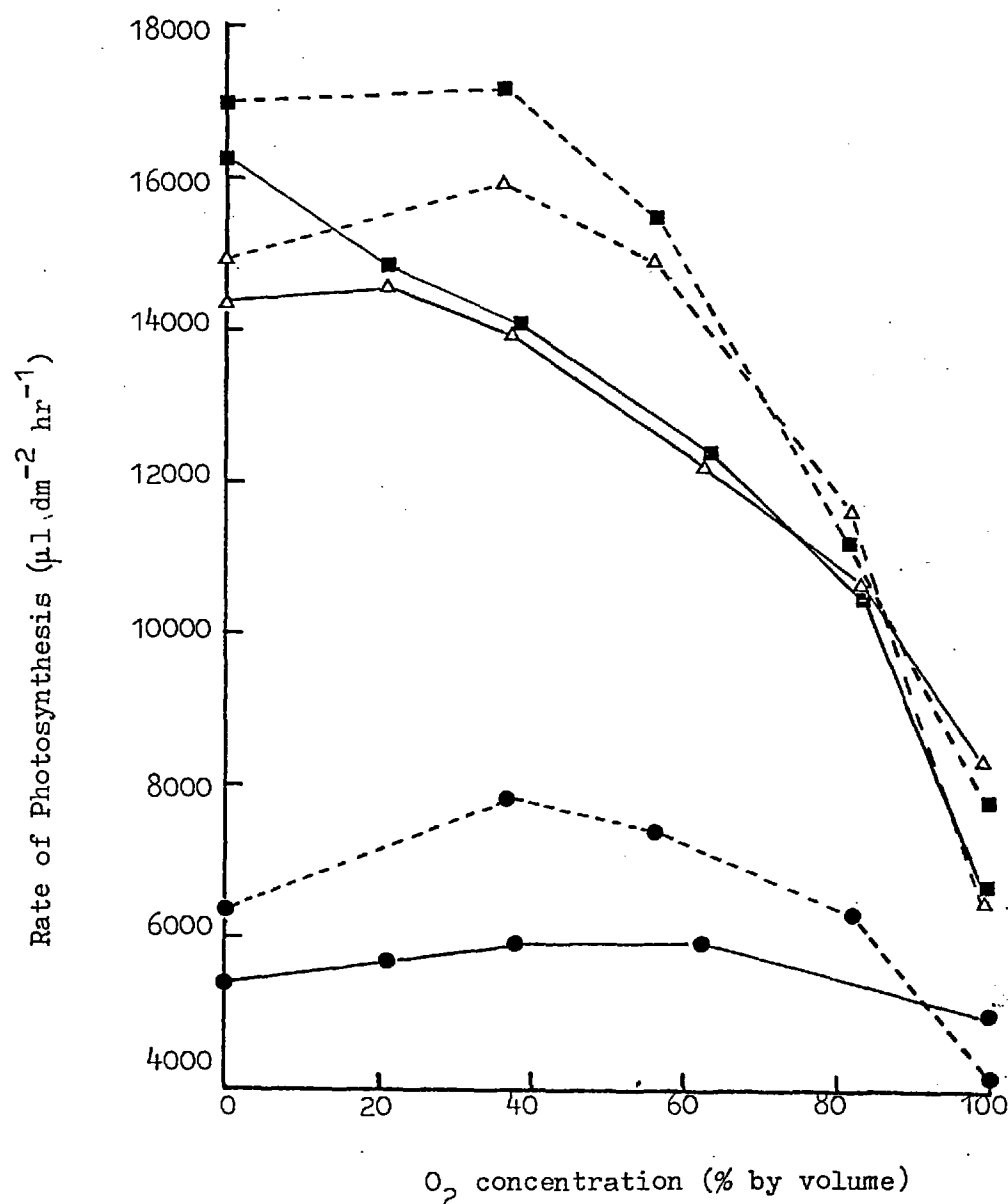
Rates of Photosynthesis by Maize Leaves Treated with Solutions of NH_4Cl in Phosphate Buffer (pH 7.6) During the first Hour After Excision.



The rates of photosynthesis by samples of maize leaves were measured at 600 ppm (Δ), 300 ppm (●) and 100 ppm (□) CO_2 in the presence of different concentrations of O_2 . The samples were floated during the first hour after excision on the following solutions: 0.2 M phosphate buffer (.....), 2.15 mM NH_4Cl solution in 0.2 M phosphate buffer (-----) or 5.12 mM NH_4Cl in 0.2 M phosphate buffer (——). The light intensity was 1950 ft candles and the temperature was 28°C.

FIGURE:12

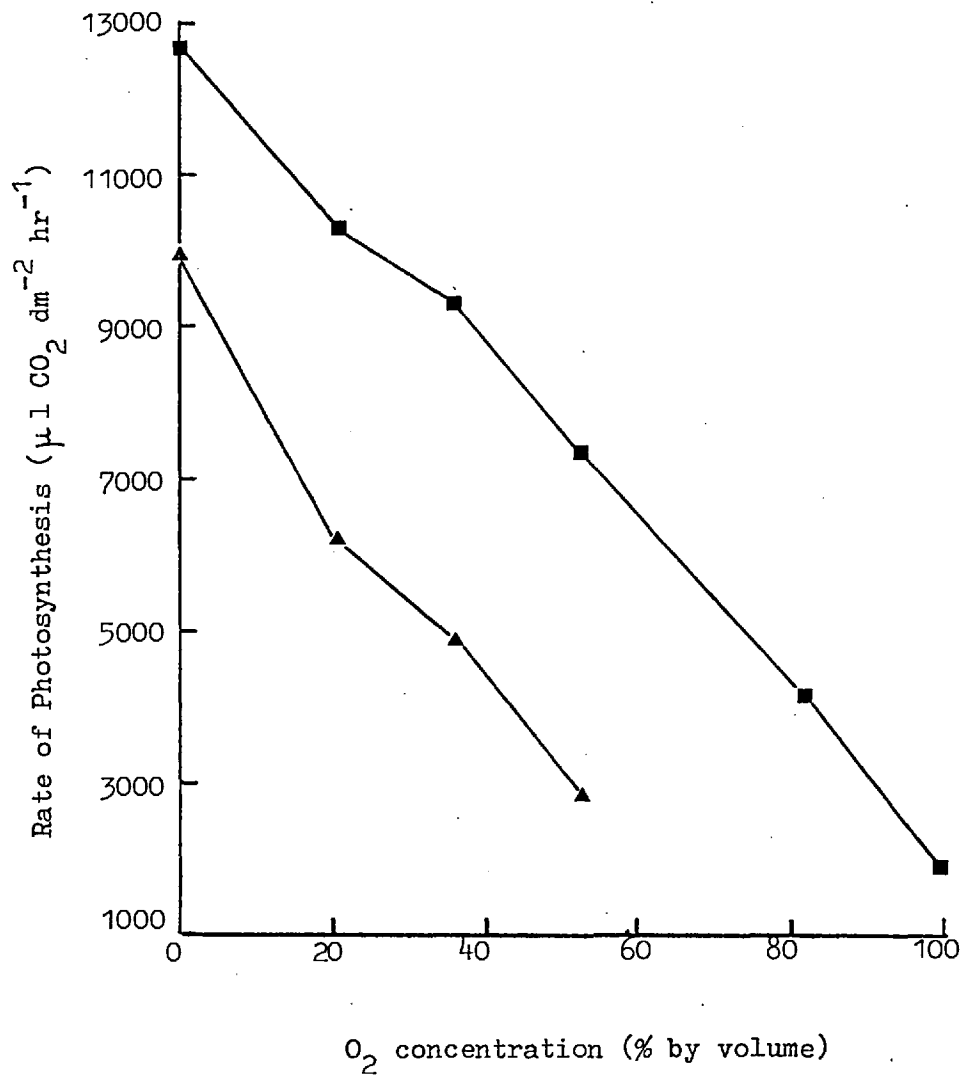
Rates of Photosynthesis by Maize Leaves Treated with Solutions of NH_4Cl in HEPES Buffer (pH 7.4) During the First Hour after Excision.



The rates of photosynthesis by samples of maize leaves were measured at 600 ppm (■), 300 ppm (Δ) and 100 ppm (●) CO_2 in the presence of different concentrations of O_2 using an I.R.G.A. The samples were floated during the first hour after excision on either 0.01 M solution of HEPES buffer (-----) or 5.12 mM solution of NH_4Cl in 0.01 M HEPES buffer (——). The light intensity was 1950 ft candles and the temperature was 28°C .

FIGURE:13

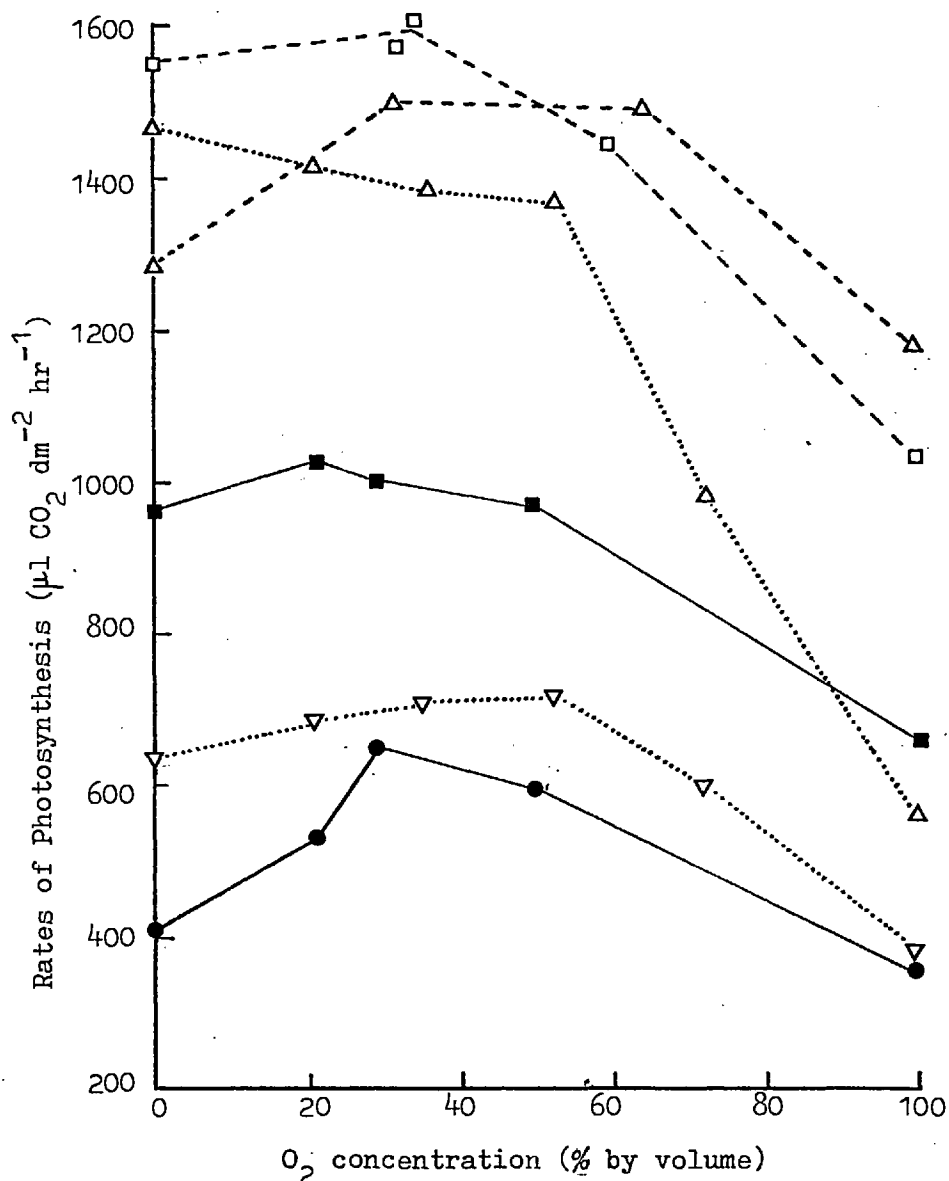
Rates of Photosynthesis by Tobacco Leaves Treated with Solutions of NH_4Cl in HEPES Buffer, pH 7.4, During the First Hour After Excision.



Rates of photosynthesis by samples of tobacco leaf were measured at 600 ppm (■—■) and 300 ppm (▲—▲) CO_2 in the presence of different concentrations of O_2 using an I.R.G.A. The samples were floated on a solution of 5.12 mM NH_4Cl in 0.01 M HEPES buffer during the first hour after excision. The light intensity was 1950 ft candles and the temperature was 28°C.

FIGURE:14

Rates of Photosynthesis by Maize and Tobacco Leaves Treated with Solutions of NaCl During the First Hour After Excision.



The rates of photosynthesis by samples of tobacco leaf were measured at 600 ppm (■—■) and 400 ppm (●—●) CO₂ in the presence of different concentrations of O₂ using an I.R.G.A. Before making the measurements the samples were floated on a solution of 5.12 mM NaCl during the first hour after excision. Samples of maize leaves were floated either on a solution of 10.2 mM NaCl (----) or a solution of 5.12 mM NaCl (.....) during the first hour after excision. Rates of photosynthesis of these samples were then measured at 600 ppm (□), 300 ppm (Δ) and 100 ppm (▽) CO₂ in the presence of different concentrations of O₂. The light intensity was 1950 ft candles and the temperature was 28°C.

III. Incorporation of ^{14}C into Ethanol/ H_2O -Soluble Products During 5 min
Photosynthesis by Maize and Tobacco Leaves in Atmospheres Containing
300 ppm ^{14}C CO_2 .

The effects of O_2 concentration on the distribution of ^{14}C among the soluble products of photosynthesis by samples of maize and tobacco leaves is shown in Table 5. There was a decrease in the proportion of ^{14}C incorporated into sucrose with increased O_2 concentration; this effect was most marked in the samples of tobacco leaves. The % ^{14}C incorporated into glycine and serine was considerably greater in tobacco than in maize; there was more ^{14}C in serine than in glycine in tobacco with all gas mixtures; conversely there was more ^{14}C in glycine than in serine in maize samples except in the presence of an atmosphere devoid of O_2 . In maize and tobacco more ^{14}C glycine was formed in the atmospheres containing O_2 than where O_2 was absent. More ^{14}C was incorporated into glutamic acid in atmospheres of O_2 -free N_2 and 39% O_2 by maize than by tobacco leaf but there was no significant difference in the amount of ^{14}C in glutamic acid between maize and tobacco samples at 76% O_2 . The amount of ^{14}C incorporated into glyceric acid was greater in tobacco than maize and, in both, the amount was increased with increasing O_2 concentration.

More ^{14}C was incorporated into alanine in maize than in tobacco leaf, and ^{14}C alanine was markedly decreased in the sample of maize leaf when the O_2 content of the atmosphere was increased to 76%. Malic and aspartic acids were much more labelled in maize than in tobacco leaves in all treatments. The % of soluble ^{14}C in phosphate esters was greater in samples of tobacco leaf than in samples of maize leaf except in the presence of a high O_2 concentration (76%) when the reverse was true.

TABLE:5

Label (^{14}C) in Various Compounds in Leaf Tissue from Maize or Tobacco Plants
Following Photosynthesis for 5 min in Atmospheres Containing $[\text{}^{14}\text{C}]\text{CO}_2$.

Leaf	Maize			Tobacco		
Pretreatment	0.2M phosphate buffer			distilled water		
O_2 concentration of atmosphere	O_2 -free N_2	38% O_2	76% O_2	O_2 -free N_2	28% O_2	76% O_2
Product	(^{14}C in the various products as a % of total ^{14}C in the extract)					
sucrose	28.40	23.92	23.19	38.36	28.35	17.30
glycine	0.002	2.70	3.74	0.60	5.78	7.72
serine	0.44	2.10	0.59	5.06	12.69	15.40
glyceric acid	0.08	0.26	0.52	0.16	0.97	3.38
glutamic acid	1.36	2.48	1.09	0.41	1.14	1.48
alanine	6.78	6.80	1.77	1.26	0.95	0.85
malic acid	9.97	9.12	11.68	0.68	1.77	1.38
aspartic acid	7.54	9.82	8.57	2.19	1.12	1.69
phosphate esters	21.07	23.87	31.74	28.19	26.27	19.45
other compounds	0.27	0.58	0.37	1.28	3.17	3.98
loss	24.10	18.48	16.82	21.81	17.79	27.37

The light intensity was 1950 ft candles, temperature 28°C , and the specific activity of the CO_2 $3.72\ \mu\text{Ci}/\mu\text{mol}$. The proportion of O_2 in the atmosphere was varied as shown and the concentration of $[\text{}^{14}\text{C}]\text{CO}_2$ was 300 ppm.

IV. Total Carbon Assimilated and the Distribution of ^{14}C between Products of 5 min Photosynthesis by Samples of Maize Leaves in 100 or 5000 ppm $[^{14}\text{C}]\text{CO}_2$ in Atmospheres of either O_2 -free N_2 , 21% O_2 or 80% O_2 .

Leaf samples were maintained at 28°C and 1950 ft candles with 100 ppm $[^{14}\text{C}]\text{CO}_2$ ($6.2\mu\text{Ci}/\mu\text{mol}$) or 5000 ppm $[^{14}\text{C}]\text{CO}_2$ ($1.89\mu\text{Ci}/\mu\text{mol}$) (see Materials and Methods sections 3, 4 and 8).

1. Total Carbon Assimilated

The total carbon assimilated by samples of maize leaf during 5 min photosynthesis is shown in Table 6. At 100 ppm CO_2 less carbon was assimilated in an atmosphere consisting of N_2 than in an atmosphere with 21% O_2 and least in an atmosphere with 80% O_2 . This confirms the results, described previously, when an I.R.G.A. was used to determine rates of photosynthesis. (see Results section I.2(b)). The results obtained at 5000 ppm CO_2 may be anomalous to the extent that the greatest uptake of CO_2 was found when the atmosphere contained the highest concentration of O_2 . The total carbon assimilated was increased by increasing the CO_2 concentration from 100 ppm to 5000 ppm and the ^{14}C incorporated into the ethanol/ H_2O soluble fractions constituted the major proportion of the total carbon assimilated.

2. Distribution of ^{14}C Between Products Extracted from Maize Leaf After 5 min Photosynthesis in $[^{14}\text{C}]\text{CO}_2$.

The total carbon fixed and the ^{14}C in compounds extracted by ethanol and water were determined in atmospheres consisting of either 100 ppm $[^{14}\text{C}]\text{CO}_2$ ($6.2\mu\text{Ci}/\mu\text{mol}$) or 5000 ppm $[^{14}\text{C}]\text{CO}_2$ ($1.89\mu\text{Ci}/\mu\text{mol}$) in O_2 -free N_2 or 21% O_2 (Experiment A). Subsequently the experiment was repeated and additional determinations of the total carbon fixed and the distribution of ^{14}C in the ethanol/ H_2O soluble fraction were carried out in atmospheres

TABLE:6

Total Carbon Assimilated by Samples of Maize Leaves During 5 min of Photosynthesis in Atmospheres Containing $[^{14}\text{C}]\text{CO}_2$ and the Distribution Between the Ethanol Soluble and Ethanol Insoluble Fractions.

CO ₂ concentration	100 ppm						5000 ppm			
O ₂ concentration (% by volume)	0	21	80	0	21	80	0	21	80	
Experiment	A	B	A	B	A	B	A	B	A	B
(carbon assimilated expressed as g atoms of carbon /g fresh wt leaf tissue)										
Total carbon uptake	6.51	10.09	7.50	12.58	- 8.70	31.90	19.99	17.36	19.77	- 22.56
Total carbon incorporated into ethanol/H ₂ O soluble fraction	6.23	9.70	7.24	12.00	- 8.30	30.35	18.90	15.80	18.90	- 21.60
Total carbon incorporated into ethanol/H ₂ O insoluble fraction	0.28	0.39	0.26	0.58	- 0.4	1.55	1.09	1.56	0.87	- 0.96

Atmospheres contained different proportions of O₂ as shown with 100 ppm CO₂ labelled with ^{14}C , 6.2 $\mu\text{Ci}/\mu\text{mol}$ or 5000 ppm CO₂ labelled with ^{14}C , 1.89 $\mu\text{Ci}/\mu\text{mol}$. The light intensity was 1950 ft candles and the temperature was 28°C.

TABLE:7

^{14}C Products Extracted with Ethanol and Water After 5 min Photosynthesis
by Samples of Maize Leaf.

CO ₂ concentration	100 ppm					5000 ppm				5000 ppm		
O ₂ concentration	21					21				0		
(% by volume)												
Experiment	A		B		A		B		A		B	
Chromatogram	a	b	c	d	e	f	g	h	j	k	l	m
Product	(¹⁴ C in the various compounds expressed as a % of the total soluble ¹⁴ C)											
sucrose	28.7	29.8	29.0	29.6	32.1	32.8	27.0	27.0	40.8	42.4	34.0	33.5
glycine + serine	-	-	3.5	3.5	2.15	1.9	2.0	1.8	0.6	0.5	0.6	0.4
glutamic acid	0.6	-	0.6	-	1.2	-	0.9	-	1.3	-	1.1	-
malic acid	4.0	4.6	5.5	5.2	9.8	9.5	8.1	8.0	7.7	7.3	5.0	5.3
aspartic acid	4.9	5.0	6.0	6.2	7.4	7.1	11.0	11.0	4.2	-	2.8	2.9
hexose + heptose	16.9	-	15.0	16.2	9.0	-	7.6	7.1	10.0	-	8.9	8.0
monophosphates												

Atmospheres contained 21% O_2 with 100 ppm CO_2 labelled with ^{14}C , $6.2 \mu\text{Ci}/\mu\text{mol}$ or 5000 ppm CO_2 labelled with ^{14}C , $1.89 \mu\text{Ci}/\mu\text{mol}$. Atmospheres of O_2 -free N_2 contained 5000 ppm CO_2 with labelled with ^{14}C , $1.89 \mu\text{Ci}/\mu\text{mol}$. The light intensity was 1950 ft candles and the temperature was 28°C .

a and b were duplicate chromatograms (0.1 ml samples) of the $\text{EtOH}/\text{H}_2\text{O}$ soluble fraction extracted from samples of maize leaf supplied with 100 ppm ^{14}C CO_2 in 21% O_2 , Experiment A. Likewise chromatograms c and d, e and f and g and h were used to separate compounds in 0.1 ml of the extracts from samples of maize leaf supplied with atmospheres of which the composition is shown above.

consisting of either 100 ppm $[^{14}\text{C}] \text{CO}_2$ or 5000 ppm $[^{14}\text{C}] \text{CO}_2$ in 80% O_2 . More results of the latter experiment (Experiment B) are presented later (see Tables 8 and 9). The reproducibility of the results was determined in two ways (see Table 7):

- (1) Two aliquots of the same extract were fractionated by 2-dimensional paper chromatography and the ^{14}C content of a selected number of the compounds that separated was determined.
- (2) The ^{14}C content of a selected number of compounds found in experiment A was compared with the ^{14}C content of the same compounds labelled under similar conditions in experiment B.

Reproducibility of results was satisfactory when comparing the ^{14}C in compounds from the same fraction separated on different chromatograms. Although, under similar conditions, there was a considerable difference in the total ^{14}C in the EtOH/ H_2O -soluble fractions in experiments A and B (Table 6) the relative distribution of ^{14}C between the compounds in the fractions compared in Table 7 was similar. It was considered therefore that the reproducibility of results was adequate for a comparison of the relative distribution of the ^{14}C assimilated between products of photosynthesis.

Distribution of carbon assimilated between the products of photosynthesis.

The calculated amounts of carbon assimilated into various products of photosynthesis is shown in Table 8, and the distribution of ^{14}C expressed as a % of the total ^{14}C in the EtOH/ H_2O soluble fraction is shown in Table 9 (Experiment B).

1. Sucrose

More carbon was incorporated into sucrose in 5000 ppm CO_2 than in 100 ppm CO_2 ; the increase was approximately proportional to the increased rate of photosynthesis at the higher CO_2 concentration. At 100 ppm CO_2 ,

incorporation into sucrose was greater in air than in either N_2 or 80% O_2 . At 5000 ppm CO_2 the greatest assimilation into sucrose was in an atmosphere of N_2 . The amount of new carbon in sucrose after photosynthesis in atmospheres containing different O_2 and CO_2 concentrations therefore reflected the overall rate of photosynthesis. The % of the total carbon assimilated that was found in sucrose was similar following photosynthesis in atmospheres with either 100 or 5000 ppm CO_2 .

2. Glycine and Serine

The ^{14}C incorporated into glycine plus serine was increased with increased O_2 concentration at both high and low CO_2 concentrations, but less glycine and serine were formed when atmospheres contained 5000 ppm CO_2 . When glycine and serine were separated (see Table 10) a different response was seen between ^{14}C entering these compounds depending on changes in O_2 concentration in the atmosphere containing 100 ppm CO_2 . Glycine synthesis was increased with increasing O_2 concentration but most carbon entered serine in air; a similar response to that of the overall rate of photosynthesis. With 5000 ppm CO_2 both compounds increased with increased O_2 concentration in the atmosphere. The incorporation of carbon into glycine was greater than into serine in atmospheres containing O_2 ; in N_2 more serine than glycine was formed.

