GLYCOLLATE METABOLISM

IN DETACHED WHEAT LEAVES

by

Kumarasinghege Saliya Kumarasinghe

Thesis submitted to the University of London

for the degree of

Doctor of Philosophy

Department of Botany,

Rothamsted Experimental Station,

Harpenden,

Herts.

November, 1975

ABSTRACT

The metabolism of carbon in detached wheat leaves was investigated to establish: (1) the immediate precursor of photorespiratory CO_2 and (2) the rate of flow of carbon through the glycollate pathway.

Wheat leaves were allowed to assimilate $^{14}CO_2$ during steady-state photosynthesis in 1% or 21% oxygen and 80, 150, 325 or 1000 vpm carbon dioxide. Amounts of ^{14}C in the products were determined at various times during the assimilation of $^{14}CO_2$ and after subsequent exposure to atmospheres containing 0, 80, 150, 325 or 1000 vpm $^{12}CO_2$ in either 1% or 21% oxygen and in either light or darkness.

The metabolically active pools of glycine, serine and phosphate esters were saturated with ^{14}C after 15 minutes exposure to $^{14}CO_2$; specific radioactivity measurements showed that half of the glycine and serine in the leaf was metabolically active. During subsequent metabolism in the light in 21% O_2 without $^{14}CO_2$, the decrease of ^{14}C in glycine and serine was accounted for increased ^{14}C in sucrose and $^{14}CO_2$ evolved. Isonicotinyl hydrazide decreased incorporation of radioactivity from 150 vpm $^{14}CO_2$ into serine and sucrose and upon changing to $^{12}CO_2$ less $^{14}CO_2$ was released, ^{14}C in glycine and serine decreased less and little ^{14}C-sucrose was formed. After steady-state photosynthesis for 15 minutes in air containing $^{14}CO_2$ much ^{14}C was lost from glycine during the first minute in darkness in air without $^{14}CO_2$; approximately 75% of this ^{14}C appeared in serine and 25% was evolved as $^{14}CO_2$. These results suggest that glycine is the main substrate for photorespiratory evolution of CO_2.

Serine formed was metabolized to sucrose or its carbon re-cycled through photosynthetic intermediates into the glycollate pathway. In the dark little further metabolism of serine occurred. Both ^{14}C-serine supplied and that formed by $^{14}CO_2$ assimilation were metabolized most rapidly with CO_2 and O_2 concentrations close to those in normal air.

Upon transfer to CO_2-free 1% O_2, ^{14}C decreased in glycine, increased in serine and little $^{14}CO_2$ was released. However, in 1% O_2 containing 1000 vpm $^{12}CO_2$, ^{14}C decreased faster from glycine and much $^{14}CO_2$ was evolved. Therefore, in CO_2-free 1% O_2 much of the $^{14}CO_2$ produced was probably reassimilated by the leaf.

Rates of carbon flow through the glycollate pathway were deduced from the carbon flow out of ^{14}C - saturated pools of glycine under conditions that curtailed the formation of new glycine, e.g. in 1000 vpm CO_2 or darkness. The rate of loss of radioactivity from glycine exceeded the rate of carbon assimilation during the preceding period of photosynthesis. This suggests that <u>in vivo</u> all the carbon fixed during photosynthesis could be metabolized through glycine to serine.

CONTENTS

Materials and Methods

Experimental and Results

ABBREVIATIONS

ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
EDTA	Ethylene diamine tetraacetic acid
FDP	Fructose diphosphate
α-HPMS	α-hydroxypyridinemethanesulphonate
INH	Isonicotinyl hydrazide
SHB	Sodium-2-hydroxy-3-butynoate
GAP	Glyceraldehyde phosphate
DHAP	Dihydroxy acetone phosphate
PGA	3-phosphoglyceric acid
NADH	Nicotinamide adenine dinucleotide (reduced)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
UDPG	Uridine diphosphate glucose
UTP	Uridine triphosphate
RuDP	Ribulose diphosphate
DHETPP	Dihydroxyethylthiamine pyrophosphate
FMN	Flavin mononucleotide
F.6.P	Fructose-6-phosphate
G.6.P	Glucose-6-phosphate
G.1.P	Glucose-1-phosphate
P_i	Inorganic orthophosphate

INTRODUCTION

Glycollate has been known to be an early product of photosynthesis since the work of Calvin and co-workers (1948, 1949). Although unicellular green algae like Chlorella were the experimental material in these early experiments, details of the metabolic fate of this compound in photosynthetic tissues were sought primarily in higher plants. Such investigations led to the establishment of a sequence of reactions whereby glycollate is converted via glyoxylate, glycine and serine into glycerate and ultimately into sucrose (Rabson, Tolbert and Kearney, 1962; Wang and Waygood, 1962; Tolbert, 1963). This metabolic sequence is now generally referred to as the C-2 or the glycollate pathway of carbon metabolism.

In recent years it has become increasingly evident that the glycollate pathway is responsible for photorespiration. Photorespiration, in its simplest sense, can be defined as the release of carbon dioxide, with a concomitant uptake of oxygen by green plants in the light. Often, photorespiration is used in a more restricted sense, referring to a distinct respiratory process taking place in the light, having properties (response to oxygen and temperature) different from respiration in the dark. Most higher plants native to temperate climates (C_3 plants) are readily shown to photorespire but some plants native to drier hotter climates (C_4 plants) photorespire only very slowly. Some evidence suggests that light restricts metabolism responsible for dark respiration but induces the light dependent release of CO_2 (Jackson and Volk, 1970). Under normal conditions the photorespiratory loss of CO_2 in C_3 plants probably accounts for at least 25% of the carbon fixed during photosynthesis (Atkins and Canvin, 1971) and this loss is regarded as the most important single factor responsible for observed lower rates of net photosynthesis in C_3 plants compared to C_4 species (Zelitch, 1971), when measured at high temperatures (i.e. above 25° C).

In algae and in higher plants a significant proportion of the carbon assimilated during photosynthesis is metabolized by the glycollate pathway. Warburg and Krippahl (1960) showed that Chlorella, during a 10 min. period, converted as much as 92% of the total photosynthetic carbon into glycollate while in higher plant leaves it has been estimated that nearly 50% of the carbon fixed during photosynthesis may be metabolized by way of the glycollate pathway (Zelitch, 1959; Tolbert and Yamazaki, 1969). In sunflower leaves, Fock, Höhler, Canvin and Grant (1971) demonstrated that the flow of carbon through glycine and serine was respectively 23-29% and 62-77% of the apparent rate of carbon - assimilation. These observations suggest that the glycollate pathway is more than a mere side reaction to the Calvin cycle and should be considered a major route of carbohydrate production during photosynthesis.

1. (a) Effects of external factors on glycollate synthesis and photorespiration.

Many workers have shown that glycollate is produced in the light and that its production is stimulated by lower CO_2 and higher O_2 concentrations (Benson and Calvin, 1950; Wilson and Calvin, 1955; Coombs and Whittingham, 1966). In several unicellular green algae, depending on growth and experimental conditions, a large part of the glycollate produced may be excreted into the external medium (Tolbert and Zill, 1956; Whittingham and Pritchard, 1963; Miller, Meyer and Tanner, 1963), whereas in higher plants glycollate is further metabolized rapidly within the plant. It is believed that glycollate is formed within the chloroplasts during photosynthesis and Kearney and Tolbert (1962) and Ellyard and Gibbs (1969) have isolated chloroplasts capable of synthesizing glycollic acid in vivo.

Whittingham and Pritchard (1963) found that only a small amount of glycollic acid was excreted by Chlorella during photosynthesis below

1500 lux although this light intensity was sufficient to half saturate photosynthesis. However, glycollate production increased with increasing light intensity far beyond the value needed to saturate photosynthesis. There is evidence that relatively weak illumination curtails dark respiration and induces a different respiratory process (Hoch, Owens and Kok, 1963; Ozbun, Volk and Jackson, 1964; Poskuta, Nelson and Krotkov, 1967) and Holmgren and Jarvis (1967) showed that this efflux of CO_2 in the light at low ambient CO_2 concentrations was strongly dependent on the irradiance. These results therefore suggest that both glycollate production and CO_2 evolution are stimulated by increasing light intensities.

Warburg and Krippahl (1960) demonstrated that in light glycollate production in whole plants is stimulated by increased O_2 concentrations in the atmosphere. Further, in Chlorella fed with $^{14}CO_2$, ^{14}C-glycollate formation was greatest in 100% O_2, less in 21% O_2 and least in 0% O_2 (Bassham and Kirk, 1962). At air levels of CO_2 32% of the $^{14}CO_2$ assimilated by Chlorella accumulated in glycollate when the atmosphere contained 100% O_2 but only 2% accumulated when the atmosphere contained 21% O_2 (Whittingham, Coombs and Marker, 1967). Also, Whittingham, Coombs and Marker (1967) demonstrated that in Chlorella the percentage radioactivity in glycollate after 3 min. photosynthesis in 300 vpm $^{14}CO_2$ increased from 5 to about 38% with an increase in O_2 concentration from 0-100% O_2.

Respiratory processes in plants are also markedly affected by the O_2 concentration. Whereas dark respiration is saturated at O_2 concentrations as low as 2% (Beevers, 1960), photorespiration at this O_2 concentration occurs only at a very slow rate. However, photorespiration increases with O_2 concentrations up to 100% (Tregunna, Krotkov and Nelson, 1966; Forrester, Krotkov and Nelson, 1966 ; Poskuta, 1968). In 1920 Warburg reported the inhibition of photosynthesis in Chlorella by high partial pressures of oxygen, a phenomenon now generally referred to as

the Warburg effect. In the absence of O_2 the net CO_2 assimilation was enhanced in several species of higher plants also (Björkman, 1966; Hesketh, 1967). Björkman, Gauhl, Hiesey, Nicholson and Nobs (1969) grew Mimulus plants (C_3) with their roots in normal air and their aerial parts in an atmosphere containing various concentrations of CO_2 in 4% or 21% O_2. After 10 days of continuous illumination, measurement of the increases in dry weight showed that the growth of Mimulus was inhibited by higher O_2 concentrations whereas that of a maize plant (C_4) similarly treated was not affected. In a similar study Gauhl and Björkman (1969) demonstrated that lowering the O_2 level from 21% to less than 2% increased net photosynthesis 53% in Atriplex patula (high photorespiration) and only 4% in Atriplex rosea (low photorespiration). Such results have been widely interpreted as showing that if photorespiration were decreased the net rate of photosynthesis and the growth rate of C_3 plants would be much improved.

Benson and Calvin (1950), Wilson and Calvin (1955) and Tolbert and Zill (1956), showed convincingly that glycollate production during photosynthesis is favoured at lower CO_2 concentrations. In Chlorella maximum rates of glycollate synthesis were observed at CO_2 concentrations between 0.1% and 0.2% (Warburg and Krippahl, 1960). Pritchard, Griffin and Whittingham (1962) also demonstrated a 60% decrease in glycollate synthesis by Chlorella when the CO_2 concentration was increased from 0.1% to 0.2% and in higher plants also, a decreased percentage of newly assimilated ^{14}C from $^{14}CO_2$ appears in intermediates of the glycollate pathway in atmospheres with high compared to low concentrations of CO_2 (Lee and Whittingham, 1974; Tolbert, 1973; Zelitch, 1971). It therefore appears that glycollate is formed faster at lower than at higher concentrations of CO_2.

Studies on the effect of atmospheric CO_2 concentration on the magnitude of photorespiration have provided conflicting data. Measurements using isotopic methods have shown that the rate of CO_2 evolution is unchanged

by CO_2 concentrations from 0 up to 300 vpm although the specific radioactivity of the CO_2 evolved, after exposure to $^{14}CO_2$, depended upon the CO_2 concentration (Ludwig and Canvin, 1971). Bishop and Whittingham (1968) demonstrated that in a light intensity of 2.2×10^4 erg. $cm.^{-2}s.^{-1}$ the rate of photosynthesis of a tomato plant grown and assimilating in air enriched to 0.1% CO_2 is three times that of a plant grown and assimilating in air. The increased photosynthesis was thought to be partly due to decreased rate of photorespiration. In similar experiments tomato plants were exposed in the light to atmospheres containing between 40 and 1400 parts/10^6 $^{14}CO_2$ and the incorporation of radioactive carbon into various photosynthetic products was investigated (Lee and Whittingham, 1974). At high CO_2 concentrations a significantly greater proportion of ^{14}C entered sucrose and alcohol insoluble material but, at lower CO_2 concentrations the incorporation of ^{14}C into glycine and serine was much greater. They pointed out that whilst the increase in plant growth resulting from increasing the concentration of CO_2 is mainly to be attributed to an increased rate of photosynthesis, it also in part arises from the suppression of photorespiration. Goldsworthy (1968), in order to eliminate the effects of photorespiration on photosynthesis, inhibited photorespiration by using atmospheres containing little or no O_2. He found that if net CO_2 uptake was measured at about 2% O_2 in the ambient atmosphere, there was little difference in the apparent Michaelis constant (K_m) between maize or sugar cane and tobacco; the values were all around 300 vpm CO_2. However, although the K_m for maize in 2% O_2 or in air and for tobacco in 2% O_2 was about the same, the K_m for tobacco in air was appreciably higher, 488 vpm CO_2. Goldsworthy concluded that the decreased apparent photosynthesis in air by C_3 plants compared to C_4 species was mainly due to photorespiration. Björkman (1971) also reported that the differences in the rate of net CO_2 uptake between Atriplex rosea (C_4 species) and Atriplex patula (C_3 species) disappeared when the O_2 concentration was decreased to less than 1.5% compared to 21%. However, he pointed out that when CO_2 uptake

was expressed as a function of the intercellular CO_2 concentration there were significant differences in calculated values of apparent K_m between A. rosea (75 vpm) and A. patula (150 vpm) even at 0.15% O_2. This is consistent with the idea that the C_4 species havean initial carboxylating mechanism different from RuDP carboxylase. When photorespiration was suppressed (0.15% O_2) photosynthesis in the C_3 species A. patula was saturated around 400 vpm CO_2 (intercellular concentration). However, at atmospheric levels of O_2 when photorespiration is not inhibited, rate of net photosynthesis in C_3 plants increases with increase in CO_2 concentrations even up to 1000 vpm CO_2 (Zelitch, 1971). High external CO_2 concentrations ensure a steep CO_2 concentration gradient, so that the RuDP carboxylase is more nearly saturated with CO_2 and formation of substrate of photo-respiration is decreased; stomatal and other resistances to gaseous diffusion into the leaf determine the steady-state concentration of CO_2 at the sites of carboxylation and this is affected also by the affinity of the carboxylase for CO_2, the light intensity, and temperature.

It is often accepted that temperature also affects the metabolism of glycollate in plants, but such conclusions were made largely from effects of temperature on photorespiration. At constant light intensities assimilation of CO_2 by a leaf in a closed system decreases the CO_2 concentration in the atmosphere until a CO_2 level is reached at which there is no net gas exchange, the CO_2 compensation point. The CO_2 compensation point is mainly a function of the relative rates of true photosynthesis and photo-respiration; plants with high rates of photorespiration have a high CO_2 compensation point. Zelitch (1966) found that at 25°C the CO_2 compensation point of tobacco was 48 whereas at 35°C it was 80 vpm CO_2. He suggested that the Q_{10} of photorespiration was higher than the Q_{10} of true photo-synthesis. Hew, Krotkov and Canvin (1969) also demonstrated that in sunflower leaves the Q_{10} (20-30°) of CO_2 evolution in the light was 1.45 whereas that of photosynthesis was 0.92.

Similar observations have been made for Atriplex hastata, Pelargonium and wheat (Tregunna and Downton, 1967; Osmond, Troughton and Goodchild, 1969; Egle and Schenk, 1953; Moss et al, 1969). At 35°C the CO_2 compensation points were 109, 170 and 180 vpm CO_2, whereas at 25° they were only 35, 80 and 52 vpm CO_2 suggesting that in these species the Q_{10} for photorespiration is greater than for photosynthesis. Normally photosynthesis increases with increase in temperature up to an optimum; while this may be partly because increased temperature stimulates photorespiration, relatively more than photosynthesis, many other factors are involved and it is doubtful whether any useful conclusions can be reached with regard to values of the Q_{10} for the individual processes.

1. (b) The mechanism of glycollate biosynthesis

While the factors affecting the biosynthesis and further metabolism of glycollate are reasonably well understood, the mechanism of synthesis of this two-carbon acid has remained enigmatic.

Two contrasting mechanisms have been proposed for the biosynthesis of glycollic acid. In one, glycollate is synthesized de novo from CO_2 without the involvement of the Calvin cycle (Warburg and Krippahl, 1960; Stiller, 1962). According to the other, a phosphorylated intermediate of the Calvin cycle is considered as the precursor.

Tanner, Brown, Elyster and Treharne (1960) proposed a mechanism for the de novo synthesis of glycollate involving the condensation of C-1 free radicals made directly from CO_2. Kinetic evidence in support of this proposal was presented by Anderson and Fuller (1967) who showed that in the photoheterotrophically grown Rhodospirillum rubrum, the ^{14}C present in glycollate during the first few seconds of photosynthesis in $^{14}CO_2$ was greatly in excess of that found in phosphate esters. Zelitch (1965) claimed that the carboxyl carbon atom of glycollic acid had a higher specific

activity than the carboxyl carbon atom of 3-phosphoglyceric acid in tobacco leaves exposed to $^{14}CO_2$ for 5 seconds. The specific activity data alone can be of limited value in deciding the origin of glycollic acid since leaves probably contain one or more pools of glycollic acid and 3-phosphoglyceric acid that are not directly related to the process of CO_2 fixation (Hatch and Slack, 1970). Furthermore, opposite results were obtained by Hess and Tolbert (1966) who thought that Zelitch's results had been due to an artifact.

From the distribution of ^{14}C following short periods of photosynthesis in $^{14}CO_2$ Wilson and Calvin (1955) suggested that glycollate might be formed by oxidation of a C-2 fragment derived from the sugar phosphate intermediates of the photosynthetic carbon reduction cycle. Griffiths and Byerrum (1959) have indeed shown that tobacco leaves incorporated radioactivity from ribose-1-^{14}C, mainly into the α-carbon of glycollate, and transketolase, an enzyme requiring thiamine pyrophosphate catalysed the formation of glycollate from fructose-6-phosphate (Bradbeer & Racker, 1961). Subsequently Bradbeer and Anderson (1967) demonstrated that chloroplast preparations from spinach, spinach beet and meteor pea made glycollate from various sugar phosphates including a commercial preparation of ribulose diphosphate; however the highest yield of glycollate was obtained with fructose diphosphate. Previously, Tolbert (1963) discussed evidence that phosphoglycollate was an intermediate for glycollate synthesis and suggested that phosphoglycollate could be formed by the action of transketolase on the diphosphates of xylulose, fructose or sedoheptulose though not from ribulose diphosphate. Tolbert pointed out that ribulose diphosphate does not have the necessary trans-configuration of the hydroxyl groups between carbons 3 and 4 for the aldolase, transaldolase or transketolase activity. More recently, evidence has accumulated that ribulose diphosphate is in fact the most likely precursor of phosphoglycollate. It has become evident that RuDP carboxylase has catalytic activity both as a carboxylase and an oxygenase (Ogren and Bowes, 1971; Bowes and Ogren, 1972; Laing, Ogren and Hageman, 1974). Ogren and Bowes proposed that ribulose

diphosphate is oxidized by molecular oxygen in the presence of RuDP carboxylase thus producing phosphoglycollate and 3-phosphoglycerate (1).

$$\text{RuDP} + O_2 \xrightarrow{\text{RuDP oxygenase}} \text{3-P-glycerate} + \text{P-glycollate} \quad (1)$$

With preparations of the enzyme RuDP oxygenase from both spinach and soybean, Andrews, Lorimer and Tolbert (1973) demonstrated the formation of phosphoglycollate from ribulose-1,5-diphosphate in the presence of O_2. Lorimer, Andrews and Tolbert (1973) provided further evidence in support of this mechanism. When oxygen-18 was present as molecular oxygen, the label was incorporated into one of the carboxyl oxygen atoms of phosphoglycollate while no label appeared in 3-phosphoglycerate. Although the oxygenase and the carboxylase activities were found to co-purify and all other attempts to separate them have failed, the activities showed differences in a number of respects. The oxygenase was more stable than the carboxylase and the activity ratio of oxygenase to carboxylase increased from 0.25 in the crude extract to 0.59 in the final fractions. The pH optima also differed. Oxygenation of RuDP is now accepted as a reaction by which phosphoglycollate and thus glycollate may be synthesized during photosynthesis (Laing et al., 1974). This mechanism in which CO_2 and O_2 compete for reaction with RuDP in the presence of RuDP carboxylase (Andrews et al., 1973; Lorimer et al., 1973) would also account for the interaction of oxygen and CO_2 concentration on glycollate production in vivo.

2. The glycollate pathway

(a) Intermediates

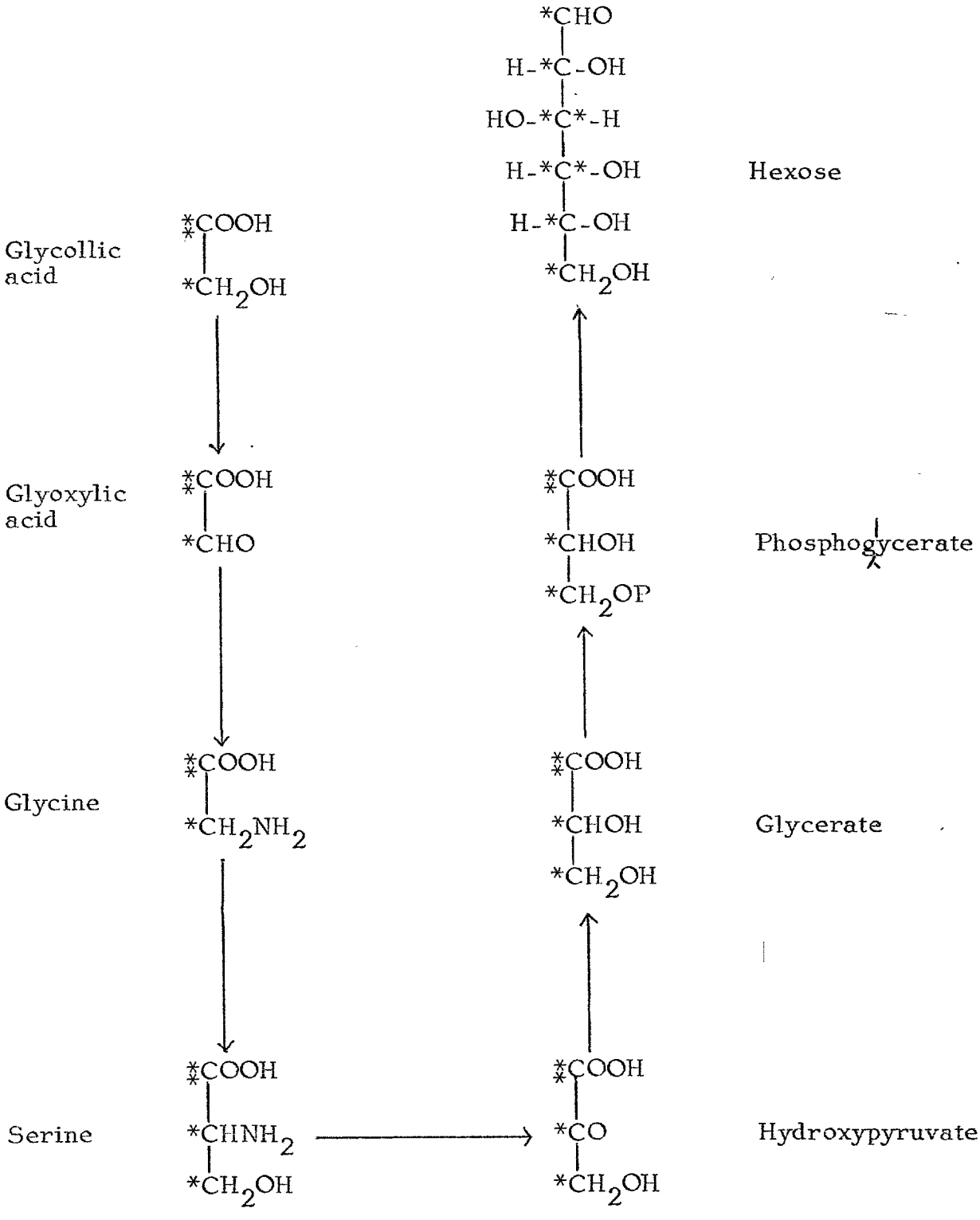
Benson and Calvin (1950) found that when barley leaves, after a 30-second exposure to a low concentration of $^{14}CO_2$, were transferred to CO_2-free air for 2 min. 23% of the ^{14}C in the soluble products was in glycollate and 14% in glycine and serine. Subsequently, Tolbert and Cohan (1953) established that when ^{14}C-labelled glycollate was supplied to barley and wheat leaves the major products formed were glycine and serine. The carboxyl and the α-carbon atom of glycollate became the carboxyl and the

α-carbon atom of glycine and serine respectively. The β-carbon atom of serine was also formed from the α-carbon atom of glycollic acid. When glycollate-2-^{14}C was fed to maize leaves, radioactive serine and glycerate were formed and were labelled predominantly in the α and β carbon atoms. Further, Rabson, Tolbert and Kearney (1962) showed that when glycollate-2-^{14}C was supplied to Thatcher wheat leaves Serine-2,3-^{14}C and glycerate-2-3-^{14}C were the major products. When serine-3-^{14}C was fed glycerate-3-^{14}C was formed. In a time course study, glycine and serine were the only products formed at first, from glycollate-^{14}C; subsequently glycerate, hexose phosphates and sucrose also became labelled. It was concluded that the glycollate pathway started with glycollate and led to the synthesis of glycine, serine and glycerate.