3. Glutamic acid

More carbon was incorporated into glutamic acid in atmospheres containing 5000 ppm CO_2 than in atmospheres containing 100 ppm CO_2 . The amount of carbon entering glutamic acid reflected the trend in the overall rate of photosynthesis i.e. with 100 ppm CO_2 most incorporation into glutamic acid was in an atmosphere of air, whilst at 5000 ppm CO_2 most ^{14}C was incorporated into glutamic acid in an atmosphere of O_2 -free N_2 .

4. Glyceric acid

At low CO_2 ^{14}C incorporation into glyceric acid, like that into glycine and serine, was increased at increased O_2 concentrations. In atmospheres of N_2 or air more glyceric acid was formed with 5000 ppm than with 100 ppm CO_2 , but in 80% O_2 the reverse was true.

5. Threonine

In atmospheres containing 100 ppm CO_2 the incorporation of C into threonine was increased with increased O_2 concentrations; at 5000 ppm CO_2 the most synthesis of threonine took place with 21% O_2 present (Table 8).

6. Alanine

There was a greater incorporation of C into alanine in atmospheres with 5000 than with 100 ppm CO_2 and a marked decrease in synthesis of alanine was observed with 5000 ppm CO_2 in 80% O_2 . At 100 ppm CO_2 most alanine was formed in O_2 -free N_2 and least in 80% O_2 (Tables 8 and 9).

7. Malic acid

The incorporation into malic acid was increased in atmospheres of 80% O_2 containing 100 ppm CO_2 or 5000 ppm CO_2 (Tables 8 and 9). Synthesis of malic acid was also greater in air containing 5000 ppm CO_2 than in N_2 containing 5000 ppm CO_2 . At 100 ppm there was no significant increase in malic acid in air compared with N_2 (Table 9).

8. Aspartic acid

In atmospheres containing 100 ppm CO_2 the incorporation of carbon into aspartic acid was greatest in N_2 ; conversely, in atmospheres containing 5000 ppm CO_2 more carbon was incorporated into aspartic acid when the concentration of O_2 in the atmosphere was increased (Tables 8 and 9).

9. Citric acid

^{14}C incorporation into citric acid was somewhat increased at the higher

CO₂ concentration but was not significantly affected by the O₂ concentration in the atmosphere (Table 8).

10. 3-phosphoglyceric acid. 3 PGA.

Changes in the concentration of CO₂ and O₂ in the atmosphere had little effect on incorporation of carbon into 3-phosphoglyceric acid.

11. Phosphoenolpyruvate

At 100 ppm CO₂ the incorporation of ¹⁴C into phosphoenolpyruvate was increased by increased O₂ concentration; at 5000 ppm incorporation into phosphoenolpyruvate was greatest in N₂ and least in 21% O₂ (Table 8).

12. Phosphate esters and Sugar phosphates

With the low concentration of CO₂ in the atmosphere the synthesis of triose and pentose phosphates decreased with increased O₂ concentration, but with 5000 ppm CO₂ in the atmosphere no clear trend was seen (Tables 8 and 9). The hexose and heptose monophosphates together with sucrose, malic, aspartic and 3-phosphoglyceric acids accounted for approximately 60% of the carbon assimilated (Table 9). The synthesis of sugar diphosphates was influenced by O₂ concentration at low CO₂ and increased with increased O₂ at high CO₂ (Tables 8 and 9).

Summary of Results

Compounds the synthesis of which responded to changes in O_2 and CO_2 concentration in a similar manner to the rate of photosynthesis.

sucrose

glutamic acid

serine (in presence of 100 ppm CO_2)

Compounds into which more carbon was incorporated as the O_2 concentration was increased at 100 ppm CO_2 .

glycine

malic acid

glyceric acid

glycollic acid

threonine

phosphoenolpyruvate

Compounds into which less carbon was incorporated as the O_2 concentration was increased at 100 ppm CO_2 .

alanine

aspartic acid

triose and pentose phosphates

Only two compounds were found to have an inverse relationship with the rate of photosynthesis at 100 ppm CO_2 i.e. less formed in air than in N_2 and 80% O_2 , these were fumaric acid and an unknown compound.

Compounds into which more carbon was incorporated as the O_2 concentration was increased at 5000 ppm CO_2 .

glycine plus serine

hexose and heptose monophosphates

aspartic acid

glycollic acid

malic acid

3-phosphoglyceric acid.

Compounds into which less carbon was incorporated as the O_2 concentration was increased at 5000 ppm CO_2 .

sucrose

alanine

glyceric acid

TABLE:8

Amount of Carbon Incorporated into Various Compounds in Maize Leaf During
5 min Photosynthesis (1950 ft candles, 28°C)

CO ₂ concentration	100 ppm			5000 ppm		
O ₂ concentration	0	21	80	0	21	80
(% by volume)						
Product	(carbon in the various compounds as μg atoms C/g fresh wt)					
sucrose	2.45	3.38	2.13	6.57	5.59	5.10
glycine + serine	0.09	0.34	0.38	0.08	0.43	0.52
glutamic acid	0.03	0.07	0.04	0.15	0.19	0.16
glyceric acid	0.001	0.006	0.01	0.01	0.02	0.008
threonine	0.007	0.02	0.06	0.07	0.18	0.07
alanine	0.30	0.09	0.04	1.32	1.35	0.19
malic acid	0.50	0.64	1.05	0.96	1.69	4.01
aspartic acid	1.56	0.70	0.46	0.54	2.29	2.69
citric acid	0.03	0.03	0.04	0.20	0.19	0.13
fumarate +	0.02	0.02	0.17	0.08	0.03	0.14
glycollate						
phosphoenolpyruvate	0.02	0.03	0.04	0.21	0.03	0.10
3-PGA	1.02	1.23	0.86	1.42	1.80	1.95
triose + pentose	0.23	0.16	0.12	0.24	0.18	0.32
phosphates						
hexose + heptose	1.23	1.77	1.28	1.71	1.59	4.42
phosphates						
sugar diphosphates	0.16	0.13	0.08	0.22	0.25	0.60
unknown compounds	0.04	0.04	0.08	0.05	0.12	0.12
insoluble	0.38	0.27	0.11	0.22	0.10	0.11
polysaccharides						

Atmospheres contained different proportions of O₂ as shown with 100 ppm
CO₂ labelled with ¹⁴C, 6.2 $\mu\text{Ci} / \mu\text{mol}$ or 5000 ppm CO₂ labelled with ¹⁴C,
1.89 $\mu\text{Ci} / \mu\text{mol}$.

TABLE:9

Label (^{14}C) in Various Compounds in Maize Leaf During 5 min Photosynthesis
(1950 ft candles, 28°C).

CO_2 concentration		100 ppm			5000 ppm	
O_2 concentration	0	21	80	0	21	80
(% by volume)						
Product	(^{14}C in the various compounds as a % of total soluble ^{14}C)					
sucrose	25.00	29.00	24.50	34.00	27.00	24.00
glycine + serine	1.01	3.50	4.40	0.60	2.00	2.50
glutamic acid	0.32	0.60	0.40	1.10	0.90	0.80
glyceric acid	0.01	0.05	0.14	0.06	0.08	0.04
alanine	3.10	0.80	0.38	6.80	6.50	0.90
malic acid	5.20	5.50	12.00	4.96	8.10	19.00
aspartic acid	16.00	6.00	5.20	2.80	11.00	13.00
3-PGA	10.40	10.60	9.90	7.30	8.60	9.40
triose + pentose	2.26	1.40	1.26	1.28	0.85	1.07
phosphates						
hexose + heptose	12.50	15.00	15.00	8.90	7.60	11.00
phosphates						
sugar diphosphates	1.60	1.10	0.90	1.10	1.20	1.50
other compounds	4.81	3.72	4.48	4.21	3.21	3.21
loss	17.39	22.73	20.44	26.89	22.96	13.58

Atmospheres contained different proportions of O_2 as shown, with 100 ppm CO_2 labelled with ^{14}C , $6.12 \mu\text{Ci}/\mu\text{mol}$ or 5000 ppm CO_2 labelled with ^{14}C , $1.89 \mu\text{Ci}/\mu\text{mol}$.

TABLE:10

^{14}C Carbon Incorporated into Glycine and Serine in Maize Leaves during 5 min Photosynthesis. (1950 ft candles, 28°C)

Concentration of CO_2 ppm	100			5000		
Atmosphere	N_2	21% O_2	80% O_2	N_2	21% O_2	80% O_2
^{14}C as a percentage of the total ^{14}C in the EtOH/ H_2O soluble extract.						
Total glycine plus serine	1.00	3.50	4.40	0.60	2.00	2.50
Glycine	0.20	1.60	2.30	0.06	0.70	0.90
Serine	0.40	0.98	0.24	0.20	0.50	0.60

Atmospheres contained different proportions of oxygen and nitrogen with 100 ppm CO_2 labelled with ^{14}C ($6.2\mu\text{Ci}/\mu\text{mol}$) or 5000 ppm CO_2 labelled with ^{14}C ($1.89\mu\text{Ci}/\mu\text{mol}$).

V. ^{14}C in Various Compounds Formed by Samples of Maize Leaf During 5 min Photosynthesis in Atmospheres Containing $[\text{}^{14}\text{C}]\text{CO}_2$.

The samples of leaf were supplied with 100, 300 or 5000 ppm $[\text{}^{14}\text{C}]\text{CO}_2$ in gas mixtures containing different proportions of O_2 for 5 min (1950 ft. candles, 28°C); see Table:11. The samples of leaf supplied with 300 ppm $[\text{}^{14}\text{C}]\text{CO}_2$ had been floated on 0.2 M phosphate buffer for the first hour after excision; the O_2 concentration in atmospheres with 300 ppm CO_2 were different from those with 100 ppm and 5000 ppm CO_2 . In spite of these differences in treatment the amounts of ^{14}C in various compounds in samples supplied with 300 ppm CO_2 are intermediate between those in samples supplied with 100 ppm and 5000 ppm CO_2 .

Sucrose, alanine, malate, aspartate and the phosphate esters contained at least 60% of the total soluble ^{14}C extracted from samples of maize leaves treated with the gas mixtures containing $[\text{}^{14}\text{C}]\text{CO}_2$. The % ^{14}C in sucrose following photosynthesis in O_2 -free N_2 was increased with increasing CO_2 concentration in the atmosphere. In the presence of O_2 however increased incorporation into sucrose with increased CO_2 concentration was not observed. Following photosynthesis in atmospheres containing O_2 less ^{14}C was found in glycine and serine when the CO_2 concentration was high. The % ^{14}C incorporated into alanine was least in atmospheres containing 100 ppm CO_2 whatever the O_2 concentration. At any CO_2 concentration $[\text{}^{14}\text{C}]$ malate was greatest in the presence of high O_2 concentrations (76% or 80%). Under anaerobic conditions the % ^{14}C present in aspartate was decreased with increased CO_2 concentration; in the presence of O_2 the reverse was true. In the presence of N_2 or 21% and 38% O_2 the % ^{14}C in phosphate esters was decreased with high (5000 ppm) CO_2 ; but in the presence of a high O_2 concentration there was little effect of CO_2 concentration.

TABLE:11

^{14}C in Various Compounds Following Photosynthesis by Samples of Maize Leaves in Gas Mixtures Containing 100 ppm, 300 ppm or 5000 ppm $[^{14}\text{C}]\text{CO}_2$ (1950 ft candles, 28°C).

O_2 concentration (% by volume)	0	0	0	21	38	21	80	76	80
CO_2 concentration (ppm)	100	300	5000	100	300	5000	100	300	5000
Product	(^{14}C in the various compounds extracted expressed as % of total soluble ^{14}C)								
sucrose	25.00	28.40	34.00	29.00	23.92	27.00	24.50	23.19	24.00
glycine + serine	1.00	0.44	0.60	3.50	4.80	2.00	4.40	4.30	2.50
alanine	3.10	6.78	6.80	0.80	6.80	6.50	0.38	1.77	0.90
malic acid	5.20	9.97	4.96	5.50	9.12	8.10	12.00	11.68	19.00
aspartic acid	16.00	7.54	2.80	6.00	9.82	11.00	5.20	8.57	13.00
phosphate esters	27.85	28.19	19.58	28.35	26.27	18.45	27.40	19.45	23.43
other compounds	21.85	18.68	31.26	26.85	19.27	26.95	26.12	31.04	17.53

Atmospheres contained different proportions of O_2 as shown above with 100 ppm CO_2 labelled with ^{14}C , $6.2\mu\text{Ci}/\mu\text{mol}$
 300 ppm CO_2 labelled with ^{14}C , $3.72\mu\text{Ci}/\mu\text{mol}$ or 5000 ppm CO_2 labelled with ^{14}C , $1.89\mu\text{Ci}/\mu\text{mol}$,

VI Photosynthesis by Segments of Maize Leaf in O₂-free N₂ or Air Containing 100 ppm [¹⁴C] CO₂

In earlier experiments analyses were made after photosynthesis for 5 min atmospheres containing [¹⁴C] CO₂. Differences were described in amounts of ¹⁴C in various compounds depending on the composition of the atmosphere containing [¹⁴C] CO₂. The object of the experiment described now was to show to what extent some of the differences observed were caused by differences in the pool-size of photosynthesis intermediates rather than rates of photosynthesis. The segments of maize leaf were supplied with 100 ppm [¹⁴C] CO₂ (specific activity 18.98 μCi/μ mol) in air or O₂-free N₂ for 20, 40, 60, 120, 300 or 660 sec in the light (1950 ft candles; 28°C) (see Materials and Methods Sections 7 and 8).

1. Total Carbon Assimilated

Total carbon assimilated, ¹⁴C in compounds soluble in ethanol and water and the ¹⁴C in insoluble compounds are shown in Tables 12 and 13. Most of the ¹⁴C assimilated was present in the soluble fractions. The rate at which [¹⁴C] CO₂ was assimilated was greater in air than in O₂-free N₂. This confirms conclusions reached from gas exchange measurements made using an I.R.G.A. see Fig. 8.

2. Amounts of ¹⁴C in Various Compounds Extracted with Ethanol and H₂O Following Photosynthesis in Atmospheres Containing 100 ppm [¹⁴C] CO₂.

The ¹⁴C incorporated into various compounds soluble in ethanol and water after exposure of samples of leaf to [¹⁴C] CO₂ for periods of 20, 40, 60, 120, 300 or 660 sec are shown in Table 14 and Fig 15 in the presence of O₂-free N₂ and in Table 15 and Fig 16 in the presence of air, expressed as ng atoms of carbon/2 cm² leaf.

The carbon incorporation into malic plus aspartic acids was greater in air than in O_2 -free N_2 (Figs 15 and 16) and appeared to have saturated after 300 to 660 sec. After 20 sec these acids contained over 60% of the ^{14}C incorporated, ^{14}C in malic acid decreased with increased exposure to $[^{14}C]CO_2$ after 300 sec in both air and O_2 -free N_2 (see Tables 14 and 15); this was the only compound constituting a major proportion of the total soluble ^{14}C which showed this trend. In the presence of air or O_2 -free N_2 the ^{14}C present in aspartic acid was usually greater than that present in malic acid.

After 600 sec exposure to $[^{14}C]CO_2$ in the presence of air or O_2 -free N_2 sucrose contained the greatest amount of ^{14}C (Tables 14 and 15) and there was no indication that ^{14}C incorporation was saturated (Figs 15 and 16). $[^{14}C]$ sucrose was present after only a 20 sec exposure to $[^{14}C]CO_2$ in the presence of either O_2 -free N_2 or air. After 20 sec photosynthesis in the presence of O_2 -free N_2 the ^{14}C incorporated into sucrose exceeded that present in either glycine, serine or alanine (Table 14). In the presence of air however more ^{14}C was present in alanine than sucrose after 20 sec photosynthesis (Table 15).

The carbon incorporation into glycine plus serine was greater in the presence of air than in O_2 -free N_2 (Figs 15 and 16). The ^{14}C present in glycine plus serine was saturated after 300 sec photosynthesis in the presence of O_2 -free N_2 (Fig 15); in the presence of air there was no indication that ^{14}C incorporation into these amino-acids had saturated after 660 sec photosynthesis. The amount of $[^{14}C]$ glycine was generally greater than $[^{14}C]$ serine in the presence of either O_2 -free N_2 or air.

The ^{14}C assimilated into alanine increased with increased duration of exposure to $[^{14}C]CO_2$ up to at least 660 sec in the presence of either O_2 -free N_2 or air (Tables 14 and 15). The pool of alanine accessible to recently

assimilated carbon was not therefore saturated after 5 min photosynthesis in $[^{14}\text{C}]\text{CO}_2$ (Figs 15 and 16). The carbon incorporated into alanine after 660 sec exposure to $[^{14}\text{C}]\text{CO}_2$ was markedly greater in the presence of N_2 than air (Tables 14 and 15).

The amount of $[^{14}\text{C}]$ glyceric acid was generally greater in the presence of air than of O_2 -free N_2 and in the presence of air the incorporation of ^{14}C into glyceric acid was saturated after 120 sec photosynthesis. In the presence of O_2 -free N_2 or air the carbon assimilated into glutamic acid increased with increased time of exposure to $[^{14}\text{C}]\text{CO}_2$; there was no indication therefore that carbon incorporation into this compound had saturated after 120 sec photosynthesis. After 660 sec photosynthesis there was more ^{14}C in glutamic acid in the presence of air than O_2 -free N_2 . With the exception of samples exposed for 20 sec only the ^{14}C incorporated into sugar phosphates including PGA was greater in the presence of air than O_2 -free N_2 . These compounds were a major proportion of the total carbon assimilated even after only 20 sec exposure (see Tables 14 and 15).

TABLE:12

Total Carbon Assimilated by Segments of Maize Leaf in an Atmosphere of O₂-free N₂ Containing [¹⁴C]CO₂ for Various Periods of Time.

Time (sec)	20	40	60	120	300	600
Carbon Assimilated	(expressed as ng atoms of C/2cm ² leaf tissue)					
Total Carbon	6.513	13.05	20.10	50.16	94.37	304.79
Carbon present in						
EtOH/H ₂ O soluble fraction	6.500	13.00	20.00	50.00	90.00	300.00
Carbon present in						
EtOH/H ₂ O insoluble fraction	0.013	0.05	0.10	0.16	4.37	4.79

Atmospheres of O₂-free N₂ containing 100 ppm CO₂ labelled with ¹⁴C, 18.98 μ Ci / μ mol were supplied to segments of maize leaf (1 x 2 cm) for various periods of time as shown above. The light intensity was 1950 ft candles and the temperature was 28°C.

TABLE:13

The Total Carbon Assimilated by Segments of Maize Leaf in an Atmosphere of Air Containing $[^{14}\text{C}]\text{CO}_2$ for Various Periods of Time.

Time (sec)	20	40	60	120	300	660
Carbon Assimilated	(expressed as ng atoms of C/2 cm ² leaf tissue)					
Total carbon	-	17.51	31.54	126.20	200.70	513.60
Carbon present in						
EtOH/H ₂ O soluble fraction	8.00	17.50	31.50	126.00	200.00	510.00
Carbon present in						
EtOH/H ₂ O insoluble fraction	-	0.01	0.04	0.18	0.72	3.56

Atmospheres of 21% O₂ containing 100 ppm CO₂ labelled with ¹⁴C, 18.98 μCi/μmol were supplied to segments of maize leaf (1 x 2 cm) for various periods of time as shown above. The light intensity was 1950 ft candles and the temperature was 28°C.

TABLE:14

Carbon Incorporated into Various Compounds in Segments of Maize Leaf During Photosynthesis in Atmospheres Containing 100 ppm $[^{14}\text{C}]\text{CO}_2$ (specific activity $18.98\mu\text{Ci}/\mu\text{mol}$) in O_2 -free N_2 .

Time of exposure to $[^{14}\text{C}]\text{CO}_2$ (sec)	20	40	60	120	300	600
Product	(carbon from CO_2 in various compounds expressed as ng atoms of C/2 cm^2 leaf)					
sucrose	0.07	0.61	1.35	4.58	13.33	106.1
glycine	0.02	0.05	0.06	0.10	0.85	) 1.59
serine	0.01	0.04	0.05	0.06	0.98	
glutamic acid	0.03	0.05	0.06	0.41	0.90	1.65
glyceric acid	0.01	0.04	0.04	0.06	1.05	0.17
alanine	0.04	0.12	0.37	-	2.32	41.41
malic acid	0.94	2.72	2.90	6.46	18.77	17.75
aspartic acid	1.73	1.45	3.24	15.05	-	28.08
citric + fumaric acids	0.03	0.08	0.09	0.24	0.51	0.74
glycollic acid	0.0001	0.03	0.12	0.24	0.75	0.91
erythronic +	0.001	0.04	0.06	0.20	4.66	3.10
α -ketoglutaric acids						
phosphoenol pyruvate	0.02	0.05	0.04	0.18	1.24	0.59
sugar phosphates + PGA	1.00	2.56	4.56	10.27	-	52.71
unknown compounds	0.02	0.01	0.06	0.13	1.46	0.73
origin	1.41	4.70	6.26	10.42	23.14	39.05
other compounds	1.27	0.50	0.74	1.60	-	5.42

The light intensity was 1950 ft candles and temperature was 28°C .

TABLE:15

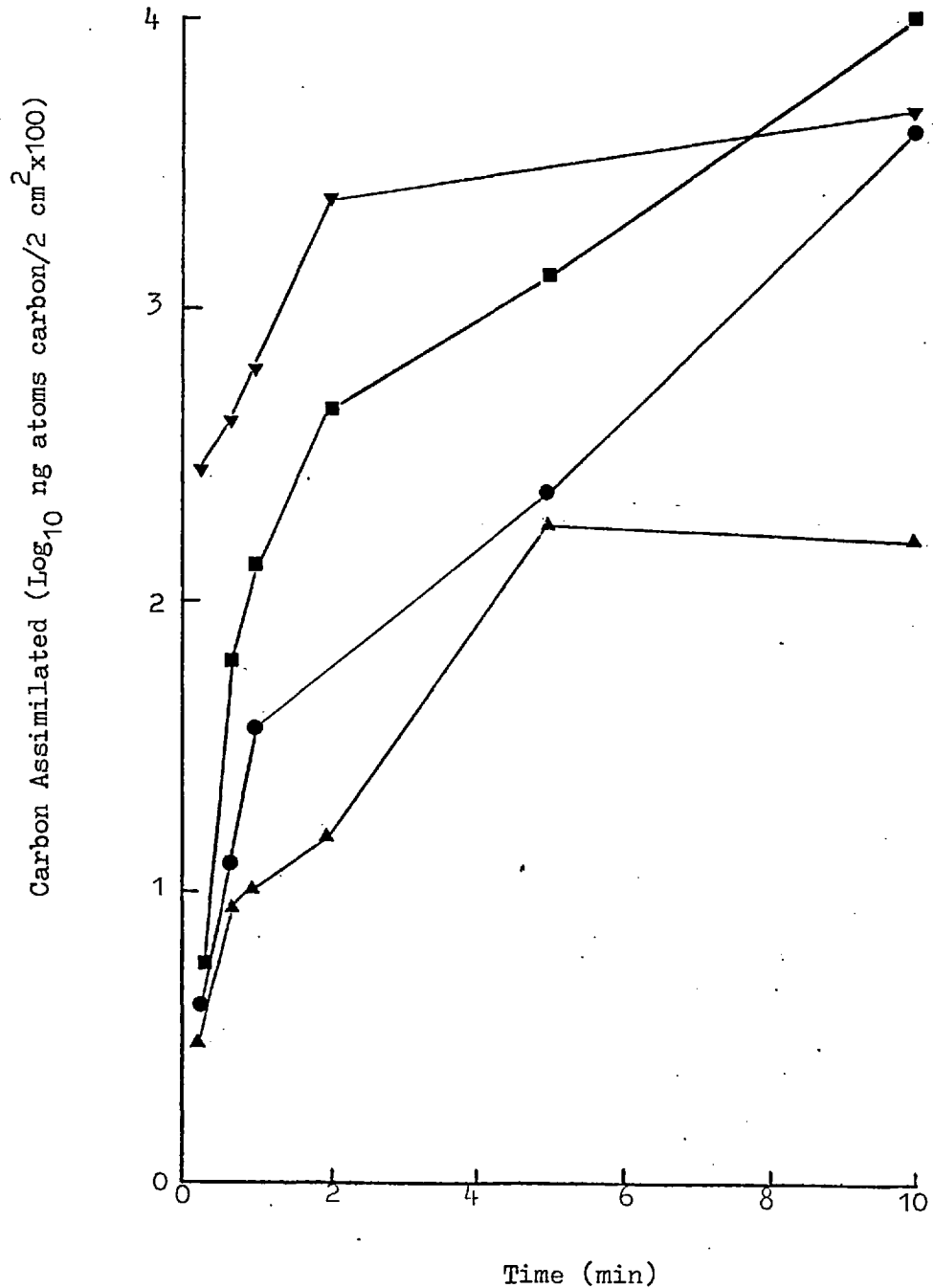
Carbon Incorporated into Various Compounds in Segments of Maize Leaf During Photosynthesis in Atmospheres Containing 100 ppm $[^{14}\text{C}]\text{CO}_2$ (specific activity $18.98 \mu\text{Ci}/\mu\text{mol}$) in Air.