When glycollate-2-^{14}C was supplied to wheat leaves in the light, it was metabolized to sucrose predominantly labelled in the carbon atoms 1,2,5 and 6 of the hexose molecules. With serine-3-^{14}C the hexose moieties of sucrose were predominantly labelled in carbon atoms 1 and 6 (Jimenez, Baldwin, Tolbert and Wood, 1962; Wang and Waygood, 1962). According to previous work (Rabson et al., 1962) serine-3-^{14}C gave rise to glycerate-3-^{14}C. These labelling patterns therefore suggested that glycerate was incorporated directly into hexose by a reversal of the Embden-Meyerhof pathway (Jimenez et al., 1962).

Another observation made by the use of specifically labelled substrates concerns differences in the degree of randomization of the ^{14}C-label among the six carbon atoms of the hexoses. In young soybean leaves hexose formed from glycollate-1-^{14}C was predominantly labelled in carbon atoms 3 and 4 whereas this was much less so in the case of wheat leaves. Nevertheless, with both plants randomization of ^{14}C among all carbon atoms of the sugars occurred to some extent (Jimenez et al., 1962). When glycine-1-^{14}C was fed to wheat leaves a higher degree of randomization between the carbon atoms of the glucose moieties of sucrose was observed than with

glycine-2-^{14}C as substrate (Wang and Waygood, 1962). This contrast in distribution of the ^{14}C-label among carbon atoms of the glucose units of sucrose was explained partly in terms of a refixation of the $^{14}CO_2$ produced by decarboxylation of glycine to give a one carbon unit and CO_2 as proposed by Sagers and Gunsalus (1961) or due to recycling of the intermediates and end products through other metabolic pathways. Marker and Whittingham (1967) demonstrated that in excised pea leaves carboxyl labelled glycollic acid and glycine formed carboxyl-labelled serine, whereas glycollic acid and glycine labelled in the α -carbon atom gave rise to serine labelled in the α and β positions. These results are consistent with the labelling patterns shown by Rabson et al.(1962). Moreover, the distribution of ^{14}C within the glucose from sucrose and insoluble polyglucan formed from glyoxylic acid-2-^{14}C or glycine-2-^{14}C again showed heavier labelling in carbon atoms 1,2,5 and 6 whereas carboxyl labelled glycollic acid and glycine was metabolized to hexose labelled almost uniformly. Similar observations were made by Miflin, Marker and Whittingham (1966). Two explanations for the randomization of radioactivity in carbon atoms of the hexoses formed from the carboxyl labelled intermediates of the glycollate pathway were considered by Marker and Whittingham (1967). Firstly, a complete oxidation of glycollate or glycine followed by refixation of the $^{14}CO_2$ produced. However, such a mechanism is unlikely to occur, since then the level of randomization of the ^{14}C in the hexoses should be the same, irrespective of the initial position of the ^{14}C in glycollate and glycine. Secondly, the conversion of glycine-1-^{14}C to serine results in the production of$^{14}CO_2$ with a portion of it being refixed immediately as suggested by Wang and Waygood (1962) and Wang and Burris (1963). Such refixation would result in uniformly labelled glycollate and hence hexose made from this glycollate would be uniformly labelled. However, the serine would be labelled in the carboxyl carbon and hexose made from it would be labelled only in carbons 3 and 4. The label in hexose derived from both routes would result in relatively much heavier labelling in carbons 3 and 4 (33% of ^{14}C in each) than was observed in practice (17 to 21% of ^{14}C in each). A satisfactory


Glycollic acid
Glyoxylic acid
Glycine
Serine
Hexose
Phosphoglycerate
Glycerate
Hydroxypyruvate

SCHEME 1

explanation for this discrepancy has not been found.

Wang and Waygood (1962) have further confirmed the reaction sequence of the glycollate pathway by means of isotopic competition experiments. In wheat leaves addition of glycollate or glyoxylate did not lower the incorporation of radioactivity into sugars from glycine-^{14}C or serine-^{14}C, but the formation of ^{14}C-labelled sugars from glyoxylate-^{14}C was drastically decreased by the addition of either glycine or serine. The synthesis of sugars from glycine-^{14}C was diminished by the addition of serine-^{12}C whereas the addition of glycine-^{12}C did not lower the incorporation of radioactivity into sugars from serine-^{14}C. These results are consistent with those of Rabson et al. (1962) and Jimenez et al. (1962) in that glycollate, glyoxylate, glycine and serine appear in a sequence in the glycollate pathway. Wang and Waygood's scheme also suggested that glycerate is an intermediate between serine and sucrose. Scheme 1 summarizes the sequence of reactions by which glycollate may be metabolized to sucrose as revealed by isotopic studies.

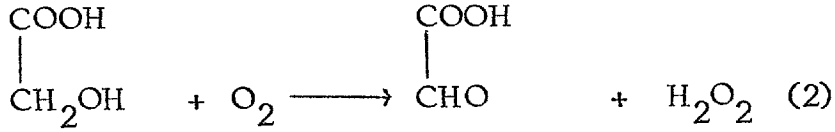
2. (b) Enzymes and reactions involved in metabolism of glycollate

Clagett, Tolbert and Burris (1949) found that extracts of a number of leaves were capable of catalysing the oxidation of glycollate and lactate. They partially purified this enzyme from extracts of tobacco leaves. Zelitch and Ochoa (1953) isolated the enzyme from spinach leaves in a highly purified form and established that it is a flavoprotein with FMN as the prosthetic group. Tolbert and Cohan (1953) and Delavan and Benson (1958) reported that glycollic acid oxidase is associated with the chloroplasts. However, Thompson and Whittingham (1968) suggested that this activity could be attributed to cytoplasmic contamination and enzyme adsorption on to the chloroplasts. They could neither find glycollate oxidase activity in a chloroplast preparation where 50-90% of the chloroplasts were intact nor was there any activity associated with free lamellar systems prepared from a glycerol gradient. The subsequent discovery by electron microscopy and

isolation by density gradient centrifugation of the sub-cellular organelles designated as microbodies led to a clarification of the location of some of the enzymes involved in the glycollate pathway. De Duve (1966, 1969) called these organelles from rat liver and kidney, peroxisomes as they were originally assayed in vitro by peroxidative release of $^{14}CO_2$ from labelled formate. The name is now widely used for similar microbodies from plants because their enzyme content is similar to that of liver peroxisomes (Tolbert, Oeser, Kisaki, Hageman and Yamazaki, 1968; Tolbert and Yamazaki, 1969).

Peroxisomes have now been found in the leaves of all plants that have been examined (Tolbert, Oeser, Yamazaki, Hageman and Kisaki, 1969). They are bounded by a single membrane and have a granular matrix without lamellae. Large dense crystalline inclusions are often seen in the microbodies (Frederick and Newcombe, 1969). Peroxisomes are often found in close proximity to chloroplasts and mitochondria. This may be of special relevance to the metabolic sequence of the glycollate pathway. Among the enzymes detected in the peroxisomal fraction isolated by isopycnic density gradient centrifugation of spinach leaf extracts are glycollate oxidase, catalase, hydroxypyruvate reductase, glutamate glyoxylate aminotransferase, serine glyoxylate aminotransferase, glutamate-oxalo-acetate aminotransferase, NAD-malate dehydrogenase and NADP-isocitric dehydrogenase (Yamazaki and Tolbert, 1970).

The conversion of glycollate to glyoxylate which is catalysed by the enzyme glycollate oxidase can be written as follows (2)



$$\begin{array}{l} \text{COOH} \\ | \\ \text{CH}_2\text{OH} \end{array} + O_2 \longrightarrow \begin{array}{l} \text{COOH} \\ | \\ \text{CHO} \end{array} + H_2O_2 \quad (2)$$

Goulding, Lord and Merrett (1969) reported that Chlorella fusca var. vacuolata did not excrete glycollate under conditions known to stimulate

glycollate excretion in other species of Chlorella. This was not due to an inability to synthesize glycollate but due to the presence of an enzyme which oxidized glycollate to glyoxylate (Lord and Merrett, 1968; Zelitch and Day, 1968). This enzyme is not an oxidase (Nelson and Tolbert, 1969) and has been designated glycollate dehydrogenase in order to distinguish it from the higher plant enzyme.(Nelson and Tolbert, 1970). Nevertheless, a colourless Chlorella mutant (Schmid and Schwarze, 1969) and a wild type Chlorella strain (Kowallik and Schmid, 1971) have been reported to contain the typical glycollate oxidase.

Glyoxylate produced in reaction (2) can be further oxidized non-enzymatically by hydrogen peroxide to yield formic acid and CO_2 (3) (Zelitch, 1964, 1967).

$$\begin{array}{l} \text{COOH} \\ | \\ \text{CHO} \end{array} + H_2O_2 \longrightarrow H.COOH + H_2O + CO_2 \quad (3)$$

This further oxidation is thought to be unlikely in the peroxisomes due to the presence of excess catalase which would rapidly destroy the H_2O_2 (Tolbert and Yamazaki, 1969; Kisaki and Tolbert, 1969).

Wilson, King and Burris (1954) described a transaminase in plant tissues which catalysed the conversion of glyoxylate to glycine in the presence of glutamate. Although the glyoxylate glutamate aminotransferase is located in the peroxisomes its activity has been found to be only 2% of that of glycollate oxidase (Kisaki and Tolbert, 1969). Subsequent work (Tolbert and Yamazaki, 1969) showed a much higher rate of 30%. Isolated peroxisomes do not convert glycine to glyoxylate because the equilibrium of the transamination is strongly in favour of glycine.

$$\begin{array}{l} \text{COOH} \\ | \\ \text{CHO} \end{array} + \text{glutamate} \longrightarrow \begin{array}{l} \text{COOH} \\ | \\ CH_2NH_2 \end{array} + \alpha\text{-ketoglutarate} \quad (4)$$

A number of investigators have studied the conversion of glycine to serine using both animal tissues (Sakami, 1955; Huennekens and Osborn, 1959) and plant tissues (Wilkinson and Davies, 1958; McConnell and Bilinski, 1959; Wang and Waygood, 1962; Sinha and Cossins, 1964). There is considerable evidence that the reaction involves the participation of the two enzymes glycine decarboxylase and serine hydroxymethyltransferase. A glycine decarboxylase has been reported in Rhodopseudomonas spheroides and in rat liver mitochondria. The enzyme serine hydroxymethyltransferase has also been extensively studies in mammalian and avian tissues (Sakami, 1955; Hatefi, Osborn, Kay and Huennekens, 1957), in bacterial preparations (Wright, 1955) and in some plant tissues (Wilkinson and Davies, 1960). The cleavage of glycine to form a one-carbon unit has been demonstrated by Sagers and Gunsalus (1961) in Diplococcus glycinophilus and by Pitts and Crosbie (1962) in Escherichia coli. The reaction requires the co-factors tetrahydrofolate, pyridoxal phosphate and NAD DPN (Sagers and Gunsalus, 1961; Cossins and Sinha, 1966). Glycine is first decarboxylated to form CO_2, NH_3 and an activated one-carbon unit N^5N^{10}-methylenetetrahydrofolate which in turn reacts with another molecule of glycine to form serine. The overall reaction could be written as follows (5, 5a)

$$\begin{array}{l} \mathrm{COOH} \\ | \\ \mathrm{CH_2NH_2} \end{array} + \text{tetrahydrofolate} \longrightarrow \mathrm{CO_2} + \mathrm{NH_3} + N^5N^{10}\text{-methylenetetrahydrofolate} \qquad (5)$$

$$\begin{array}{l} \mathrm{COOH} \\ | \\ \mathrm{CH_2NH_2} \end{array} + N^5N^{10}\text{-methylenetetrahydrofolate} \longrightarrow \begin{array}{l} \mathrm{COOH} \\ | \\ \mathrm{CHNH_2} \\ | \\ \mathrm{CH_2OH} \end{array} + \text{tetrahydrofolate} \qquad (5a)$$

The first step of the reaction (5) in which CO_2 is produced is catalysed by glycine decarboxylase and the second part where a C-1 unit is transferred to another molecule of glycine to form serine is catalysed by the enzyme serine hydroxymethyltransferase. These observations were further substantiated by the use of specifically labelled substrates. A wheat leaf extract converted glycine-1-C^{14} to serine-1-^{14}C and $^{14}CO_2$; glycine-2-^{14}C formed exclusively serine-2,3-^{14}C (Cossins and Sinha, 1966). The CO_2 produced during the conversion of glycine to serine was therefore derived from the carboxyl carbon atom of glycine. These results are consistent with the idea that two molecules of glycine are converted to one each of serine, CO_2 and NH_3.

When sub-cellular fractions of green leaves were prepared by non-linear sucrose isopycnic centrifugation the glycine decarboxylase was found to be located in the mitochondria. Although serine hydroxymethyltransferase was also principally located in mitochondria some activity was found in chloroplasts as well. Shah and Cossins (1970) demonstrated the presence of serine hydroxymethyltransferase in chloroplasts isolated from pea leaves by the non-aqueous method, but the activity detected was inadequate to account for the rates of serine biosynthesis observed in vivo and Bird, Keys and Whittingham (1971) have shown conclusively that such chloroplasts are unique.

However, Kisaki and Tolbert (1970) showed that tobacco leaf discs decarboxylated glycine faster than glycollate, glyoxylate or serine. Kisaki, Imai and Tolbert (1971) and Kisaki, Yoshida and Imai (1971) isolated mitochondria from spinach leaves which catalysed the formation of serine, CO_2 and NH_3 from glycine. The in vitro rates of glycine decarboxylation were comparable to known rates of photorespiration in vivo. Bird, Cornelius, Keys and Whittingham (1972) continued the investigation into the decarboxylation of glycine by mitochondria. They separated particles from extracts of tobacco leaves by differential centrifugation which catalysed

the conversion of two molecules of glycine to one each of serine and CO_2. They found that the reaction was slow in the absence of O_2, but a maximum rate was attained at 3% by volume in the atmosphere. Potassium cyanide, O-phenanthraline and antimycin-A, which are known inhibitors of the mitochondrial electron transport, also inhibited the synthesis of serine from glycine. These data suggested that the conversion of glycine to serine was in some way connected with the cytochrome system of the mitochondrion. Addition of ADP stimulated O_2 uptake and the conversion of glycine to serine. Also, although the mitochondrial preparations contained other substrates that were oxidized and resulted in phosphorylation of ADP, phosphorylation rates increased upon addition of glycine. In the presence of isonicotinyl hydrazide (INH) an inhibitor of serine formation from glycine, phosphorylation in the absence of glycine was not affected but the increased phosphorylation caused by adding glycine was less than in the absence of the inhibitor. Other experiments confirmed that oxidation of glycine to serine was coupled to ATP synthesis in mitochondria.

The series of reactions responsible for the conversion of glycollate to serine has been shown to require no light, but the further metabolism of serine is light dependent (Miflin et al., 1966; Ongun and Stocking, 1965). The requirement for light was confirmed by Waidyanatha, Keys and Whittingham (1974a,b) who also found that oxygen and carbon dioxide were needed for synthesis of sucrose from serine in wheat leaves. Three separate factors seemed to control sucrose synthesis from serine in wheat leaves in the light: a keto acid was required for the deamination of serine, ATP and reduced pyridine nucleotides were needed in the cytoplasm and a sufficient input of new carbon into the leaf was also needed. It was concluded that photosynthesis, in addition to a supply of keto acids, may also provide substrates that can be degraded and provide energy in the cytoplasm for the conversion of glycerate to sugar phosphates and sucrose.

Yamazaki and Tolbert (1970) reported a serine-pyruvate aminotransferase in peroxisomes which catalysed the conversion of serine to hydroxypyruvate and Stafford, Magaldi and Vennesland (1954) characterized a NAD-linked D-glycerate dehydrogenase from parsley leaves which catalysed the reduction of hydroxypyruvate to glycerate. Tolbert, Yamazaki and Oeser (1970) also located a NADH-hydroxypyruvate reductase which catalysed the conversion of hydroxypyruvate to glycerate in peroxisomes of spinach leaves. A D-glycerate kinase which catalysed the conversion of glycerate to 3-phosphoglycerate by ATP in plant tissues has been described by Cheung, Rosenblum and Sallach (1968) and Hatch and Slack (1966). Recently, Heber, Kirk, Gimmler and Schafer (1974) investigated the distribution of glycerate kinase between chloroplasts and the cytoplasm. Of the total activity 50-70% was found in the chloroplasts and 30-50% in the cytoplasm. From these data it is evident that formation of 3-PGA from glycerate can take place to a considerable extent in the cytoplasm.

Serine + Keto acid $\xrightleftharpoons{\text{transaminase}}$ Hydroxypyruvate + amino acids (6)

Hydroxypyruvate + NADH $\xrightleftharpoons{\text{Hydroxypyruvate reductase}}$ Glycerate + NAD (7)

Glycerate + ATP $\xrightleftharpoons{\text{Glycerate kinase}}$ PGA + ADP (8)

PGA + ATP $\xrightleftharpoons{\text{Phosphoglyceryl kinase}}$ 1,3-di PGA + ADP (9)

1,3 di PGA + NAD(P)H $\xrightleftharpoons{\text{Triose phosphate dehydrogenase}}$ GAP + NAD(P) + Pi (10)

GAP $\xrightleftharpoons{\text{Triose phosphate isomerase}}$ DHAP (11)

GAP + DHAP $\xrightleftharpoons{\text{Aldolase}}$ FDP (12)

FDP $\xrightleftharpoons{\text{F. Diphosphatase}}$ F.6.P. + pi (13)

F.6.P. $\xrightleftharpoons{\text{Isomerase}}$ G.6.P. (14)

G.6.P. $\xrightleftharpoons{\text{Phosphoglucomutase}}$ G.1.P. (15)

ATP + UDP $\xrightleftharpoons{\text{UDP kinase}}$ UTP + ADP (16)

UTP + G.1.P. $\xrightleftharpoons{\text{UDPG pyrophosphorylase}}$ UDPG + PiPi (17)

UDPG + F.6.P. $\xrightleftharpoons[\text{glucosyl transferase}]{\text{UDPG fructose phosphate}}$ sucrose-P+UDP (18)

Sucrose-P $\xrightleftharpoons{\text{Sucrose phosphatase}}$ sucrose + Pi (19)

A number of reactions are involved in the conversion of PGA to sucrose. The enzymes known to catalyse these reactions are shown above (reaction 9-19). Heber, Gimmler and Schafer (1974) reported that in spinach leaves, NAD dependent glyceraldehyde phosphate dehydrogenase was distributed to an equal extent between the cytoplasm and the chloroplasts. Stocking (1959) and Heber (1960) demonstrated a high activity of Aldolase and triosephosphate isomerase in the cytoplasm and also in the chloroplasts. Fructose diphosphatase which catalyses the conversion of FDP to F.6.P has been reported to be mainly associated with the cytoplasm (Racker and Schroeder, 1958), although subsequent work (Smillie, 1960; Latzko and Gibbs, 1968) showed appreciable activity in chloroplasts as well. Smillie (1963) also reported that phosphohexoisomerase, the enzyme responsible for the conversion of G.6.P to G.1.P, is mainly associated with the cytoplasm.

Recently, Bird, Cornelius, Keys and Whittingham (1974) investigated the distribution of two key enzymes responsible for sucrose synthesis, UDPG pyrophosphorylase which catalyses the formation of UDPG from UTP and G.1.P and UDPG fructose phosphate glucosyl transferase which catalyses

the formation of sucrose phosphate from UDPG and F.6.P. Sucrose was synthesized from UDPG plus F.6.P at rates of 17.9, 25.0, 9.2 and 27.7 μmol/hr./g.fr.wt respectively with extracts obtained from pea shoots, spinach, wheat and bean leaves. Unwashed chloroplasts isolated from pea shoots, in which half the plastids were intact, contained less than 4% of the UDPG fructose phosphate glucosyltransferase activity. These results clearly show that the two enzymes mentioned are not associated with the chloroplasts and further, that if this enzyme localization observed in vitro occurs also in vivo, then sucrose synthesis in leaves cannot take place in the chloroplast. The conclusion was that sucrose synthesis in leaves occurs mainly in the cytoplasm.

2. (c) Inhibitor Studies

Zelitch (1966) blocked the oxidation of glycollate in tobacco leaf discs with α-HPMS and found, especially at high temperatures (35°C) that net photosynthetic CO_2 assimilation was increased about 3-fold. However, no such increase in net photosynthesis was observed at 30°C (Zelitch, 1965) or at 25°C (Zelitch, 1966). Further, it must be pointed out that for the two observations made at 25° (Zelitch, 1966), different results were obtained. Net photosynthesis was decreased by more than 50% in one case, but unaffected in the other. More recently, Jewess, Kerr and Whitaker (1974) described the efficiency of acetylenic substrate analogues as irreversible inhibitors of certain flavoproteins (Hellerman and Erwin, 1968). Sodium salt and the methyl ester of the acetylenic analog 2-hydroxy 3-butynoic acid (HBA) were effective inhibitors of glycollic oxidase. In 5 hours 3×10^{-4}M methyl-HBA caused a 99% inhibition of glycollate oxidase activity in pea leaf discs and also caused nearly twice as much glycollate to accumulate as in untreated tissue. However, no detectable inhibition of photorespiration was observed and also there was no stimulation of net CO_2 assimilation (Kerr, personal communication).

In the presence of INH, which inhibits the conversion of glycine to serine, the specific radioactivity of CO_2 released by tobacco leaf segments into CO_2-free air, following photosynthesis in $^{14}CO_2$, was much less, than in the absence of the inhibitor (Goldsworthy, 1966). However, the presence of the inhibitor had little effect on the rate of $^{14}CO_2$ uptake.

Zelitch (1974) reported the effect of the inhibitor, potassium glycidate which is an epoxide similar in structure to glycollate, on the rate of photorespiration and CO_2 uptake in illuminated tobacco leaf discs. It inhibited glycollate synthesis 40-50%, decreased the release of photorespiratory CO_2 by about 40% and, of special significance, it increased net photosynthesis by 40-50%. It is however not confirmed that this increase in net photosynthesis is not an artifact of experimental technique because no such increase was observed in two experiments, especially under conditions where α-HPMS, an inhibitor which was previously claimed to increase the net photosynthesis in tobacco leaf discs by about 3-fold (Zelitch, 1966), was supplied after the supply of potassium glycidate. It is therefore not certain whether blocking the glycollate pathway at normal temperatures and at normal levels of CO_2 and O_2 consistently results in any increase in net photosynthesis.

3. Measurement of photorespiration

The term photorespiration was used in its current sense by Decker and Tio (1959) to explain the outburst of CO_2 by leaves of many species, when they were suddenly transferred from light to darkness. Photorespiration is a process by which green plants take up O_2 and release CO_2 in the light and it can be measured by measuring either of these two processes. Jackson and Volk (1970) reviewed various techniques for measuring photorespiration, but difficulties were encountered with each method which makes precise measurement impossible. The main problem is that under normal conditions photosynthesis and photorespiration occur simultaneously in the same tissue and carry out opposite overall reactions. Decker (1955)

demonstrated that in the hybrid tobacco Nicotiana sanderae there is a rapid evolution of CO_2 immediately following the darkening of the photosynthesizing tissue. He suggested that this CO_2 outburst resulted from a slight delay between the rate of deceleration of photosynthesis and the rate of deceleration of photorespiration. After about 2 min. the evolution of CO_2 decreased to a level equal to the rate of dark respiration. It was pointed out that the magnitude of the post-illumination outburst relative to the subsequent rate of dark respiration is a measure of the extent to which light stimulates CO_2 production (Decker, 1959). With leaves of tobacco, pea bean, castor bean, tulip, poplar and white ash the photorespiratory rate estimated by the post-illumination outburst of CO_2 was about 4-5 fold greater than the dark respiratory rate (Decker and Wein, 1958; Decker, 1959). The post-illumination CO_2 outburst depended on the light intensity of the previous period of photosynthesis (Tregunna, Krotkov and Nelson, 1961; Egle and Fock, 1967). Further, Forrester, Krotkov and Nelson (1966) observed no CO_2 outburst in leaves kept in 1% O_2, but it occurred in 20% O_2 and was 3 times as large in atmospheres of 100% O_2. Dark respiration was, however, unaffected by increase in O_2 concentration around the leaf from 2-47% (Krotkov, 1963; Tregunna et al., 1966). The partial pressure of CO_2 in the atmosphere also markedly affect the post-illumination CO_2 outburst. In bean and sunflower leaves and in the thalli of the liverwort Concephalum conicum (Egle and Fock, 1967) the post-illumination CO_2 outburst which was detected in normal air was completely inhibited even at 99% O_2 when the concentration of CO_2 was high (1200 vpm). Moreover, in tobacco leaves the outburst of CO_2 was larger at 33.5°C than at 26°C (Decker, 1959). At 33.5° the photorespiratory rate measured by the post-illumination CO_2 outburst represented 66% of net photosynthesis whereas at 26° it was only 45%. Bulley and Tregunna (1971) showed that soybean leaves photorespired at a rate 75% of the rate of net CO_2 assimilated. This high rate was calculated when a correction was made for the failure of the infra-red gas analyser to measure the maximum CO_2 concentration in the post-illumination burst because of the rather large volume of the

measuring cell. Measurement of photorespiration by the post-illumination CO_2 outburst could however lead to an under-estimate if the cessation of photosynthesis is not as abrupt as assumed and some of the outburst is refixed.