Time of exposure to $[^{14}\text{C}]\text{CO}_2$ (sec)	20	40	60	120	300	660
Product	(Carbon from CO_2 in various compounds expressed as ng atoms of C/2 cm^2 leaf)					
sucrose	0.01	0.06	0.76	14.45	37.45	199.7
glycine	0.004	0.03	0.24	4.89	9.30	34.55
serine	0.009	0.02	0.10	0.69	3.45	16.38
glutamic acid	0.01	0.02	0.10	1.03	0.88	3.68
glyceric acid	0.01	0.06	0.03	0.18	0.31	0.22
alanine	0.02	0.10	0.40	3.37	10.81	23.71
malic acid	1.06	3.40	2.86	17.51	43.87	33.92
aspartic acid	1.06	-	14.33	36.46	41.98	58.77
citric + fumaric acids	0.01	0.05	0.10	0.32	0.36	1.16
glycollic acid	0.003	0.007	0.05	0.15	0.07	0.24
erythronic +	0.004	0.02	0.08	0.35	1.00	4.10
α -ketoglutaric acids						
phosphoenol pyruvate	0.02	0.04	0.05	0.21	0.25	1.94
sugar phosphates + PGA	0.59	3.55	5.38	31.24	48.40	109.30
unknown compounds	0.009	0.02	0.19	0.80	1.42	3.26
origin	1.02	1.68	6.45	4.98	0.06	5.00
other compounds	4.16	8.44	0.38	3.37	0.39	3.24

The light intensity was 1950 ft candles and temperature was 28°C .

FIGURE:15

Carbon Incorporated into Various Compounds in Segments of Maize Leaf during Photosynthesis in Atmospheres Containing 100 ppm $[^{14}\text{C}] \text{CO}_2$ (specific activity $18.98 \mu\text{Ci}/\mu\text{mol}$) in O_2 -free N_2 .

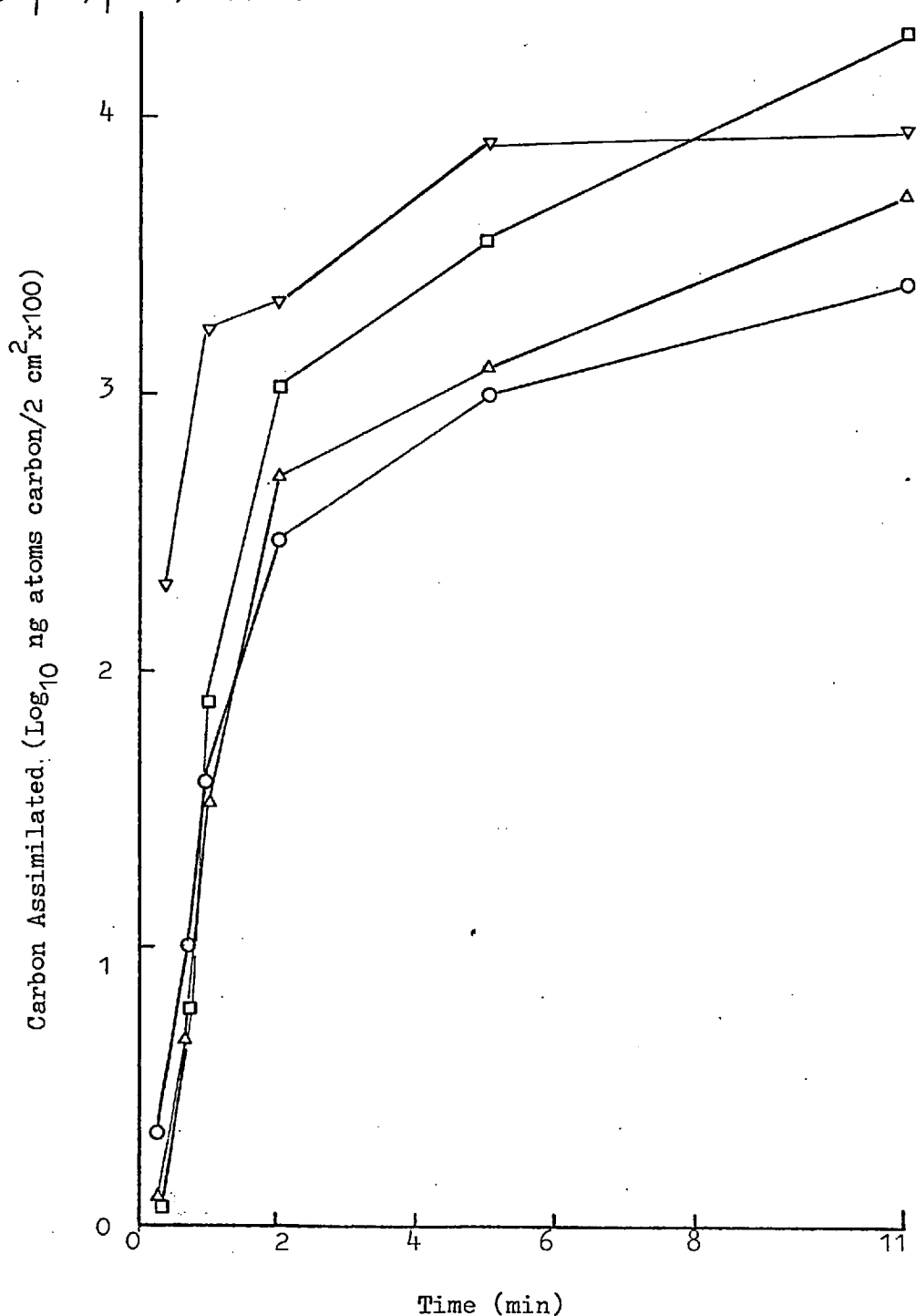


Carbon assimilated into sucrose (■), aspartate plus malate (▼), alanine (●) and glycine plus serine (▲) during various periods of photosynthesis from $[^{14}\text{C}] \text{CO}_2$. The light intensity was 1950 ft candles and the temperature was 28°C .

FIGURE:16

Carbon Incorporated into Various Compounds in Segments of Maize Leaf

During Photosynthesis in Atmospheres Containing 100 ppm $[^{14}\text{C}]\text{CO}_2$ (specific activity $18.98 \mu\text{Ci}/\mu\text{mol}$) in Air.



Carbon assimilated into sucrose (□), aspartate plus malate (▽), alanine (○) and glycine plus serine (△) during various periods of photosynthesis from $[^{14}\text{C}]\text{CO}_2$. The light intensity was 1950 ft candles and the temperature was 28°C .

VII Total Carbon Assimilated and Amounts of ^{14}C in Products of 5 min Photosynthesis by Maize Leaves treated with 5.12 mM Solutions of NH_4Cl in 0.2 M Phosphate Buffer or 0.2 M Phosphate Buffer

Samples of maize leaves were treated with the solution of NH_4Cl in phosphate buffer or with phosphate buffer as above for 1 hr after excision. After a further 40 min floating on water samples that had been treated with NH_4Cl in phosphate buffer were supplied with 300 ppm ^{14}C CO_2 ($3.72\mu\text{Ci}/\mu\text{mol}$) in O_2 -free N_2 , 42% O_2 or 78% O_2 and those treated with phosphate buffer supplied with 300 ppm ^{14}C CO_2 ($3.72\mu\text{Ci}/\mu\text{mol}$) in O_2 -free N_2 , 38% O_2 or 76% O_2 (see Materials and Methods Sections 5 and 8).

The total carbon assimilated by the samples of maize leaf is shown in Table 16. The total ^{14}C in soluble products decreased with increasing O_2 concentration when leaf samples were treated with buffer containing NH_4Cl (Table 16). Total carbon assimilated by the control samples was not as expected from the I.R.G.A. measurements (Fig. 11); most carbon was incorporated when O_2 was absent from the atmosphere.

Distribution of ^{14}C among products of 5 min photosynthesis by samples of maize leaves is shown in Tables 17 and 18. Results are expressed as μg atoms of C/g fresh wt in Table 17 and in Table 18 the ^{14}C in each compound is expressed as a % of the total ^{14}C incorporated into the fraction.

1. Sucrose

The % of total ^{14}C incorporated into sucrose was decreased when the O_2 concentration was increased especially in samples treated with NH_4Cl (Table 18).

2. Glycine and Serine

More carbon was incorporated into glycine plus serine in control samples when the O_2 concentration was highest but in samples treated with NH_4Cl most carbon was incorporated into glycine and serine when the

atmosphere contained 42% O_2 (Table 17). Glycine and serine were separated and the % ^{14}C incorporated into each is shown in Table 19. There was more ^{14}C in glycine than in serine after photosynthesis in atmospheres containing O_2 . When photosynthesis took place in an atmosphere without O_2 more ^{14}C was present in serine than in glycine.

3. Glutamic acid

Less $[^{14}C]$ glutamate was formed by samples of leaves treated with NH_4Cl than by control samples (Tables 17 and 18). Both treated and control samples contained most $[^{14}C]$ glutamate after photosynthesis in the atmosphere containing the intermediate O_2 concentration (Table 18).

4. Glyceric acid

The carbon incorporated into glyceric acid was increased with increasing O_2 concentration whether or not samples of leaves had been treated with NH_4Cl (Tables 17 and 18). Less glyceric acid was synthesised in samples of leaves which had been treated with NH_4Cl (Table 17).

5. Alanine

With increased O_2 concentration in the atmosphere both control samples and those treated with NH_4Cl incorporated less carbon into alanine. There was less ^{14}C in alanine in samples of leaves treated with NH_4Cl than in the controls (Tables 17 and 18).

6. Malic acid

In samples of leaves treated with NH_4Cl the ^{14}C incorporated into malic acid was increased when O_2 concentration in the atmosphere was increased. In the control samples most ^{14}C was incorporated into malic acid in the atmosphere with the highest concentration of O_2 and least in the atmosphere with the intermediate O_2 concentration (Tables 17 and 18).

7. Aspartic acid

In samples of leaf treated with NH_4Cl [^{14}C] aspartate synthesis was decreased with increased O_2 concentration (Tables 17 and 18) but the effect was less in magnitude than that observed in alanine. The ^{14}C in aspartic acid in the control samples was not greatly affected by the O_2 concentration of the atmosphere but the greatest incorporation into aspartic acid was in 38% O_2 (Table 18).

8. Phosphate esters

The % of soluble ^{14}C incorporated into phosphate esters (Table 18) was decreased with increased O_2 in the atmosphere in the control samples. In samples of leaves treated with NH_4Cl the % of soluble ^{14}C found in phosphate esters was very high after photosynthesis in the atmosphere containing 78% O_2 .

TABLE:16

Carbon Assimilated by Samples of Maize Leaf During 5 mins of Photosynthesis in Atmospheres Containing $[^{14}\text{C}]\text{CO}_2$.

Pretreatment	5.12 mM NH_4Cl in 0.2 M phosphate buffer			0.2 M phosphate buffer		
O_2 concentration (% by volume)	0	42	75	0	38	76
Carbon incorporated into ethanol/ H_2O soluble fraction	(carbon assimilated expressed as μ atoms of carbon/g fresh wt leaf tissue)					
	10.2	8.4	6.3	11.4	9.0	10.9

Samples of leaf were floated on either 5.12 mM NH_4Cl in 0.2 M phosphate buffer (pH 7.6) or on 0.2M phosphate buffer (pH 7.6) during the first hour after excision.

Subsequently atmospheres containing different proportions of O_2 , as shown with 300 p.p.m. CO_2 labelled with ^{14}C , 3.72 $\mu\text{Ci}/\mu\text{mol}$. The light intensity was 1950 ft candles and the temperature was 28°C.

TABLE:17

Total Amounts of Carbon Incorporated into Compounds Soluble in Ethanol and Water During 5 min Photosynthesis by Samples of Maize Leaf in Atmospheres Containing 300 ppm $[^{14}\text{C}]\text{CO}_2$ ($3.72\mu\text{Ci}/\mu\text{mol}$) and Different Concentrations of O_2 .

Solution used to pretreat samples	5.12 mM NH_4Cl in 0.2 M phosphate buffer			0.2 M phosphate buffer (control)		
O_2 concentration	O_2 -free N_2	42% O_2	78% O_2	O_2 -free N_2	38% O_2	76% O_2
Product	(carbon in the various products as $\mu\text{g atoms C/g fresh wt}$)					
sucrose	2.99	1.75	0.86	3.09	1.88	2.28
glycine + serine	0.08	0.31	0.18	0.05	0.37	0.42
glutamic acid	0.08	0.08	0.03	0.15	0.19	0.11
glyceric acid	0.01	0.01	0.02	0.008	0.02	0.05
alanine	0.59	0.25	0.06	0.74	0.53	0.17
malic acid	0.74	0.99	1.21	1.08	0.72	1.15
aspartic acid	0.79	0.74	0.39	0.82	0.77	0.84
phosphate esters	2.88	1.75	3.33	2.13	1.79	3.04
glycollic acid	0.0006	0.003	0.003	0.0003	0.003	0.009
unknown compounds	0.02	0.13	0.06	0.01	0.04	0.02

Samples of leaf were floated on 5.12 mM NH_4Cl in 0.2 M phosphate buffer or on 0.2 M phosphate buffer during the first hour after excision. The light intensity was 1950 ft candles and the temperature 28°C .

TABLE: 18

^{14}C Incorporated into Compounds Soluble in Ethanol and Water During 5 min Photosynthesis by Samples of Maize Leaf in Atmospheres Containing 300 ppm $[^{14}\text{C}]\text{CO}_2$ ($3.7\mu\text{Ci}/\mu\text{mol}$) and Different Concentrations of Oxygen.

Solution used to pretreat samples	5.12 mM NH_4Cl in			0.2 M phosphate buffer		
	0.2 M phosphate buffer			(control)		
O_2 concentration	O_2 -free N_2	42% O_2	78% O_2	O_2 -free N_2	38% O_2	76% O_2
Product	(^{14}C in the various products as a % of total ^{14}C in the extract)					
sucrose	30.43	21.04	13.91	28.40	23.92	23.19
glycine + serine	0.80	3.80	2.90	0.44	4.80	4.30
glutamic acid	0.79	1.02	0.41	1.36	2.48	1.09
glyceric acid	0.09	0.18	0.31	0.08	0.26	0.52
alanine	6.05	2.96	0.96	6.78	6.80	1.77
malic acid	7.55	11.91	19.55	9.97	9.12	11.68
aspartic acid	8.06	8.95	6.29	7.54	9.82	8.57
phosphate esters	29.75	21.97	54.53	28.19	26.27	19.45
other compounds	0.21	0.55	1.12	1.28	3.17	3.98
loss	16.27	27.62	0.02	15.96	13.36	25.45

Samples of leaf were floated on 5.12 mM NH_4Cl in 0.2 M phosphate buffer or on 0.2 M phosphate buffer during the first hour after excision. The light intensity was 1950 ft candles and the temperature was 28°C .

TABLE:19

Label (^{14}C) in Glycine and Serine in Maize Leaf During 5 min Photosynthesis in Atmospheres Containing 300 ppm [^{14}C] CO_2 ($3.72\mu\text{Ci} / \mu\text{mol}$) and Different Concentrations of O_2 .

Solution used to pretreat samples	5.12 mM NH_4Cl in			0.2 M phosphate buffer		
	0.2 M phosphate buffer			(control)		
O_2 concentration	O_2 -free N_2	42% O_2	78% O_2	O_2 -free N_2	38% O_2	76% O_2
Product	(^{14}C in the products as a % of total ^{14}C in the extract)					
glycine	0.34	2.66	2.50	0.002	2.70	3.74
serine	0.44	1.11	0.50	0.44	2.10	0.59

VIII Total Carbon Assimilated and Amounts of ^{14}C in Products of 5 min
Photosynthesis by Tobacco Leaves treated with 5.12 mM Solutions of
NaCl or Distilled Water.

For 1 hr after excision samples of tobacco leaf were floated on a solution of NaCl or distilled water as above; after a further 40 min floating on distilled water the samples were supplied with 300 ppm ^{14}C CO_2 (3.72 $\mu\text{Ci}/\mu\text{mol}$) in O_2 -free N_2 , 25% O_2 or 28% O_2 , or 76% O_2 (see Materials and Methods Sections 5 and 8).

The total carbon assimilated by samples of tobacco leaf during 5 min photosynthesis is shown in Table:20. The total carbon incorporated into the ethanol soluble fraction was greatest in the absence of O_2 . Measurements of rates of photosynthesis using an I.R.G.A. (see Fig. 14) showed that the rates for samples of tobacco leaf which had been pretreated with NaCl were greatest in the presence of approximately 25% O_2 . This effect was not demonstrated by the measurement of ^{14}C CO_2 uptake; however the carbon incorporated by samples of tobacco leaf which had been pretreated with NaCl, in the absence of O_2 was much decreased compared to the control sample.

The interpretation of this experiment is difficult, therefore because of differences between samples of leaf which were not due entirely to the different treatments given. In Table:21 the carbon incorporated into various compounds separated from the ethanol and water soluble extract is expressed as μg atoms of carbon/g fresh wt of leaf and in Table:22 results are expressed as a % of the total ^{14}C present in the EtOH/ H_2O -soluble fraction. Most of the carbon assimilated was present in sucrose, PGA, hexose and heptose monophosphates and the sugar diphosphates. Little effect of pretreatment was found upon the % of ^{14}C in sucrose after 5 min photosynthesis in ^{14}C CO_2 in O_2 -free N_2 or in 25%/28% O_2 but in 76% O_2 pretreatment with

TABLE:20

Carbon Assimilated by Samples of Tobacco Leaf During 5 mins of Photosynthesis in Atmospheres Containing $[^{14}\text{C}]\text{CO}_2$.

Pretreatment	5.12 mM NaCl			distilled water		
O_2 concentration (% by volume)	0	25	80	0	28	80
(carbon assimilated expressed as μg atoms of carbon/g fresh wt leaf tissue)						
Carbon incorporated into ethanol/ H_2O soluble fraction	5.1	4.5	1.5	16.0	4.3	2.9

Samples of leaf were floated on 5.12 mM NaCl or distilled water during the first hour after excision. Subsequently atmospheres containing different proportions of O_2 , as shown, were supplied with 300 ppm CO_2 labelled with ^{14}C , $3.72\mu\text{Ci}/\mu\text{mol}$. The light intensity was 1950 ft candles and the temperature was 28°C .

NaCl resulted in decreased incorporation of carbon into sucrose with an increase of the % of ^{14}C present in phosphate esters (Table 22).

The total carbon incorporated into sucrose (Table 21) was decreased by pretreatment in solutions of NaCl compared to H_2O especially when photosynthesis with $[\text{}^{14}\text{C}]\text{CO}_2$ was in an atmosphere of O_2 -free N_2 . The carbon incorporated into serine exceeded that into glycine in all samples; synthesis of glycine, serine, glutamic acid and glyceric acid was increased in the presence of increased O_2 concentration. Pretreatment with NaCl solution caused a decrease compared to control leaf, pretreated in H_2O , in the proportion of ^{14}C from $[\text{}^{14}\text{C}]\text{CO}_2$ incorporated into glycine; this trend was greatest where photosynthesis took place in an atmosphere containing 76% O_2 . More of the ^{14}C metabolized appeared in aspartic acid in samples of leaf pretreated with NaCl solution than in samples pretreated with water. In the presence of O_2 the ^{14}C incorporated into malic acid was less in the salt treated samples than in the control samples (Table 22). The carbon incorporated into triose and pentose phosphates was decreased with an increase in O_2 concentration from 0 to 76% (Table 21) especially in those samples pretreated with NaCl solution.

TABLE:21

The Amounts of Carbon Incorporated into Products of Photosynthesis by Tobacco Leaf.

Pretreatment Solution	5.12 mM NaCl			distilled water (control)		
O ₂ concentration (% by volume)	0	25	76	0	28	76
Product	(carbon incorporated into the various compounds expressed as μg atoms of C/g fresh wt)					
sucrose	1.82	0.87	0.10	5.25	0.83	0.39
glycine	0.03	0.20	0.13	0.08	0.17	0.18
serine	0.25	0.42	0.20	0.69	0.37	0.35
glutamic acid	0.02	0.11	0.02	0.06	0.03	0.03
glyceric acid	0.003	0.05	0.04	0.02	0.05	0.08
alanine	0.07	0.04	0.01	0.17	0.03	0.04
malic acid	0.04	0.03	0.009	0.09	0.05	0.03
aspartic acid	0.16	0.10	0.045	0.30	0.03	0.04
citric acid	0.04	0.04	0.04	0.11	0.02	0.04
glycollic acid	0.003	0.009	0.03	0.02	0.005	0.007
triose phosphates	0.02	0.02	0.006	0.08	0.01	0.01
pentose phosphates	0.04	0.02	0.01	0.46	0.02	0.04
3 PGA + hexose and heptose phosphates + sugar diphosphates	0.98	0.73	0.32	3.32	0.74	0.39
unknown compounds	0.06	0.15	0.06	0.17	0.05	0.07

Samples of leaf were floated on 5.12 mM NaCl solution or distilled water during the first hour after excision and then supplied for 5 min with atmospheres containing 300 ppm CO₂ labelled with ¹⁴C, 3.72 $\mu\text{Ci}/\mu\text{mol}$ in O₂-free N₂, 25 or 28% O₂ or 76% O₂. The light intensity was 1950 ft candles and the temperature was 28°C.

TABLE:22

The Amounts of ^{14}C in Various Compounds After Photosynthesis by Tobacco Leaf.

Pretreatment Solution	5.12 mM NaCl			distilled water (control)		
O_2 concentration (% by volume)	0	25	76	0	28	76
Product	(^{14}C in the various compounds expressed as a % of total soluble ^{14}C)					
sucrose	43.42	26.04	7.90	38.36	28.35	17.30
glycine	0.79	6.03	10.08	0.60	5.78	7.72
serine	6.05	12.45	15.39	5.06	12.69	15.40
glutamic acid	0.46	0.90	1.27	0.41	1.14	1.48
glyceric acid	0.07	1.53	3.01	0.16	0.97	3.38
alanine	1.68	1.21	0.79	1.26	0.95	0.85
malic acid	0.95	0.80	0.73	0.68	1.77	1.38
aspartic acid	3.70	2.98	3.48	2.19	1.12	1.69
triose phosphates	0.60	0.45	0.50	0.58	0.45	0.45
pentose phosphates	1.06	0.58	0.74	3.36	0.56	1.46
3-PGA + hexose and heptose phosphates + sugar diphosphates	23.36	21.73	24.26	24.25	25.26	17.54
other compounds	1.22	3.66	5.98	1.28	3.17	3.98
Loss	16.64	22.74	23.87	21.81	17.79	27.37

Samples of leaf were floated on 5.12 mM NaCl solution or distilled water during the first hour after excision and then supplied for 5 min with atmospheres containing 300 ppm CO_2 labelled with ^{14}C , $3.72 \mu\text{Ci}/\mu\text{mol}$ in O_2 -free N_2 , 25% or 28% O_2 or 76% O_2 . The light intensity was 1950 ft candles and the temperature was 28°C .

IX. Metabolism of $[U - ^{14}C]$ Malate by Maize Leaf

Samples of maize leaf lamina, 2 cm^2 , were supplied with $[U - ^{14}C]$ malate ($4\text{ }\mu\text{Ci}$, $0.4\text{ }\mu\text{mol}$ in 0.05 ml) for 40 min in the light (1950 ft candles) or in darkness in atmospheres of O_2 -free N_2 or air containing 5000 ppm or 100 ppm CO_2 , the temperature was $28^\circ C$ (see Materials and Methods Sections 6 and 8). The ^{14}C compounds extracted after supplying the labelled substrate in the light are shown in Table 23, the ^{14}C in the various compounds being expressed as a % of the total soluble ^{14}C , and in Table 25 the ^{14}C content of the extracted compounds is expressed as d.p.m. $\times 10^{-3}/\text{cm}^2$ leaf tissue. In Table 24 the ^{14}C in the various compounds extracted after supply of the labelled substrate in darkness is shown expressed as a % of the total soluble ^{14}C and the ^{14}C content of the extracted compounds expressed as d.p.m. $\times 10^{-3}/\text{cm}^2$ leaf tissue is shown in Table 26.

The total ^{14}C extracted in ethanol and water from samples of maize leaf supplied with $[U - ^{14}C]$ malate in light and darkness is shown in Tables 23 and 24. Except with samples of leaf kept in air containing 5000 ppm CO_2 more malate was taken up in light than in darkness and in air than in O_2 -free N_2 . In the light the total $[^{14}C]$ malate absorbed was greatest in samples in atmospheres containing 100 ppm CO_2 . In the dark the concentration of CO_2 had little effect.

^{14}C Products formed in the Light.

The amounts of ^{14}C in individual compounds extracted from segments of maize leaf are shown in Tables 23 and 25. The major product of $[U - ^{14}C]$ malate metabolism in the light was sucrose. A greater proportion of absorbed malate was converted to sucrose in air than in nitrogen (Table 23) especially in the presence of a low CO_2 concentration (100 ppm) (Table 25).