Another way to measure photorespiration by inhibiting photosynthesis is to measure the CO_2 output in the light into CO_2-free air. Moss (1966) measured the amount of CO_2 evolved into CO_2-free air in both light and darkness and found that for many species light increased CO_2 evolution above normal dark respiration. Hew and Krotkov (1968) found that with sunflower and geranium leaves the rate of CO_2 evolution in the light into CO_2-free 100% O_2 was 1.8 to 2 times greater than into CO_2-free 21% O_2. Further, when the illuminance was increased from 500 to 1000 f.c. the ratio of the CO_2 evolved into CO_2-free air in the light to darkness also increased from 0.83 to 1.5 in sunflower leaves at 20°C (Hew, Krotkov and Canvin, 1969). The CO_2 evolved in the light into CO_2-free air under such conditions therefore represented photorespiration and not dark respiration. From such measurements, it was calculated that in soybean 46% (Samish, Pallas, Dornhoff and Shibles, 1972) Sunflower 27% (Ludwig and Canvin, 1971) Sugar beet 40% (Hofstra and Hesketh, 1969) and in tobacco 55% (Kisaki, 1973) of net CO_2 assimilated was concurrently evolved as CO_2. This again is not an accurate measurement of photorespiration because some of the CO_2 released by photorespiration would be refixed by photosynthesis and the rate of photorespiration would be underestimated. Because of the criticism that in the above two methods photorespiration was measured when photosynthesis was inhibited and that its rate may not be normal under these circumstances, attempts have been made to measure photorespiration during normal photosynthesis by the use of radioisotopes. Volk and Jackson (1972) estimated photorespiration by measuring rate of $^{18}O_2$ uptake from an atmosphere containing this isotope. They found that in maize photorespiration represented only 6% of the net CO_2 uptake. This method again could lead to an underestimate of photorespiration because some of the $^{16}O_2$

released by photosynthesis could be taken in by the tissue together with the supplied $^{18}O_2$.

Another method employed to estimate photorespiration is to measure the dilution of specific activity of the $^{14}CO_2$ supplied to an illuminated leaf in a closed system. Under such conditions the $^{12}CO_2$ produced from the unlabelled photorespiring substrates will diffuse out of the leaf and during the first few seconds would lower the specific activity of the $^{14}CO_2$ in the system. The decrease in the specific activity of the $^{14}CO_2$ supplied can be used to estimate the rate of photorespiration. This method was used by Krotkov, Runeckles and Thimann, (1958) to demonstrate photorespiration in wheat leaves and subsequently in tobacco and sunflower leaves (Krotkov, 1963). However, Ludwig and Krotkov (1967)reported that in sunflower leaves detectable incorporation of ^{14}C into photorespiratory substrates occurs within the tissue in less than 15 to 45 seconds so that the photo-respired CO_2 is quickly labelled. In this species the method therefore underestimates photorespiration if more than 15 seconds elapses before the specific activity of CO_2 in the system is determined.

All assays of photorespiration therefore underestimate its magnitude to some extent. Despite this it has been found that measured rates of photo-respiration are generally high ranging between 25-60% of net photosynthesis. These results clearly indicate that there is considerable variation in estimates depending on the method of estimation and the plant species, even at similar temperatures.

4. Reaction responsible for photorespiratory CO_2 evolution

Tregunna, Krotkov and Nelson (1964) demonstrated that the $^{14}CO_2$ released immediately upon darkening of tobacco leaves had the same specific activity as the $^{14}CO_2$ supplied during a previous light period. They concluded that some recent product of photosynthesis was used as substrate for photorespiration. From similar measurements Goldsworthy (1966) also

concluded that illumination brings about a change of substrate for CO_2 production; recent assimilates being preferentially oxidized in the light. Ludwig and Canvin (1971) similarly measured the $^{14}CO_2$ and CO_2 evolution into CO_2-free air over a long period, from sunflower leaves previously allowed to photosynthesize in $^{14}CO_2$. The high specific radioactivity of the $^{14}CO_2$ evolved during photosynthesis or in the early period in CO_2-free air showed that the substrate for light respiration was an early product of photosynthesis. However, after longer periods in CO_2-free air, the picture was more complex; the CO_2 evolution did not cease completely even after prolonged exposure to CO_2-free air and different amounts of $^{14}CO_2$ were released depending on the length of the previous period of photosynthesis in $^{14}CO_2$. This was taken as evidence that when the rate of photosynthesis is adequate, the substrate for photorespiration is supplied from the immediate products of photosynthesis, but in CO_2-free air when photosynthesis is restricted the substrate would be generated from storage products including soluble forms of carbohydrates. Waidyanatha, Keys and Whittingham (1974), showed that in the absence of CO_2, exogenously supplied glucose could provide the substrate for both photorespiration and sucrose synthesis.

Downton and Tregunna (1968) supplied glycollate exogenously to shoots of corn, oats and wheat and found that the CO_2 evolved in the dark was greater at 21% O_2 than at 2% O_2. Since photorespiration is also increased at 21% O_2 compared to 2% O_2, they proposed that glycollate is a substrate for CO_2 evolution in the light. Zelitch (1958) found that α-HPMS was an active inhibitor of glycollate oxidation; it caused glycollate to accumulate in leaf tissue in the light and decreased the release of CO_2 (photorespiration). Unfortunately α-HPMS does not inhibit only glycollate oxidase (Osmund and Avadhani, 1970) but also decrease the rate of net photosynthesis (Moss, 1968). Moss (1968) found that added glycollate increased the CO_2 production by tobacco leaves in the light in CO_2-free air. Illuminated barley leaves

converted ^{14}C-glycollate to $^{14}CO_2$ and to normal products of photosynthesis (Tamas and Bidwell, 1971). In similar experiments where glycollate-1-^{14}C was supplied to tobacco leaf discs Zelitch (1966) observed an increased evolution of $^{14}CO_2$ in the light compared to darkness. From these results he deduced that glycollate is the primary substrate of CO_2 evolution during photorespiration and that the CO_2 was derived from the carboxyl position of glycollic acid. Zelitch (1972) conducted further experiments to determine whether glycine or glyoxylate was the more immediate precursor of photorespiratory CO_2. Tobacco leaf discs were illuminated while floating on 18 mM solutions of glycollate-1-^{14}C or glycine-1-^{14}C in CO_2-free air. The $^{14}CO_2$ released was measured when these radioactive substrate solutions were provided alone or with 9mM α-HPMS or INH. α-HPMS inhibited $^{14}CO_2$ production from glycollate-1-^{14}C. INH decreased the formation of serine from glycine-1-^{14}C but did not significantly decrease the $^{14}CO_2$ production from either glycollate-1-^{14}C or glycine-1-^{14}C. Zelitch concluded that the glycollate pathway of carbon metabolism does not produce sufficient CO_2 during the synthesis of serine from glycine to account for observed rates of photorespiration and that the non-enzymic oxidation of glyoxylate by H_2O_2 (Zelitch, 1967) was probably the reaction responsible for CO_2 evolution during photorespiration. The H_2O_2 needed for this oxidation was thought to be that produced during the oxidation of glycollate by the enzyme glycollate oxidase situated in the peroxisomes. However, Tolbert et al. (1969) showed that peroxisomes contain sufficient catalase to destroy completely all the H_2O_2 produced in the peroxisomes. It was concluded that a decarboxylation of glyoxylate by H_2O_2 is unlikely to occur in the peroxisomes. Further, Kisaki and Tolbert (1969) demonstrated that rates of light driven CO_2 evolution from glyoxylate-^{14}C catalysed by broken chloroplast fractions were at least a thousand times less than rates of glycine formation from glyoxylate in peroxisomes in the dark.

Marker and Whittingham (1967) demonstrated that 25.5% and 28.3% respectively of the radioactivity in glycollic acid-1-^{14}C and glycine-1-^{14}C fed to excised leaves of Pisum sativum was evolved as CO_2. Glycine-2-^{14}C produced only 9.5% CO_2. They concluded that the $^{14}CO_2$ produced during photorespiration is derived from the carboxyl carbon of glycollic acid or glycine and that both these compounds were equally effective precursors of photorespiratory CO_2. INH is known to inhibit the conversion of glycine to serine in Chlorella (Pritchard et al., 1962) and in higher plant leaves (Wade, 1969) and caused the accumulation of glycollate and glycine in Chlorella (Pritchard et al., 1962) and glycine in leaves. Goldsworthy (1966) allowed tobacco leaf segments treated with 10^{-2}M α-HPMS or 10^{-2}M INH, to assimilate $^{14}CO_2$ in the light and found that during a subsequent period in light $^{14}CO_2$ released into CO_2-free air had a lower specific activity than that from untreated leaves, the specific activity was, in fact, similar to that of CO_2 released in darkness. It appeared that both inhibitors decreased photorespiration and therefore that glycine is the immediate precursor of photorespired CO_2 and that the inhibition by α-HPMS was the result of decreased conversion of glycollate to glycine. Further, Wade (1969) found that whereas α-HPMS inhibited $^{14}CO_2$ production from glycollate-1-^{14}C but not from glycine-1-^{14}C, INH in addition inhibited the $^{14}CO_2$ release from glycine-1-^{14}C. Kisaki and Tolbert (1970) obtained more extensive similar evidence in support of this by supplying specifically ^{14}C labelled substrates to tobacco and corn leaf segments and measuring the $^{14}CO_2$ evolution in the light and darkness. Carboxyl labelled glycine was more rapidly decarboxylated than were glycollate, glyoxylate or serine. α-HPMS inhibited CO_2 evolution from glycollate but not from glycine. INH severely inhibited the CO_2 release from both glycollate and glycine. They therefore concluded that CO_2 production during photorespiration occurs during the conversion of glycine to serine.

Results inconsistent with glycine as the immediate substrate of photorespiration were reported by Atkins, Canvin and Fock (1971). Sunflower

leaf discs were placed in CO_2-free air with 21% O_2, after 15 min. photosynthesis in $^{14}CO_2$ (270 vpm CO_2, 21% O_2). Radioactivity in glycine decreased rapidly and $^{14}CO_2$ was evolved. In a similar experiment when leaf discs were placed in CO_2-free air with 1% O_2, the radioactivity in glycine decreased as before but an equivalent amount of $^{14}CO_2$ was not evolved and there was very little change in the amount of ^{14}C in serine. Atkins et al. suggested that in CO_2-free 1% O_2 either $^{14}CO_2$ was produced and not evolved or $^{14}CO_2$ production was not associated with the observed decrease of ^{14}C in glycine. Further, since the radioactivity accumulated in serine when there was little or no evolution of $^{14}CO_2$, it appeared that the further metabolism of serine might be important as a source of CO_2 during photorespiration.

5. Metabolic pools and the rate of carbon flow through the glycollate pathway.

Smith, Bassham and Kirk (1961) measured the concentration of carbon in a rapidly turning over metabolic pool of a photosynthetic intermediate in Chlorella, from the level of radioactivity it attained as a function of time of exposure of the algae to $^{14}CO_2$. Finally, the specific activity of the active pool of the given compound should approach that of the administered $^{14}CO_2$ so that an estimate of the amount of carbon in it can be made. Atkins et al.(1971) fed sunflower leaf discs with $^{14}CO_2$ gas of known specific activity and found that ^{14}C in 3-PGA, glycine and serine reached a constant level after 10 min photosynthesis in $^{14}CO_2$. The fact that these labelled pools were actively turning over was confirmed by the finding that after 15 min in $^{14}CO_2$, when the gas stream was changed to $^{12}CO_2$, the radioactivity from these compounds disappeared quickly. Smith et al.(1961) also observed that the incorporation of ^{14}C into the pool of glutamic acid continued to increase throughout the entire experimental period of 110 minutes. It was therefore suggested that in Chlorella there were active and less active pools of glutamic acid. The existence of multiple pools of amino acids in plants has also been emphasised by many others (Hellebust and

Bidwell, 1963; Miflin et al., 1966; Oaks and Bidwell, 1970). The size of the active metabolic pools of amino acids vary considerably depending on the environmental conditions. In 1% O_2 compared to 21% O_2, the pool size of glycine and also of serine in sunflower leaves (Atkins et al. 1971) and in bean leaves (Voskresenskaya, Wiil, Grishina and Parnik, 1970) decreased considerably. Further, the incorporation of radioactivity into glycine and serine in tomato leaves was markedly decreased at 950 vpm (Lee and Whittingham, 1974) and at 1000 vpm (Bishop and Whittingham, 1968) CO_2 in the atmosphere. Similar decreases in pool size of glycine and serine were observed in sunflower leaves at high CO_2 concentrations by Mahon, Fock and Canvin (1974). A diurnal fluctuation in the pool size of glycine and serine in tobacco leaves has been reported by Noguchi and Tamaki (1962). Thus, in addition to partial pressures of CO_2 and O_2 in the atmosphere, various other factors affect the metabolic pools of amino acids within the cell.

Smith et al. (1961) using Chlorella, calculated the rate of flow of carbon through the active pools of several amino acids including glycine and serine, by determining the maximum slope of the curves for the incorporation of radioactivity at times between 5 to 16 min. after the introduction of $^{14}CO_2$. This method however would lead to inaccurate measurements, mainly due to the presence of multiple pools of these amino acids. Fock, Höhler, Canvin and Grant (1971) conducted similar experiments with illuminated sunflower leaf discs. They suggested that between the first and the second minute of $^{14}CO_2$ assimilation the best estimate of rate of carbon flow into glycine and serine was possible since during this period the specific activity of the amino acids and therefore the outflow of ^{14}C, is relatively low. Similar reasoning could be used to calculate the efflux of carbon from the amino acids during the subsequent period without $^{14}CO_2$ provided that the active pools had become during the $^{14}CO_2$ assimilation period, saturated with ^{14}C. From their measurements Fock et al. (1971) estimated that the carbon flow through glycine and serine in 21%

O_2 was 23 to 29% and 62 to 77% respectively of the apparent rates of photosynthesis and concluded that both glycine and serine were important intermediates located on the main route of carbon metabolism during photosynthesis. It has been suggested (Tolbert and Yamazaki, 1969; Zelitch, 1971) that in the normal leaf in sunlight more than half the carbon fixed may be metabolized by the glycollate pathway.

The investigations reported in this thesis were made mainly to establish the immediate substrate for photorespiration and to estimate the proportion of carbon fixed during photosynthesis, that is metabolized by way of the glycollate pathway in excised wheat leaves.

MATERIALS AND METHODS

1. Plant Material

One variety of wheat (Triticum aestivum), viz. Kolibri, was used in all experiments reported here. Wheat seeds selected for uniformity in size and soundness were sown in 6 inch plastic pots containing a commercial compost (75% peat and 25% sand) enriched with inorganic fertilizer. Twelve seeds were sown per pot, two in the centre and the remaining ten in pairs about an inch away from the periphery. The plants were grown in a growth room under artificial illumination, of about 1000 f.c. at the level of the leaves, supplied by a mixture of fluorescent and tungsten lamps for 16 hours each day. The temperature was maintained throughout at $20^{\circ} \pm 1^{\circ}C$.

Before each experiment 13-day old plants were transferred to a dark room at 5 p.m. The following morning (14th day) the lights in the dark room were switched on at 10 a.m. so that the plants had a total dark period of 17 hours. Leaves (first leaf) of comparable size were excised from the plants and a 4 cm segment of the lamina was cut under water with a sharp blade discarding about 2 cm from the base and the remaining portion of the apex. The leaf segments were quickly placed in slots made in a perspex disc so that the basal cut ends were dipping either in water or in a solution of an inhibitor depending on the experiment. The leaf segments were held in an up-right position by a wire framework, (see Figs. 1a and 1b). Each perspex disc carrying leaf segments was transferred to a leaf chamber of the apparatus (Fig. 2). Leaf samples were not cut in the growth room where the apparatus was set up, because the rapid flow of air through the growth room caused leaf segments to wilt before they could be transferred to the apparatus.

2. Measurement of leaf area

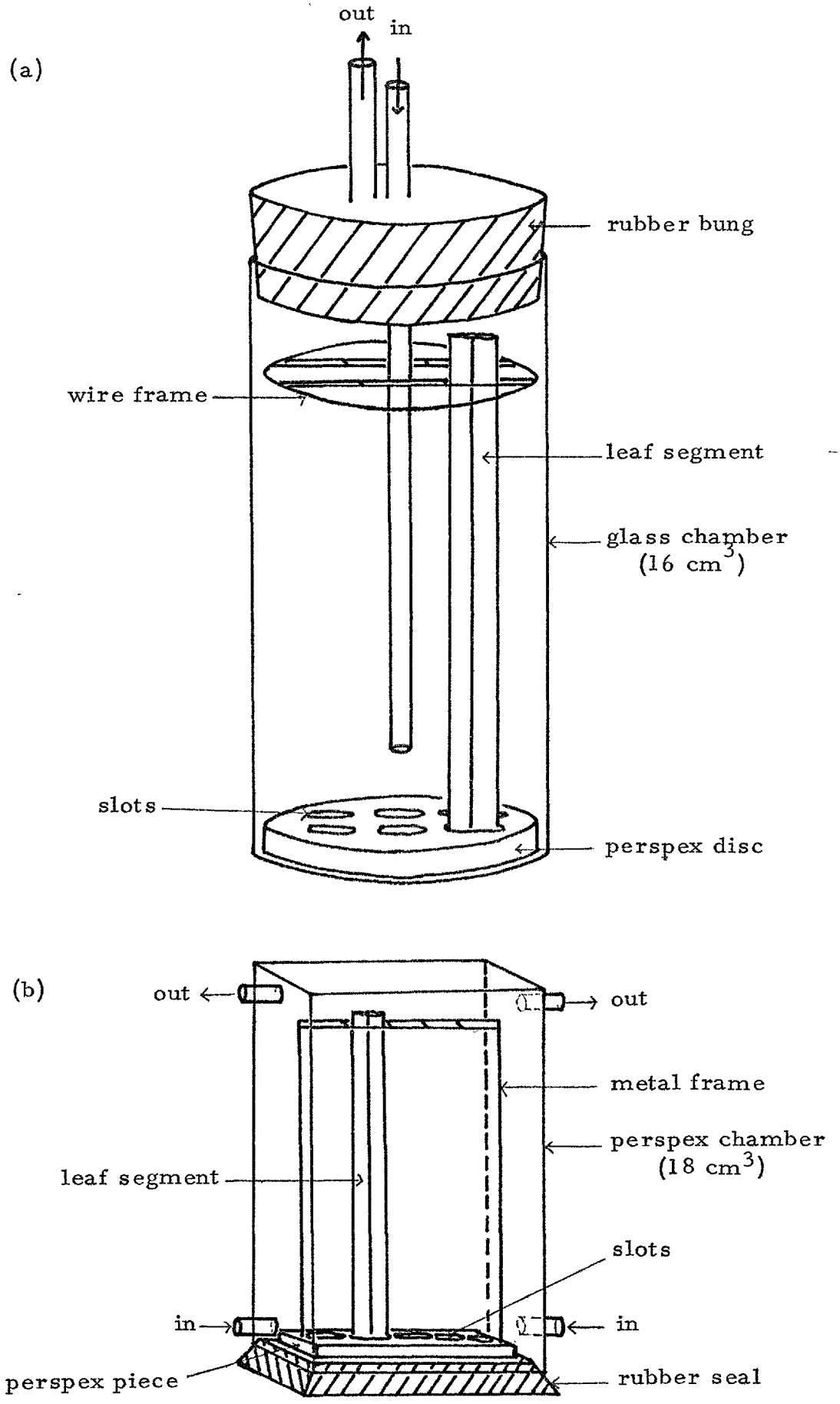
The areas of 20 samples, each composed of five 4 cm leaf segments and cut from leaves comparable in size and age to those normally used in experiments, were counted using a Paton electronic planimeter (Paton Industries Ltd., Australia). The measured average area of 5 leaf segments was 9.96 ± 0.25 cm^2. Since the standard error calculated was small, being 2.5% of the sample mean, a uniform value of 0.1 dm^2 per 5 leaf segments was used for all the relevant calculations in this thesis.

3. Leaf chambers

Two sizes of leaf chamber were used. One, a rectangular perspex chamber of volume 18 cm^3 (Fig. 1b). A rectangular piece of rubber, carefully ground at the edges and smeared with vaseline, sealed the bottom of this feeding chamber. The five leaf segments were placed in slots in a piece of perspex which was glued to the upper surface of the rubber seal. Air-tightness of the seal was checked before and during the experiment by partially immersing the bottom of the feeding chamber in a petri dish containing water. Small rubber bands held the base to the body of the chamber. These feeding chambers were used in the first experiment only. They were discarded because they were difficult to use.

The second type of feeding chamber (Fig. 1a) was much simpler and more easily obtained. It comprised of a glass specimen tube of volume 16 cm^3. A perspex disc with slots in it (see Fig. 1a) carried the 5 leaf segments which, as before, were held up-right by a wire framework. A rubber bung fitted with two glass tubes (0.3 cm in diameter) provided the inlet and outlet for the gas mixtures. These chambers were easily

Fig. 1. Leaf chambers



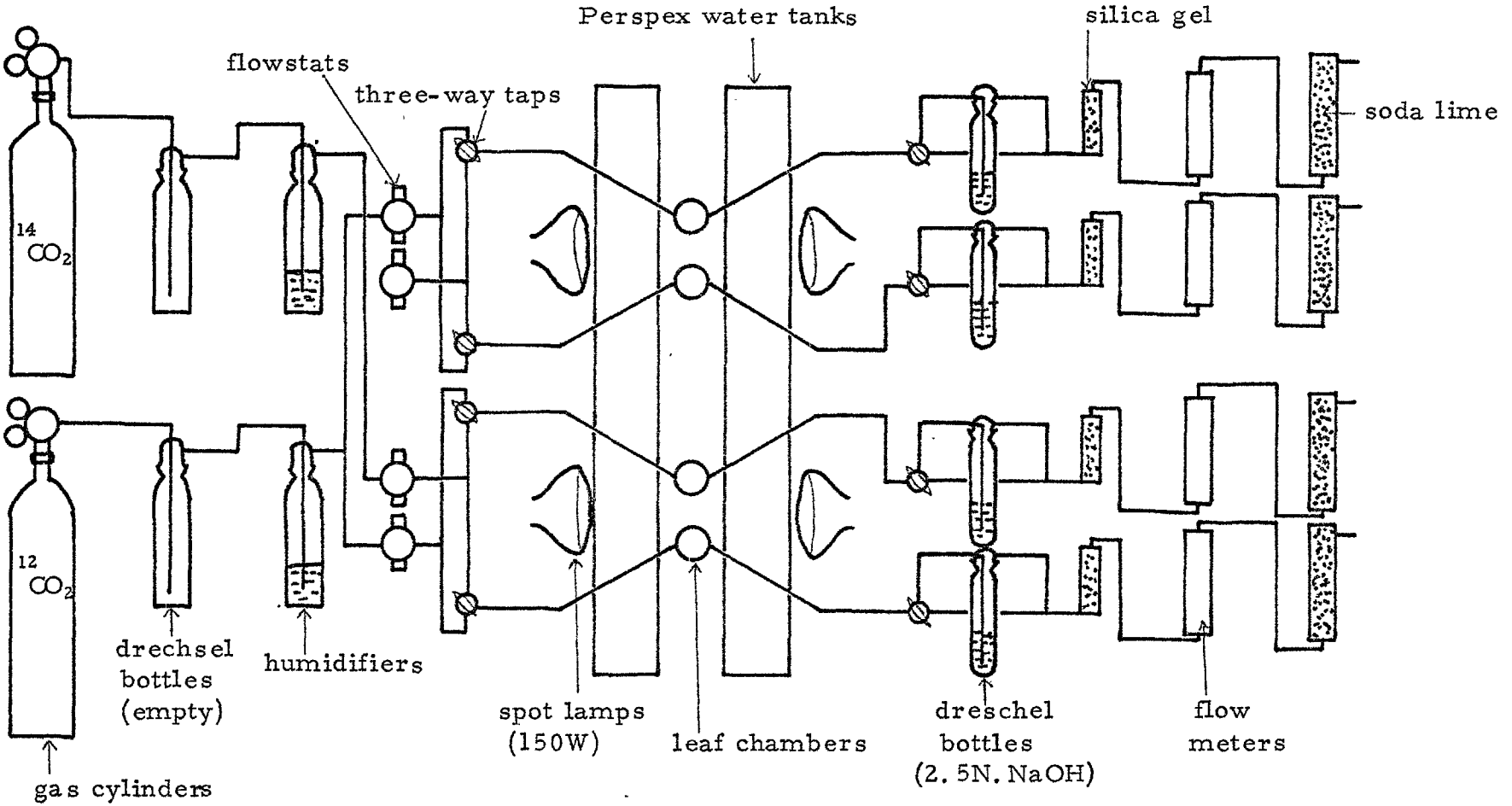


Fig. 2. A diagram of the apparatus used for supplying various gas mixtures to leaf segments.

made air-tight without vaseline and fitting the rubber bung was quicker and easier than with the rectangular chambers. They were therefore used in all the other experiments described in this thesis.

4. Apparatus to supply gas mixtures with and without $^{14}CO_2$ -

The apparatus used for supplying the gas mixtures is illustrated in Fig. 2. All the gas mixtures were supplied from cylinders. In one experiment only, where CO_2-free air was used during the preincubation period, atmospheric air was passed through the system by a pump (Model AP - 220, J.I. Wade & Son Ltd.), after the CO_2 in the air had been removed by a tower (30 x 5 cm) containing soda-lime ('Carbosorb'). During the experiments, the flow rate of gas mixtures through the system was maintained at 430 ml/min (27 chamber volumes of gas per minute); with this flow depletion of CO_2 within the leaf chamber because of photosynthesis did not exceed 15% of the CO_2 supplied. The flow rate was controlled by flow-stats (G.A. Platon Ltd.) and measured by flow meters (G.A. Platon Ltd.; Mete rate, Jencons Scientific Ltd.). The air streams were humidified by bubbling through water contained in a Drechsel bottle (Quickfit and Quartz Ltd.) placed between gas cylinders and the flowstats. A second empty Drechsel bottle between the humidifier and the gas cylinder protected the cylinder from water in the humidifier. A three way tap between the leaf chamber and the flowstats selected the gas mixture. The change over from one gas mixture to another did not take more than 1 - 2 seconds. The outlet of the leaf chamber was also connected to a 3 - way tap which directed gas leaving the leaf chamber either directly to a tower of soda-lime (to absorb the $^{14}CO_2$ so that the radioactive gas did not contaminate the experimental room) or first through a Drechsel bottle fitted with a sintered bubbler (see Fig. 2) (Quickfit and Quartz Ltd.) and containing 25 ml of a 2.5 N solution of NaOH to absorb

the $^{14}CO_2$ released. Internal volume of the plastic and glass tubes between the two 3-way taps connected to the inlet and the outlet of the leaf chamber was kept to a minimum to reduce the time taken to flush each gas mixture from that part of the system. In practice the residual labelled gas in the leaf chamber could be flushed out in 5 to 8 seconds but to ensure complete removal of residual $^{14}CO_2$ a period of 15 seconds was allowed before the tap was turned for the outgoing gas to pass through NaOH. A column of silica jel (6 x 2 cm) dried the gas stream before it passed through the flow meters. This prevented condensation of moisture within the flow meters and so maintained their accurate measurement of the flow rate through the system.

5. Illumination and heat filter

Four 150 w reflector spot lamps (Mazda, Philips or Cryselco) two on either side were used to illuminate the four leaf chambers (see Fig. 2). A light intensity of 3000 $\pm$ 100 f.c. was obtained at the surface of the leaf chambers by placing the lamps about 12 inches away. The intensity of illumination was kept uniform by varying the distance, when necessary, between the lamps and the leaf chambers. A light meter with a barrier type photocell (Evans Electroselenium Ltd.) was used to measure the light intensity. Some of the heat generated by the spot lamps was prevented from reaching the leaf chambers by having perspex water tanks (8 x 22 x 52 cm) (Fig. 2) between the leaf chambers and the lamps. The inlet of the water tank was connected to a cold water tap and the outlet, an inch below the top of the perspex tank, was directed to waste so that there was a continuous flow of cold water through the cooling tank. This arrangement allowed leaf chambers to be maintained at 20 $\pm$ 1.0°C throughout the course of experiments.

6. Preparation of gas mixtures

Gas mixtures containing CO_2 (^{14}C or ^{12}C) O_2 and N_2 were made using pure gases obtained in high-pressure cylinders (British Oxygen Company). The required gas mixtures were prepared in steel gas cylinders with an internal volume of 9.5 litres by filling with CO_2-free air and nitrogen to the appropriate amounts of CO_2. Calculated amounts of sodium carbonate solutions ($Na_2{}^{12}CO_3$ or $Na_2{}^{12}CO_3 + Na_2{}^{14}CO_3$) were pipetted into the three necked round bottom flask D (Fig. 3). One opening of this flask was covered with a suba seal. Of the other two, one was connected to a tower containing soda-lime and through this route CO_2-free air could be admitted. The third opening was connected to an evacuated cylinder through a system (C) of pressure gauges as shown in Fig. 3. High-pressure cylinders (A) containing CO_2-free air, O_2 or N_2 could be connected to this system. Carbon dioxide ($^{12}CO_2$ or $^{12}CO_2 + {}^{14}CO_2$) was released by injecting a solution of 50% lactic acid from a syringe into the proper amount of sodium carbonate in solution in the flask. The acid was added dropwise until all the effervescence stopped. CO_2-free air admitted from the soda-lime tower (E) was allowed to flush through the flask so that the CO_2 released was carried into the evacuated cylinder. The supply of CO_2-free air was continued until atmospheric pressure was reached. This was indicated by the lack of bubbling in the Drechsel bottle (G). When 1% O_2 was required, N_2 (supplied by a separate high pressure cylinder) was passed through the flask during this period instead of CO_2-free air. CO_2-free air was admitted from cylinder A until the appropriate pressure increase was reached. When necessary, e.g. to obtain CO_2 in 1% O_2, small cylinder was brought to the final pressure with pure N_2. Otherwise the small cylinder containing the required amount of $^{12}CO_2$ or $^{12}CO_2 + {}^{14}CO_2$ was brought to the final pressure with the CO_2-free air. The amounts

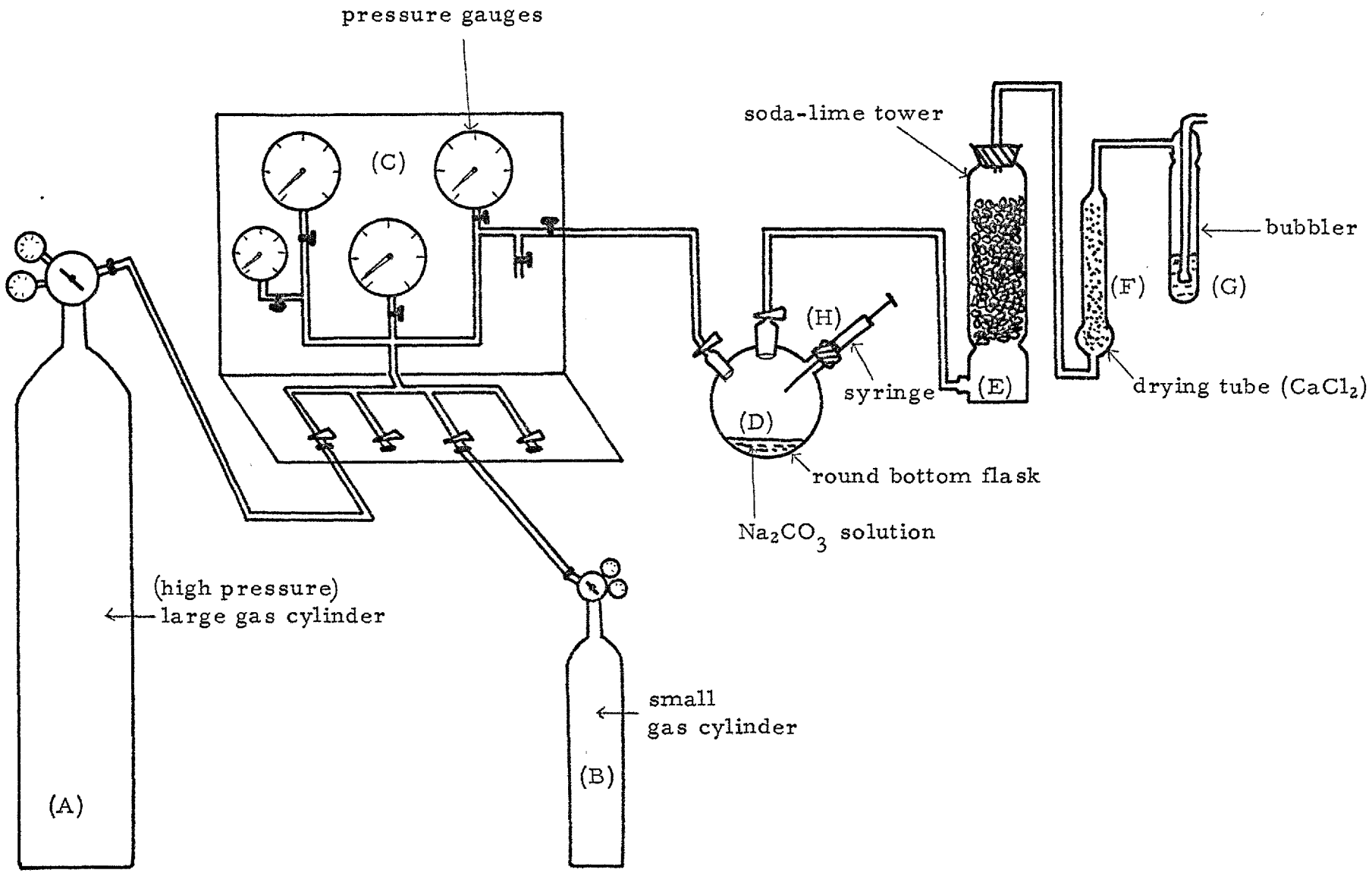


Fig. 3. A diagram of the apparatus used in the preparation of gas mixtures.

of CO_2 in the gas mixtures were checked by measurements made with infra-red gas analyser (Grubb Parsons Model SB 2). The errors observed were usually less than 5%.

7. Supply of labelled substrates and inhibitors

Radioactive solutions (serine-U-^{14}C and D-glycerate-U-^{14}C) were diluted to the desired specific activity and concentration by the addition of the appropriate unlabelled compound and water; the solutions were stored in a deep freezer until required. Solutions of inhibitors and substrates (0.05 ml) were carefully pipetted into the slots made in the perspex discs in experiments in which these solutions were used. The controls were provided with water instead. When labelled substrates were used only three leaf segments (instead of 5) were employed. In such experiments one row of three slots contained water or a solution of the inhibitors and the other row the substrate. This arrangement facilitated the rapid transfer of leaves from one slot containing water or the inhibitor to the other containing the substrate without having to change over from one perspex disc to another.

8. Absorption of $^{14}CO_2$ in 2.5 N NaOH solution

The efficiency of absorption was tested as follows. Gas mixtures containing different concentrations of CO_2 (150, 325 or 1000 vpm) were allowed to bubble through the 2.5 N NaOH (25 ml) in the Drechsel bottles for 10 minutes at the usual flow rate of 430 ml per minute. The gas from the outlet of the bottles was collected in polythene coated aluminium foil bags (Soplaril (Great Britain) Ltd.) and the concentration of CO_2 in these bags was subsequently measured by passing through an IRGA (Grubb Parsons, Model SB 2). NaOH depleted the CO_2 in the 150 vpm CO_2 air

stream to less than 5 vpm, 325 vpm CO_2 to less than 10 vpm and 1000 vpm CO_2 to less than 15 vpm. That is, more than 96% of the CO_2 in the gas supplied was absorbed by NaOH. The efficiency of the method was further checked when $^{14}CO_2$ was being collected in experiments. Two Drechsel bottles containing 2.5 N NaOH were connected in series in the gas stream; in 10 such tests more than 99% of the radioactivity recovered was found in the first Drechsel bottle and less than 1% in the second. Since the flow rate was always 430 ml/min and CO_2 concentrations higher than 1000 vpm were not used, collection in a single Drechsel bottle was judged sufficiently accurate for the estimation of respired $^{14}CO_2$.

In order to ensure that the $^{14}CO_2$ evolved was derived from the leaves and not from the system (which might absorb some $^{14}CO_2$ during the supply of $^{14}CO_2$) $^{14}CO_2$ was passed through the leaf chambers without leaves in them, for 15 minutes. During a subsequent period in 1000 vpm $^{12}CO_2$ (a concentration of CO_2 in which a comparatively large amount of $^{14}CO_2$ was often evolved; see Experimental and Results) the air which came out of the leaf chambers was bubbled through NaOH for various times up to 10 minutes and the radioactivity estimated. The amount of $^{14}CO_2$ recovered was insignificant.

9. Killing and extraction of leaves

After the various experimental treatments leaf segments were withdrawn from the leaf chambers, each cut rapidly into two and plunged into boiling absolute ethanol (4 ml) which was boiled for a further 2 minutes. A pair of forceps, to the gripping end of which was fitted two flat metal plates, facilitated the rapid removal of two or three leaf segments at a time from the leaf chambers. The whole procedure of removing the leaves from the leaf chamber and dropping them into boiling ethanol did not take more than 10 - 12 seconds.

The pieces of leaf were withdrawn from the ethanol extract and ground with acid washed sand using a glass mortar and a pestle. The ground leaves were transferred into a 10 ml glass centrifuge tube using 5 ml of 50% ethanol and the suspension centrifuged (MSE minor) at 3000 x g for 10 minutes. The supernatant liquid was combined with the absolute ethanol in which the tissue was originally boiled; the residue was re-extracted with 1 ml of water by mixing, using a Whirlimixer (Fisons scientific apparatus), for 1 to 2 minutes and the suspension centrifuged. The supernatant liquid was added to the rest of the extract and the volume made up to 10 ml.

10. Two-dimensional thin-layer chromatography of leaf extracts

Cellulose powder (Whatman thin-layer chromedia CC 41; 40 g) was thoroughly shaken, in a conical flask (500 ml) fitted with a ground glass stopper, with water (92 ml) for about 4 to 5 minutes. The slurry was sufficient to spread over five glass plates (20 x 20 cm) using a spreader (Quickfit and Quartz Ltd.) set for a layer thickness of 0.5 mm. The plates were allowed to dry on the spreader in still air for up to 1 hour and then overnight by blowing air over them with an electric fan or by placing the plates in a fume cupboard with the extractor fan on.

In the first experiment the leaf extracts (0.1 ml) were applied to the starting point on a thin-layer chromatographic plate using an Agla micrometer syringe (Wellcome reagents Ltd.) but this method was very time consuming and was therefore soon discontinued. More commonly, aliquots (1.0 ml) of extracts were dried down overnight in vacuo in a desiccator over anhydrous $CaCl_2$. The residue was redissolved in 50% ethanol (0.1 ml) and an aliquot of the concentrate was applied to the starting point on the TLC plate using either 5 or 10 µl disposable glass

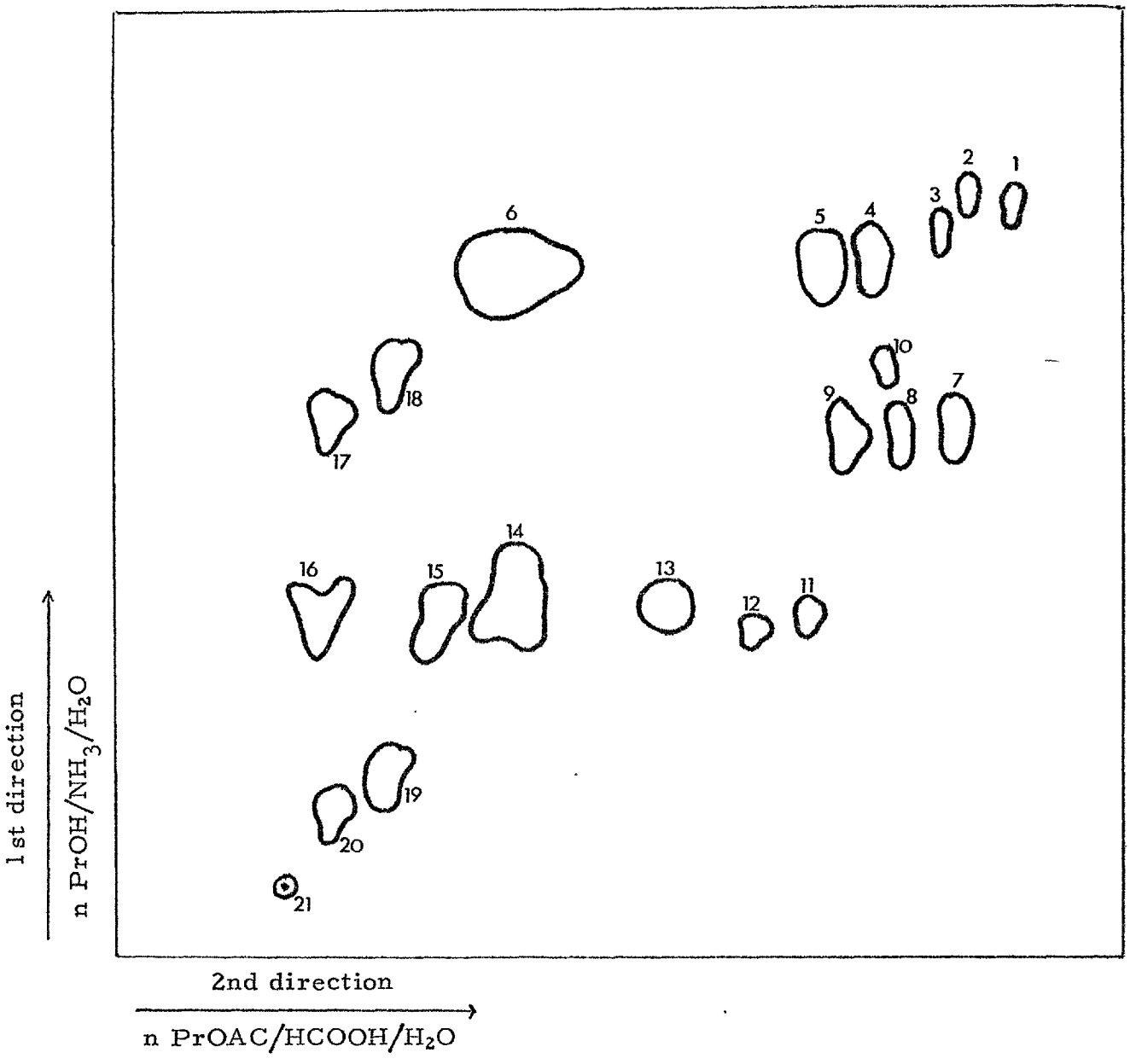
pipettes ('Microcaps'; Drummond Scientific Co.). Over 98% of the total radioactivity in the original extract was normally recovered after concentration and in comparison to the plates developed after applying the extract using Agla micrometer syringes, there was no evidence of an apparent breakdown or loss of any compounds.

The plates were developed in Shandon-multiplate tanks or Shandon thin-layer tanks using n-propanol : ammonia (sp.gr.O.88) : water (6 : 3 : 1 v/v) containing 2 g. EDTA per litre twice in the first direction and n-propylacetate ; formic acid (90%) : water (11 : 5 : 3 v/v) twice in the second direction. These solvent systems are essentially similar to those of Bieleski (1965) and Waidyanatha (1973). The plates were withdrawn from the tank when the solvent front reached the upper end of the cellulose layer. The developing time was approximately two hours with the first solvent and one hour with the second solvent. The plates were dried by a fan in the fume cupboard for at least 3 hours between each development. This system of TLC separated out glycollate, alanine, glycerate, glycine, serine, sucrose, PGA, PEP and UDPG very satisfactorily.

Although sucrose tended to trail in the basic solvent (Figs. 5 and 6) the estimation of its radioactivity was not affected because the trail did not overlap any other radioactive products. Separation of malate, aspartate, glutamate and various other phosphate esters was not always satisfactory.

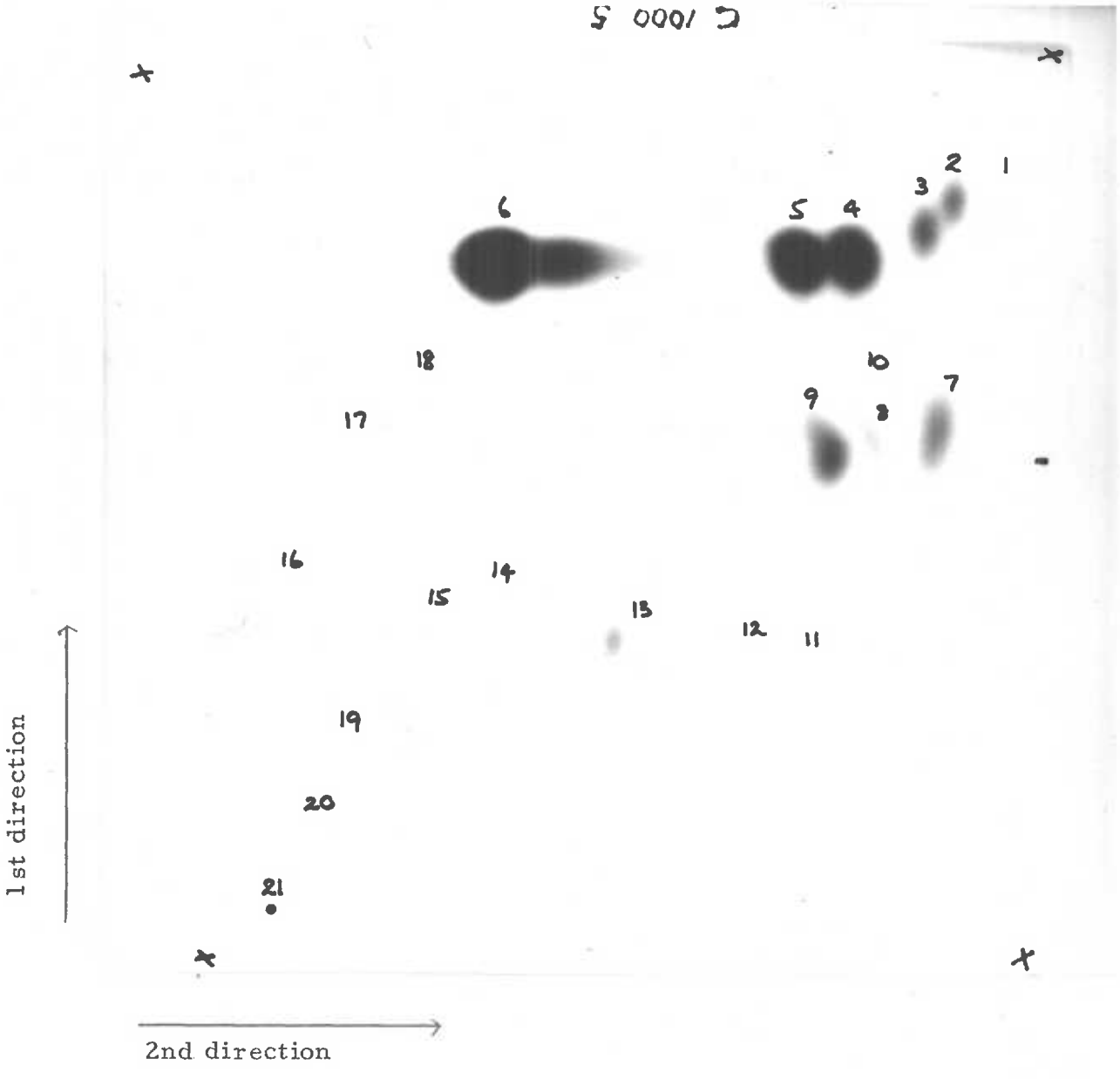
The positions of the compounds separated on the chromatograms were checked with those of Waidyanatha (1973) and by Co-chromatography of leaf extracts with authentic radioactive or non-radioactive compounds. The radioactive compounds were located by autoradiography and the non-radioactive compounds by spraying with appropriate reagents (Dawson, Elliot, Elliot and Jones, 1969). Amino acids on the chromatograms were identified by the purple colour produced when sprayed with ninhydrin

Fig. 4. A map showing the relative location of compounds identified on TLC developed in the solvent system described in the text.