The ^{14}C incorporated into glycine and serine at low CO_2 was greater when malate was supplied in air than when it was supplied in O_2 -free N_2 . These amino acids were almost equally labelled after supply of $[\text{U} - ^{14}\text{C}]$ malate (Tables 23 and 25). More ^{14}C was incorporated into alanine in N_2 than in air especially with 100 ppm CO_2 . The synthesis of ^{14}C aspartate from $[\text{U} - ^{14}\text{C}]$ malate was greater in the absence of O_2 with 100 ppm CO_2 but this was not true in the presence of 5000 ppm CO_2 . More glutamine was formed from $[\text{U} - ^{14}\text{C}]$ malate in aerobic than in anaerobic conditions. The formation of ^{14}C glutamate was greater in the presence of 5000 ppm CO_2 than in the presence of 100 ppm CO_2 . More phosphate ester compounds were synthesised from malate in the presence of low CO_2 (100 ppm) than high CO_2 (5000 ppm).

^{14}C Products Formed in the Dark.

The amounts of ^{14}C in compounds from segments of maize leaf supplied with $[\text{U} - ^{14}\text{C}]$ malate in the dark are shown in Tables 24 and 26. No sucrose was synthesised from $[\text{U} - ^{14}\text{C}]$ malate in the dark and in anaerobic conditions the metabolism of malate was severely limited. The ^{14}C incorporated into glycine and serine was greater in air than in N_2 and under aerobic conditions the synthesis of glycine and serine was greater at low CO_2 (100 ppm). Conversely in O_2 -free N_2 the synthesis of these compounds was greater at high CO_2 (5000 ppm). In the presence of N_2 there was a greater synthesis of glycine from $[\text{U} - ^{14}\text{C}]$ malate than serine, however the reverse was true in the presence of air. Alanine, aspartate and glutamate were major products of $[\text{U} - ^{14}\text{C}]$ malate metabolism in air; more aspartate and glutamate were formed in the presence of 5000 ppm CO_2 compared with 100 ppm CO_2 . Glutamine synthesis was little affected by the gaseous environment except in air containing 5000 ppm CO_2 where most was formed from $[\text{U} - ^{14}\text{C}]$ malate. The greater aerobic synthesis of amino acids (including threonine Table 26) and amides from $[\text{U} - ^{14}\text{C}]$ malate was

accompanied by synthesis of acids of the Krebs cycle. For example more succinate + citrate and fumarate were present when malate was supplied in air than when it was supplied in O_2 -free N_2 (see Table 26).

Differences in Metabolism of $[U - ^{14}C]$ Malate in Light and Darkness.

The ^{14}C content of compounds extracted from segments of maize leaf supplied with $[U - ^{14}C]$ malate in the light and dark may be compared in Tables 23 and 24. Synthesis of sucrose was light-dependent. Glycine and serine were formed from malate both in the light and in the dark and more of each was formed under aerobic than under anaerobic conditions with 100 ppm CO_2 . Alanine synthesis was greater in O_2 -free N_2 than in air in the light but greater in air than in N_2 in the dark. The ^{14}C incorporated into aspartate was markedly greater in the presence of O_2 in the dark and constituted one of the major products of malate metabolism. Under anaerobic conditions there was a much greater synthesis of $[^{14}C]$ aspartate in the light than the dark. Aspartate synthesis in the light therefore was less O_2 -dependent than in the dark. The production of $[^{14}C]$ glutamate from malate was primarily affected by O_2 concentration in darkness whereas the concentration of CO_2 was a greater influence in the light. In both light and darkness the synthesis of glutamine was greater in the presence of air than in the presence of N_2 . The metabolism of $[U - ^{14}C]$ malate to Krebs cycle acids, fumarate, succinate and citrate was greater in the dark than the light especially under aerobic conditions (Tables 25 and 26).

TABLE:23

Metabolism of [U - ^{14}C] Malate in the Light by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 .

CO_2 Concentration	100 ppm		5000 ppm	
O_2 Concentration	O_2 -free N_2	21%	O_2 -free N_2	21%
Product	(^{14}C in the various compounds expressed as a % of total soluble ^{14}C)			
sucrose	20.0	37.9	20.7	32.6
glycine	0.5	2.7	-	0.7
serine	0.3	2.2	-	0.8
alanine	15.0	3.9	9.5	6.2
malate	43.4	36.3	40.5	34.4
aspartate	2.8	1.3	1.8	2.2
glutamine	0.5	0.6	0.3	0.7
glutamate	0.5	0.8	3.9	3.0
phosphate esters	5.0	4.1	1.3	3.2
other compounds	12.0	10.2	14.3	16.2
dpmx $10^{-6}/\text{cm}^2$ leaf				
Total soluble ^{14}C	1.26	1.76	0.74	0.32

Analysis of the ^{14}C compounds extracted after supplying the labelled substrate ($4 \mu\text{Ci}$, $0.4 \mu\text{mol}$ in 0.05 ml) for 40 min in the light (1950 ft candles) at 28°C to segments of lamina, 2 cm^2 .

TABLE:24

Metabolism of $[U - ^{14}C]$ Malate in the Dark by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 .

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration	0	21	0	21
(% by volume)				
Product	(^{14}C in the various compounds expressed as a % of the total soluble ^{14}C)			
glycine	0.15	1.48	0.25	0.25
serine	0.02	2.76	0.18	0.40
alanine	3.14	21.98	5.43	-
malate	78.44	24.73	72.70	-
aspartate	0.71	15.69	1.19	33.37
glutamine	0.14	0.94	0.16	2.17
glutamate	0.27	9.12	0.22	15.09
fumarate	0.17	4.18	0.15	5.05
other compounds	17.40	19.12	19.72	43.67
+ loss				
	d.p.m. $\times 10^{-6}/cm^2$ leaf			
Total soluble ^{14}C	0.58	0.82	0.40	0.90

Analysis of ^{14}C compounds extracted after supplying the labelled substrate ($4 \mu Ci$, $0.4 \mu mol$ in $0.05 ml$) for 40 min in the dark at $28^\circ C$ to segments of lamina, $2 cm^2$.

TABLE:25

Metabolism of $[U - ^{14}C]$ Malate in the Light by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 .

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration	0	21	0	21
(% by volume)				
Product	(^{14}C in the various compounds expressed as d.p.m. $\times 10^{-3}/cm^2$ leaf)			
sucrose	185.5	532.1	120.3	96.9
glycine	4.5	37.9	-	2.1
serine	2.65	30.7	-	2.7
alanine	138.7	55.1	55.0	18.3
malate	402.9	513.7	234.9	102.7
aspartate	25.8	18.8	10.5	6.4
glutamine	4.8	8.1	2.1	1.8
glutamate	4.6	10.9	22.5	8.9
fumarate	2.5	-	6.9	4.2
succinate and citrate	20.1	-	9.3	4.1
phosphate esters	48.3	60.95	9.62	9.9
other compounds	73.5	105.2	36.3	30.1
insoluble polysaccharides	16.7	25.8	7.2	8.9

Analysis of the ^{14}C compounds extracted from segments of lamina, 2 cm^2 , after supplying the labelled substrate ($4\text{ }\mu\text{Ci}$, $0.4\text{ }\mu\text{mol}$ in 0.05 ml) for 40 min in the light (1950 ft candles) at $28^\circ C$.

TABLE:26

Metabolism of [U - ^{14}C] Malate in the Dark by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 .

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration (% by volume)	0	21	0	21
Product	(^{14}C in the various compounds expressed as d.p.m. $\times 10^{-3}/\text{cm}^2$ leaf)			
glycine	0.88	12.13	1.02	2.44
serine	0.11	22.64	0.74	3.85
alanine	18.11	180.32	21.67	-
malate	451.90	202.88	290.09	-
aspartate	4.11	128.71	4.78	322.24
glutamine	0.79	7.71	0.64	19.74
glutamate	1.58	74.83	0.87	144.95
fumarate	1.00	34.29	0.61	48.50
succinate + citrate	15.23	22.34	11.80	37.84
threonine	3.79	10.27	2.17	13.35
α -ketoglutarate	5.94	10.91	3.56	14.76
phosphoenol- pyruvate	13.97	52.97	7.75	38.40
other compounds	19.40	40.71	19.91	52.47
insoluble polysaccharide	2.21	13.26	3.06	21.36

Analysis of the ^{14}C compounds extracted from segments of leaf lamina, 2 cm^2 , after supplying the labelled substrate ($4\text{ }\mu\text{Ci}$, $0.4\text{ }\mu\text{mol}$ in 0.05 ml) for 40 min in darkness.

X. Metabolism of [U - ^{14}C] Alanine by Maize Leaf.

Segments of maize leaves (1 x 2 cm) were supplied with [U - ^{14}C] alanine (4 μCi , 0.4 μmol in 0.05 ml) for 40 min in the light (1950 ft candles) or in darkness, at 28°C in the presence of O_2 -free N_2 or 21% O_2 containing either 100 ppm or 5000 ppm CO_2 (see Materials and Methods Sections 6 and 8). The total ^{14}C extracted in ethanol and water from the segments of leaf in the light is shown in Table 27 and in the dark in Table 28. More [U - ^{14}C] alanine was taken up in the light than in the dark and in both light and darkness more was taken up in N_2 than in air. The presence of a high CO_2 concentration (5000 ppm) decreased uptake compared to the presence of low CO_2 (100 ppm).

^{14}C Products Formed in the Light

The ^{14}C compounds extracted after supplying the labelled substrate in the light are shown in Table 27, the ^{14}C in the various compounds being expressed as a % of the total soluble ^{14}C and in Table 29 the ^{14}C content of the extracted compounds is expressed as d.p.m. $\times 10^{-3}/\text{cm}^2$ leaf tissue. Although the total soluble ^{14}C extracted was greater from those samples supplied with the labelled substrate in the presence of N_2 containing 100 ppm CO_2 . The ^{14}C content of compounds other than malate was greater from samples supplied with the substrate in the presence of 21% O_2 containing 100 ppm CO_2 . In all treatments the major product of [U - ^{14}C] alanine metabolism in the light was sucrose; the synthesis of sucrose was greater in air than N_2 (Table 27). The ^{14}C incorporated into sucrose was decreased by the presence of 5000 ppm CO_2 compared with 100 ppm CO_2 in both N_2 and 21% O_2 (Table 29). The synthesis of [U - ^{14}C] glycine and serine was considerably increased by the presence of O_2 in the presence of both 5000 and 100 ppm CO_2 . In atmospheres devoid of O_2 synthesis of glycine and serine was greater at low CO_2 (Tables 27 and 29). The ^{14}C incorporated into

serine was less than into glycine except when the atmosphere was 21% O_2 with 100 ppm CO_2 when the two compounds were almost equally labelled. The concentration of CO_2 had very little effect on the incorporation of ^{14}C into malate but the presence of O_2 increased malate synthesis (Table 27). The synthesis of [^{14}C]aspartate however was markedly increased by the presence of 100 ppm CO_2 compared with 5000 ppm CO_2 , but the incorporation of ^{14}C into aspartate was decreased by the presence of O_2 (Tables 27 and 29). [^{14}C]glutamine was synthesised from [U - ^{14}C]alanine to a greater extent in N_2 than in air especially with 100 ppm CO_2 . The ^{14}C incorporated into glutamate from [U - ^{14}C]alanine was not affected by CO_2 concentration in the presence of O_2 , but in N_2 there was a greater synthesis with 5000 ppm CO_2 . In an atmosphere of N_2 the synthesis of phosphate esters was greater in the presence of 100 ppm CO_2 compared with 5000 ppm CO_2 but in an atmosphere containing 21% O_2 the reverse was true (Tables 27 and 29).

^{14}C Products Formed in the Dark

The ^{14}C content of compounds extracted from segments of maize leaf supplied with the labelled substrate in darkness is shown in Table 28, the ^{14}C incorporated into the various compounds being expressed as a % of the total soluble ^{14}C and in Table 30 the ^{14}C content of the extracted compounds is expressed as d.p.m. $\times 10^{-3}/cm^2$ leaf tissue. Metabolism of alanine in darkness was stimulated by the presence of an atmosphere containing O_2 and malate, aspartate, glutamate, and fumarate were among the major products of the increased metabolism. This effect of O_2 concentration was particularly noticeable on the synthesis of aspartate; in the absence of N_2 the ^{14}C content of aspartate was negligible but in the presence of 21% O_2 aspartate was one of the two major products of alanine metabolism (Table 30). The synthesis of [^{14}C]glutamate was influenced by the gaseous environment in a similar manner to aspartate, being the major product of aerobic alanine metabolism (Tables 28 and 30). The incorporation of ^{14}C into glutamine was little affected by the concentration of O_2 or CO_2

present in the atmosphere; it was the major product of alanine metabolism in the absence of O_2 . More $[^{14}C]$ malate was produced aerobically, and in the presence of high CO_2 (5000 ppm) than in low CO_2 (100 ppm) (Table 28). The amount of ^{14}C incorporated into glycine and serine was greater in the presence of 21% O_2 than in O_2 -free N_2 and the metabolism of alanine to glycine and serine in both N_2 and air was greater with 5000 ppm than 100 ppm CO_2 (Table 28). The amount of $[^{14}C]$ serine produced was greater than $[^{14}C]$ glycine in all treatments (Table 30). The ^{14}C incorporated into fumarate and α -ketoglutarate + erythronate was greater under aerobic than anaerobic conditions (Table 30), though the amount of $[^{14}C]$ threonine was greater in the presence of N_2 than 21% O_2 .

Differences in $[U - ^{14}C]$ Alanine Metabolism in Light and Darkness.

The ^{14}C content of compounds extracted from segments of maize leaf supplied with $[U - ^{14}C]$ alanine in the light and in the dark may be compared in Tables 27 and 28. The synthesis of sucrose from alanine was wholly light-dependent and in the light constituted the major product of alanine metabolism. The ^{14}C incorporated into glycine and serine was considerably decreased in the dark, though as in the light the presence of O_2 increased the synthesis of these amino acids. Synthesis of serine from alanine in darkness was faster than synthesis of glycine; in the light more glycine than serine was formed. The ^{14}C incorporated into malate in the presence of O_2 -free N_2 in darkness was less than in the light, but in the presence of 21% O_2 there was little difference in the amount of ^{14}C incorporated into malate in the light or in the dark. The synthesis of aspartate in the light was affected mainly by the concentration of CO_2 in the atmosphere; there was greater synthesis of $[^{14}C]$ aspartate in the presence of 100 ppm CO_2 compared to 5000 ppm CO_2 . In darkness

however more aspartate was produced under aerobic than anaerobic conditions. The % soluble ^{14}C in glutamine was increased when alanine was supplied in the dark compared to the light. The synthesis of $[\text{}^{14}\text{C}]$ glutamate from $[\text{U} - \text{}^{14}\text{C}]$ alanine in the presence of O_2 -free N_2 was greater in the light than in darkness but in the presence of 21% O_2 the reverse was true.

TABLE:27

Metabolism of [U - ^{14}C] Alanine in the Light by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 .

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration	O_2 -free N_2	21%	O_2 -free N_2	21%
Product	(^{14}C in the various compounds expressed as a % of total soluble ^{14}C)			
sucrose	27.9	38.7	18.5	28.2
glycine	0.5	3.1	0.3	11.3
serine	0.4	3.7	0.2	7.8
alanine	45.7	28.2	62.0	27.9
malate	3.4	5.0	3.6	5.3
aspartate	7.4	5.4	2.6	2.4
glutamine	2.2	1.1	1.6	1.4
glutamate	1.7	3.0	3.8	2.8
phosphate esters	3.8	4.4	1.6	5.8
other compounds	7.0	7.4	5.8	7.1
	d.p.m. $\times 10^{-6}/\text{cm}^2$ leaf tissue			
Total soluble ^{14}C	1.06	0.95	0.63	0.25

Analysis of the ^{14}C compounds extracted after supplying the labelled substrate ($4\mu\text{Ci}$, $0.4\mu\text{mol}$ in 0.05 ml) for 40 min in the light (1950 ft candles) at 28°C to segments of lamina 2 cm^2 .

TABLE:28

Metabolism of $[U - ^{14}C]$ Alanine in the Dark by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 .

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration (% by volume)	0	21	0	21
Product	(^{14}C in the various compounds expressed as a % of total soluble ^{14}C)			
glycine	00.02	0.10	0.03	0.23
serine	0.15	0.56	0.24	1.02
alanine	76.76	49.79	73.01	35.42
malate	0.73	5.03	2.34	4.40
aspartate	0.03	10.02	0.04	13.66
glutamine	4.18	4.30	3.82	4.19
glutamate	0.24	14.22	0.19	20.00
fumarate	0.05	7.96	0.08	6.29
other compounds	17.84	8.03	20.25	14.79
	d.p.m. $\times 10^{-6}/cm^2$ leaf tissue			
Total soluble ^{14}C	0.67	0.48	0.44	0.14

Analysis of the ^{14}C compounds extracted after supplying the labelled substrate ($4 \mu Ci$, $0.4 \mu mol$ in $0.05 ml$) for 40 min in the dark at $28^\circ C$ to segments of lamina $2 cm^2$.

TABLE:29

Metabolism of [U - ^{14}C] Alanine in the Light by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 .

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration	0	21	0	21
(% by volume)				
Product	(^{14}C in the various compounds expressed as d.p.m. $\times 10^{-3}/\text{cm}^2$ leaf tissue)			
sucrose	308.12	367.84	114.49	71.69
glycine	5.41	29.10	1.91	28.71
serine	4.63	34.50	1.41	19.82
alanine	507.45	268.10	387.19	70.84
malate	37.64	47.65	22.57	13.36
aspartate	81.83	52.11	16.30	6.02
glutamine	24.28	10.56	9.83	3.62
glutamate	19.07	28.14	23.81	7.04
fumarate	4.34	2.10	2.31	0.74
α -ketoglutarate + erythronate	6.55	15.92	4.40	5.14
phosphate esters	42.42	42.18	10.18	14.66
other compounds	29.23	20.40	23.57	9.64
insoluble polysaccharide	30.41	34.19	10.38	11.76

Analysis of the ^{14}C compounds extracted from segments of lamina, 2 cm^2 , after supplying the labelled substrate ($4\mu\text{Ci}$, $0.4\mu\text{ mol}$ in 0.05 ml) for 40 min in the light (1950 ft candles) at 28°C .

TABLE:30

Metabolism of [U - ^{14}C] Alanine in the Dark by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 .

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration (% by volume)	0	21	0	21
Product	(^{14}C in the various compounds expressed as d.p.m. $\times 10^{-3}/\text{cm}^2$ leaf tissue)			
glycine	0.14	0.48	0.14	0.35
serine	1.03	2.70	1.07	1.46
alanine	511.96	239.13	319.97	50.74
malate	4.86	24.16	10.26	6.30
aspartate	0.17	48.11	0.19	19.57
glutamine	27.88	20.67	16.76	6.01
glutamate	1.61	68.29	0.84	28.66
fumarate	0.36	38.25	0.35	9.02
succinate	0.66	0.72	0.94	0.04
threonine	10.65	7.27	11.10	2.50
α -ketoglutarate + erythronate	0.75	6.84	0.55	2.64
phosphoenolpyruvate	-	1.87	0.15	0.14
other compounds	23.71	29.18	13.70	15.19
insoluble polysaccharides	2.60	3.65	-	0.92

Analysis of the ^{14}C compounds extracted from segments of lamina, 2 cm^2 , after supplying the labelled substrate for 40 min in the dark at 28°C .

XI Metabolism of [2 - ^{14}C] Glycine by Maize Leaf.

Segments of maize leaf (1 x 2 cm) were supplied with [2 - ^{14}C] glycine (4 μCi , 0.4 μmol in 0.05 ml) for 40 min in the light (1950 ft candles) or in darkness, at 28°C in atmospheres of either O_2 -free N_2 or 21% O_2 containing either 100 ppm or 5000 ppm CO_2 (see Materials and Methods sections 6 and 8). The total ^{14}C extracted in ethanol and water from the segments of leaf in the light is shown in Table:31 and in the dark in Table:32. The total ^{14}C absorbed was greater in the light than in darkness, except with air containing 5000 ppm CO_2 when there was little effect of light on the uptake of glycine. The presence of O_2 increased the uptake of [2 - ^{14}C] glycine especially in darkness. In the light more ^{14}C was present in segments of leaf supplied with the labelled substrate in the presence of low (100 ppm) CO_2 than in the presence of high (5000 ppm) CO_2 ; the reverse was true in darkness.

^{14}C Products formed in the Light.

The ^{14}C compounds extracted after supplying the labelled substrate in the light are shown in Table:31, the ^{14}C in the various compounds being expressed as a % of the total soluble ^{14}C and in Table:33 the ^{14}C content of the extracted compounds is expressed as d.p.m. x $10^{-3}/\text{cm}^2$ leaf tissue. In all samples glycine and serine accounted for most of the soluble ^{14}C . In the presence of O_2 -free N_2 containing either 5000 ppm or 100 ppm CO_2 the amount of ^{14}C in serine was greater than that in glycine; but in the presence of 21% O_2 the reverse was true.

Sucrose, second to serine, was the major product of [2 - ^{14}C] glycine metabolism and the synthesis of sucrose was greater in aerobic than in anaerobic conditions (Tables 31 and 33). In air the % ^{14}C incorporated into sucrose was greater with 100 ppm CO_2 than with 5000 ppm CO_2 but in N_2 the

% ^{14}C in sucrose was greatest at 5000 ppm CO_2 . The amount of ^{14}C in alanine was greater with 5000 ppm than with 100 ppm CO_2 but there was no affect of O_2 concentration on the synthesis of [^{14}C] alanine. Incorporation of ^{14}C into malate was not affected by O_2 concentration in the presence of 5000 ppm CO_2 but with 100 ppm CO_2 more [^{14}C] malate was formed in air than in O_2 -free N_2 . More [^{14}C] glutamate was synthesised in the presence of N_2 than in the presence of 21% O_2 especially with 5000 ppm CO_2 . The production of ^{14}C labelled glycerate and phosphate esters was greater in aerobic than in anaerobic conditions (Table 33).

^{14}C Products Formed in the Dark.

The amounts of ^{14}C in various compounds extracted from segments of maize leaf supplied with [$2 - ^{14}\text{C}$] glycine for 40 min in darkness are shown in Table 32, the ^{14}C in the various compounds being expressed as a % of the total soluble ^{14}C and in Table 34 the ^{14}C content of the extracted compounds is expressed as d.p.m. $\times 10^{-3}/\text{cm}^2$ leaf tissue. Glycine and serine accounted for most of the soluble ^{14}C in the leaf samples. There was more ^{14}C in glycine than in serine except in the sample supplied with [$2 - ^{14}\text{C}$] glycine in 21% O_2 containing 5000 ppm CO_2 . In the presence of O_2 there was a greater synthesis of alanine, malate, aspartate, glutamate and glycerate and α -ketoglutarate from [$2 - ^{14}\text{C}$] glycine than in the presence of N_2 . There was a greater incorporation of ^{14}C into alanine and glutamate in the presence of 5000 ppm CO_2 compared to 100 ppm CO_2 (Tables 32 and 34); this was true of the ^{14}C content of fumarate (Table 34).

Differences in the Metabolism of [$2 - ^{14}\text{C}$] Glycine in Light and Darkness.

The ^{14}C content of compounds extracted from segments of maize leaf supplied with the labelled substrate in the light and in the dark may be compared in Tables 31 and 32. Serine was the major product of [$2 - ^{14}\text{C}$]

glycine metabolism in both the light and the dark. The [^{14}C]serine was greater than [^{14}C]glycine in anaerobic conditions in the light but in darkness [^{14}C]glycine was generally greater than [^{14}C]serine. The synthesis of [^{14}C]sucrose was light-dependent. The % of the total soluble ^{14}C in alanine and malate was greater in the presence of 21% O_2 in the dark than in the light but in the presence of N_2 the reverse was true. In darkness the % [^{14}C]glutamate and aspartate was greater than in the light. [^{14}C]glutamine was not synthesised from [2 - ^{14}C]glycine under any of the conditions studied. The % of the total soluble ^{14}C present in compounds other than glycine and serine was greater under anaerobic conditions in the dark than in the light, but under aerobic conditions this was greater in the light than in the dark.

TABLE:31

Metabolism of $[2 - ^{14}\text{C}]$ Glycine in the Light by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 .

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration	0	21	0	21
(% by volume)				
Product	(^{14}C in the various compounds expressed as a % of the total soluble ^{14}C)			
sucrose	2.0	20.9	8.3	15.5
glycine	30.0	54.5	37.3	43.2
serine	61.9	13.5	44.2	21.8
alanine	0.4	0.4	1.3	1.0
malate	0.1	1.1	0.6	0.5
aspartate	0.2	0.3	0.3	0.5
glutamate	0.4	0.3	1.1	0.5
phosphate esters	1.1	3.3	2.4	2.4
other compounds	3.9	5.7	4.5	14.6
	d.p.m. $\times 10^{-6}/\text{cm}^2$ leaf tissue			
Total soluble ^{14}C	1.82	2.01	1.16	1.20

Analysis of the ^{14}C compounds extracted after supplying the labelled substrate ($4 \mu\text{Ci}$, $0.4 \mu\text{mol}$ in 0.05 ml) for 40 min in the light (1950 ft candles) for 40 min.

TABLE:32

Metabolism of $[2 - ^{14}\text{C}]$ Glycine in Darkness by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 and CO_2 .

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration	0	21	0	21
(% by volume)				
Product	(^{14}C in the various compounds expressed as a % of the total soluble ^{14}C)			
glycine	60.39	38.11	57.17	34.78
serine	13.00	33.95	13.37	45.36
alanine	0.23	1.22	1.22	1.75
malate	0.18	0.51	0.16	0.49
aspartate	0.58	1.65	0.35	2.13
glutamate	0.79	1.40	1.01	2.72
glycerate + α -ketoglutarate	3.27	3.43	2.01	2.97
other compounds	21.56	19.73	27.99	9.80
	d.p.m. $\times 10^{-6}/\text{cm}^2$ leaf tissue			
Total soluble ^{14}C	0.36	1.08	0.46	1.17

Analysis of the ^{14}C compounds extracted after supplying the labelled substrate ($4 \mu\text{Ci}$, $0.4 \mu\text{mol}$ in 0.05ml) for 40 min in darkness at 28°C to segments of lamina, 2 cm^2 .

TABLE:33

Metabolism of [2 - ^{14}C] Glycine in the Light by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 and CO_2

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration	0	21	0	21
(% by volume)				
Product	(^{14}C in the various compounds expressed as d.p.m. $\times 10^{-3}/\text{cm}^2$ leaf tissue)			
sucrose	29.4	327.4	73.1	165.3
glycine	452.2	858.2	328.4	461.4
serine	936.3	210.9	389.3	232.4
alanine	6.0	7.0	10.8	10.3
malate	1.8	17.2	5.1	4.9
aspartate	3.2	5.1	2.7	4.9
glutamate	5.4	4.2	9.3	5.6
glyceric acid	1.9	10.7	6.6	14.3
succinic acid	9.1	2.5	1.2	2.0
fumaric + erythronic acid	1.9	6.9	4.1	4.5
phosphate esters	13.5	51.5	21.3	25.4
other compounds	35.0	47.8	30.1	25.6
insoluble polysaccharides	9.2	30.0	7.3	13.2

Analysis of the ^{14}C compounds extracted after supplying the labelled substrate ($4\ \mu\text{Ci}$, $0.4\ \mu\text{mol}$ in $0.05\ \text{ml}$) for 40 min in the light (1950 ft candles) for 40 min.

TABLE:34

Metabolism of $[2 - ^{14}\text{C}]$ Glycine in Darkness by Segments of Maize Leaf in Atmospheres Containing Different Concentrations of O_2 and CO_2 .

CO_2 concentration	100 ppm		5000 ppm	
O_2 concentration	0	21	0	21
(% by volume)				
Product	(^{14}C in the various compounds expressed as d.p.m. $\times 10^{-3}/\text{cm}^2$ leaf tissue)			
glycine	218.1	411.9	262.3	405.3
serine	46.9	366.9	61.3	528.6
alanine	0.8	13.2	5.6	20.4
malate	0.7	5.6	0.7	5.7
aspartate	2.1	17.8	1.6	24.9
glutamate	2.9	15.1	4.6	31.8
fumarate	0.2	1.6	0.05	0.8
glycerate + α -ketoglutarate	11.8	37.1	21.6	34.6
other compounds	75.6	203.6	98.8	93.1
insoluble polysaccharides	2.1	8.1	3.5	13.4

Analysis of the ^{14}C compounds extracted after supplying the labelled substrate ($4 \mu\text{Ci}$, $0.4 \mu\text{mol}$ in 0.05 ml) for 40 min in darkness at 28°C to segments of lamina, 2 cm^2 .

DISCUSSION

Photosynthesis by leaves of tobacco was decreased progressively with increased O_2 concentrations in the range from 0 to 100% by volume at various concentrations of CO_2 (Fig. 10). If the concentration of CO_2 was greater than 300 ppm the rate of photosynthesis by maize was also affected by O_2 in the same way (Figs. 7 and 8). Even with concentrations of CO_2 less than 300 ppm the rate of photosynthesis by leaves of maize decreased as the O_2 concentration in the atmosphere was increased in the range 40 to 100% by volume (Figs. 8 and 9). However, in this case (Fig. 8) there was an optimum concentration of O_2 for photosynthesis in the range 21 to 41% by volume; between 0 and 21% O_2 there was an increase in photosynthesis. In other words photosynthesis by maize, unlike tobacco, was limited by the absence of O_2 at low CO_2 concentrations. The results of Forrester *et al* (1966b) also showed an enhancement by O_2 of the rate of photosynthesis by maize leaves at low CO_2 concentration. The rate of photosynthesis by maize in the presence of O_2 -free N_2 was less than with 10% O_2 at 600 ft candles and this effect was even more marked at 1000 ft candles. Downes and Hesketh (1968) found with leaves of several varieties of maize and other C_4 -plants, the rate of photosynthesis was the same in air as in O_2 -free N_2 but in a few cases photosynthesis was slower when O_2 was absent, and by as much as 6% for a particular sample of maize that was studied.

Maize differs from tobacco in possessing the C_4 -acid cycle as well as the Calvin cycle or photosynthetic carbon reduction pathway. Differences in the effects of O_2 and CO_2 on the rate of photosynthesis by the two species may, therefore, be caused by specific effects on the C_4 -dicarboxylic acid pathway in maize. For the purpose of this discussion it is accepted that the C_4 -dicarboxylic acid pathway consists of a metabolic cycle into which CO_2 is fixed before it is available to the Calvin cycle (Hatch and Slack, 1970). Fixation of atmospheric CO_2 is

catalyzed by PEP carboxylase and results in production of oxaloacetate. Aspartate is formed from oxaloacetate by transamination and malate by reduction of oxaloacetate; CO_2 is released from the C_4 -acids by decarboxylation and phosphoenolpyruvate is regenerated from the three carbon compound that remains. The CO_2 is re-fixed by a carboxylation mediated by RuDP carboxylase and in this way enters the Calvin cycle. In maize the C_4 -substrate that is decarboxylated is probably malate (Berry et al, 1970).

Synthesis of C_4 -acids (malate plus aspartate) during photosynthesis by maize in 5000 ppm $[^{14}\text{C}]\text{CO}_2$ was least in O_2 -free N_2 but in 100 ppm $[^{14}\text{C}]\text{CO}_2$ synthesis was least in the presence of 21% O_2 (Table:35). Incorporation of ^{14}C into malate plus aspartate was, therefore, inversely related to the overall rate of photosynthesis (Table:35 and Figs:7 and 8). This suggests that, in maize, the limitation by O_2 of the rate of carbon metabolism is subsequent to the synthesis of the C_4 -acids and that a direct inhibition of PEP carboxylase by O_2 is not significant in accounting for inhibition of photosynthesis by high concentrations of O_2 . Bowes and Ogren (1972) found that PEP carboxylase isolated from maize was not inhibited by O_2 . However, the accumulated C_4 -acids might inhibit the carboxylation catalyzed by PEP carboxylase since L-malate and L-aspartate do inhibit PEP carboxylase in vitro (Ting, 1968). Also, a decrease in the rate of metabolism of the C_4 -acids would be associated with slower regeneration of the acceptor, PEP.

The increased ^{14}C present in malate plus aspartate after photosynthesis in 5000 ppm $[^{14}\text{C}]\text{CO}_2$ with increased concentrations of O_2 is consistent with a decreased rate of entry of carbon into the Calvin cycle from the C_4 -cycle. The same conclusion might be reached regarding increased $[^{14}\text{C}]\text{malate plus aspartate}$ synthesised during photosynthesis in the

TABLE:35

^{14}C Carbon Incorporated into Malate plus Aspartate by Leaves of Maize During 5 min Photosynthesis from $[^{14}\text{C}]\text{CO}_2$ (1950 ft candles, 28°C).

CO_2 concentration (ppm)	100			5000		
O_2 concentration (% by volume)	0	21	80	0	21	80
(^{14}C expressed as % of total soluble ^{14}C)						
C_4 -acids (malate + aspartate)	21.20	11.50	17.20	7.76	19.10	32.00

Atmospheres contained different proportions of O_2 and O_2 -free N_2 with 100 ppm CO_2 with ^{14}C ($6.2\mu\text{Ci}/\mu\text{mol}$) or 5000 ppm CO_2 with ^{14}C ($1.89\mu\text{Ci}/\mu\text{mol}$).

presence of 80% O₂ compared to 21% O₂ containing 100 ppm [¹⁴C] CO₂ (Table:35 and Fig:8). It was not possible to judge from the data obtained whether O₂ affected also the transport of C₄-acids from the site of synthesis to the site of decarboxylation, especially, since there is conflicting evidence in the literature as to where the loci of synthesis and decarboxylation are. Some workers have proposed that RuDP carboxylase activity, and therefore possibly decarboxylation, is restricted to the chloroplasts of the cells of the bundle sheath, whilst others have suggested that the enzyme is also active in chloroplasts of the cells of the mesophyll. Both the intercellular and intracellular location of PEP carboxylase are in doubt. The present study was not directly concerned with these controversies, although the availability, in particular of O₂, may well affect the movement of carbon compounds between the sites of carboxylation. On the other hand inhibition by O₂ of the further metabolism of the C₄-acids might be due to a direct inhibition by O₂ of RuDP carboxylase. Bowes and Ogren (1972) showed that carboxylation catalyzed by RuDP carboxylase from maize was inhibited by O₂ in vitro.

Inhibition by O₂ of the rate of photosynthesis by C₃-plants may also be due partly to an effect of O₂ on RuDP carboxylase (Bowes and Ogren, 1972; Ludwig, 1972). However, with C₃-plants there is much evidence that stimulation of photorespiration by O₂ is of major importance in explaining the inhibition by O₂ of photosynthesis (Jackson and Volk, 1970). It is not clear at the present time how far stimulation of photorespiration and inhibition of RuDP carboxylase by O₂ may be caused by the same basic mechanism (Berry, 1971). If either of these mechanisms were operating in maize it would be expected that the effects of O₂ and CO₂ would be at least partly competitive. At any particular concentration of CO₂ an increase of O₂ concentration may cause some inhibition of photosynthesis;

the relative extent of this inhibition at different CO_2 concentrations will depend on the relative affinities of the reacting system for CO_2 and O_2 . Between 40 and 100% O_2 the % inhibition of photosynthesis is not significantly changed by changes in CO_2 concentration in the range 0.001 to 0.6% (Figs: 7, 8 and 9). Clearly a partial pressure of 0.6% CO_2 is not sufficient to overcome inhibitory effects of O_2 on photosynthesis by maize.

Until recently it was not generally accepted that the photorespiratory pathway, the glycolate pathway, was active to a significant extent in maize and other C_4 -plants. However, glycine and serine, which are intermediates of the C2 pathway, were ^{14}C labelled during photosynthesis from $[\text{}^{14}\text{C}]\text{CO}_2$ by leaves of maize (Tables: 5 and 9). Incorporation of ^{14}C into glycine was increased by increasing the O_2 content of the atmosphere (Tables: 5 and 9) and this was true during photosynthesis by maize whether atmospheres contained 100, 300 or 5000 ppm $[\text{}^{14}\text{C}]\text{CO}_2$ (Table:11). Increasing the concentration of CO_2 decreased incorporation of ^{14}C into glycine (Table:10). These effects of O_2 and CO_2 on glycine synthesis in maize suggest that the C2 pathway is operating in a manner similar to its operation in tobacco and other C_3 -plants. Furthermore, the major product of $[\text{}^{14}\text{C}]$ glycine metabolism in light and darkness was $[\text{}^{14}\text{C}]$ serine (Tables:31 and 32). In the light sucrose became extensively ^{14}C labelled (Table:31) but no sucrose was formed in the dark so production of sucrose from glycine was light-dependent as it is in leaves of pea, a C_3 plant (Mifflin *et al*, 1966). To confirm that the serine was synthesised from $[\text{}^{14}\text{C}]$ glycine by the C2 pathway it would be necessary to show that the ^{14}C in serine was mainly in carbon atoms 2 and 3; serine made by the C2 pathway during photosynthesis from $[\text{}^{14}\text{C}]\text{CO}_2$ should be uniformly labelled in all three carbon atoms. The intra-

molecular distribution of ^{14}C was not studied during the present work but the data of Rabson et al (1962) suggest that serine is synthesised by the C2 pathway in leaves of maize.

Synthesis of $[^{14}\text{C}]$ sucrose and $[^{14}\text{C}]$ glycerate from $[2-^{14}\text{C}]$ glycine was greater in 21% O_2 than in O_2 -free N_2 (Tables: 31 and 33). This might be because the synthesis of serine from glycine by maize is O_2 -dependent as it is in tobacco (Bird et al, 1972a). Although evidence for this dependence was found in darkness (Table:32), in the light less serine was present in aerobic than in anaerobic conditions (Table:31). Either serine produced by maize in the absence of O_2 was not synthesised from glycine by a reaction similar to that investigated by Bird et al (1972a) or there was sufficient O_2 present endogenously to satisfy the O_2 requirement for serine synthesis. The latter is probable since the synthesis of serine from glycine has a high affinity for O_2 ; the presence of 3% by volume O_2 was sufficient for maximum serine synthesis from glycine by a system isolated from leaves of tobacco (Bird et al, 1972a) and, indeed, some serine synthesis was found in vitro even when rigorous attempts were made to exclude O_2 . In the light increased $[^{14}\text{C}]$ serine and decreased $[^{14}\text{C}]$ sucrose synthesised from $[2-^{14}\text{C}]$ glycine by leaves of maize in the presence of O_2 -free N_2 compared to 21% O_2 may be due to an anaerobic inhibition of the further metabolism of serine to sucrose. The O_2 -dependent reaction involved may have a lower affinity for O_2 than the system synthesising serine from glycine. Only in the absence of O_2 was the ^{14}C present in serine greater than in glycine after photosynthesis from $[^{14}\text{C}] \text{CO}_2$ by leaves of maize (Table:10); suggesting that its further metabolism is O_2 -dependent. Further work is required to ascertain whether this additional O_2 -dependent reaction of the C2 pathway is also found in C_3 -plants.

In maize the existence of a C2 pathway similar to that in tobacco appears probable. However the CO_2 produced by the C2 pathway in maize must be re-fixed by virtue of the highly active PEP carboxylase system so that photorespiration is not easily detected. Tobacco, having relatively little PEP carboxylase, loses much of the CO_2 produced by the C2 pathway to the atmosphere (photorespiration). ATP and reduced pyridine nucleotides would be required in maize for re-fixation of CO_2 produced by operation of the C2 pathway; hence, stimulation of the C2 pathway by increased O_2 would decrease the photosynthetic capacity available for fixation of new CO_2 . Decreased apparent photosynthesis by maize caused by increased O_2 , as with C_3 -plants, may be the result partly of increased metabolism by the C2 pathway. It would be necessary to study the rate of carbon flow through the C2 pathway intermediates as well as the rate of photosynthesis in order to judge to what extent the stimulation of C2 metabolism by O_2 accounts for the decreased rates of apparent photosynthesis by maize in the presence of high O_2 concentrations with both low and high concentrations of CO_2 .

There is evidence for an alternative mechanism by which O_2 might inhibit photosynthesis. By oxidising directly a component of the photosynthetic electron transport chain e.g. as in the-Mehler reaction, it could decrease the reducing power available for CO_2 fixation. Although studies of this reaction have been largely in vitro using isolated chloroplasts, Brown and Good (1955) found evidence for an active Mehler reaction in Chlorella in vivo. It would seem unlikely that this reaction could account for an inhibition of photosynthesis by concentrations of O_2 from 40 to 100% by volume since Heber and French (1968) found that maximum O_2 uptake by chloroplasts isolated from spinach was in the presence of about 7% O_2 . They concluded that O_2 uptake by isolated chloroplasts was already saturated at low O_2 concentrations compared to inhibition by

O_2 of photosynthesis. On the other hand these observations were made in the absence of CO_2 and competition by CO_2 for reductant may modify the affinity for O_2 . It is also possible that the O_2 -dependent Mehler reaction and the stimulation by O_2 of photorespiration are related. Coombs and Whittingham (1966) proposed that the oxidation of a sugar phosphate to glycollate might be enhanced by peroxide formed by the Mehler reaction.

It is concluded that the stimulation of the C2 pathway together with increased direct inhibition by O_2 of RuDP carboxylase are the main factors responsible for the greater accumulation of $[^{14}C]C_4$ -acids after photosynthesis from $[^{14}C]CO_2$, and for decreased rates of photosynthesis by leaves of maize, that were observed when O_2 concentrations were increased from 40 to 100% by volume.

With maize it is now necessary to explain the stimulation of photosynthesis by O_2 (0 to 21%, by volume) when the CO_2 concentration is low. After photosynthesis by leaves of maize from 100 ppm $[^{14}C]CO_2$ there was an accumulation of $[^{14}C]$ malate plus aspartate in O_2 -free N_2 compared with 21% O_2 (Table:35). This may indicate that with low CO_2 concentrations the rate of photosynthesis is limited by an O_2 -dependent process subsequent to the synthesis of these acids. However in 100 ppm $[^{14}C]CO_2$ there was a change with O_2 concentration in the relative proportions of $[^{14}C]$ malate and $[^{14}C]$ aspartate synthesised, so that most aspartate and least malate were formed in O_2 -free N_2 (Table:9). Therefore, with low concentrations of CO_2 removal of O_2 from the atmosphere decreased the rate of photosynthesis because more carbon was incorporated into aspartate rather than malate, the substrate from which carbon is transferred to the Calvin cycle. The accumulation of recently fixed carbon in aspartate, if this represents a loss of carbon from the C_4 -cycle, would also limit the rate of regeneration of phosphoenolpyruvate and therefore the rate of fixation of CO_2 . Synthesis of aspartate rather than malate from

oxaloacetate may result from an increase in the availability of amino donors. This may arise in the following manner. The first amino acid into which inorganic nitrogen was incorporated during photosynthesis by Chlorella was glutamate produced by reductive amination of α -ketoglutarate (Bassham and Kirk, 1964); other amino acids were thought to be synthesised either directly or indirectly by transamination from glutamate. If this situation also exists in maize, since glutamate is not rapidly labelled during photosynthesis from $[^{14}\text{C}]\text{CO}_2$ (Tables: 14 and 15), the α -ketoglutarate needed for glutamate synthesis must be made from carbohydrates or other reserves in the leaf by a pathway involving the Krebs cycle. Synthesis of α -ketoglutarate would be decreased therefore if the partial pressure of O_2 was low. However, the rate of turnover of glutamate by transamination to other keto acids may be increased so that even when the supply of new α -ketoglutarate is limited inorganic nitrogen can be incorporated into amino acids. Reduced NADP may accumulate in the chloroplasts when the supply of the natural oxidant, CO_2 , is limited. In anaerobic conditions, the extra NADPH may be partly used to increase the rate of reductive amination of α -ketoglutarate while oxaloacetate provides, directly or indirectly, a ketoacid acceptor to maintain the turn-over of glutamate. In this way diversion of carbon into aspartate occurs. O_2 could decrease the available reducing power by stimulating the C2 pathway or by the Mehler reaction.

Stimulation of the C2 pathway by increased concentrations of O_2 from 0 to 21% by volume, would remove excess reducing power because the CO_2 released must be re-fixed, thereby consuming NADPH and ATP. The Mehler reaction would continue, or even be increased (Whitehouse et al. 1971), in the presence of an uncoupler, for example NH_4Cl . The rate of photosynthesis by leaves of maize pretreated with solutions of 5.12mM NH_4Cl in 0.2M phosphate buffer, pH 7.6, or 5.12mM NH_4Cl in 0.01M HEPES buffer,

pH 7.4 was greater or not significantly less in O_2 -free N_2 than in 21% O_2 (Figs: 11 and 12). The requirement for O_2 was now less apparent, therefore the function of O_2 may not be the oxidation of excess reducing power by the Mehler reaction. Pretreatment of samples of tobacco leaves with NH_4Cl did not affect the response of the rate of photosynthesis to different concentrations of O_2 . Both with or without exposure to NH_4Cl the rate of assimilation was greatest in N_2 and decreased with increased concentrations of O_2 (Fig: 13).

Further evidence concerning the effect of ammonia on photosynthesis by maize was sought by studying its effect on the products of $[^{14}C]CO_2$ fixation in the presence of different concentrations of O_2 . Unfortunately these data are not very helpful (Table:18). The results obtained in 78% O_2 are suspect because of the exceptionally high value for $[^{14}C]$ phosphate esters and low value for loss of ^{14}C during chromatography. Increasing the concentration of O_2 in the atmosphere from 0 to 40% (by volume) caused a greater decrease in synthesis of $[^{14}C]$ -sucrose and phosphate esters after treatment with NH_4Cl (Table:18). This could be caused by a decrease in the ratio of ATP:NADPH resulting from uncoupling of photophosphorylation. The increased synthesis of $[^{14}C]$ malate caused by O_2 after treatment with NH_4Cl indicates a decreased rate of decarboxylation or an inhibition of re-fixation of the resulting CO_2 into the Calvin cycle (Table:18). NH_4Cl increased carbon incorporation into malate and alanine during photosynthesis by Chlorella (Kanazawa et al, 1970). They proposed that this effect was due to a stimulation of pyruvate kinase by NH_4Cl . Stimulation of this enzyme in a C_4 -plant, like maize, might decrease the availability of PEP for carboxylation, resulting in less malate synthesis but increased formation of alanine. This might be greater as O_2 concentration was increased due to decreased metabolism of malate. In the presence of O_2 more $[^{14}C]$ malate, but much less $[^{14}C]$ alanine, were formed

in leaves pretreated with ammonia than in untreated leaves (Table:18). Therefore, the activity of pyruvate kinase in leaves of maize was probably not increased in the presence of ammonia.

The effect of ammonia on photosynthetic carbon metabolism by maize was therefore, consistent with its action as an uncoupler of photophosphorylation. The rate of photosynthesis, and the effect on it of O_2 , were not modified in the way that was expected, probably because the uncoupler caused the rate of photosynthesis to be limited at all concentrations of O_2 by a shortage of ATP. This shortage of ATP was enhanced in the presence of O_2 because of the stimulation of the C2 pathway (Table:17).

Confirmation that the effect of NH_4Cl on photosynthesis by maize resulted from uncoupling was that pretreatment with NaCl had no effect. However, whilst NH_4Cl did not affect the response to O_2 of the photosynthetic rate of tobacco leaf NaCl did (Figs: 13 and 14). Good (1962) showed that chloride ions were feeble uncouplers. If the effect of Cl^- ions on leaves of tobacco were due to uncoupling the effect should be increased in the presence of ammonia. This was not found to be the case. The ^{14}C incorporated into the soluble products was not greatly affected by pretreatment of leaves with solutions of 5.12mM NaCl (Table:22). The results must be interpreted with caution because the untreated sample in O_2 -free N_2 showed an exceptionally high fixation of $[^{14}C]CO_2$. The significant result was that incorporation into malate was decreased and incorporation into aspartate was increased. Hiatt and Evans (1960) reported that the activity of malic dehydrogenase was increased in vitro by the presence of NaCl. An alternative explanation for the decrease in $[^{14}C]$ malate present may be that carboxylation mediated by PEP carboxylase was inhibited in the presence of NaCl (Osmond and Greenway, 1972).

Although NaCl apparently modified the response to O_2 of the rate of photosynthesis by tobacco leaf so that it resembled the response of maize at low concentrations of CO_2 , there was no close similarity in the carbon metabolism. Aspartate synthesis was increased in tobacco leaf with NaCl but increase occurred whether O_2 was present or absent from the atmosphere.

An alternative explanation of the stimulation by O_2 of photosynthesis by leaves of maize in the presence of low concentrations of CO_2 is that photosynthesis depends on energy (ATP) generated by an O_2 -dependent process. The stimulation of photosynthesis by increasing O_2 concentration throughout the range, 0 to 21% by volume, could not be accounted for by a stimulation of oxidative phosphorylation in mitochondria, as this is known to be saturated by approximately 2% by volume O_2 . The observed effect of pretreatment of leaves of maize with NH_4Cl (Figs:11 and 12) may suggest that O_2 is needed to increase photophosphorylation. This could be of particular importance to a C_4 -plant, such as maize, in which more ATP is required for each molecule of CO_2 assimilated than in C_3 -plants. The additional energy required by C_4 -plants compared to C_3 -plants is for regeneration of the carbon acceptor, phosphoenolpyruvate. If O_2 were required for extra ATP synthesis the effects of O_2 depletion should be most apparent under conditions where the demand for energy was greatest, for example, in the presence of high CO_2 concentrations. This was not the case, since the presence of O_2 decreased the rate of photosynthesis from high concentrations of CO_2 (Fig:7). Furthermore, stimulation of photosynthesis by increasing concentrations of O_2 from 0 to 21-40% probably indicates that the reaction involved has a relatively low affinity for O_2 compared to the O_2 affinity of pseudo-cyclic phosphorylation. Heber (1969) found that the affinity for O_2 of pseudo-cyclic photophosphorylation in leaves of spinach was high, half maximal stimulation being observed with 0.1% O_2 . It seems probable therefore that the increased rate of photo-

synthesis with low concentrations of CO_2 in the presence of 21 compared to 0% O_2 was not due to a requirement for pseudo-cyclic photophosphorylation.