1	Glycollate	8	Glutamate	15	G-6-P
2	Alanine	9	Aspartate	16	UDPG
3	Glycerate	10	Glutamine	17	Raffinose
4	Glycine	11	PEP	18	Maltose
5	Serine	12	Phosphoglycollate	19	RUDP
6	Sucrose	13	PGA	20	FDP
7	Malate	14	F-6-P	21	Origin

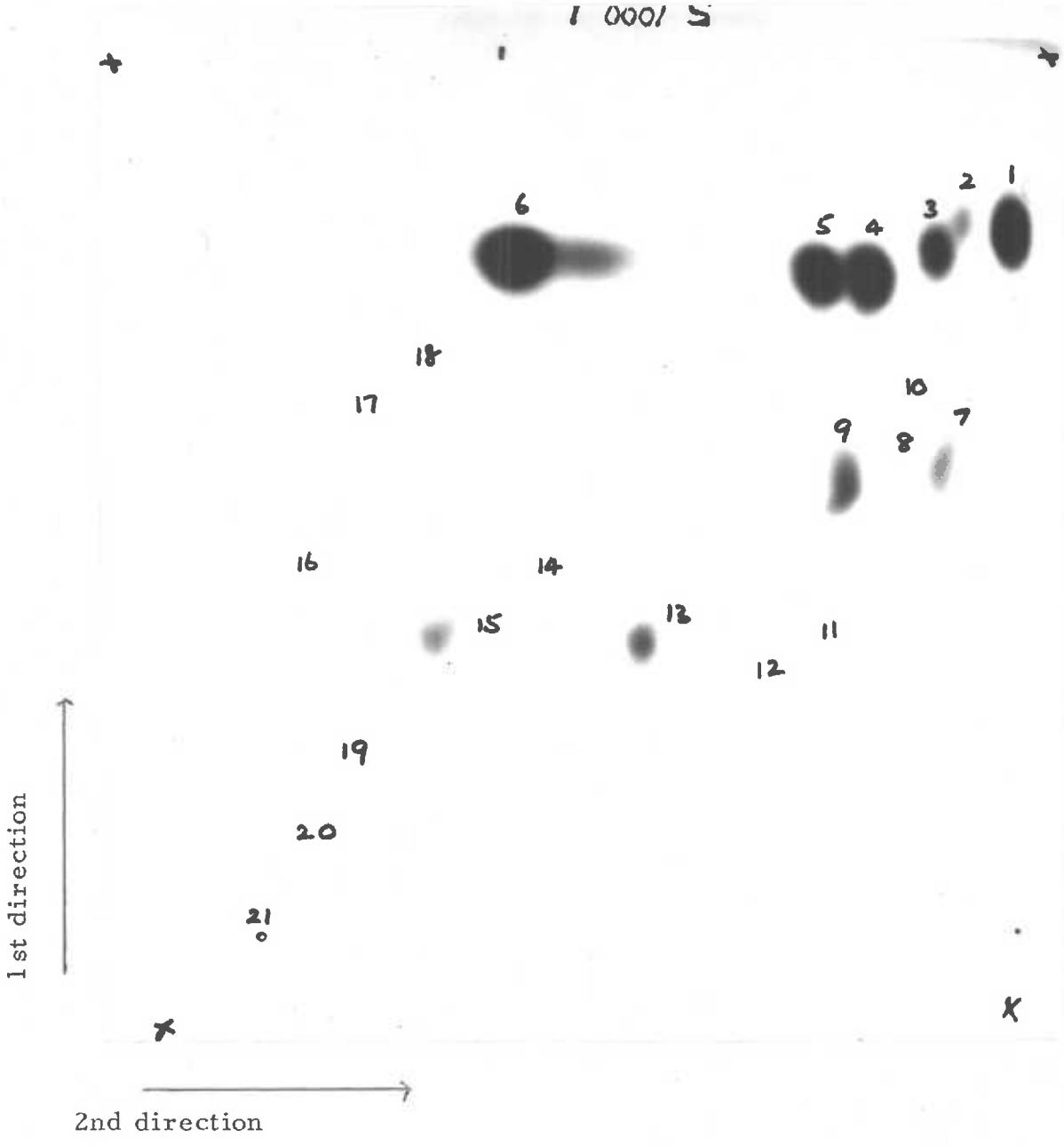
Fig. 5.



An autoradiograph illustrating the separation of ^{14}C-labelled compounds by two-dimensional thin layer chromatography (Bieleski, 1965).

Distribution of radioactivity in various compounds after photosynthesis in 150 vpm $^{14}CO_2$ for 15 min. followed by 1000 vpm $^{12}CO_2$ for a further 5 min.

Fig. 6.



An autoradiograph illustrating the separation of various ^{14}C-labelled compounds by two-dimensional thin layer chromatrography (Bieleski, 1965).

Leaves supplied with sodium hydroxybutynoate (an inhibitor of glycollate oxidase) were extracted and analysed after photosynthesis in 150 vpm $^{14}CO_2$ for 15 min. followed by 1000 vpm $^{12}CO_2$ for a further min.

reagent; and sucrose by its reaction with aniline diphenylamine or p-anisidine diphenylamine reagents. The position of phosphoglycollate was established by the yellow area produced when sprayed with an acid solution of ammonium molybdate reagent, the phosphate also showed up as a blue area when the chromatogram was viewed in ultra-violet light. The other phosphate esters were also detected with ammonium molybdate in a similar manner. The location of various compounds on the chromatograms are shown in Fig. 4.

11. Autoradiography of thin-layer chromatograms

Marks were made at the four corners of the cellulose layer with radioactive ink. Letters or numbers referring to the leaf extract used were also marked on one side of the cellulose layer. This facilitated the subsequent identification and superimposition of the autoradiograms. Kodak x-ray film (Crystallex CR 54 or Industrex C54 or D54) were cut when necessary to the required size and were placed directly on the cellulose layer. Several plates with films against the cellulose layers were stacked interspersed with sheets of paper one above the other and fastened together with rubber bands. A blank plate was placed on the uppermost plate to avoid any damage to the topmost film and cellulose layer during the fastening of the rubber bands. The period of exposure of the plates to the x-ray films depended on the amount of ^{14}C applied to the chromatograms; the time was usually less than two weeks. The films were then removed and were developed (using Kodak processing unit P3) and dried (using Kodak drying cabinet, model B).

12. Measurement of radioactivity

All measurements of radioactivity were made by liquid scintillation counting using a Packard Tri-carb liquid scintillation spectrometer (Model 3320). The scintillator contained 0.4% PPO (2,5 - diphenyloxazole) and 0.01% POPOP (1,4 bis - (2,5 phenyl) oxazolyl benzene) both obtained

from Packard Ltd., in AR toluene.

a) Aqueous solutions

For estimation of radioactivity in aqueous solutions the toluene scintillator described above was mixed with the surfactant Triton x 100 (2 : 1 by volume). 10 ml of this mixture was added to a vial containing 0.1 ml of the sample and 1 ml of water. Vials were then capped and shaken thoroughly before placing in the counter. The efficiency of counting was determined by the use of ^{14}C-toluene of known specific activity. The efficiency was usually between 83 and 88% with a gain setting of 14 and a window setting of 50 to 1000. Blank vials were always counted in order to determine the background count (usually 20 to 30 counts/minute). To estimate respired $^{14}CO_2$ in aqueous solutions containing NaOH it was necessary to leave the mixture with triton and scintillator for several hours, usually overnight, before counting began so as to obtain a correct count; the alkali caused a high background count initially probably due to chemiluminescence.

b) Thin-layer chromatograms

A solution (0.1% w/v) of pyroxylin (Cellulose nitrate, BDH chemicals) in equal volumes of absolute ethanol and diethyl ether was spread uniformly over the surface of the chromatograms using a glass rod. Air was blown over the surface and when it was sufficiently dry (about 2 minutes) the autoradiograph was superimposed with the markings made by radioactive ink on the chromatogram exactly coincident with the corresponding images on the autoradiograph. The position of the radioactive compounds on the cellulose layer were marked by running a pencil firmly round the images on the autoradiograph so that an impression was made on the thin layer of cellulose on the plate. The autoradiograph was then gently removed from

the pyroxylin layer and the plate dried again in air for a short while. The marked areas of the cellulose layer bound with pyroxylin were cut out with a sharp scalpel and each was transferred to a scintillation vial containing 10 ml of the solution of PPO and dimethyl POPOP in toluene. The method described above for the removal of radioactive spots from the cellulose layer is essentially similar to that of Caballero and Cossins (1970). The radioactivity in the vials were counted at a gain setting of 5.5; this gave the maximum efficiency. The efficiency of counting the radioactivity in these cellulose layers treated with pyroxylin was estimated by applying a sample of a solution containing a known amount of radioactivity on to a TLC plate. The plate was dried and after treating with pyroxylin, was cut and counted as above; the efficiency was usually 65 to 70%.

In later experiments the cellulose pieces were subsequently removed from the vials and the scintillator was reused to count more cellulose pieces. The vials and scintillator, however, were checked by measurements in the scintillation spectrometer to ascertain that they were free of ^{14}C. Approximately 98 to 99% of the vials had the normal background count. This procedure was repeated three or four times before fresh scintillator was needed; the practice proved economical and time saving.

13. Reproducibility of measurements of ^{14}C in photosynthetic intermediates

Unlike cultures of unicellular green plants from which samples with many similar individuals are easily obtained, leaves of higher plants have to be used in small numbers per sample. Consequently a certain amount of variation between samples has to be tolerated. The values shown in Table 1 have been obtained from experiment 8 (see Experimental and Results). The standard error as a percentage of sample means (for

Table 1. Standard errors of sample means of various compounds formed during photosynthesis in 325 vpm $^{14}CO_2$ or $^{14}CO_2$ – followed by 325 vpm $^{12}CO_2$ in the dark

	15 min. $^{14}CO_2$, 0 min. $^{12}CO_2$		15 min. $^{14}CO_2$, 1 min. $^{12}CO_2$	
	*Sample means (dpm x 10^{-4})	S.E. as % of sam. mean	*Sample means (dpm x 10^{-4})	S.E. as % of sam. mean
Glycollate	13.5 ± 0.5	3.5	10.3 ± 0.6	6.0
Alanine	15.8 ± 0.7	4.5	16.8 ± 1.1	6.6
Glycerate	29.7 ± 2.2	7.3	28.9 ± 0.9	3.2
Glycine	490.2 ± 14.4	2.9	355.8 ± 8.4	2.5
Serine	116.5 ± 4.2	3.6	222.4 ± 6.7	2.9
Sucrose	1459.5 ± 15.0	1.0	1450.8 ± 19.5	1.3
Malate	87.0 ± 5.8	6.6	88.9 ± 8.9	10.1
Glutamate	33.3 ± 1.4	4.3	30.8 ± 1.9	6.4
Aspartate	29.1 ± 1.1	3.8	31.8 ± 1.7	5.5
PEP	5.5 ± 0.4	6.5	16.0 ± 0.6	3.4
PGA	49.2 ± 2.2	4.5	62.8 ± 2.9	4.8
Sug. mono. ph.	53.8 ± 2.4	4.4	35.4 ± 1.2	3.4
UDPG	9.8 ± 0.3	3.1	10.2 ± 0.4	3.7
Unknowns	22.1 ± 0.5	2.1	21.2 ± 0.8	8.6
Origin	4.0 ± 0.2	5.5	4.9 ± 0.4	8.6
$^{14}CO_2$	-	-	35.9 ± 1.6	4.4

*Means for 10 samples (50 leaf segments) ± standard error (S.E.)

10 samples, each consisting of 5 leaf segments) for ^{14}C in each compound vary between 1.0 and 10.1.

14. Quantitative estimation of amino acids

The method of estimation was based on that of Atfield and Morris (1961). The ethanol extracts (240 mg fresh weight of leaf) were evaporated to dryness over a steam bath and the residue redissolved in 1 ml. of water and 3 ml of chloroform. The water and the chloroform extracts were well mixed and centrifuged at 3000 x g for 10 minutes. Chloroform layer contained all the plastid pigments which were removed by a pasteur pipette. The aqueous layer was further extracted twice with 1 ml portions of chloroform. The combined chloroform fractions were also extracted twice with 1 ml portions of water and all the water extracts were finally combined and evaporated to dryness. The residue was redissolved in 1 ml of water and passed through a 1 ml. (4 cm long) column of (Amberlite 1 G 120) cation exchange resin. The effluent contained acidic and neutral fractions. The column was then washed with 3 ml of distilled water and the amino acids were finally eluted with 3 ml of 2 N ammonium hydroxide solution. The eluate was dried down *in vacuo* over $CaCl_2$ and concentrated H_2SO_4 in a desiccator. The residue was redissolved in 0.1 ml of water and 0.035 ml was applied to form a streak 0.25 inches long on Whatman No. 1 chromatographic paper (46 x 52 cm). Standard solutions of glycine (0.24 μmoles) and serine (0.28 μmoles) were also applied separately on the same paper. The amino acids were separated by one-dimensional paper chromatography using n-butanol, acetone, diethylamine and water (10 : 10 : 1 : 5 v/v) for 15 to 18 hours. Developed chromatograms were dried in air in a fume cupboard for about 4 hours and transferred again to a chromatographic tank for further drying (overnight) over fresh concentrated H_2SO_4.

Cadmium acetate (0.15 g) dissolved in 7.5 ml of water was added to 1.5 ml of glacial acetic acid. To this solution was added 75 ml of acetone and 0.75 g of ninhydrin. The mixture was shaken until the nihydrin had dissolved. The chromatograms were dipped in the solution, dried in air, then hung overnight in a chromatographic tank over fresh concentrated H_2SO_4 and NaOH pellets. This increased the intensity of the colour of the amino acid spots. Glycine and serine in the extracts were identified by the position on the chromatograms of glycine and serine from the standard solutions. These coloured areas of paper were cut out and immersed in methanol (4 ml for glycine and 8 ml for serine) contained in test tubes. The tubes were covered with aluminium foil to prevent evaporation of methanol; the contents of the tubes were occasionally mixed using a Whirlimixer. After about 30 minutes the methanol extracts were transferred to a cuvette and the extinction at 500 nm was measured using a spectrophotometer (Unicam, SP 500). Suitable blanks were prepared with each set of samples analysed and the extinction corrected for any background colour produced.

Except for D-glyceric acid-U-^{14}C which was obtained from Calbiochem Ltd. all other radiochemicals were purchased from the Radiochemical Centre, Amersham, Bucks. Sodium 2-hydroxy 3-butynoate and methyl 2-hydroxy 3-butynoate were kindly provided by Dr. M.W. Kerr (Shell research laboratory, Sittingbourne, Kent, U.K.) and Dr. I. Zelitch (Connecticut agricultural experiment station, New Haven, Connecticut, U.S.A.) provided the sample of potassium glycidate.

EXPERIMENTAL AND RESULTS

Except where otherwise stated the data presented in this thesis were obtained using five leaf segments, each 4 cm in length. The radioactivity in leaf extracts and the ^{14}C in individual compounds including $^{14}CO_2$ that was evolved are usually expressed as dpm. Where $^{14}CO_2$ feeding was followed by various periods in $^{12}CO_2$ or CO_2-free conditions it was assumed that the same amount of ^{14}C should be present initially in all the leaf samples of the series so radioactivity in leaf extract plus $^{14}CO_2$ evolved was corrected to an average of total radioactivity in all the samples involved. For convenience in comparison the results of some experiments are given as a percentage of the total ^{14}C in leaf extract. The term phosphate esters refers to the total of PGA, PEP, UDPG and other sugar phosphates.

1. Incorporation of radioactivity into various compounds during steady-state photosynthesis in 80 vpm $^{14}CO_2$ with 21% O_2.

Illuminated (2900 - 3000 f.c.) wheat leaf segments were preincubated in a gas stream containing 80 vpm $^{12}CO_2$ in air for 40 min. The gas stream was then changed to 80 vpm $^{14}CO_2$ in air and samples were taken after 1, 3, 5, 10, 15, 20, 30, 45 or 60 min.

The results presented in Fig. 1a show that the rate of photosynthesis was constant during 60 minutes implying that a 40 minute preincubation period was sufficient to attain steady-state photosynthesis. Analysis of radioactivity in various products of photosynthesis in $^{14}CO_2$ revealed that after 1 minute (Table 1) 37% of the radioactivity was found in phosphate esters whereas only 19%, 18% and 12% were found in glycine, serine and sucrose respectively. After 15 minutes photosynthesis in

$^{14}CO_2$ (Table 1) only 15% of the radioactivity was found in phosphate esters 24%, 30% and 27% in glycine, serine and sucrose respectively. These results suggest that glycine, serine and sucrose are derived from phosphate esters which are intermediates of the Calvin cycle.

Fig. 1b shows also that the incorporation of radioactivity into glycine and serine was rapid for 15 minutes and then proceeded more slowly. Since steady-state conditions prevailed and the $^{14}CO_2$ had a constant specific activity, it is concluded that certain metabolically active pools of glycine and serine became saturated with ^{14}C after 15 minutes (Smith et al., 1961). The existence of further less active pools is indicated by the slower increase of ^{14}C in glycine and serine during the further period from 15 to 60 minutes; an alternative explanation for this slow increase could be an increase in pool size caused by the senescence of leaf segments.

The metabolically active pool or pools of PGA (Fig. 1b) appeared to be saturated with ^{14}C, within about 1 - 5 minutes of photosynthesis in $^{14}CO_2$, but a slower incorporation of ^{14}C followed implying the existence of other less active secondary pools of this compound also. In similar experiments with sunflower leaf discs Atkins et al. (1971) showed by a direct specific activity determination that the entire PGA pool was saturated with ^{14}C after 10 minutes photosynthesis in $^{14}CO_2$ but subsequent work also with sunflower leaf discs (Mahon et al. 1974) showed that the relative specific activity of PGA did not reach 100% even after 15 minutes photosynthesis in $^{14}CO_2$. Mahon et al. explained these results as due to dilution of the PGA pool by carbon derived from intermediates of the glycollate pathway not saturated with ^{14}C. The times required for saturation of the more active pools of other compounds were not clear due either to the existence of multiple pools or to synthesis from carbon sources that were being only slowly labelled.

Fig. 1a. Net $^{14}CO_2$ assimilation by illuminated wheat leaf segments in air containing 80 vpm $^{14}CO_2$. (1.5 μCi/μmole)

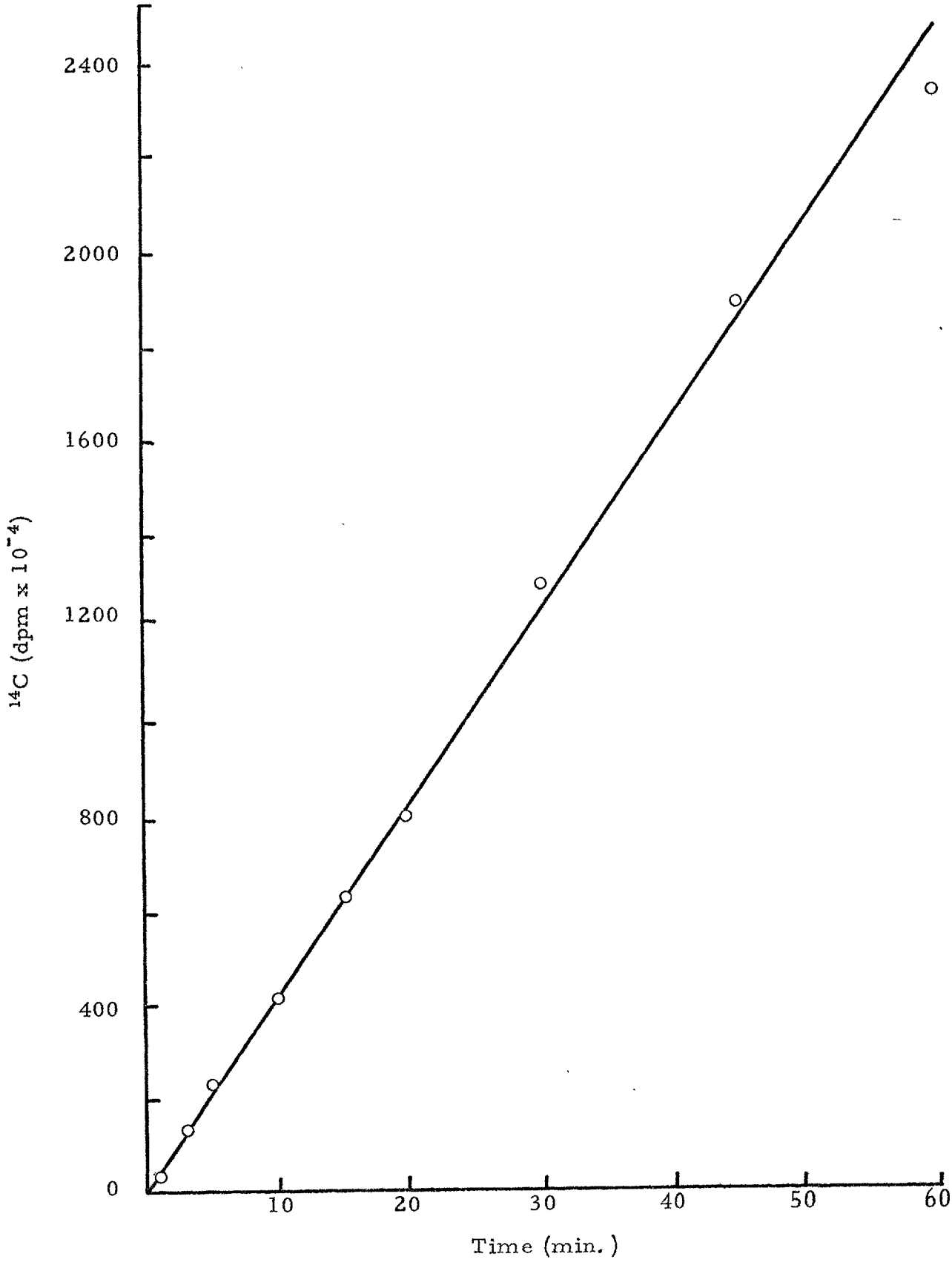


Fig. 1b. The incorporation of ^{14}C into PGA, glycine, serine and sucrose during steady-state photosynthesis in air containing 80 vpm $^{14}CO_2$

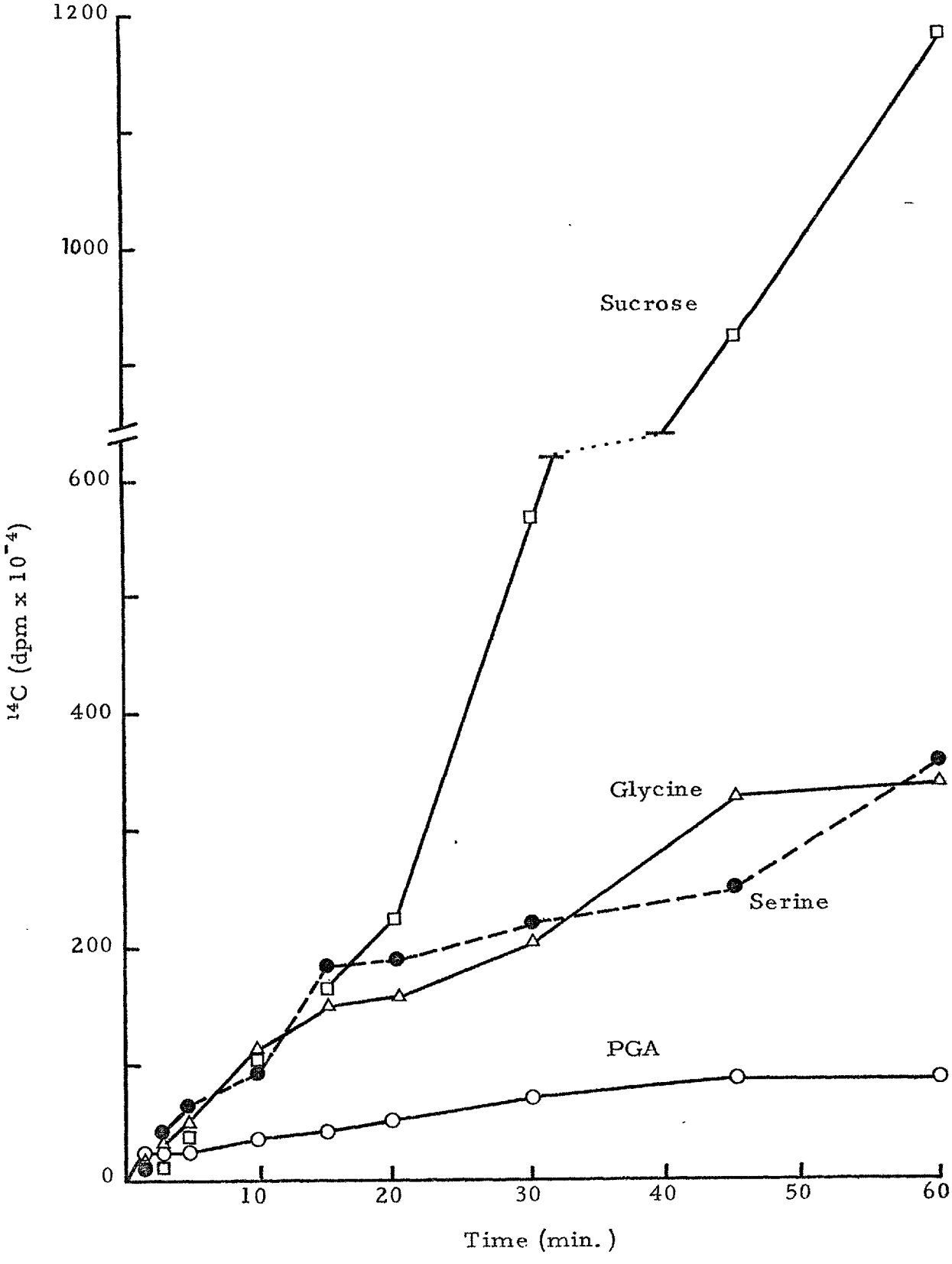


Table 1

Radioactivity in phosphate esters, glycine, serine and sucrose as a percentage of total ^{14}C assimilated during steady-state photosynthesis in 80 vpm $^{14}CO_2$

Compound	Time of exposure to $^{14}CO_2$ (min.)								
	1	3	5	10	15	20	30	45	60
Phosphate esters	36.66	30.47	19.86	18.82	15.44	15.32	11.47	10.48	9.31
Glycine	19.01	19.18	21.26	26.91	23.99	20.72	16.01	17.54	14.80
Serine	18.27	22.19	28.68	24.29	30.02	24.23	17.01	13.28	15.85
Sucrose	11.85	14.67	19.51	25.67	26.95	28.38	43.89	48.74	51.73

2. Changes of radioactivity in various compounds during photosynthesis in 80 or 150 vpm $^{14}CO_2$ and subsequently in 80 or 150 vpm $^{12}CO_2$.

Wheat leaf segments were preincubated in 80 or 150 vpm $^{12}CO_2$ air for 40 minutes at a light intensity of 2900 - 3000 f.c. The gas stream was then changed to 80 or 150 vpm $^{14}CO_2$ in air and after 1, 5 or 15 minutes the atmosphere was again changed to one of similar composition but $^{12}CO_2$ instead of $^{14}CO_2$. Samples were taken after 1, 3, 5, 10 and 20 minutes, and the $^{14}CO_2$ evolved was also estimated.