There is evidence that the C2 pathway in leaves of tobacco may provide ATP outside the chloroplast as a result of the reaction in which glycine is converted to serine (Bird et al, 1972b). The amount of carbon flowing through the C2 pathway, and therefore the associated ATP synthesis, depends on a reaction having a low affinity for O_2 . If the synthesis of serine from glycine by leaves of maize is accompanied by ATP synthesis as it is in tobacco, then photosynthesis by maize in low CO_2 without O_2 may be limited by decreased synthesis of this ATP. If this were the explanation at low CO_2 concentrations, the limitation imposed by low O_2 might still be expected when the concentration of CO_2 was increased. The active pools of glycine and serine are probably of similar size at low and high CO_2 (Table:8). Therefore rates of metabolism by the C2 pathway, and associated ATP synthesis may be similar under anaerobic conditions at high and low CO_2 . One would have to suppose that ATP outside the chloroplasts becomes especially important when the CO_2 concentration is low. In conclusion, it is considered that stimulation by O_2 of photosynthesis by maize in low concentrations of CO_2 is unlikely to be explained by any mechanism of O_2 -dependent ATP synthesis.

More information concerning the rate-limiting process of photosynthesis by maize in anaerobic conditions was obtained by studying the metabolism of $[\text{U} - ^{14}\text{C}]$ malate and $[\text{U} - ^{14}\text{C}]$ alanine supplied to segments of maize leaf. The information is not inconsistent with the conclusions set out above but does raise some additional points concerning the effect of the gaseous environment on the metabolism of these compounds by leaves of maize. In the light synthesis from $[\text{U} - ^{14}\text{C}]$ malate of $[\text{U} - ^{14}\text{C}]$ alanine and $[\text{U} - ^{14}\text{C}]$ aspartate was increased with O_2 -free N_2 compared to 21% O_2 ,

especially with 100 ppm CO_2 (Table:23). Under anaerobic conditions with low CO_2 considerably more $[^{14}\text{C}]$ aspartate than $[^{14}\text{C}]$ malate was synthesised from $[\text{U} - ^{14}\text{C}]$ alanine, though this effect was less marked than after photosynthesis from 100 ppm $[^{14}\text{C}] \text{CO}_2$ in O_2 -free N_2 (Tables:27 and 9). These data (Tables:23 and 27) are consistent with the proposal that transamination reactions are enhanced by low CO_2 in the absence of O_2 and result in the accumulation of carbon in aspartate and also alanine.

The $[^{14}\text{C}]$ aspartate synthesised from $[\text{U} - ^{14}\text{C}]$ malate in the light (Table:23) was less than might have been expected from consideration of its synthesis from $[^{14}\text{C}] \text{CO}_2$ (Table:9). This is perhaps because it is made only after decarboxylation of $[\text{U} - ^{14}\text{C}]$ malate to pyruvate, conversion of this pyruvate to PEP and carboxylation of PEP to oxaloacetate. Direct oxidation of $[\text{U} - ^{14}\text{C}]$ malate to oxaloacetate is assumed to be either contrary to the direction of flow of carbon or not to occur because the malate does not reach the malic dehydrogenase in the tissue. The C_4 -cycle is thought to operate mainly in the cells of the mesophyll but the metabolism of substrates supplied exogenously may take place in the cells of the bundle sheath since these are closer to the vascular tissue. Thus malate supplied exogenously would not enter the pools of C_4 -acids associated with the C_4 -cycle and ^{14}C from $[\text{U} - ^{14}\text{C}]$ malate could not enter the C_4 -cycle itself but must be converted to pyruvate by decarboxylation in the bundle sheath cells.

The pyruvate produced by decarboxylation of $[\text{U} - ^{14}\text{C}]$ malate supplied exogenously would be ^{14}C labelled. The transamination of this $[^{14}\text{C}]$ pyruvate probably accounts for the considerable synthesis of $[^{14}\text{C}]$ alanine from $[\text{U} - ^{14}\text{C}]$ malate especially in the absence of O_2 and in low CO_2 (Table:23). If such a synthesis of alanine also occurs from malate during photosynthesis in O_2 -free N_2 containing low CO_2 , the C_4 -cycle may be

depleted not only by the removal of carbon as aspartate but also by removal as alanine. However, alanine made from malate synthesised by carboxylation of PEP should be initially unlabelled during photosynthesis from $[^{14}\text{C}]\text{CO}_2$. Thus the alanine and PEP that was ^{14}C -labelled (Table:8) must have been made by metabolism of intermediates of the Calvin cycle. The formation of alanine may be dependent on pyruvate concentration so the ^{14}C incorporated into alanine by photosynthesis from $[^{14}\text{C}]\text{CO}_2$ might indicate the amount of $[^{14}\text{C}]$ pyruvate present in the tissue (Kanazawa et al, 1970;1972). Increased formation of alanine, and therefore presumably of pyruvate, by Chlorella and leaves of bean during photosynthesis in anaerobic conditions was reported by Bassham and Kirk (1962) and Voskresenskaya et al (1970). Bassham (1968) reported that $[^{14}\text{C}]$ pyruvate was never observed as a product of $[^{14}\text{C}]\text{CO}_2$ fixation by Chlorella in the light. However, pyruvate is labile during extraction of tissues using boiling ethanol and water mixtures. By extracting the tissues with solutions containing 2, 4-dinitrophenylhydrazine Johnson and Hatch (1969) demonstrated that $[^{14}\text{C}]$ pyruvate, isolated as the 2, 4-dinitrophenylhydrazone, was synthesised during photosynthesis from $[^{14}\text{C}]\text{CO}_2$ by sugarcane.

With low concentrations of CO_2 , $[\text{U} - ^{14}\text{C}]$ alanine was a better precursor of $[^{14}\text{C}]$ aspartate than was $[\text{U} - ^{14}\text{C}]$ malate in the light (Tables:23 and 27). It has already been pointed out that when malate was supplied exogenously, synthesis of aspartate probably resulted from pyruvate produced by decarboxylation of malate. Thus alanine is more readily assimilated into the C_4 -cycle than malate, when these metabolites are supplied exogenously. This may result because alanine is easily translocated to the locus of the C_4 -cycle. However $[\text{U} - ^{14}\text{C}]$ malate was a rather better precursor of alanine, than $[\text{U} - ^{14}\text{C}]$ alanine was of malate. These data may indicate that alanine is an intermediate of the C_4 -cycle because it is the form in which pyruvate is translocated back to the locus

of PEP regeneration. The possibility that alanine could be the precursor of phosphoenolpyruvate has already been suggested by Hatch (1971) who considered that this function might be restricted to particular C_4 -species in which aspartate may be decarboxylated to alanine while supplying CO_2 to the Calvin cycle. The evidence supporting this involvement of aspartate and alanine in the C_4 -cycle is the activity of the aminotransferases which would be necessary, and also the suggestion by Berry et al (1970) that C_4 -plants could be divided into two groups according as to whether malate or aspartate was the major C_4 -acid produced during photosynthesis. Maize was considered to belong to the group in which malate was the predominant C_4 -acid but Table:9 shows that amounts of malate and aspartate produced during photosynthesis by maize are dependent upon the composition of the gaseous environment. Furthermore, alanine may be a precursor of phosphoenolpyruvate during photosynthesis by maize (Table:27), but its involvement is perhaps restricted to certain circumstances, for example when synthesis of alanine from the Calvin cycle is enhanced. Utilization of alanine would result in an accumulation of amino-groups at the locus of PEP regeneration. It would then be necessary to postulate a counter-movement of an amino acid, for example aspartate, into the cells of the bundle sheath. There is as yet no evidence that aspartate can be decarboxylated to supply CO_2 to the Calvin cycle and it has already been pointed out that synthesis of [^{14}C]aspartate from [U - ^{14}C] malate in the light probably does not occur in the cells of the bundle sheath.

The metabolism of [U - ^{14}C] malate or [U - ^{14}C] alanine by segments of maize was severely restricted in darkness in the absence of O_2 . In the presence of O_2 the products were such as to suggest that the substrates were being oxidized by the Krebs cycle (Tables:24 and 28). In the light the effect of O_2 on the metabolism of these substrates might also be partly explained by the effect on Krebs cycle activity. However, the major

product of the light-dependent metabolism of $[U - ^{14}C]$ malate and $[U - ^{14}C]$ alanine was sucrose, and there was much less evidence of metabolism by the Krebs cycle in the light. Although glycine and serine were synthesised from both metabolites in light and darkness, there was a clear dependence on both light and O_2 for maximum synthesis to occur (Tables: 23 and 24; 27 and 28). The data are consistent with the entry of ^{14}C from malate and alanine into intermediates of the Calvin cycle in the light and from there into the C2 pathway. Except for glutamine, the same range of compounds were synthesised from malate, alanine and CO_2 in the light (Tables: 9, 23 and 27). Glutamine was not found to be ^{14}C labelled after photosynthesis from $[^{14}C]CO_2$ and its synthesis from $[U - ^{14}C]$ malate and $[U - ^{14}C]$ alanine suggests some metabolism of these compounds in the light by reactions in the mitochondria. The total soluble ^{14}C present in samples of leaf given either $[U - ^{14}C]$ malate or $[U - ^{14}C]$ alanine for 40 min in light was less in an atmosphere of 5000 ppm than in 100 ppm CO_2 (Tables: 23 and 27). This was especially true in 21% O_2 . Uptake of these substrates may have been affected by an adverse pH change in the tissue.

It has been noted that glycine and serine were synthesised from $[U - ^{14}C]$ malate and $[U - ^{14}C]$ alanine in darkness, and the synthesis of these amino acids was increased in the presence of O_2 (Tables: 24 and 28). The flow of carbon through the C2 pathway is light-dependent. Thus the synthesis of glycine and serine from glycollate in darkness is very unlikely. More $[^{14}C]$ serine than $[^{14}C]$ glycine was present after metabolism of $[U - ^{14}C]$ alanine in darkness (Table: 28) or after metabolism of $[U - ^{14}C]$ malate in darkness in the presence of O_2 (Table: 24). Glycine synthesis from serine is unlikely to occur by reversal of the reactions by which serine is synthesised from glycine in the glycollate pathway (Bird et al, 1972b). It may be that in darkness, when $[U - ^{14}C]$ alanine and malate are partly metabolised by the Krebs cycle, glycine and serine are made from

glyoxylate derived from isocitrate by the action of isocitrate lyase (Godavari, Badour and Waygood, 1972). In the presence of O_2 , synthesis of these amino acids from $[U - ^{14}C]$ malate in darkness was greater in low CO_2 than in high CO_2 (Table:24) whereas the reverse was true from $[U - ^{14}C]$ alanine (Table:28). It was not clear why the concentration of CO_2 should affect synthesis of glycine and serine from malate by way of the Krebs cycle and isocitrate lyase. Entry of carbon from alanine into the cycle by carboxylation of pyruvate may be enhanced with high CO_2 .

The metabolism of malate supplied exogenously can be considered as 'by-passing' the carboxylation mediated by PEP carboxylase and supplying carbon directly to the Calvin cycle. Since the metabolism of malate was increased if O_2 was present in the atmosphere (Table:23) any decrease in photosynthesis by maize caused by anaerobiosis may be because of an O_2 requirement by a process occurring after the synthesis of the C_4 -acids. Furthermore the malate supplied accumulated in the tissue under anaerobic conditions so perhaps the rate of decarboxylation of malate may, under certain circumstances, limit the CO_2 available for re-fixation into the Calvin cycle. On the other hand, under anaerobic compared to aerobic conditions, more ^{14}C from $[U - ^{14}C]$ malate accumulated in alanine (Table: 23). Therefore, the absence of O_2 suppresses not only the decarboxylation of exogenous malate but also further metabolism of the products. To supply alanine can be considered as 'by-passing' both the C_4 -cycle and the entry of carbon from it into the Calvin cycle, or, alternatively, as providing a close precursor of PEP, the CO_2 acceptor of the C_4 -cycle. The results of Table:27 show that added alanine is used for both sucrose synthesis and malate synthesis. Presumably the initial reaction in the metabolism of $[U - ^{14}C]$ alanine is its conversion to $[^{14}C]$ pyruvate and thence to PEP. 3-PGA, formed from the PEP, may then be metabolised by the carbon reduction cycle to sucrose. Thus the synthesis of sucrose from

[U - ^{14}C] alanine would be light-dependent as is shown in Tables: 27 and 28.

The data presented in this thesis raise some interesting questions concerning the synthesis of sucrose, which is a major product of photosynthesis by maize (Table:9). Decreased synthesis of sucrose is accompanied by an accumulation of ^{14}C from [U - ^{14}C] CO_2 in the C_4 -acids and a decreased rate of photosynthesis (Tables: 8 and 6). With low CO_2 , the absence of O_2 decreased synthesis of sucrose; also, synthesis of sucrose from substrates supplied exogenously, malate, alanine and glycine, was always greater in aerobic than in anaerobic conditions (Tables: 23, 27 and 31). [U - ^{14}C] alanine and [U - ^{14}C] malate were better precursors of sucrose than [2 - ^{14}C] glycine, especially in the absence of O_2 (Tables: 23, 28 and 31). It may be that ^{14}C from [U - ^{14}C] alanine and [U - ^{14}C] malate can be metabolised to sucrose by an O_2 -independent pathway whereas that from [2 - ^{14}C] glycine cannot. Alternatively the O_2 -dependent reaction that limits the production of sucrose from malate and alanine may have a higher affinity for O_2 than that limiting synthesis of sucrose from glycine. Thus removal of O_2 from the atmosphere would decrease the rate of sucrose synthesis from glycine more than that from malate and alanine.

It seemed possible that the activity of the C2 pathway in maize may be important in sucrose synthesis with low CO_2 because in the absence of O_2 , when metabolism by the C2 pathway would be decreased, synthesis of sucrose was also less (Table:9). On the other hand tobacco, a C_3 -plant, has an active C2 pathway to sucrose but even with low CO_2 the rates of photosynthesis and sucrose production are fastest in an atmosphere without O_2 (Fig: 10 and Table:5). The amount of ^{14}C incorporated into glycine and serine after 5 min photosynthesis from [U - ^{14}C] CO_2 was less by leaves of maize than by leaves of tobacco (comparing Tables: 5 and 10). This may show that the flow of carbon through the C2 pathway, and hence any sucrose

synthesis by this route must be slower in leaves of maize than in tobacco. On the other hand, the data could also be explained if there were a faster flow of carbon through smaller 'active' pools of intermediates of the C2 pathway in maize compared to tobacco. This alternative was tested when samples of maize leaves were supplied with 100 ppm [^{14}C] CO_2 in either O_2 -free N_2 or 21% O_2 for various periods of time. The magnitude of the active pools can be determined by how quickly they became saturated with ^{14}C .

In 21% O_2 the ^{14}C present in glycine and serine increased for more than 5 min but in O_2 -free N_2 there was apparently no further increase after 5 min (Tables: 14 and 15; Figs: 15 and 16). The data do provide some evidence that in O_2 -free N_2 there is a more rapid turnover of slightly smaller pools than in 21% O_2 . Although the data were not entirely satisfactory, it was concluded that the flow of carbon through the C2 pathway in maize is slower than in tobacco. The active pools of glycine and serine were smaller in maize than in tobacco but saturation with ^{14}C took about the same time in the two species. It seems unlikely, therefore, that the C2 pathway functions as a major route of sucrose synthesis in maize even in the presence of low CO_2 . The results do not preclude the possibility that more than one pool of these amino acids is involved in photosynthesis. Carbon might move from small active pools of glycine and serine into associated larger pools that were metabolised more slowly. The saturation of a small pool could be masked by the accumulation of carbon in the larger pool. There must be more than one active pool of glycine associated with the C2 pathway, since the enzymes mediating the metabolism of glycollate to glycine are in the peroxisomes (Tolbert, 1971) and synthesis of serine from glycine is effected in the mitochondria (Kisaki *et al*, 1971b). All the photosynthesising cells of leaves of a C_3 -plant, such as tobacco, are capable of C2 pathway metabolism by virtue of the presence of peroxisomes and mitochondria. However studies of the distribution of these organelles, especially the peroxisomes (Frederick and Newcomb, 1971), have shown that

they are present in greater numbers in the cells of the bundle sheath than in those of the mesophyll in C_4 -plants. The activity of the C2 pathway must therefore be largely restricted, in C_4 -plants, to the cells of the bundle sheath. It may be that during photosynthesis by leaves of maize with low CO_2 the activity of the C2 pathway as a route of sucrose synthesis becomes important in the cells of the bundle sheath.

During photosynthesis from CO_2 glycine may not be an important intermediate of sucrose synthesis. The importance of malate and alanine supplied exogenously to leaves of maize as precursors of sucrose must be considered further. It is suggested that the results in Tables: 23 and 24 are explained by decarboxylation of $[U - ^{14}C]$ malate to form CO_2 from C-4 and pyruvate from C-1, C-2 and C-3. The CO_2 is assimilated into intermediates of the Calvin cycle and the $[^{14}C]$ pyruvate should be metabolised similarly to that produced from alanine when $[U - ^{14}C]$ alanine was the substrate supplied. The apparent O_2 -requirement for sucrose synthesis from $[U - ^{14}C]$ malate may be due only to the effect of O_2 on subsequent metabolism of the pyruvate since the effect of O_2 on the photosynthetic metabolism of C-4 of malate should be the same as that from CO_2 in photosynthesis.

The decreased synthesis of sucrose from alanine when O_2 is removed from the atmosphere suggests that the reaction limited by the absence of O_2 is one of the steps in the synthesis of PGA from alanine. However, it was not possible to assess whether production of sucrose from alanine is of significance during photosynthesis from CO_2 or if it merely indicates the reversibility of the reactions leading from 3-PGA, in the Calvin cycle, to pyruvate. There may, however be an O_2 -dependent route of sucrose synthesis in which pyruvate is an intermediate. The increased $[^{14}C]$ alanine present after photosynthesis with $[^{14}C]CO_2$ (Table:9) in the

absence of O_2 is consistent with this suggestion. But during photosynthesis by maize from 5000 ppm $[^{14}C]CO_2$ sucrose synthesis was greater with O_2 -free N_2 than with 21% O_2 (Table:9). However this was not the case with sucrose synthesis from malate and alanine with high CO_2 , sucrose synthesis from these metabolites was stimulated by O_2 . Malate and alanine may not have reached the proper sites of the tissues. On the other hand, it may be that with high CO_2 in the absence of O_2 no reaction between malate or alanine and sucrose became rate limiting during photosynthesis because there was sufficient endogenous O_2 . Evidence from studies of photosynthesis by C_3 -plants and from high CO_2 by maize suggests that sucrose synthesis under anaerobic conditions is more efficient than under aerobic conditions and may take place in the chloroplasts with energy derived from photophosphorylation. Sucrose synthesis that is dependent on O_2 , may utilize energy produced by mitochondrial oxidative phosphorylation and take place outside the chloroplast. With high CO_2 , anaerobic and aerobic routes of sucrose production may still both function since there is sufficient endogenous O_2 to satisfy the O_2 requirement. On the other hand with high CO_2 direct fixation of atmospheric CO_2 mediated by RuDP carboxylase may be possible, as suggested by Edwards and Black (1971). Hence, with high CO_2 the greater rate of carbon assimilation and sucrose synthesis in the presence of O_2 -free N_2 compared to 21% O_2 might be explained, as it is in leaves of tobacco and other C_3 -plants, by the increased efficiency of Calvin cycle metabolism. However, the work by Farineau (1971) indicated that during photosynthesis by maize from 0.5% CO_2 in N_2 most of the carbon assimilated entered the Calvin cycle via the C_4 -acids and not by direct fixation of atmospheric CO_2 .

Synthesis of sucrose from malate might be expected because this acid is an intermediate of photosynthetic carbon metabolism by maize. However,

the increased sucrose present after metabolism of [U - ^{14}C] malate under aerobic compared to anaerobic conditions was unexpected, especially with high CO_2 . It would be of interest to study the metabolism of [4 - ^{14}C] malate under similar conditions. It is not possible to assess the significance of the production of sucrose from alanine and glycine supplied exogenously with regard to sucrose synthesis from CO_2 . However it may be that sucrose production during photosynthesis can be effected by either an anaerobic process, which appears to be the more efficient, or an aerobic one.

REFERENCES

- ANDERSEN, K.S., BAIN, J.M., BISHOP, D.G. & SMILLIE, R.M. (1972) Photosystem II activity in agranal bundle sheath chloroplasts from Zea mays. Pl. Physiol., Lancaster 49, 461.
- ANDERSON, J.M., WOO, K.C., & BOARDMAN, N.K. (1971) Photochemical systems in mesophyll and bundle sheath chloroplasts of C₄ plants. Biochim. biophys. Acta 245, 398.
- ANDERSON, J.M., BOARDMAN, N.K., & SPENCER, D. (1971) Phosphorylation by intact bundle sheath chloroplasts from maize. Biochim. biophys. Acta 245, 21.
- ANDREWS, T.J., & HATCH, M.D. (1969) Properties and mechanism of action of pyruvate, phosphate dikinase from leaves. Biochem. J. 114, 117.
- ARNON, D.I., ALLEN, M.B., & WHATLEY, F.R. (1954) Photosynthesis by isolated chloroplasts. Nature, Lond. 174, 394.
- ARNON, D.I., LOSADA, M., WHATLEY, F.R., TSUJIMOTO, H.Y., HALL, D.O., & HORTON, A.A. (1961) Photosynthetic phosphorylation and molecular oxygen. Proc. natn. Acad. Sci. U.S.A. 47, 1314.
- ASADA, K., SAITO, K., KITTOCH, S., & KASAI, Z. (1965) Photosynthesis of glycine and serine in green plants. Pl. Cell Physiol., Tokyo 6, 47.
- ATKINS, C.A., CANVIN, D.T., & FOCK, H. (1971) Intermediary metabolism of photosynthesis in relation to carbon dioxide evolution in sunflower. In Photosynthesis and Photorespiration, p.497. Ed. by Hatch, M.D., Osmond, C.B., & Slatyer, R.O. New York: Wiley-Interscience.
- BACHMANN, M.D., ROBERTSON, D.S., & BOWEN, C.C. (1969) Thylakoid anomalies in relation to grana structure in pigment-deficient mutants of Zea mays. J. Ultrastruct. Res. 28, 435.
- BALDREY, C.W., BUCKE, C., & COOMBS, J. (1969) Light/phosphoenolpyruvate dependent carbon dioxide fixation by isolated sugar cane chloroplasts. Biochem. biophys. Res. Commun. 37, 828.
- BALDREY, C.W., BUCKE, C., & COOMBS, J. (1971) Progressive release of carboxylating enzymes during mechanical grinding of sugar cane leaves. Planta 97, 310.
- BASSHAM, J.A. (1964) Kinetic studies of the photosynthetic carbon reduction cycle. A. Rev. Pl. Physiol. 15, 101.
- BASSHAM, J.A. (1968) The Calvin cycle in algae and leaves. In Photosynthesis in Sugar Cane, p.1. Ed. Coombs, J. Proceedings of an International Symposium sponsored by Imperial College, University of London & Tate and Lyle Ltd. 12th and 13th June, 1968.
- BASSHAM, J.A., BENSON, A.A., & CALVIN, M. (1950) The path of carbon in photosynthesis VIII. The role of malic acid. J. biol. Chem. 185, 781.
- BASSHAM, J.A., BENSON, A.A., KAY, L.D., HARRIS, A.Z., WILSON, A.T., & CALVIN, M. (1954) The path of carbon in photosynthesis XXI. The cyclic regeneration of carbon dioxide acceptor. J. Am. chem. Soc. 76, 1760.