The results are illustrated in Figs. 2a to 7b and Table 2 and 3. During steady-state photosynthesis in $^{14}CO_2$ the incorporation of radioactivity into sucrose (Figs. 4a and 7a) showed a definite lag compared to glycine and serine. This suggests that carbon for sucrose synthesis is probably derived from glycine and serine.

Following photosynthesis in 80 or 150 vpm $^{14}CO_2$ for 5 or 15 minutes when the atmosphere was changed to $^{12}CO_2$ (Figs. 3a, 4a, 6a and 7a) the initial rates of decrease of ^{14}C from glycine were similar (Table 2). Further these rates of decrease of ^{14}C in glycine were not very different from the rate of incorporation of ^{14}C into glycine during the preceding period of photosynthesis in $^{14}CO_2$. It thus seems likely that a metabolically active pool of glycine was saturated in much less than 15 minutes photosynthesis in $^{14}CO_2$, because if this were not so, and a main active pool was uniformly mixed and saturated after 15 minutes the rate of decrease of ^{14}C from glycine during the initial period in $^{12}CO_2$ would be expected to be slower (approximately $\frac{1}{3}$ the rate) after 5 minutes than after 15 minutes exposure to $^{14}CO_2$. Following 1 minute photosynthesis in $^{14}CO_2$ there was a net increase of ^{14}C glycine during the first minute in 80 vpm $^{12}CO_2$ followed by a rapid decrease (Fig. 2a);

Fig. 2 (a & b). Changes of radioactivity in various compounds during and following steady-state photosynthesis in 80 vpm $^{14}CO_2$ for 1 min.

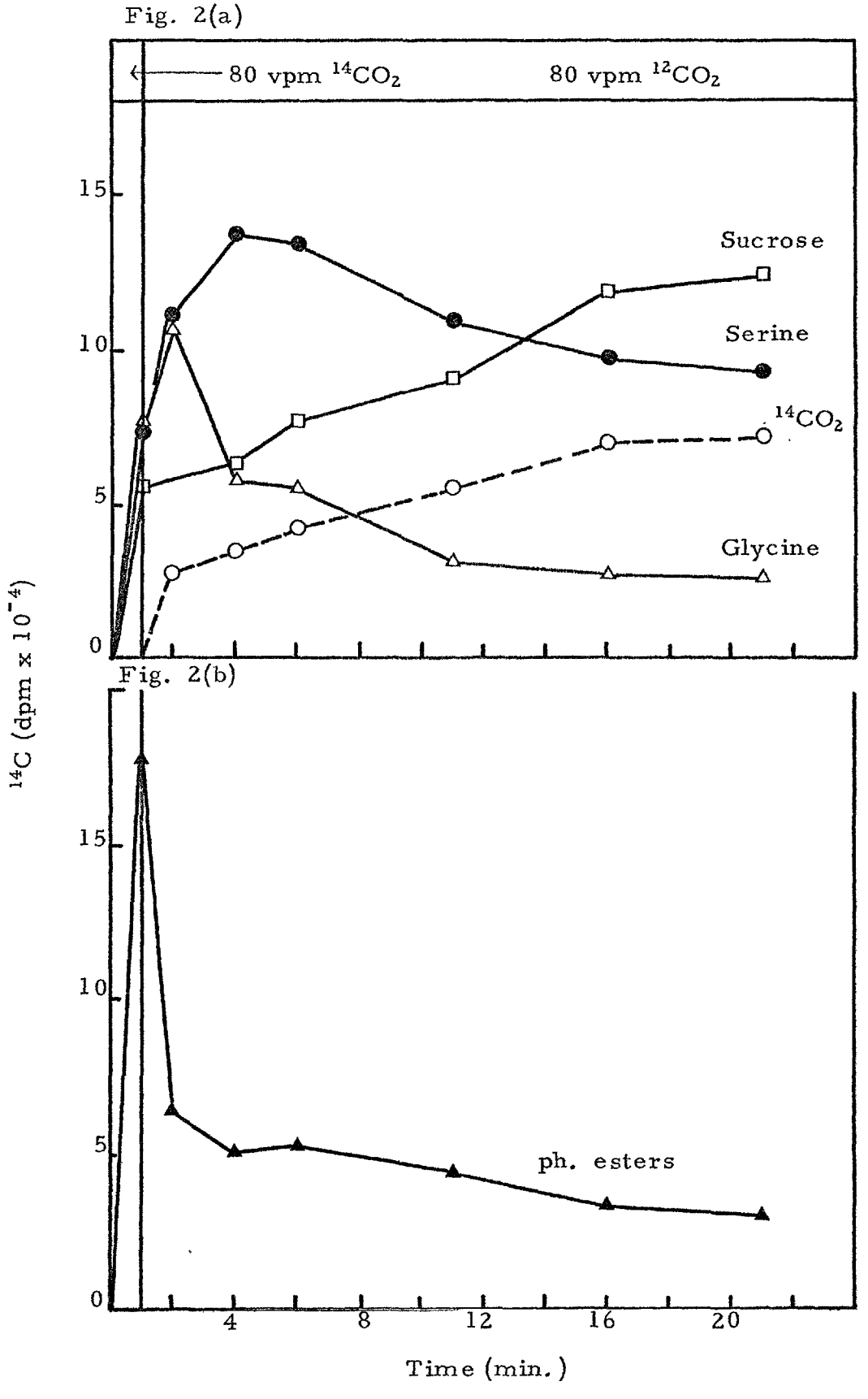


Fig. 3 (a & b). Changes of radioactivity in various compounds during and following 5 min. steady-state photosynthesis in 80 vpm $^{14}CO_2$.

Fig. 3(a)

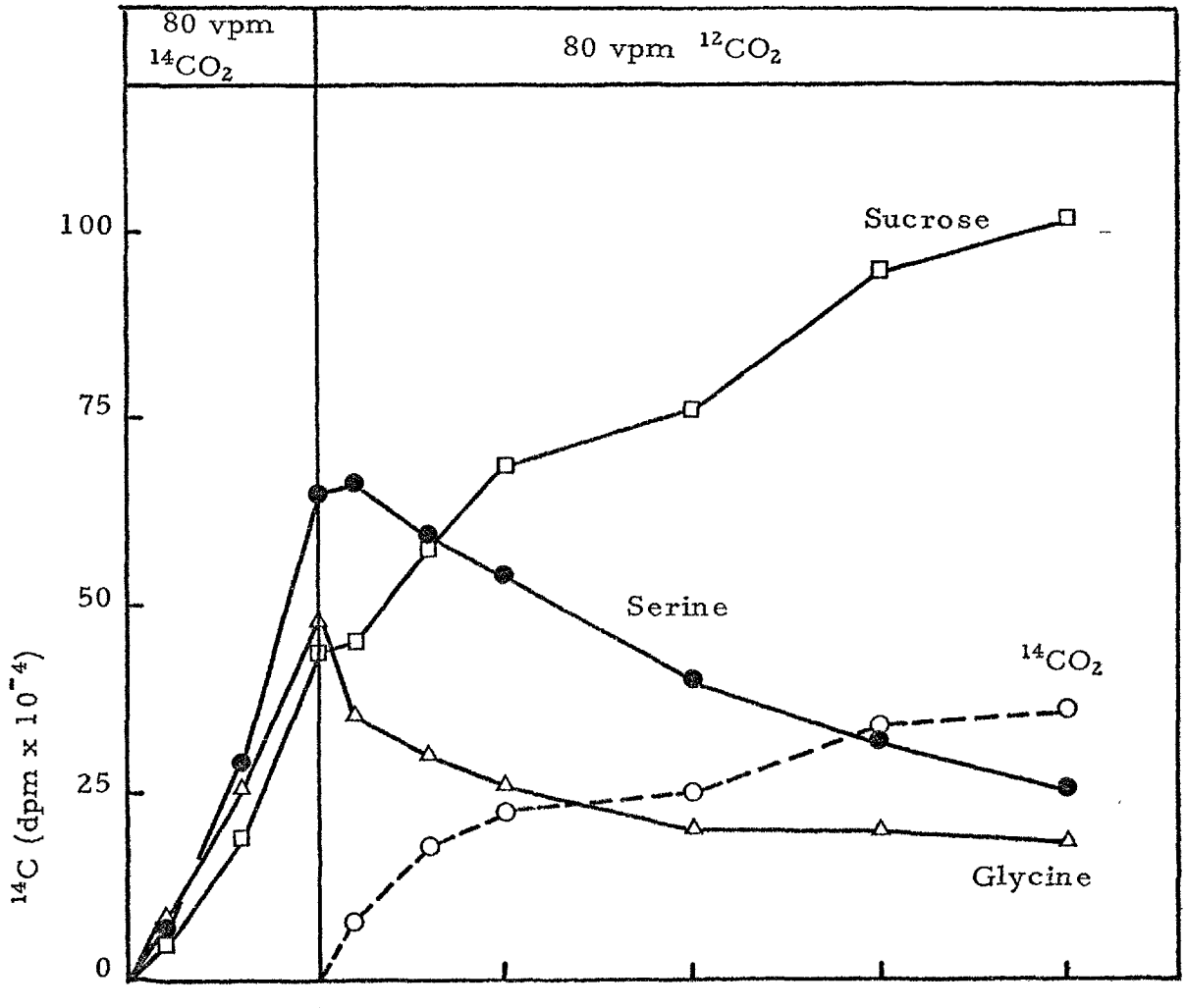


Fig. 3(b)

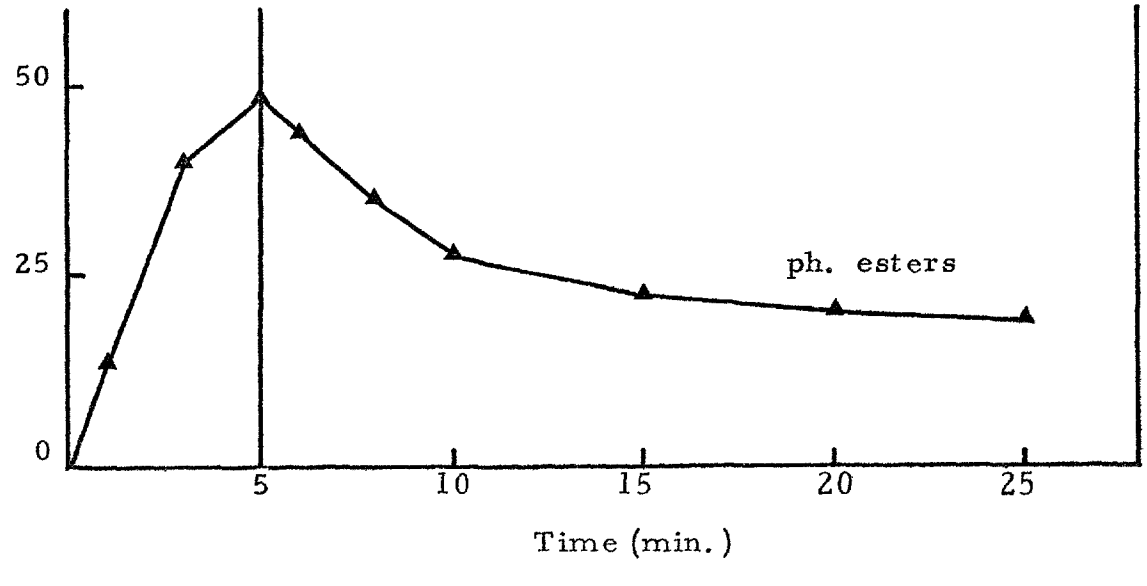


Fig. 4 (a & b). Changes in radioactivity of various compounds during and following steady-state photosynthesis in 80 vpm $^{14}CO_2$ for 15 min.

Fig. 4(a)

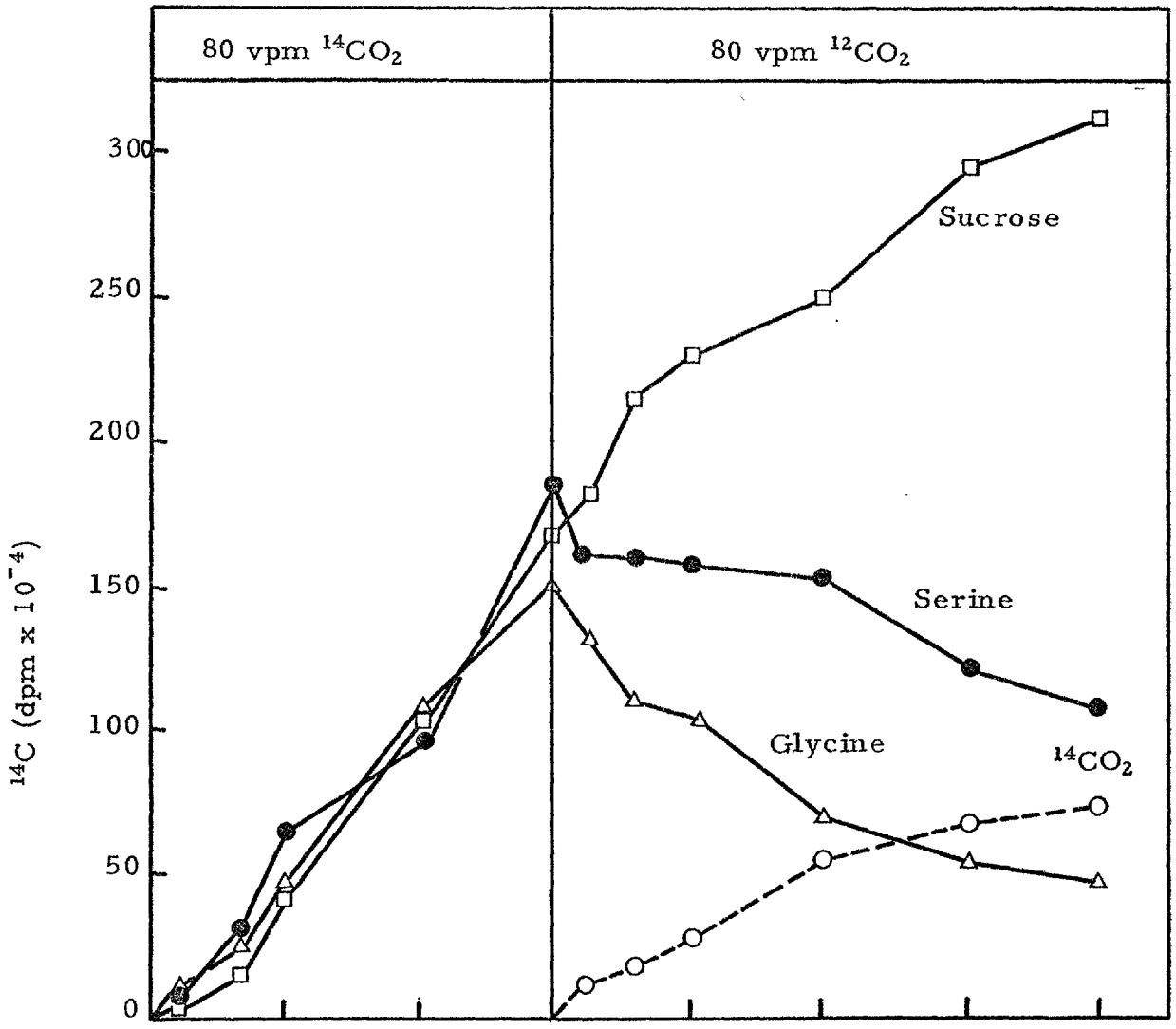


Fig. 4(b)

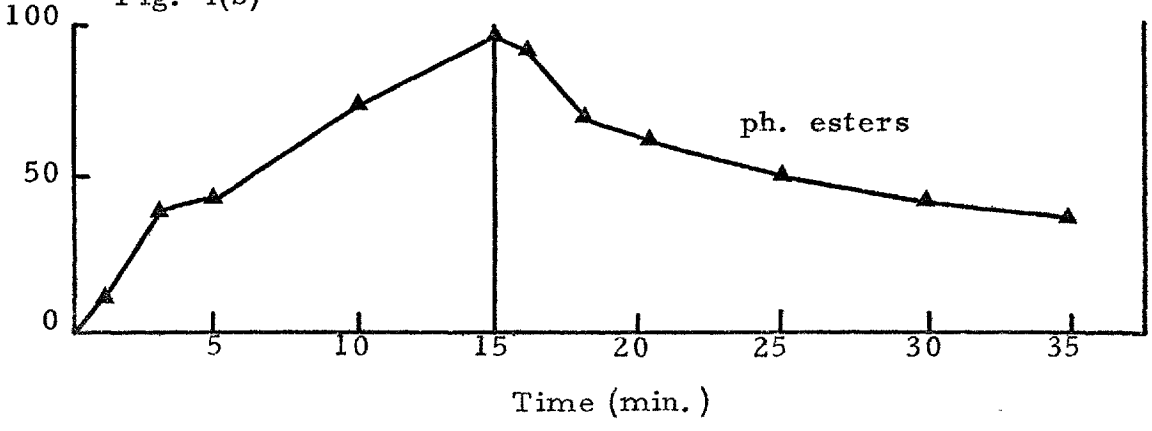


Table 2 Rates of change of ^{14}C in glycine and serine during photosynthesis in $^{14}CO_2$ or $^{14}CO_2$ followed by $^{12}CO_2$

CO_2 Conc. (vpm)	Rate of Ps.	Max. rate of incorporation of C. Glycine ($\mu gC.dm.^{-2} min.^{-1}$)	Max. rate of incorporation of C. Serine ($\mu gC.dm.^{-2} min.^{-1}$)	Time of exposure to $^{14}CO_2$ (min.)	Rate of change of C during 1st min. in $^{12}CO_2$ Glycine ($\mu gC.dm.^{-2} min.^{-1}$)	Rate of change of C during 1st min. in $^{12}CO_2$ Serine ($\mu gC.dm.^{-2} min.^{-1}$)
80	15.7	4.4	6.2			
				1	+ 1.3	+ 1.2
				5	- 4.7	- 0.6
				15	- 5.1	- 9.8
150	31.6	11.6	5.0			
				1	- 2.1	+ 1.4
				5	- 10.0	+ 4.1
				15	- 10.6	- 8.7

in 150 vpm $^{12}CO_2$ there was no increase of ^{14}C in glycine following the change to $^{12}CO_2$ (Fig. 5a). The most active pool of glycine was probably not saturated during this relatively short period of exposure to $^{14}CO_2$. However, if serine is made from glycine, an active pool of glycine must have become saturated with ^{14}C in much less than 15 minutes because there was no discernible lag in ^{14}C incorporation into serine.

It is concluded that there is a pool of glycine very rapidly saturated with ^{14}C and that this pool is sited in the cell close to the site of serine synthesis. The data would seem therefore to show at least three categories of metabolic pools of glycine; those saturated with ^{14}C in much less than 15 minutes, those saturated with ^{14}C in some 15 minutes (see Fig. 1 b) and those which turn over very slowly.

During the 20 minutesof flushing in 80 vpm $^{12}CO_2$ air following photosynthesis in 80 vpm $^{14}CO_2$ for 1, 5 or 15 minutes the radioactivity from glycine, serine and phosphate esters decreased whilst that of sucrose increased. $^{14}CO_2$ was also evolved during this period. Since glycine and serine are both closely related intermediates of the glycollate pathway (Wang and Waygood, 1962; Robson et al., 1962) a similar rate of decrease of ^{14}C would be expected from both during flushing in $^{12}CO_2$. However the results show that the rate of decrease of ^{14}C from serine compared to that from glycine was hindered to a considerable extent during the initial period of flushing. Waidyanatha et al. (1974) have shown that serine metabolism is suppressed in CO_2-free air due to a dearth of keto acids and at low CO_2 concentrations (80 vpm CO_2) decreased photosynthetic activity causes a decreased supply of keto acids at the site of deamination of serine resulting in its decreased metabolism. In 150 vpm $^{12}CO_2$ following photosynthesis in 150 vpm

Fig. 5 (a & b). The effect of INH on changes in radioactivity in various compounds during and following steady-state photosynthesis in 150 vpm $^{14}CO_2$ for 1 min.

Fig. 5(a)

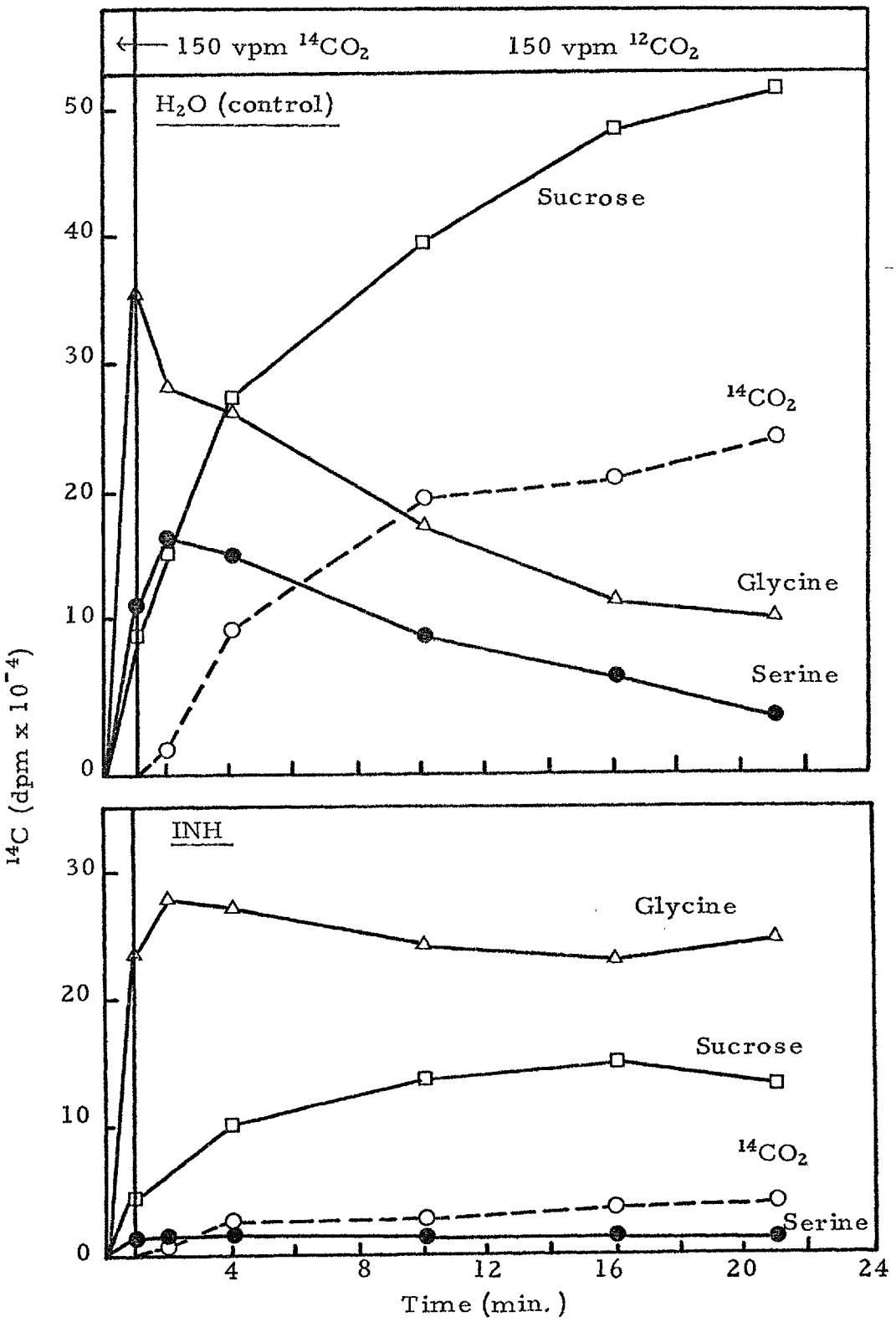


Fig. 5(b)

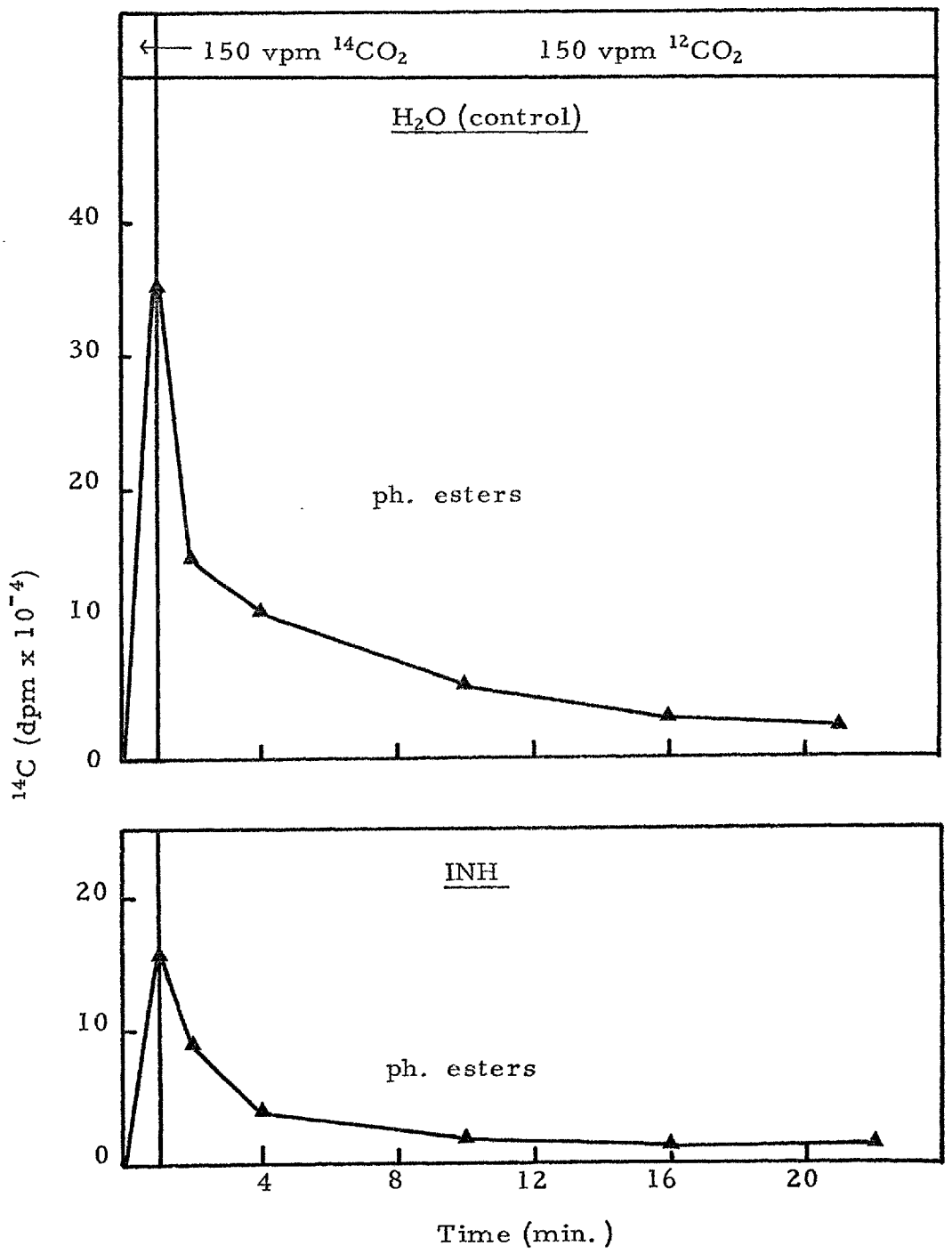

← 150 vpm $^{14}CO_2$
150 vpm $^{12}CO_2$
H_2O (control)
ph. esters
INH
ph. esters
^{14}C (dpm x 10^{-4})
0
10
20
30
40
4
8
12
16
20
Time (min.)