- BASSHAM, J.A., & CALVIN, M. (1957) In The Path of Carbon in Photosynthesis. p.23 Englewood Cliffs, N.J.: Prentice-Hall, Inc.
- BASSHAM, J.A., & KIRK, M. (1962) The effect of oxygen on the reduction of CO_2 to glycolic acid and other products during photosynthesis by Chlorella. Biochem. biophys. Res. Commun. 9, 376.
- BASSHAM, J.A., & KIRK, M. (1964) Photosynthesis of amino acids. Biochim. biophys. Acta 90, 553.
- BAZZAZ, M., & GOVINDJEE. (1971) Studies on the photochemical activities and spectral characteristics of bundle sheath and mesophyll chloroplasts in Zea mays. Pl. Physiol., Lancaster 47, S 189.
- BEDNARZ, R.M., & RASMUSSEN, H.P. (1972) CO_2 -fixation sites in leaves of maize and oats. J. exp. Bot. 23, 415.
- BENSON, A.A., BASSHAM, J.A., CALVIN, M., GOODALE, T.C., HASS, V.A., & STEPKA, W. (1950) The path of carbon in photosynthesis V. Paper chromatography and radioautography of products. J. Am. chem. Soc. 72, 1710.
- BENSON, A.A., & CALVIN, M. (1950) The path of carbon in photosynthesis VII. Respiration and photosynthesis. J. exp. Bot. 1, 63.
- BENSON, A.A., KAWAUCHI, S., HAYES, P., & CALVIN, M. (1952) The path of carbon in photosynthesis. XVI. Kinetic relationships of the intermediates in steady state photosynthesis. J. Am. chem. Soc. 74, 4477.
- BERRY, J.A., (1970) The effect of oxygen on CO_2 fixation by carboxydismutase in vitro and an examination of a possible reaction of ribulose diphosphate with oxygen. Carnegie Inst. Washington Year Book 70, p.526.
- BERRY, J.A., DOWNTON, W.J.S., & TREGUNNA, E.B. (1970) The photosynthetic carbon metabolism of Zea mays and Gomphrena globosa: the location of the CO_2 fixation and the carboxyl transfer reactions. Can. J. Bot. 48, 777.
- BIELESKI, R.L., & YOUNG, R.E. (1963) Extraction and separation of phosphate esters from plant tissues. Analyt. Biochem, 6, 54.
- BIRD, I.F., CORNELIUS, M.J., KEYS, A.J., & WHITTINGHAM, C.P. (1972a) Oxidation and phosphorylation associated with the conversion of glycine to serine. Phytochemistry 11, 1587.
- BIRD, I.F., CORNELIUS, M.J., KEYS, A.J., & WHITTINGHAM, C.P. (1972b) Adenosine triphosphate synthesis and the natural electron acceptor for synthesis of serine from glycine in leaves. Biochem. J. 128, 191.
- BIRD, I.F., PORTER, H.K., & STOCKING, C.R. (1965) Intracellular localisation of enzymes associated with sucrose synthesis in leaves. Biochim. biophys. Acta. 100, 366.
- BISALPUTRA, T., DOWNTON, W.J.S., & TREGUNNA, E.B. (1969) The distribution and ultrastructure of chloroplasts in leaves differing in photosynthetic carbon metabolism. I. Wheat, Sorghum and Aristida (Gramineae). Can. J. Bot. 47, 15.

- BJÖRKMAN, O. (1965) Photosynthetic inhibition by oxygen in higher plants. Carnegie Inst. Washington, Year Book 65, p.446.
- BJÖRKMAN, O. (1966) Effect of oxygen concentration on photosynthesis in higher plants. *Physiologia Pl.* 19, 618.
- BJÖRKMAN, O. (1969) Carboxydismutase activity in plants with and without β -carboxylation photosynthesis. *Planta* 88, 197.
- BJÖRKMAN, O., GAUHL, E., HIESEY, W.M., NICHOLSON, F., & NOBS, M.A. (1967) Growth of Mimulus, Marchantia and Zea under different oxygen and carbon dioxide levels. Carnegie Inst., Washington. Year Book 67, p.477.
- BJÖRKMAN, O., GAUHL, E., & NOBS, M.A. (1968) Comparative studies of Atriplex species with and without β -carboxylation photosynthesis and their first-generation hybrid. Carnegie Inst. Washington, Year Book 68, p.620.
- BLACK, C.C., & MOLLENHAUER, H.H. (1971) Structure and distribution of chloroplasts and other organelles in leaves with various rates of photosynthesis. *Pl. Physiol.*, Lancaster 47, 15.
- BOVE, J.M., BOVE, C., WHATLEY, F.R., & ARNON, D.I. (1963) Chloride requirement for oxygen evolution in photosynthesis. *Z. Naturf.* 18, 683.
- BOWES, G., & OGREN, W.L. (1971) Properties of RuDP and PEP carboxylases from soybean and corn. *Pl. Physiol.*, Lancaster 47, S 58.
- BOWES, G., & OGREN, W.L. (1972) Oxygen inhibition and other properties of soybean ribulose 1, 5-diphosphate carboxylase. *J. biol. Chem.* 247, 2171.
- BRIGGS, G.E., & WHITTINGHAM, C.P. (1952) Factors affecting the rate of photosynthesis of Chlorella at low concentrations of carbon dioxide and in high illumination. *New Phytol.* 51, 236.
- BROWN, A.H. (1953) The effects of light on respiration using isotopically enriched oxygen. *Am. J. Bot.* 40, 719.
- BROWN, A.H., & GOOD, N. (1955) Photochemical reduction of oxygen in chloroplast preparations and in green cells. I. The study of oxygen exchanges in vitro and in vivo. *Archs Biochem. Biophys.* 57, 340.
- BUCHANAN, J.G., BASSHAM, J.A., BENSON, A.A., BRADLEY, D.F., CALVIN, M., DAUS, L.L., GOODMAN, M., HAYES, P.M., LYNCH, V.H., NORRIS, L.T., & WILSON, A.T. (1952) A pathway of carbon in photosynthesis XVII. The phosphorus compounds as intermediates in photosynthesis. In A Symposium on Phosphorus Metabolism. vol. II, p.440. Ed. McElroy, W.D., & Glass, H.B. Baltimore: John Hopkins Press.
- BUCKE, C., & LONG, S.P. (1971) Release of carboxylating enzymes from maize and sugar cane leaf tissue during progressive grinding. *Planta* 99, 199.
- CALVIN, M., BASSHAM, J.A., BENSON, A.A., LYNCH, V.H., OUELLET, C., SCHOU, L., STEPKA, W., & TOLBERT, N.E. (1951) Carbon dioxide assimilation in plants. *Symp. Soc. exp. Biol.* V, 284.

- CHANG, W.H., & TOLBERT, N.E. (1965) Distribution of C^{14} in serine and glycine after $C^{14}O_2$ photosynthesis by isolated chloroplasts. Modification of serine- C^{14} degradation. *Pl. Physiol.*, Lancaster, 40, 1048.
- CHANG, F.H., & TROUGHTON, J.H. (1972) Chlorophyll a/b ratios in C_3 and C_4 plants. *Photosynthetica* 6, 57.
- CHEN, T.M., BROWN, R.H., & BLACK, C.C. (1969) Photosynthetic activity of chloroplasts isolated from Bermudagrass (*Cynodon dactylon* L.) a species with a high photosynthetic capacity. *Pl. Physiol.*, Lancaster 44, 649.
- CHEN, T.M., BROWN, R.H., & BLACK, C.C. (1970) CO_2 compensation concentration, rate of photosynthesis and carbonic anhydrase activity of plants. *Weed Sci.* 18, 399.
- CHEN, T.M., BROWN, R.H., & BLACK, C.C. (1971) Photosynthetic $^{14}CO_2$ fixation products and activities of enzymes related to photosynthesis in Bermudagrass and other plants. *Pl. Physiol.*, Lancaster 47, 199.
- CHEUNG, G.P., ROSENBLUM, I.Y., & SALLACH, H.J. (1968) Comparative studies of enzymes related to serine metabolism in higher plants. *Pl. Physiol.*, Lancaster 43, 1813.
- CLAGETT, C.O., TOLBERT, N.E., & BURRIS, R.H. (1949) Oxidation of α -hydroxy acids by enzymes from plants. *J. Biol. Chem.* 178, 977.
- COOMBS, J., & WHITTINGHAM, C.P. (1966) The effect of high partial pressures of oxygen on photosynthesis in *Chlorella* - I. The effect on end products of photosynthesis. *Phytochemistry* 5, 643.
- COOPER, R.A., & KORNBERG, H.L. (1965) Net formation of phosphoenolpyruvate from pyruvate by *Escherichia coli*. *Biochim. biophys. Acta* 104, 618.
- COSSINS, E.A., & SINHA, S.K. (1965) Occurrence and properties of L-amino acid: 2-glyoxylate aminotransferase in plants. *Can. J. Biochem.* 43, 495.
- COSSINS, E.A., & SINHA, S.K. (1966) The interconversion of glycine and serine by plant tissue extracts. *Biochem. J.* 101, 542.
- CRAWFORD, C.G., & JENSEN, R.G. (1971) Isolation and partial characterization of ferredoxin from *Zea mays*. *Pl. Physiol.*, Lancaster 47, 447.
- DAWSON, R.M.C., ELLIOTT, D.C., ELLIOTT, W.H., JONES, K.M. (1959) Data for biochemical research. Clarendon Press, Oxford.
- DECKER, J.P. (1955) A rapid postillumination deceleration of respiration in green leaves. *Pl. Physiol.*, Lancaster 30, 82.
- DECKER, J.P. (1957) Further evidence of increased carbon dioxide production accompanying photosynthesis. *J. Solar Energy Sci. & Engineering* 1, 30.
- DELAVAN, L.A., AND BENSON, A.A. (1958) Light stimulation of glycolic acid oxidation in chloroplasts. *Brookhaven Symp. Biol.* 11, 259.
- DOWNES, R.W., & HESKETH, J.D. (1968) Enhanced photosynthesis at low oxygen concentrations: differential response of temperate and tropical grasses. *Planta* 78, 79.

- DOWNTON, W.J.S. (1970) Preferential C⁴-dicarboxylic acid synthesis, the postillumination CO₂ burst, carboxyl transfer step, and grana configurations in plants with C⁴-photosynthesis. *Can. J. Bot.* 48, 1795.
- DOWNTON, W.J.S. (1971) Check list of C⁴ species. In *Photosynthesis and Photorespiration*, p.554 Ed. by Hatch, M.D., Osmond, C.B., & Slatyer, R.O. New York: Wiley-Interscience.
- DOWNTON, W.J.S., BISALPUTRA, T., & TREGUNNA, E.B. (1969) The distribution and ultrastructure of chloroplasts in leaves differing in photosynthetic carbon metabolism. II *Atriplex rosea* and *Atriplex hastata* (Chenopodiaceae) *Can. J. Bot.* 47, 915.
- DOWNTON, W.J.S., & TREGUNNA, E.B. (1968a) Carbon dioxide compensation - its relation to photosynthetic carboxylation reactions, systematics of the Gramineae, and leaf anatomy. *Can. J. Bot.* 46, 207.
- DOWNTON, W.J.S., & TREGUNNA, E.B. (1968b) Photorespiration and glycolate metabolism: a re-examination and correlation of some previous studies. *Pl. Physiol.*, Lancaster 43, 923.
- EDWARDS, G.E., & BLACK, C.C. (1971) Isolation of mesophyll cells and bundle sheath cells from *Digitaria sanguinalis* (L) Scop. Leaves and a scanning microscopy study of the internal leaf cell morphology. *Pl. Physiol.*, Lancaster 47, 149.
- EDWARDS, G.E., LEE, S.S., CHEN, T.M., & BLACK, C.C. (1970) Carboxylation reactions and photosynthesis of carbon compounds in isolated mesophyll and bundle sheath cells of *Digitaria sanguinalis* (L.) Scop. *Biochem. biophys. Res. Commun.* 39, 389.
- EGLE, K., & FOCK, H. (1967) Light-respiration - correlation between CO₂ fixation, O₂ pressure and glycolate concentration. In *Biochemistry of Chloroplasts*: vol.2, p.79 Ed. by Goodwin, T.W. London: Academic Press Inc.
- ELLYARD, P.W., & GIBBS, M. (1967) The effect of oxygen on photosynthesis in chloroplasts. *Pl. Physiol.*, Lancaster 42: S-33.
- EL-SHARKAWY, M., & HESKETH, J. (1965) Photosynthesis among species in relation to characteristics of leaf anatomy and CO₂ diffusion resistances. *Crop.Sci.* 5, 517.
- EL-SHARKAWY, M.A., LOOMIS, R.S., & WILLIAMS, W.A. (1967) Apparent reassimilation of respiratory carbon dioxide by different plant species. *Physiologia Pl.* 20, 171.
- EVERSON, R.G. (1969) Bicarbonate equilibria and the apparent Km (HCO₃⁻) of isolated chloroplasts. *Nature, Lond.* 222, 876.
- EVERSON, R.G., COCKBURN, W., & GIBBS, M. (1967) Sucrose as a product of photosynthesis in isolated spinach chloroplasts. *Pl. Physiol.*, Lancaster 42, 840.
- EVERSON, R.G., & SLACK, C.R. (1968) Distribution of carbonic anhydrase in relation to the C⁴ pathway of photosynthesis. *Phytochemistry* 7, 581.

- FARINEAU, J. (1971) A comparative study of the activities of photosynthetic carboxylation in a C₄ and a Calvin-type plant (the sites of CO₂ fixation in C₄ plants). In Photosynthesis and Photorespiration, p.202 Ed. by Hatch, M.D., Osmond, C.B., & Slatyer, R.O. New York: Wiley-Interscience.
- FOCK, H., EGLE, K., SCHAUB, H., & HILGENBERG, W. (1969) The effect of oxygen on the radioactivity of the products of photosynthesis after ¹⁴CO₂ fixation. *Z. Pflanzenphysiol.* 61, 261.
- FOCK, H., KROTKOV, G., & CANVIN, D.T. (1969) Photorespiration in liverworts and leaves In Progress in Photosynthesis Research, vol.I, p.482 Ed. Metzner, H., sponsored by International Union of Biological Sciences, Tübingen.
- FORRESTER, M.L., KROTKOV, G., & NELSON, C.D. (1966a) Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. I. Soybean. *Pl. Physiol.*, Lancaster 41, 422.
- FORRESTER, M.L., KROTKOV, G., & NELSON, C.D. (1966b) Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. II. Corn and other monocotyledons. *Pl. Physiol.*, Lancaster 41, 428.
- FORTI, G., & JAGENDORF, A.T., (1961) Photosynthetic phosphorylation in the absence of redox dyes: oxygen and ascorbate effects. *Biochim. biophys. Acta* 54, 322.
- FREDERICK, S.E., & NEWCOMB, E.H. (1971) Ultrastructure and distribution of microbodies in leaves of grasses with and without CO₂-photorespiration. *Planta* 96, 152.
- FRIGERIO, N.A., & HARBURY, H.A. (1958) Preparation and some properties of crystalline glycolic acid oxidase of spinach. *J. biol. Chem.* 231, 135.
- GIBBS, M., ELLYARD, P.W., & LATZO, E. (1968) Warburg effect: control of photosynthesis by oxygen. In Comparative Biochemistry and Biophysics of Photosynthesis, p.387 Ed. Shibata, K., Takamiya, A., Jagendorf, A.T., & Fuller, R.C., University of Tokyo Press, Tokyo, University Park Press, State college, Pennsylvania.
- GODAVARI, H.R., BADOUR, S.S., & WAYGOOD, E.R., (1972) Isocitrate lyase in green leaves. *Pl. Physiol.*, Lancaster 49, S25.
- GOLDSWORTHY, A. (1966) Experiments on the origin of CO₂ released by tobacco leaf segments in the light. *Phytochemistry* 5, 1013.
- GOOD, N.E. (1960) Activation of the Hill reaction by amines. *Biochim. biophys. Acta* 40, 502.
- GOOD, N.E. (1962) Uncoupling of the Hill reaction from photophosphorylation by anions. *Archs Biochem. Biophys.* 96, 653.
- GOVINDJEE, OWENS, O.V.H., & HOCH, G. (1963) A mass-spectroscopic study of the Emerson enhancement effect. *Biochim. biophys. Acta* 75, 281.

- GRACEN, V.E., HILLIARD, J.H., BROWN, R.H., & WEST, S.H. (1972) Peripheral reticulum in chloroplasts of plants differing in CO₂ fixation pathways and photorespiration. *Planta* 107, 189.
- GRAHAM, D., & WHITTINGHAM, C.P. (1968) The path of carbon during photosynthesis in *Chlorella pyrenoidosa* at high and low carbon dioxide concentrations. *Z. Pflanzenphysiol.* 58, 418.
- GREENWAY, H., & OSMOND, C.B. (1972) Salt responses of enzymes from species differing in salt tolerance. *Pl. Physiol., Lancaster* 49, 256.
- HALLIWELL, B., LEEK, A.E., & BUTT, V.S. (1972) Oxidative decarboxylation of glycollate and glyoxylate by leaf peroxisomes. *Biochem J.* 128, 87p.
- HARTT, C.E. (1967) Effect of moisture supply upon translocation and storage of ¹⁴C in sugarcane. *Pl. Physiol., Lancaster* 42, 338.
- HARVEY, M.J., & GIBBS, M. (1970) Distribution of photosynthetic products between chloroplasts and incubation medium. *Pl. Physiol., Lancaster* 46, S6.
- HATCH, M.D. (1971) Mechanism and function of the C₄ pathway of photosynthesis. In *Photosynthesis and Photorespiration*. p. 139. Ed. Hatch, M.D., Osmond, C.B., & Slayter, R.O. New York: Wiley-Interscience.
- HATCH, M.D., & SLACK, C.R. (1966) Photosynthesis by sugar-cane leaves. *Biochem. J.* 101, 103.
- HATCH, M.D., & SLACK, C.R. (1967) The participation of phosphoenolpyruvate synthetase in photosynthetic CO₂ fixation of tropical grasses. *Arch Biochem. Biophys.* 120, 224.
- HATCH, M.D., & SLACK, C.R. (1968) A new enzyme for the interconversion of pyruvate and phosphopyruvate and its role in the C₄ dicarboxylic acid pathway of photosynthesis. *Biochem. J.* 106, 141.
- HATCH, M.D., & SLACK, C.R. (1969a) NADP-specific malate dehydrogenase and glycerate kinase in leaves and evidence for their location in chloroplasts. *Biochem. biophys. Res. Commun.* 34, 589.
- HATCH, M.D., & SLACK, C.R. (1969b) Studies on the mechanism of activation and inactivation of pyruvate, phosphate dikinase. *Biochem. J.* 112, 549.
- HATCH, M.D., & SLACK, C.R. (1970) Photosynthetic CO₂-fixation pathways. *A. Rev. Pl. Physiol.* 21, 141.
- HATCH, M.D., SLACK, C.R., & BULL, T.A. (1969) Light-induced changes in the content of some enzymes of the C₄-dicarboxylic acid pathway of photosynthesis and its effect on other characteristics of photosynthesis. *Phytochemistry* 8, 697.
- HATCH, M.D., SLACK, C.R., & JOHNSON, H.S. (1967) Further studies on a new pathway of photosynthetic carbon dioxide fixation in sugar-cane and its occurrence in other plant species. *Biochem. J.* 102, 417.
- HEATH, R.L., & HIND, G. (1969) The role of Cl⁻ in photosynthesis II. The effect of Cl⁻ upon fluorescence. *Biochim. biophys. Acta* 172, 290.

- HEBER, U. (1969) Conformational changes of chloroplasts induced by illumination of leaves in vivo. *Biochim. biophys. Acta* 180, 302.
- HEBER, U., & FRENCH, C.S. (1968) Effects of oxygen on the electron transport chain of photosynthesis. *Planta* 79, 99.
- HEBER, U., & WILLENBRINK, J. (1964) Sites of synthesis and transport of photosynthetic products within the leaf cell. *Biochim. biophys. Acta* 82, 313.
- HEICHEL, G.H., & MUSGRAVE, R.B. (1969) Relation of CO₂ compensation concentration to apparent photosynthesis in maize. *Pl. Physiol., Lancaster* 44, 1724.
- HESKETH, J.D. (1963) Limitations to photosynthesis responsible for differences among species. *Crop Sci.* 3, 493.
- HESKETH, J.D. (1967) Enhancement of photosynthetic CO₂ assimilation in the absence of oxygen, as dependent upon species and temperature. *Planta* 76, 371.
- HESS, J.L., & TOLBERT, N.E. (1966) Glycolate, glycine, serine and glycerate formation during photosynthesis by tobacco leaves. *J. biol. Chem.* 241, 5705.
- HESS, J.L., & TOLBERT, N.E. (1967) Glycolate pathway in Algae. *Pl. Physiol., Lancaster* 42, 371.
- HEW, C.S., & GIBBS, M. (1970) Light-induced O₂ evolution, triphosphopyridine nucleotide reduction and phosphorylation by chloroplasts of maize, sugarcane and sorghum. *Can. J. Bot.* 48, 1265.
- HEW, C.S., & KROTKOV, G. (1967) Effect of temperature on apparent photosynthesis, CO₂ evolution in light and in darkness by attached leaves of sunflower, soybean and egg plants. *Pl. Physiol., Lancaster* 42, S47.
- HEW, C.S., & KROTKOV, G. (1968) Effect of O₂ on the rates of CO₂ evolution in light and in darkness by photosynthesizing and non-photosynthesizing leaves. *Pl. Physiol., Lancaster* 43, 464.
- HEW, C.S., KROTKOV, G., & CANVIN, D.T. (1969) Determination of the rate of CO₂ evolution by green leaves in light. *Pl. Physiol., Lancaster* 44, 662.
- HIATT, A.J., & EVANS, H.J. (1960) Influence of salts on activity of malic dehydrogenase from spinach leaves. *Pl. Physiol., Lancaster* 35, 662.
- HILLER, R.G. (1970) Transients in the photosynthetic carbon reduction cycle produced by iodoacetic acid and ammonium chloride. *J. exp. Bot.* 21, 628.
- HILLER, R.G., & WHITTINGHAM, C.P. (1964) The synthesis of amino-acids in Chlorella in light and dark. I. Photosynthetic products in Chlorella. *J. exp. Bot.* 15, 21.
- HIND, G., NAKATANI, H.Y., & IZAWA, S. (1969) The role of Cl⁻ in photosynthesis. I. The Cl⁻ requirement of electron transport. *Biochim. biophys. Acta* 172, 277.
- HIND, G., & JAGENDORF, A.T. (1965) Effect of uncouplers on the conformational and high energy states of chloroplasts. *J. biol. Chem.* 240, 3202.

- HIND, G., & WHITTINGHAM, C.P. (1963) Reduction of ferricyanide by chloroplasts in the presence of nitrogenous bases. *Biochim. biophys. Acta* 75, 194.
- HOCH, G., OWENS, O.v.H., & KOK, B. (1963) Photosynthesis and respiration. *Archs. Biochem. Biophys.* 101, 171.
- HOFSTRA, G., & NELSON, C.D. (1969) A comparative study of translocation of assimilated ^{14}C from leaves of different species. *Planta* 88, 103.
- HOLM-HANSEN, O., NISHIDA, K., MOSES, V., & CALVIN, M. (1959) Effects of mineral salts on short-term incorporation of carbon dioxide in Chlorella. *J. exp. Bot.* 10, 109.
- HOMANN, P.H., & SCHMID, G.H. (1967) Photosynthetic reactions of chloroplasts with unusual structures. *Pl. Physiol.*, Lancaster 42, 1619.
- HOMANN, P.H., SCHMID, G.H., & GAFFRON, H. (1967) Structure and photochemistry in tobacco chloroplasts. In Comparative Biochemistry and Biophysics of Photosynthesis. Ed. Shibata, K., Takamiya, A., Jagendorf, A.T., & Fuller, R.C. Univ. of Tokyo Press. University Park Press, State College, Penns.
- IRVINE, J.E. (1970) Evidence for photorespiration in tropical grasses. *Physiologia Pl.* 23, 607.
- JACKSON, W.A., & VOLK, R.J. (1969) Oxygen uptake by illuminated maize leaves. *Nature, Lond.* 222, 269.
- JACKSON, W.A., & VOLK, R.J. (1970) Photorespiration. *A. Rev. Pl. Physiol.* 21, 385.
- JIMENEZ, E., BALDWIN, R.L., TOLBERT, N.E., & WOOD, W.A. (1962) Distribution of C^{14} in sucrose from glycolate- C^{14} and serine-3- C^{14} metabolism. *Archs Biochem. Biophys.* 98, 172.
- JOHNSON, H.S., & HATCH, M.D. (1968) Distribution of the C_4 -dicarboxylic acid pathway of photosynthesis and its occurrence in dicotyledonous plants. *Phytochemistry* 7, 375.
- JOHNSON, H.S., & HATCH, M.D. (1969) The C_4 -dicarboxylic acid pathway of photosynthesis. Identification of intermediates and products and quantitative evidence for the route of carbon flow. *Biochem. J.* 114, 127.
- JOLLIFFE, P.A., & TREGUNNA, E.B. (1968) Effect of temperature, CO_2 concentration, and light intensity on oxygen inhibition of photosynthesis in wheat leaves. *Pl. Physiol.*, Lancaster 43, 902.
- JOSHI, G., DOLAN, T., GEE, R., & SALTMAN, P. (1962) Sodium chloride effect on dark fixation of CO_2 by marine and terrestrial plants. *Pl. Physiol.*, Lancaster 37, 446.
- KANAZAWA, T., KANAZAWA, K., KIRK, M.R., & BASSHAM, J.A. (1972) Regulatory effects of ammonia on carbon metabolism in Chlorella pyrenoidosa during photosynthesis and respiration. *Biochim. biophys. Acta* 256, 656.
- KANAZAWA, T., KIRK, M.R., & BASSHAM, J.A. (1970) Regulatory effects of ammonia on carbon metabolism in photosynthesizing Chlorella pyrenoidosa. *Biochim. biophys. Acta* 205, 401.