Fig. 6 (a & b). The effect of INH on changes in radioactivity in various compounds during and following steady-state photosynthesis in 150 vpm $^{14}CO_2$ for 5 min.

Fig. 6(a)

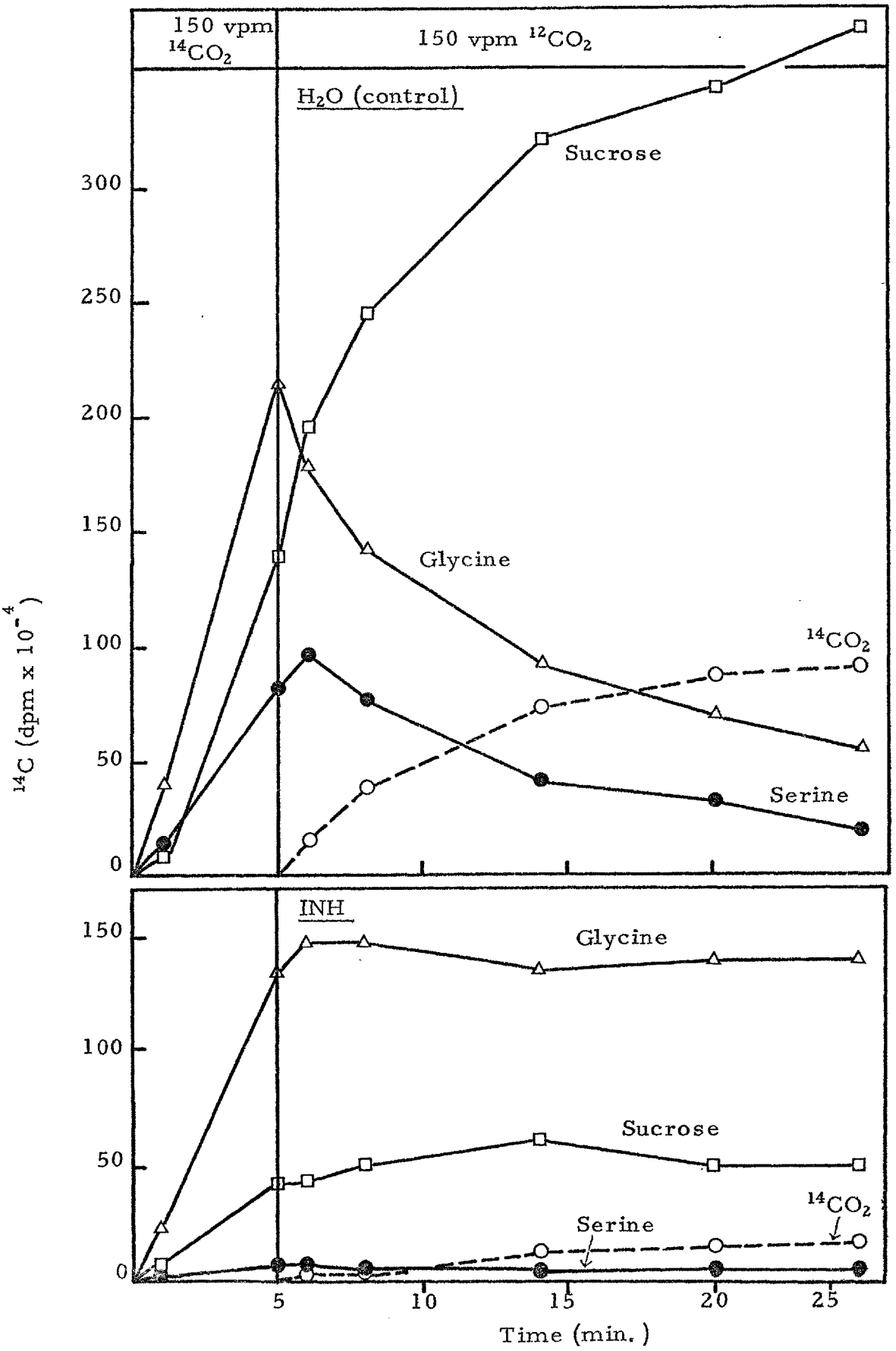


Fig. 6(b)

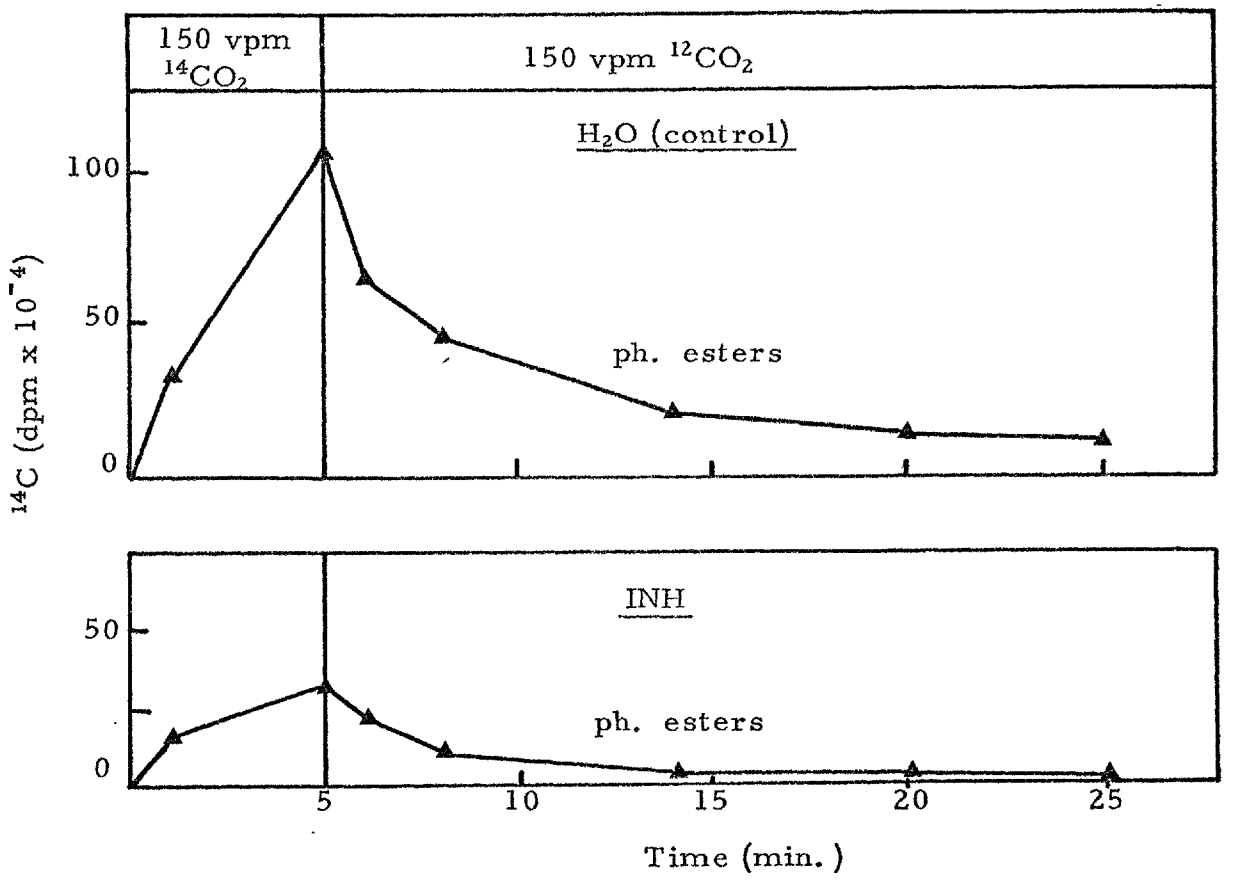

150 vpm $^{14}CO_2$
150 vpm $^{12}CO_2$
H_2O (control)
ph. esters
INH
ph. esters
100
50
0
50
0
5
10
15
20
25
Time (min.)
^{14}C (dpm x 10^{-4})

Fig. 7 (a & b). The effect of INH on changes in radioactivity of various compounds formed during steady-state photosynthesis in 150 vpm $^{14}CO_2$ for 15 min. followed by 150 vpm $^{12}CO_2$.

Fig. 7(a)

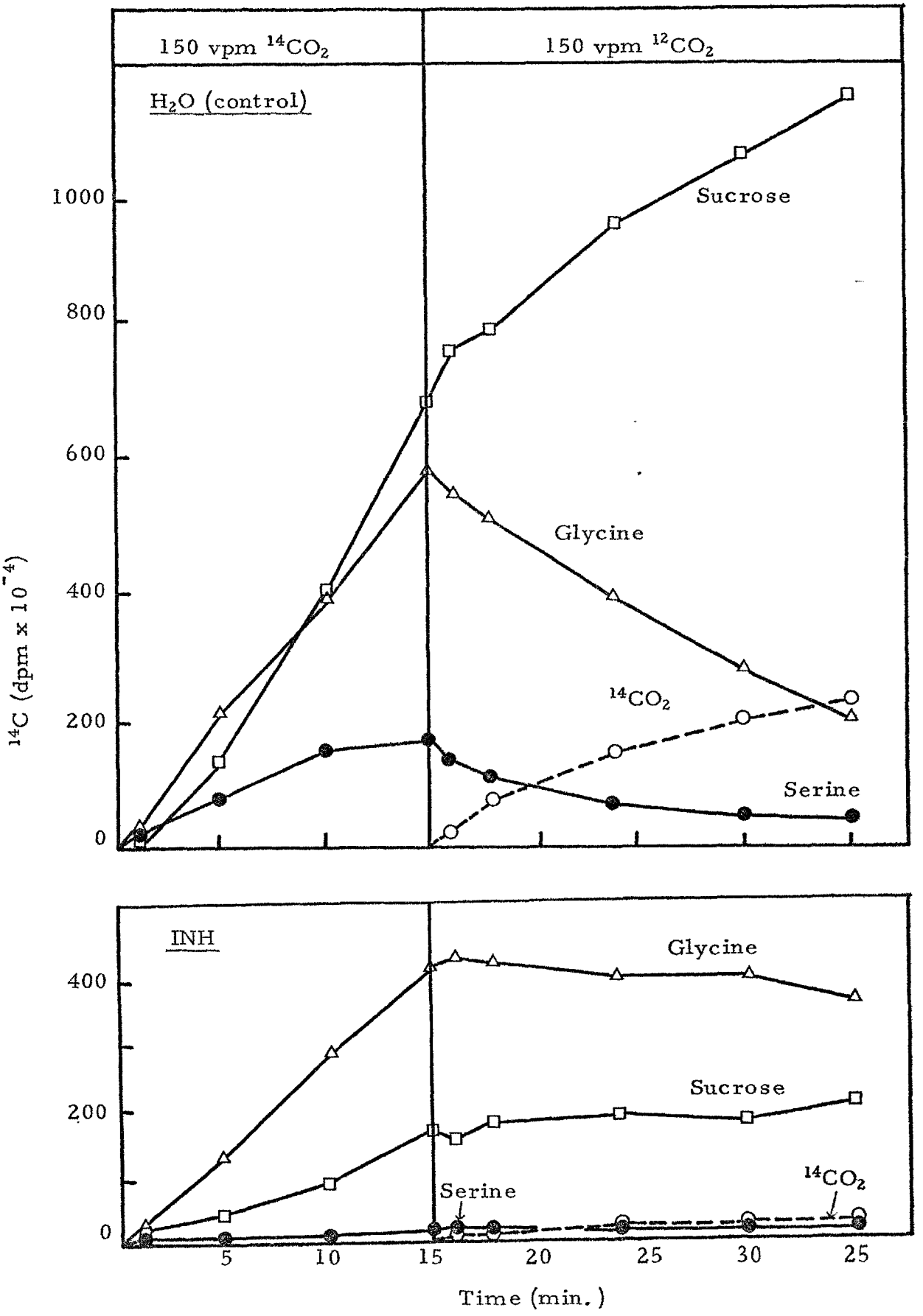


Fig. 7(b)

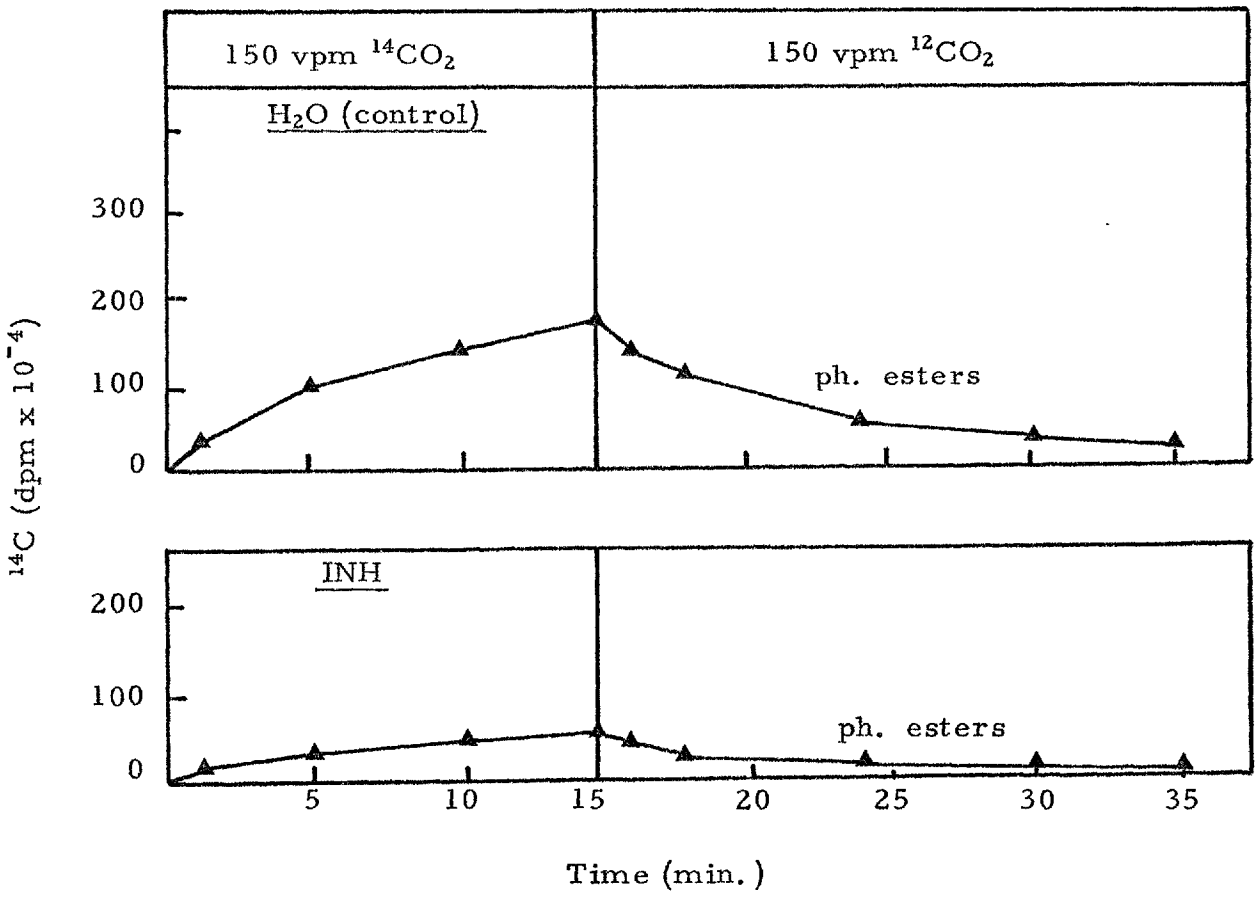

150 vpm $^{14}CO_2$
150 vpm $^{12}CO_2$
H_2O (control)
300
200
100
0
ph. esters
INH
200
100
0
ph. esters
5
10
15
20
25
30
35
^{14}C (dpm x 10^{-4})
Time (min.)

Table 3

Effect of INH on incorporation of radioactivity into glycine, serine and sucrose during steady-state photosynthesis in 150 vpm $^{14}CO_2$–

(values in parenthesis refer to % inhibition of incorporation of ^{14}C)

	Time of exposure to $^{14}CO_2$	Photosynthetic rate ($\mu gC.\ dm^2$)	Incorporation of ^{14}C into ($\mu gC.\ dm^{-2}$)		
			Glycine	Serine	Sucrose
Control	1	29.42	10.38	3.63	2.97
	5	158.35	58.41	22.17	37.68
	15	473.87	159.94	47.5	187.25
INH	1	14.01	6.46	0.39	1.17
		(52.38)	(37.82)	(87.80)	(60.60)
	5	65.20	36.86	2.07	11.69
		(58.81)	(36.94)	(90.66)	(68.97)
	15	199.09	116.63	5.42	48.78
		(57.8)	(37.13)	(88.58)	(73.95)

$^{14}CO_2$ (Figs. 5a, 6a and 7a) metabolism of ^{14}C serine was much faster. The optimum concentration of CO_2 for serine metabolism was found to be near 200 vpm (Waidyanatha, 1973).

A further experiment performed in 150 vpm CO_2 investigated the effect of isonicotinyl hydrazide (INH) on the distribution of radioactivity during steady-state photosynthesis in $^{14}CO_2$ or $^{14}CO_2$ followed by $^{12}CO_2$. Throughout the entire experimental period wheat leaf segments were allowed to take up INH (20 mg/ml) instead of water.

INH inhibited photosynthetic assimilation during 15 minutes by 58% while incorporation of radioactivity into glycine was inhibited by only 37% and that into serine and sucrose by 88 and 74% respectively (Table 3). The inhibition of incorporation of ^{14}C into serine and sucrose is consistent with the known property of INH of inhibiting the conversion of glycine to serine (Pritchard et al., 1962; Wade, 1968). It may be deduced that sucrose is made from glycine with serine as an intermediate. In leaves treated with INH the further metabolism of ^{14}C glycine during flushing in 150 vpm $^{12}CO_2$ was inhibited more than 90% compared to the control (Fig. 7a). In comparison with the controls $^{14}CO_2$ evolution was inhibited about 80% and the synthesis of new ^{14}C sucrose was inhibited to an even greater extent (97%) especially in the leaves allowed to photosynthesize in $^{14}CO_2$ for 5 or 15 minutes. Thus when the further metabolism of ^{14}C-glycine was inhibited, $^{14}CO_2$evolution and formation of new ^{14}C sucrose was also severely inhibited. In the control leaves following photosynthesis in $^{14}CO_2$ for 5 minutes (Fig. 6a) the decrease of radioactivity from glycine during the subsequent period of 10 minutes photosynthesis in $^{12}CO_2$ was 144×10^4 dpm whereas the increase of ^{14}C in CO_2evolved during the same period was 93×10^4 dpm. After 15 minutes photosynthesis in $^{14}CO_2$ (Fig. 7a) the decrease of ^{14}C-glycine was 304×10^4 dpm (in 10 minutes) while the increase of $[^{14}C]$ CO_2 during the same period was 209×10^4 dpm. The decreases of

radioactivity in serine and phosphate esters (Figs. 6b and 7b) were too small to account for ^{14}C in the CO_2 evolved and since the compounds shown in Figs. 5a and 5b or 7a and 7b represent more than 90% of the radioactivity in the extracts it is clear that the only compound whose decrease in radioactivity could account for the increase of ^{14}C in CO_2 is that of glycine.

The main conclusion from these experiments is that the metabolism of carbon through glycine is important for both sucrose synthesis and CO_2 evolution in the light.

3. Incorporation of ^{14}C during steady-state photosynthesis in air containing 150 or 325 vpm $^{14}CO_2$ and changes following transfer to different atmospheres without $^{14}CO_2$.

Wheat leaf segments were preincubated in 150 or 325 vpm $^{12}CO_2$ in air for 40 minutes at a light intensity of 2900 - 3000 f.c., 150 or 325 vpm $^{14}CO_2$ air was then supplied for 15 minutes and finally the gas stream was changed to either 150 or 325 vpm $^{12}CO_2$ in 21% O_2, CO_2-free 21% O_2, 1000 vpm $^{12}CO_2$ in 21% O_2 or CO_2-free 1% O_2. Samples were taken after 1, 2, 3, 5 and 10 minutes and the $^{14}CO_2$ evolved was also estimated. The results are illustrated in Figs. 8a to 10 and Table 4.

In CO_2-free 1% O_2 (Figs. 8a and 9a) we observe that the radioactivity is lost more rapidly from glycine than in CO_2-free 21% O_2 or 150 or 325 vpm $^{12}CO_2$ in 21% O_2 but less $^{14}CO_2$ was evolved, 40 - 50% of that to be expected from the loss of ^{14}C from glycine on the basis of reaction 5, 5a (see introduction). Either the expected amount of $^{14}CO_2$ was produced by the conversion of the glycine to serine and was mostly refixed within the tissue rather than released to the outside atmosphere or very little $^{14}CO_2$ was produced, in which case glycine can no longer

be regarded as the immediate precursor of the photorespired $^{14}CO_2$. In CO_2-free 1% O_2 (Figs. 8a and 9a), except for an initial decrease during the first minute (Fig. 8a), ^{14}C in serine accumulated to an extent not inconsistent with the precursor being the ^{14}C-glycine. Atkins et al. (1971) obtained similar results for sunflower leaf discs, and because serine accumulated when there was little CO_2 production, they concluded that the further metabolism of serine and not the conversion of glycine to serine was responsible for CO_2 production during photorespiration. However, in the present study, during flushing in CO_2-free 1% O_2, the rate of decrease of radioactivity from phosphate esters was very slow compared to that in CO_2-free air or in an atmosphere with 1000 vpm $^{12}CO_2$ in 21% O_2. Since nearly 60% of the radioactivity in phosphate esters was in PGA the maintenance of a high level of radioactivity in phosphate esters could be explained by continuous refixation of the photorespired $^{14}CO_2$. Reassimilation of respired $^{14}CO_2$ would result also in lower apparent rates of loss of ^{14}C from glycine and serine which would receive further ^{14}C from the $^{14}CO_2$ that was reassimilated. (Compare decrease of ^{14}C in glycine in air with 150 vpm $^{12}CO_2$ and air with 1000 vpm $^{12}CO_2$). The faster decrease of ^{14}C in glycine in CO_2-free 1% O_2 compared to CO_2-free 21% O_2 can also be explained on the basis of reassimilation because the higher O_2 concentrations would result in more glycollate formation from phosphate esters in the chloroplast as suggested by Andrews et al. (1973) and Lorimer et al. (1973) and therefore more $^{14}CO_2$ for recycling; in 1% O_2 phosphate esters made from the reassimilated respiratory $^{14}CO_2$ would not be so readily converted to glycollate so there would be less $^{14}CO_2$ for recycling.

That the small amount of $^{14}CO_2$ released into CO_2-free 1% O_2 can be explained by refixation, is also consistent with the fact that with increased competition in the tissue by $^{12}CO_2$ from the external atmosphere (see Fig. 10)

Fig. 8 (a & b). Incorporation of ^{14}C during steady-state photosynthesis in 150 vpm $^{14}CO_2$ with 21% O_2 and changes following transfer to different atmospheres without $^{14}CO_2$.