- KEARNEY, P.C., & TOLBERT, N.E. (1962) Appearance of glycolate and related products of photosynthesis outside of chloroplasts. *Archs Biochem. Biophys.* 98, 164.
- KENTEN, R.H., & MANN, P.J.G. (1952) Hydrogen peroxide formation in oxidations catalysed by plant α -hydroxyacid oxidase. *Biochem. J.* 52, 130.
- KISAKI, T., IMAI, A., & TOLBERT, N.E (1971a) Intracellular localization of enzymes related to photorespiration in green leaves. *Pl. Cell Physiol.*, Tokyo 12, 267.
- KISAKI, T., & TOLBERT, N.E. (1969) Glycolate and glyoxylate metabolism by isolated peroxisomes or chloroplasts. *Pl. Physiol.*, Lancaster 44, 242.
- KISAKI, T., & TOLBERT, N.E. (1970) Glycine as a substrate for photorespiration. *Pl. Cell Physiol.*, Tokyo 11, 247.
- KISAKI, T., YOSHIDA, N., & IMAI, A. (1971b) Glycine decarboxylase and serine formation in spinach leaf mitochondrial preparation with reference to photorespiration. *Pl. Cell Physiol.*, Tokyo 12, 275.
- KLUGE, M., & OSMOND, C.B. (1972) Studies on phosphoenolpyruvate carboxylase and other enzymes of Crassulacean acid metabolism of Bryophyllum tubiflorum and Sedum praealtum. *Z. Pflanzenphysiol.* 66, 97.
- KORTSCHAK, H.P., HARTT, C.E., & BURR, G.O. (1965) Carbon dioxide fixation in sugarcane leaves. *Pl. Physiol.*, Lancaster 40, 209.
- KROGMANN, D.W., JAGENDORF, A.T., & AVRON, M. (1959) Uncouplers of spinach chloroplast photosynthetic phosphorylation. *Pl. Physiol.*, Lancaster 34, 272.
- LAETSCH, W.M. (1968) Chloroplast specialization in dicotyledons possessing the C_4 -dicarboxylic acid pathway of photosynthetic CO_2 fixation. *Am. J. Bot.* 55, 875.
- LAETSCH, W.M., STETTLER, D.A., & VLITOS, A.J. (1966) The ultrastructure of sugar-cane chloroplasts. *Z. Pflanzenphysiol.* 54, 472.
- LEONARD, D.L., & BIDWELL, R.G.S. (1969) The effect of oxygen on carbon dioxide fixation. *Pl. Physiol.*, Lancaster 44, S12.
- LEWANTY, Z., MALESZEWSKI, S., & POSKUTA, J. (1971) The effect of oxygen concentration on ^{14}C incorporation into products of photosynthesis of detached leaves of maize. *Z. Pflanzenphysiol.* 65, 469.
- LUDWIG, L.J. (1972) The relationship between photosynthesis and respiration. In Crop Processes in Controlled Environments, p. 305. Ed Rees, A.R., Cockshull, K.E., Hand, D.W., & Hurd, R.G. London: Academic Press.
- LUDWIG, L.J., & CANVIN, D.T. (1971) The rate of photorespiration during photosynthesis and the relationship of the substrate of light respiration to the products of photosynthesis in sunflower leaves. *Pl. Physiol.*, Lancaster 48, 712.
- LUDWIG, L.J., & KROTKOV, G. (1967) The kinetics of labelling of the substrates for CO_2 evolution by sunflower leaves in the light. *Pl. Physiol.*, Lancaster 42, S47.

- LUDWIG, J., KROTKOV, G., & CANVIN, D.T. (1969) The relationship of the products of photosynthesis to the substrates for CO₂-evolution in light and darkness. In Progress in Photosynthesis Research Vol.I. p.494 Ed., Metzner, H. sponsored by International Union of Biological Sciences, Tubingen
- MAEBA, P., & SANWAL, B.D. (1969) Phosphoenolpyruvate carboxylase of Salmonella. Some chemical and allosteric properties. J. biol. Chem. 244, 2549
- MALESZEWSKI, S., & NELSON, C.D. (1968) The effect of oxygen concentration on the rate of formation of the ¹⁴C-products of photosynthesis in Balsam fir. Pl. Physiol., Lancaster 43, S20.
- MATHIEU, Y. (1969) Influence de l'oxygene sur les transferts d'electrons de la photosynthese. I. Influence de diverses concentrations d'oxygene sur quelques reactions de Hill. Biochim. biophys. Acta 189, 411.
- MATSUMOTO, H., WAKIUCHI, N., & TAKAHASHI, E. (1968) Changes of sugar levels in cucumber leaves during ammonium toxicity. Physiologia Pl. 21, 1210.
- MAYNE, B.C., EDWARDS, G.E., & BLACK, C.C. (1971) Spectral, physical, and electron transport activities in the photosynthetic apparatus of mesophyll cells and bundle sheath cells of Digitaria sanguinalis (L.) Scop. Pl. Physiol., Lancaster, 47, 600.
- MEHLER, A.H. (1951a) Studies on reactions of illuminated chloroplasts. I. Mechanism of the reduction of oxygen and other Hill reagents. Archs Biochem. Biophys. 33, 65.
- MEHLER, A.H. (1951b) Studies on reactions of illuminated chloroplasts. II. Stimulation and inhibition of the reaction with molecular oxygen. Archs Biochem. Biophys. 34, 339.
- MEHLER, A.H., & BROWN, A.H. (1952) Studies on reactions of illuminated chloroplasts. III. Simultaneous photoproduction and consumption of oxygen studied with oxygen isotopes. Archs Biochem. Biophys. 38, 365.
- MIFLIN, B.J., MARKER, A.F.H., & WHITTINGHAM, C.P. (1966) The metabolism of glycine and glycolate by pea leaves in relation to photosynthesis. Biochim. biophys. Acta 120, 266.
- MILLER, G., & EVANS, H.J. (1957) The influence of salts on pyruvate kinase from tissues of higher plants. Pl. Physiol., Lancaster 32, 346.
- MORTIMER, D.C. (1959) Some short-term effects of increased carbon dioxide concentration on photosynthetic assimilation in leaves. Can. J. Bot. 37, 1191.
- MOSS, D.N. (1962) The limiting carbon dioxide concentration for photosynthesis. Nature, Lond. 193, 587.
- MOSS, D.N. (1966a) Respiration of leaves in light and darkness. Crop Sci. 6, 351.
- MOSS, D.N. (1966b) Glycolate as a substrate for photorespiration. Pl. Physiol., Lancaster 41, Meetings XXXVIII.
- MOSS, D.N. (1967) High activity of the glycolic acid oxidase system in tobacco leaves. Pl. Physiol., Lancaster 42, 1463.
- MOSS, D.N. (1968) Photorespiration and glycolate metabolism in tobacco leaves. Crop Sci. 8, 71.

- MOSS, D.N., & RASMUSSEN, H.P. (1969) Cellular localization of CO₂ fixation and translocation of metabolites. *Pl. Physiol.*, Lancaster 44, 1063.
- MOSS, D.N., WILLMER, C.M., & CROOKSTON, R.K. (1971) CO₂ compensation concentration in maize (*Zea mays* L.) genotypes. *Pl. Physiol.*, Lancaster 47, 847.
- MULCHI, L., VOLK, R.J., & JACKSON, W.A. (1968) Oxygen exchange by illuminated soybean leaves at carbon dioxide concentrations limiting photosynthesis. *Pl. Physiol.*, Lancaster 43, S13.
- McALISTER, E.D., & MYERS, J. (1940) The time course of photosynthesis and fluorescence observed simultaneously. *Smithson. misc. Collns* 99 (6), 1.
- NORRIS, L, NORRIS, R.E., & CALVIN, M. (1955) A survey of the rates and products of short-term photosynthesis in plants of nine phyla. *J. exp. Bot.* 6, 64.
- OGREN, W.L., & BOWES, G. (1971) Ribulose diphosphate carboxylase regulates soybean photorespiration. *Nature, Lond. (New Biology)* 230, 159.
- ONGUN, A., & STOCKING, C.R. (1966) Effect of light and dark on the intracellular fate of photosynthetic products. *Pl. Physiol.*, Lancaster 40, 825.
- OSMOND, C.B. (1967) β -carboxylation during photosynthesis in *Atriplex*. *Biochim. biophys. Acta* 141, 197.
- OSMOND, C.B., & GREENWAY, H. (1972) Salt responses of carboxylation enzymes from species differing in salt tolerance. *Pl. Physiol.*, Lancaster 49, 260.
- OSMOND, C.B., TROUGHTON, J.H., & GOODCHILD, D.J. (1969) Physiological, biochemical and structural studies of photosynthesis and photorespiration in two species of *Atriplex*. *Z. Pflanzenphysiol.* 61, 218.
- OZBUN, J.L., VOLK, R.J., & JACKSON, W.A. (1964) Effects of light and darkness on gaseous exchange of bean leaves. *Pl. Physiol.*, Lancaster 39, 523.
- OZBUN, J.L., VOLK, R.J., & JACKSON, W.A. (1965a) Effect of potassium deficiency on photosynthesis, respiration and the utilization of photosynthetic reductant by immature bean leaves. *Crop Sci.* 5, 69.
- OZBUN, J.L., VOLK, R.J., & JACKSON, W.A. (1965b) Effect of potassium deficiency on photosynthesis, respiration and the utilization of photosynthetic reductant by mature bean leaves. *Crop Sci.* 5, 497.
- PALADINI, A.C., & LELOIR, L.F. (1952) Studies on uridine-diphosphate-glucose. *Biochem. J.* 51, 426.
- PATTERSON, M.S., & GREENE, R.C. (1965) Measurement of low energy beta-emitters in aqueous solution by liquid scintillation counting of emulsions. *Analyt. Chem.* 37, 854.
- POLYA, G.M., & OSMOND, C.B. (1972) Photophosphorylation by mesophyll and bundle sheath chloroplasts of C₄ plants. *Pl. Physiol.*, Lancaster 49, 267.

- POSKUTA, J. (1968) Photosynthesis, photorespiration and respiration of detached spruce twigs as influenced by oxygen concentration and light intensity. *Physiologia Pl.* 21, 1129.
- POSKUTA, J. (1969) Photosynthesis, respiration and post-illumination fixation of CO₂ by corn leaves as influenced by light and oxygen concentration. *Physiologia Pl.* 22, 76.
- POSKUTA, J., NELSON, C.D., & KROTKOV, G. (1966) Photosynthesis, photorespiration and dark respiration of spruce seedlings. *Proc. Canad. Soc. Pl. Physiol.* 7, 19.
- POSKUTA, J., NELSON, C.D., & KROTKOV, G. (1967) Effects of metabolic inhibitors on rates of CO₂ evolution in light and in darkness by detached spruce twigs, wheat and soybean leaves. *Pl. Physiol.*, Lancaster 42, 1187.
- PRITCHARD, G.G., WHITTINGHAM, C.P., & GRIFFIN, W.J. (1961) Effect of isonicotinyl hydrazide on the path of carbon in photosynthesis. *Nature*, Lond. 190, 553.
- RABSON, R., TOLBERT, N.E., & KEARNEY, P.C. (1962) Formation of serine and glyceric acid by the glycolate pathway. *Archs Biochem. Biophys.* 98, 154.
- RAVEN, J.A. (1970) The role of cyclic and pseudocyclic photophosphorylation in photosynthetic ¹⁴CO₂ fixation in Hydrodictyon africanum. *J. exp. Bot.* 21, 1.
- REED, M.L., & GRAHAM, D. (1968) Control of photosynthetic carbon dioxide fixation during an induction phase in Chlorella. *Pl. Physiol.*, Lancaster 43, S29.
- REHFELD, D.W., RANDALL, D.D., & TOLBERT, N.E. (1970) Enzymes of the glycolate pathway in plants without CO₂-photorespiration. *Can. J. Bot.* 48, 1219.
- RICHARDSON, K.E., & TOLBERT, N.E. (1961) Phosphoglycolic acid phosphatase. *J. biol. Chem.* 236, 1285.
- ROSADO-ALBERIO, J., ELLIOT WEIER, T., & STOCKING, C.R. (1968) Continuity of the chloroplast membrane systems in Zea mays L. *Pl. Physiol.*, Lancaster 43, 1325.
- ROSENBERG, L.L., CAPINDALE, J.B., & WHATLEY, F.R. (1958) Formation of oxaloacetate and aspartate from phospho-enol-pyruvate in spinach leaf chloroplast extract. *Nature*, Lond. 181, 632.
- SANADI, D.R., & BENNETT, M.J. (1960) Synthesis of serine from glycine in mitochondrial fragments. *Biochim. biophys. Acta* 39, 367.
- SCHÖCH, E. (1971) Discovery of a Compositae species with malate and aspartate as the major initial products of ¹⁴CO₂ fixation. *Z. Pflanzenphysiol.* 64, 367.
- SCHOU, L., BENSON, A.A., BASSHAM, J.A., & CALVIN, M. (1950) The path of carbon in photosynthesis, XI. The role of glycolic acid. *Physiologia Pl.* 3, 487.
- SCHURMANN, P., BUCHANAN, B.B., & ARNON, D.I. (1971) Role of cyclic photophosphorylation in photosynthetic carbon dioxide assimilation by isolated chloroplasts. *Biochim. biophys. Acta* 267, 111.

- SINHA, S.K., & COSSINS, E.A. (1964) The metabolism of [^{14}C]glycine by plant tissues. *Biochem. J.* 93, 27.
- SINHA, S.K., & COSSINS, E.A. (1965) The importance of glyoxylate in amino acid biosynthesis in plants. *Biochem. J.* 96, 254.
- SLACK, C.R. (1968) The photoactivation of a phosphopyruvate synthase in leaves of Amaranthus palmeri. *Biochem. biophys. Res. Commun.* 30, 483.
- SLACK, C.R. (1969) Localization of certain photosynthetic enzymes in mesophyll and parenchyma sheath chloroplasts of maize and Amaranthus palmeri. *Phytochemistry* 8, 1387.
- SLACK, C.R., & HATCH, M.D. (1967) Comparative studies on the activity of carboxylases and other enzymes in relation to the new pathway of photosynthetic carbon dioxide fixation in tropical grasses. *Biochem. J.* 103, 660.
- SLACK, C.R., HATCH, M.D., & GOODCHILD, D.J. (1969) Distribution of enzymes in mesophyll and parenchyma-sheath chloroplasts of maize leaves in relation to C_4 -dicarboxylic acid pathway of photosynthesis. *Biochem. J.* 114, 489.
- SMILLIE, R.M., ANDERSEN, K.S., TOBIN, N.F., ENTSCH, B., & BISHOP, D.G. (1972) Nicotinamide adenine dinucleotide phosphate photoreduction from water by agranal chloroplasts isolated from bundle sheath cells of maize. *Pl. Physiol., Lancaster* 49, 471.
- SMITH, I. (1960) In Chromatographic and Electrophoretic Techniques, vol.1. Chromatography. London: Heinemann.
- STOCKING, C.R., WILLIAMS, G.R., & ONGUN, A. (1963) Intracellular distribution of the early products of photosynthesis. *Biochem. biophys. Res. Commun.* 10, 416.
- TAMIYA, H., & HUZISIGE, H. (1949) Effect of oxygen on the dark reaction of photosynthesis. *Stud. Tokugawa Inst.* 6, 83.
- THOMPSON, C.M., & WHITTINGHAM, C.P. (1968) Glycollate metabolism in photosynthesising tissue. *Biochim. biophys. Acta* 153, 260.
- TING, I.P. (1968) CO_2 metabolism in corn roots. III. Inhibition of P-enolpyruvate carboxylase by L-malate. *Pl. Physiol., Lancaster* 43, 1919.
- TOLBERT, N.E. (1958) Secretion of glycolic acid by chloroplasts. *Brookhaven Symp. Biol.* 11, 271.
- TOLBERT, N.E. (1971) Microbodies-peroxisomes and glyoxysomes. *A. Rev. Pl. Physiol.* 22, 45.
- TOLBERT, N.E., & BURRIS, R.H. (1950) Light activation of the plant enzyme which oxidises glycolic acid. *J. biol. Chem.* 186, 791.
- TOLBERT, N.E., GLAGETT, C.O., & BURRIS, R.H. (1949) Products of the oxidation of glycolic acid and L-lactic acid by enzymes from tobacco leaves. *J. biol. Chem.* 181, 905.

- TOLBERT, N.E., & COHAN, M.S. (1953) Products formed from glycolic acid in plants. *J. biol. Chem.* 204, 649.
- TOLBERT, N.E., & HESS, J.L. (1966) The effect of hydroxymethanesulfonates on $^{14}\text{CO}_2$ photosynthesis by algae. *J. biol. Chem.* 241, 5712.
- TOLBERT, N.E., OESER, A., YAMAZAKI, R.K., HAGEMAN, R.H., & KISAKI, T. (1969) A survey of plants for leaf peroxisomes. *Pl. Physiol.*, Lancaster 44, 135.
- TOLBERT, N.E., & ZILL, L.P. (1956) Excretion of glycolic acid by algae during photosynthesis. *J. biol. Chem.* 222, 895.
- TREGUNNA, E.B., & DOWNTON, J. (1967) Carbon dioxide compensation in members of the Amaranthaceae and some related families. *Can. J. Bot.* 45, 2385.
- TREGUNNA, E.B., DOWNTON, J., & JOLLIFFE, P. (1969) Genetic and environmental control of photorespiration. In *Progress in Photosynthesis Research*, vol. 1, p.488. Ed. Metzner, H. sponsored by International Union of Biological Sciences, Tübingen.
- TREGUNNA, E.B., KROTKOV, G., & NELSON, C.D. (1961) Evolution of carbon dioxide by tobacco leaves during the dark period following illumination with light of different intensities. *Can. J. Bot.* 39, 1045.
- TREGUNNA, E.B., KROTKOV, G., & NELSON, C.D. (1964) Further evidence on the effects of light on respiration during photosynthesis. *Can. J. Bot.* 42, 989.
- TREGUNNA, E.B., KROTKOV, G., & NELSON, C.D. (1966) Effect of oxygen on the rate of photorespiration in detached tobacco leaves. *Physiologia Pl.* 19, 723.
- TROUGHTON, J.H. (1971) The lack of carbon dioxide evolution in maize leaves in the light. *Planta* 100, 87.
- TURNER, J.S., & BRITTAIN, E.G. (1962) Oxygen as a factor in photosynthesis. *Biol. Rev.* 37, 130.
- TURNER, J.S., TODD, M., & BRITTAIN, E.G. (1956) The inhibition of photosynthesis by oxygen. I. Comparative physiology of the effect. *Aust. J. biol. Sci.* 9, 494.
- USUDA, H., KANAI, R., & TAKEUCHI, M. (1971) Comparison of carbon dioxide fixation and the fine structure in various assimilatory tissues of Amaranthus retroflexus L. *Pl. Cell Physiol.*, Tokyo, 12, 917.
- VANDOR, S.L., & TOLBERT, N.E. (1968) Glycolate biosynthesis by isolated chloroplasts. *Pl. Physiol.*, Lancaster 43, S12.
- VINES, H.M., & WEDDING, R.T. (1960) Some effects of ammonia on plant metabolism and a possible mechanism for ammonia toxicity. *Pl. Physiol.*, Lancaster 35, 820.
- VON ARX, E., & NEHER, R. (1963) Eine multidimensionale technik zur chromatographischen identifizierung von aminosäuren. *J. Chromat.* 12, 329.
- VOSKRESENSKAYA, N.P., WILL, Y.A., GRISHINA, G.S., & PÄRNIK, T.R. (1970) Effect of oxygen concentration and light intensity on the distribution of labelled carbon in photosynthesis products in bean plants. *Photosynthetica* 4, 1.

- WAKIUCHI, N., MATSUMOTO, H., KONDO, S., & TAKAHASHI, E. (1972) Changes in nicotinamide nucleotide coenzymes in cucumber leaves during ammonium toxicity. *Physiologia Pl.* 26, 230.
- WAKIUCHI, N., MATSUMOTO, H., & TAKAHASHI, E. (1971) Changes in some enzyme activities of cucumber during ammonium toxicity. *Physiologia Pl.* 24, 248.
- WALKER, D.A. (1962) Pyruvate carboxylation and plant metabolism. *Biol. Rev.* 37, 215.
- WALSH, D.A., & SALLACH, H.J. (1966) Comparative studies on the pathways for serine biosynthesis in animal tissues. *J. biol. Chem.* 241, 4068.
- WANG, D., & BURRIS, R.H. (1963) Carbon metabolism of C^{14} -labelled amino acids in wheat leaves. II. Serine and its role in glycine metabolism. *Pl. Physiol., Lancaster* 38, 430.
- WANG, D., & HAO, M.H.S. (1960) Effect of benzimidazole on the formation of sugars from glycine-2- C^{14} in detached leaves of Khapli wheat. *Pl. Physiol., Lancaster* 35, SXV.
- WANG, D., & WAYGOOD, E.R., (1962) Carbon metabolism of C^{14} -labelled amino acids in wheat leaves. I. A pathway of glyoxylate-serine metabolism. *Pl. Physiol., Lancaster* 37, 826.
- WARBURG, O. (1920) Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen. II. *Biochem. Z.* 103, 188.
- WARBURG, O., & LÜTTIGENS, W. (1946) Photochemische reduktion des chinons in grünen Zellen und Granula. *Biokhimiya* 11, 303.
- WASSINK, E.C., VERMEULEN, D., REMAN, G.H., & KATZ, E. (1938) On the relation between fluorescence and assimilation in photosynthesizing cells. *Enzymologia* 5, 100.
- WAYGOOD, E.R., ARYA, S., & PAN, D., (1971) Intracellular localization of P-enolpyruvate acid carboxylase in C_3 and C_4 plants. *Pl. Physiol., Lancaster* 47, abstract 59.
- WHELAN, T., BENEDICT, C.R., & SACKETT, W.M. (1970) Carbon isotope discrimination in plants possessing a Hatch and Slack pathway. *Pl. Physiol., Lancaster* 46, S6.
- WHITEHOUSE, D.G., LUDWIG, L.J., & WALKER, D.A. (1971) Participation of the Mehler reaction and catalase in the oxygen exchange of chloroplast preparations. *J. exp. Bot.* 22, 772.
- WHITTINGHAM, C.P. (1952) Rate of photosynthesis and concentration of carbon dioxide in Chlorella. *Nature, Lond.* 170, 1017.
- WHITTINGHAM, C.P., COOMBS, J., & MARKER, A.F.H. (1967) The role of glycollate in photosynthetic carbon fixation. In Biochemistry of Chloroplasts, vol. II, p.154. Ed. Goodwin, T.W. London: Academic Press Inc.
- WHITTINGHAM, C.P., & PRITCHARD, G.G. (1963) The production of glycollate during photosynthesis in Chlorella. *Proc. R. Soc. B.* 157, 366.

- WILSON, A.T., & CALVIN, M. (1955) The photosynthetic cycle. CO_2 dependent transients. J. Am. chem. Soc. 77, 5948.
- WINTER, K., & VON WILLERT, D.J. (1972) NaCl induced crassulacean acid metabolism in Mesembryanthemum crystallinum. Z. Pflanzenphysiol 67, 166.
- WOO, K.C., ANDERSON, J.M., BOARDMAN, N.K., DOWNTON, W.J.S., OSMOND, C.B., & THORNE, S.W. (1970) Deficient photosystem II in agranal bundle sheath chloroplasts of C_4 plants. Proc. natn. Acad. Sci. U.S.A. 67, 18.
- YEMM, E.W. (1969) Photorespiration and photosynthesis in young leaves of barley. In Progress in Photosynthesis Research, vol.1, p.474. Ed. Metzner, H. sponsored by International Union of Biological Sciences, Tübingen.
- YEMM, E.W., & BIDWELL, R.G.S. (1969) Carbon dioxide exchanges in leaves. I. Discrimination between $^{14}\text{CO}_2$ and $^{12}\text{CO}_2$ in photosynthesis. Pl. Physiol., Lancaster 44, 1328.
- YU, Y.L., TOLBERT, N.E. & ORTH, G.M. (1964) Isolation and distribution of phosphoglycolate phosphatase. Pl. Physiol., Lancaster 39, 643.
- ZELITCH, I. (1957) α -hydroxysulfonates as inhibitors of the enzymatic oxidation of glycolic and lactic acids. J. biol. Chem. 224, 251.
- ZELITCH, I. (1958) The role of glycolic acid oxidase in the respiration of leaves. J. biol. Chem. 233, 1299.
- ZELITCH, I. (1959) The relationship of glycolic acid to respiration and photosynthesis in tobacco leaves. J. biol. Chem. 234, 3077.
- ZELITCH, I. (1965) The relation of glycolic acid synthesis to the primary photosynthetic carboxylation reaction in leaves. J. biol. Chem. 240, 1869.
- ZELITCH, I. (1966) Increased rate of net photosynthetic carbon dioxide uptake caused by the inhibition of glycolate oxidase. Pl. Physiol., Lancaster 41, 1623.
- ZELITCH, I. (1968) Investigations on photorespiration with a sensitive ^{14}C -assay. Pl. Physiol., Lancaster 43, 1829.
- ZELITCH, I. (1972) The photooxidation of glyoxylate by envelope-free spinach chloroplasts and its relation to photorespiration. Archs Biochem. Biophys. 150. 698.
- ZELITCH, I., & DAY, P.R. (1968) Variation in photorespiration. The effect of genetic differences in photorespiration on net photosynthesis in tobacco. Pl. Physiol., Lancaster 43, 1838.
- ZELITCH, I., & OCHOA, S. (1953) Oxidation and reduction of glycolic and glyoxylic acids in plants. J. biol. Chem. 201, 707.

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