Fig. 9 (a & b). Incorporation of ^{14}C during steady-state photosynthesis in air containing 325 vpm $^{14}CO_2$ and changes following transfer to different atmospheres without $^{14}CO_2$.

Fig. 8(a)

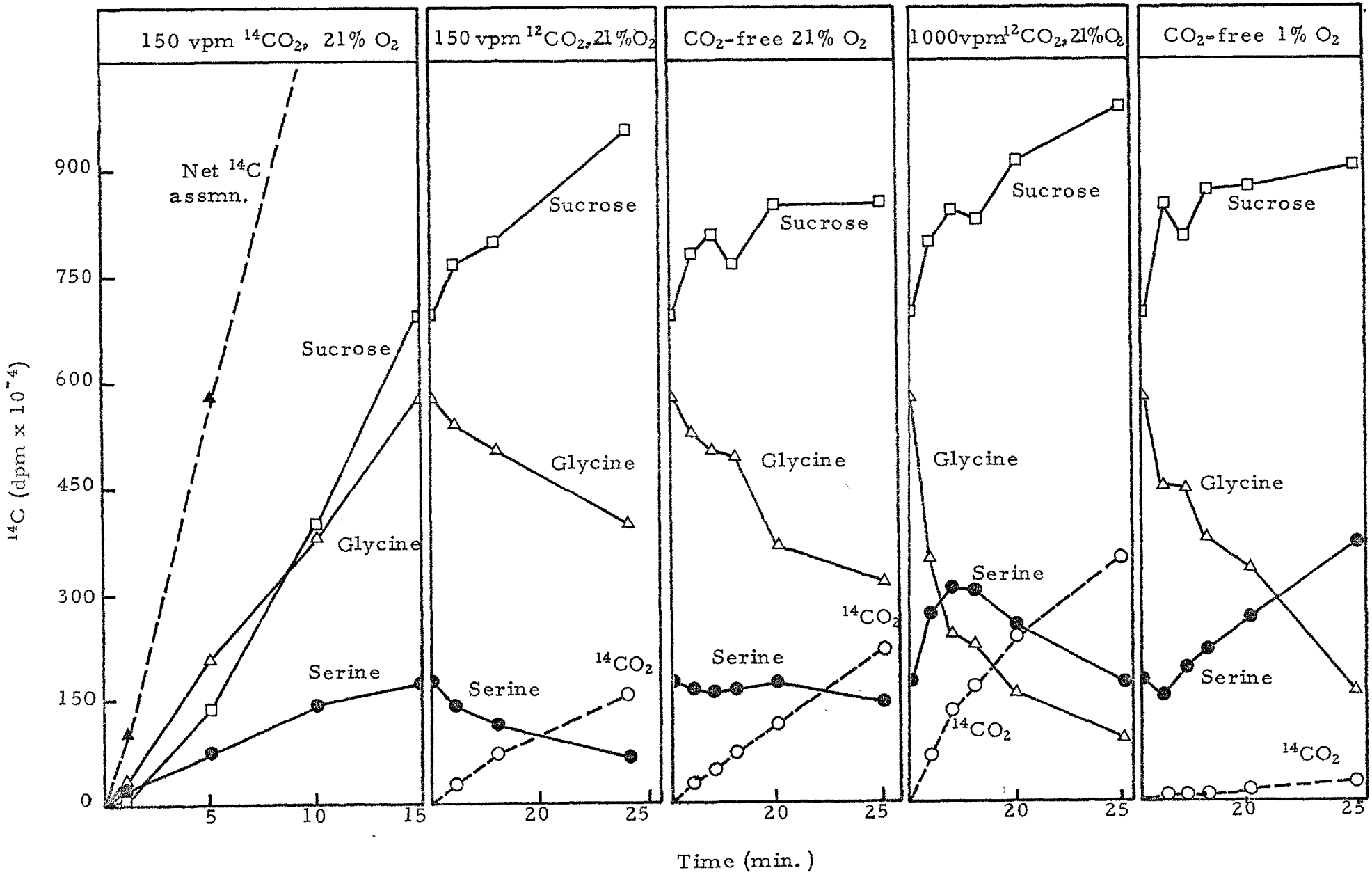

150 vpm $^{14}CO_2$, 21% O_2
150 vpm $^{12}CO_2$, 21% O_2
CO_2-free 21% O_2
1000vpm $^{12}CO_2$, 21% O_2
CO_2-free 1% O_2
Net ^{14}C assmn.
Sucrose
Glycine
Serine
$^{14}CO_2$
^{14}C (dpm x 10^{-4})
0
150
300
450
600
750
900
5
10
15
20
25
Time (min.)

Fig. 8(b)

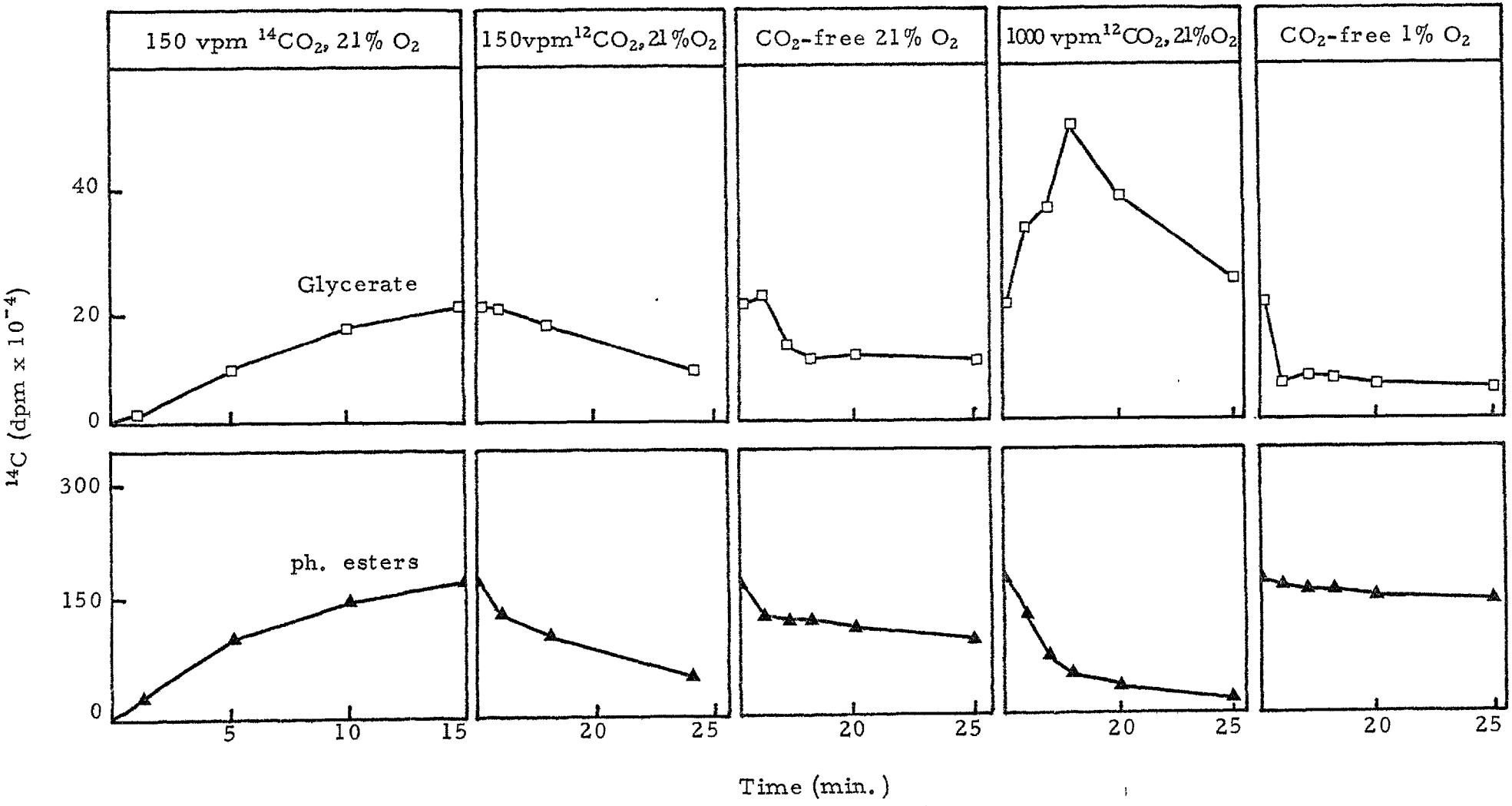

150 vpm $^{14}CO_2$, 21% O_2
150vpm $^{12}CO_2$, 21% O_2
CO_2-free 21% O_2
1000 vpm $^{12}CO_2$, 21% O_2
CO_2-free 1% O_2
Glycerate
ph. esters
^{14}C (dpm x 10^{-4})
40
20
0
300
150
0
5
10
15
20
25
Time (min.)

Fig. 9(a)

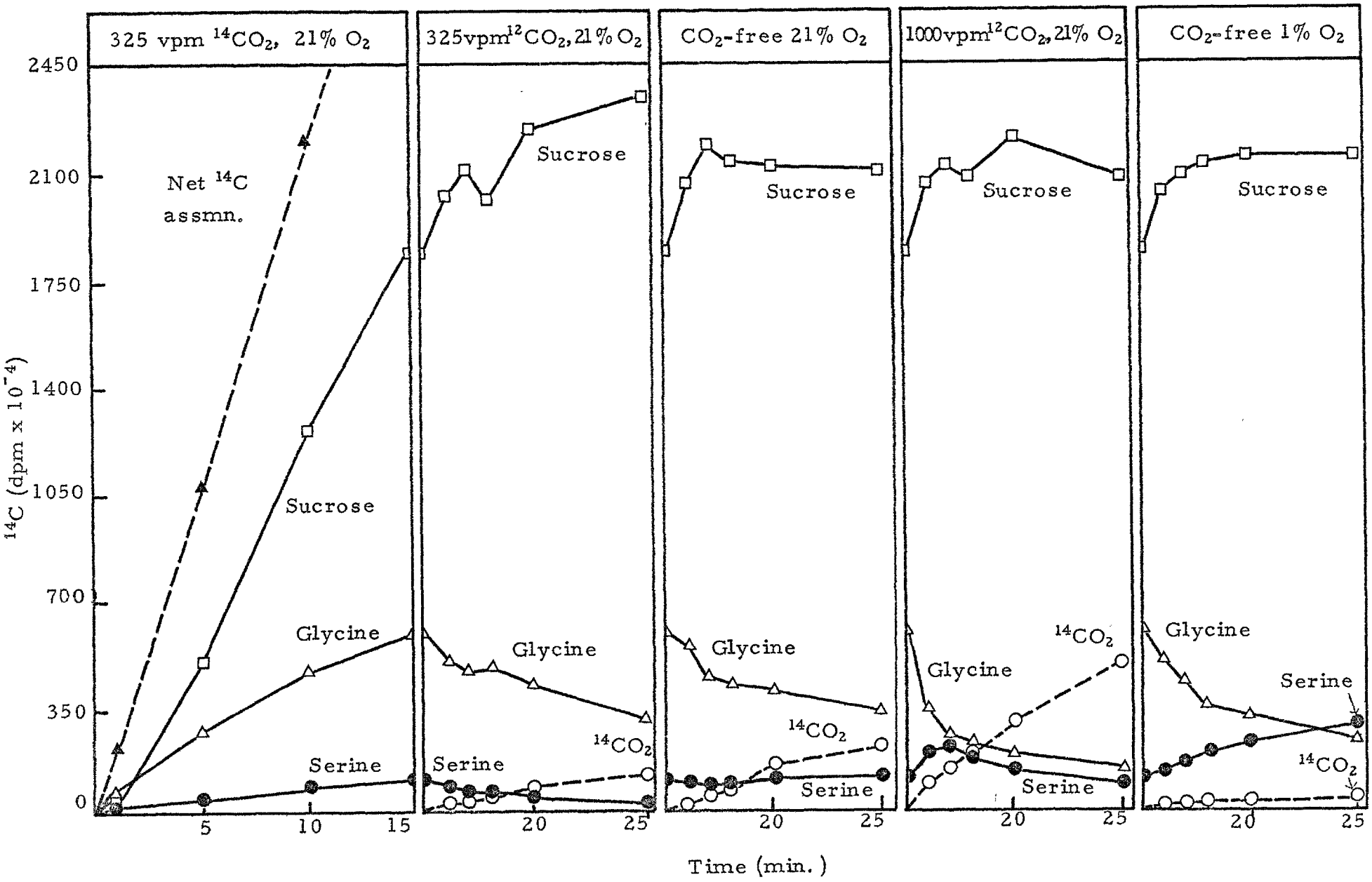

325 vpm $^{14}CO_2$, 21% O_2
325vpm $^{12}CO_2$, 21% O_2
CO_2-free 21% O_2
1000vpm $^{12}CO_2$, 21% O_2
CO_2-free 1% O_2
^{14}C (dpm x 10^{-4})
2450
2100
1750
1400
1050
700
350
0
Net ^{14}C assmn.
Sucrose
Glycine
Serine
$^{14}CO_2$
5
10
15
20
25
Time (min.)

Fig. 9(b)

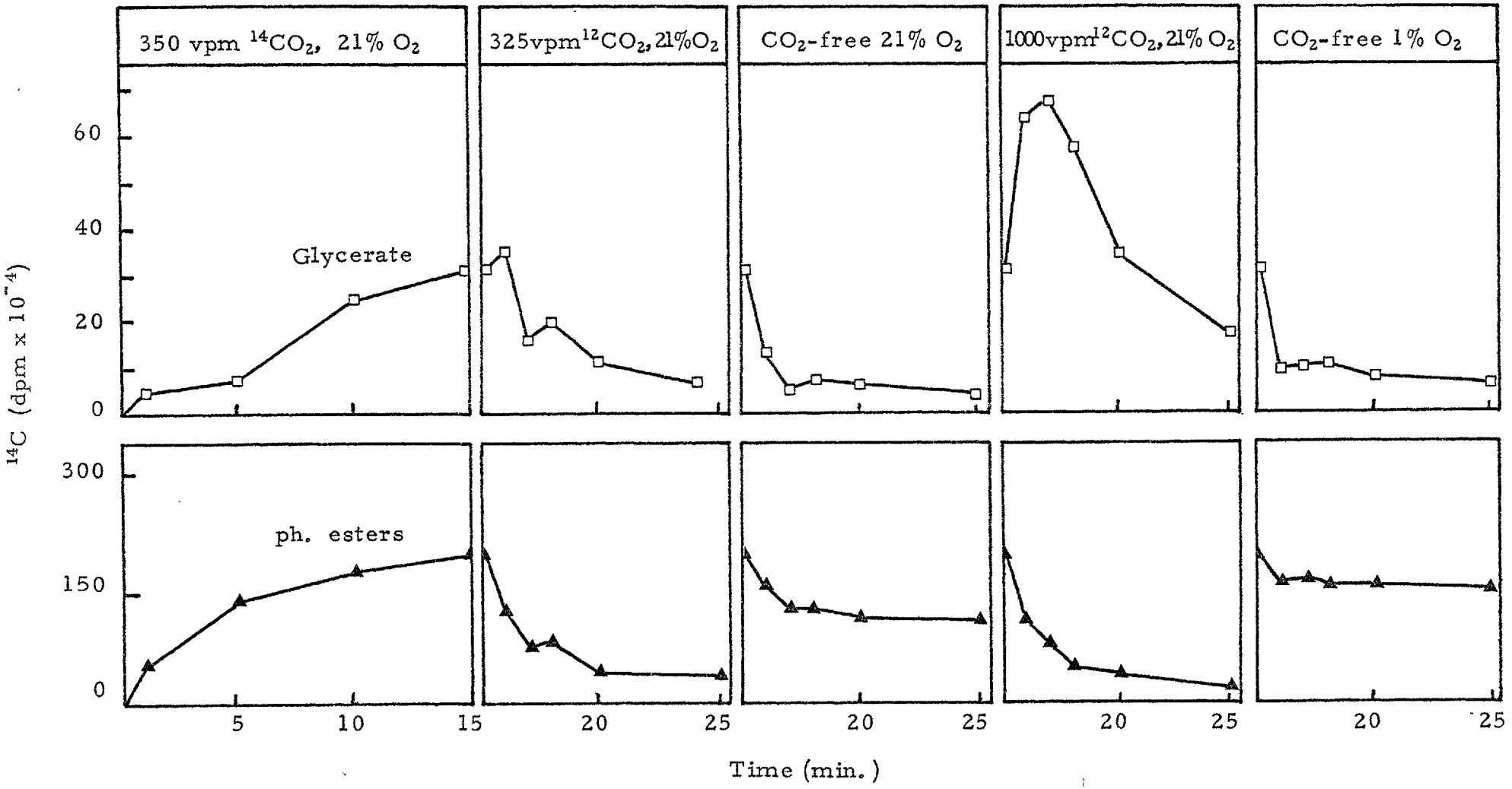
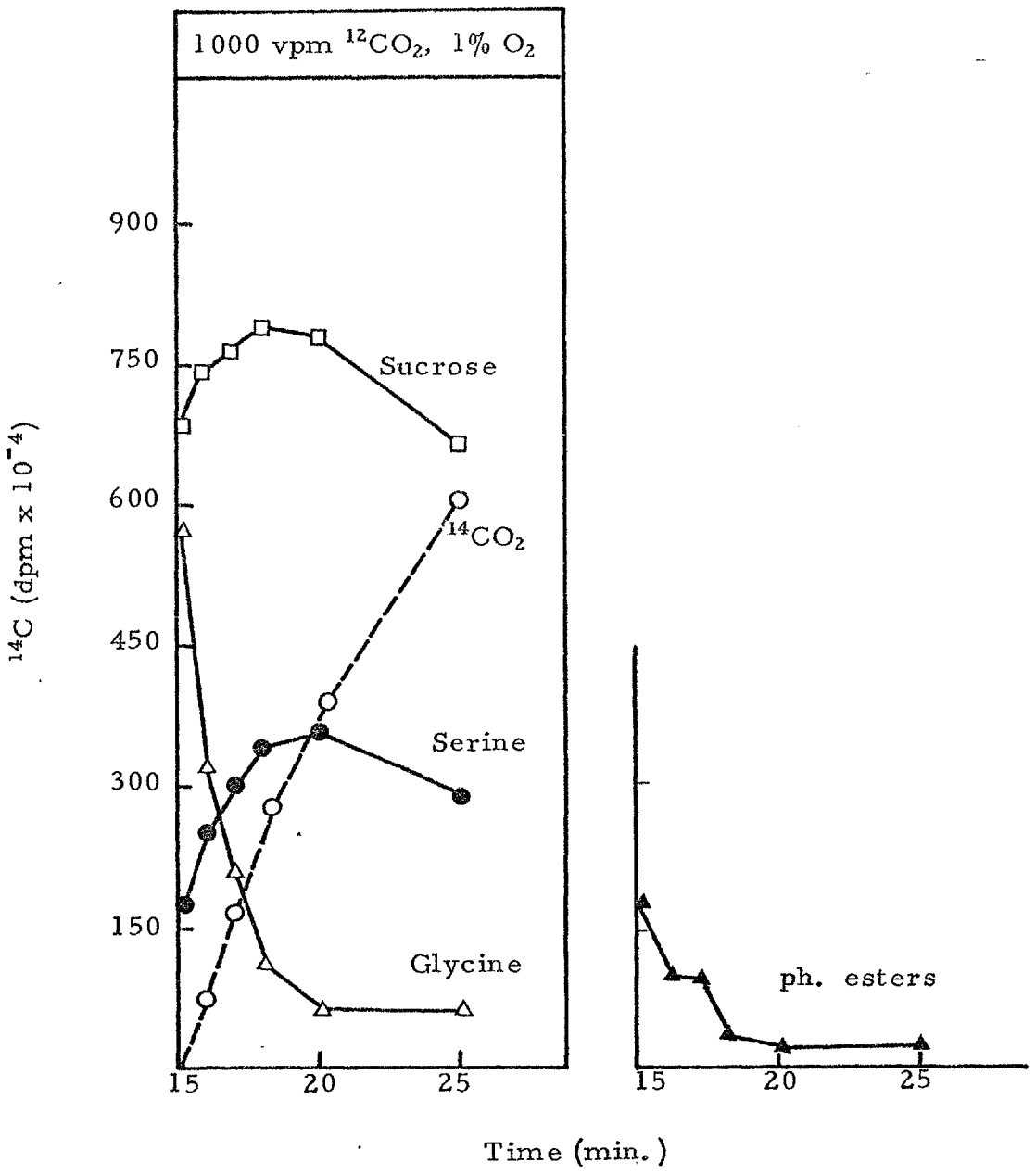

350 vpm $^{14}CO_2$, 21% O_2
325vpm $^{12}CO_2$, 21% O_2
CO_2-free 21% O_2
1000vpm $^{12}CO_2$, 21% O_2
CO_2-free 1% O_2
Glycerate
ph. esters
^{14}C (dpm x 10^{-4})
60
40
20
0
300
150
0
5
10
15
20
25
Time (min.)

Fig. 10. The further metabolism of ^{14}C-labelled compounds in 1000 vpm $^{12}CO_2$ and 1% O_2, following steady-state photosynthesis for 15 min. in 150 vpm $^{14}CO_2$ with 21% O_2.



increased amounts of $^{14}CO_2$ are evolved. The reason why more CO_2 is released in CO_2-free air than in 150 or 325 vpm CO_2 in air (Figs. 8a and 9a) is probably because there is less dilution by $^{12}CO_2$ from outside of intermediates giving rise to the $^{14}CO_2$; in CO_2-free 1% O_2 where the rate of photosynthetic fixation is increased and refixed $^{14}CO_2$ cannot give rise to a new supply of glycerine because synthesis of glycollate is stopped, less $^{14}CO_2$ appears externally. Also in CO_2-free air the intracellular concentration of CO_2 will be higher than in CO_2-free 1% O_2 because of photorespiration and diffusion outwards of CO_2 will therefore be faster.

As already mentioned the slower decrease of ^{14}C in glycine in 150 vpm $^{12}CO_2$ and 325 vpm $^{12}CO_2$ compared to 1000 vpm $^{12}CO_2$ is partly the result of reassimilation of photorespiratory $^{14}CO_2$; it is also caused by recycling of carbon from photorespiratory intermediates for Waidyanatha et al. (1974a) and Fig. 12a show that ^{14}C-serine supplied externally is metabolized to give $^{14}CO_2$ with labelling of glycollate and glycine. Nevertheless ^{14}C-serine in air was mostly converted to sucrose. Especially in treatments following photosynthesis in 150 vpm $^{14}CO_2$ serine metabolism in CO_2-free 1% O_2 and CO_2-free air appeared to be slow (Fig. 8a) (see Waidyanatha et al., 1974a), and there was less incorporation of ^{14}C into sucrose than in 150 or 325 vpm CO_2, 21% O_2.

Comparing the four different conditions (Figs. 8a and 9a) the fastest rate of decrease of radioactivity from glycine and fastest $^{14}CO_2$ evolution were observed in 1000 vpm $^{12}CO_2$ with 21% O_2. Thus when the rate of loss of ^{14}C from glycine was high more $^{14}CO_2$ was evolved suggesting that carbon flow through glycine was closely related to photorespiratory CO_2 evolution. These results are in accordance with the contention

that glycine (Goldswarthy, 1966; Kisaki and Tolbert, 1970: Bird et al., 1972) is the immediate precursor of photorespired CO_2.

When the atmosphere around the leaf segments was changed from 150 vpm $^{14}CO_2$ to 1000 vpm $^{12}CO_2$ (Fig. 8a) the intermediates of the Calvin cycle would be rapidly saturated with ^{12}C. Therefore little new ^{14}C glycine would be formed. Also it is believed that high CO_2 decreases formation of glycollate (Lee and Whittingham, 1974). When the atmosphere was changed to 1000 vpm $^{12}CO_2$ following photosynthesis in $^{14}CO_2$ there was an initial accumulation of ^{14}C in serine for about 3 minutes and some ^{14}C also accumulated in glycerate (Figs. 8b and 9b). However after 3 minutes the radioactivity in serine again declined suggesting that the metabolism of ^{14}C serine had not been completely suppressed. Either the flow of carbon from glycine to serine increased in rate or the rate of metabolism of serine was decreased. Bird et al. (unpublished) have found no effect of increasing CO_2 on rate of conversion of glycine to serine in vitro. On the other hand the increased rate of photosynthesis caused by raising the CO_2 from 150 to 1000 vpm would mean competition for available energy and reducing power between ^{12}C products of photosynthesis and [^{14}C] carbon labelled serine already in the tissue. Therefore, ^{14}C accumulates in serine in 1000 vpm CO_2 because of decreased metabolism of serine. Since 1000 vpm CO_2 decreases glycollate formation and does not seem to stimulate conversion of glycine to serine the rate of decrease of ^{14}C from glycine in 1000 vpm CO_2 may be equated with the rate of conversion of glycine to serine in the preceding steady-state. If this is accepted the rate of loss of ^{14}C from glycine can be calculated and compared to the rate of photosynthesis to give a measure of the proportion of carbon fixed in photosynthesis and metabolized by way of the glycollate pathway. Calculation of the rates (Table 4) assuming saturation during the preceding period of photosynthesis in $^{14}CO_2$ shows

Table 4

Rates of carbon incorporation and flow calculated from changes in ^{14}C during and following steady-state photosynthesis by wheat leaves in 150 or 325 vpm $^{14}CO_2$ in 21% O_2

Photosynthesis in $^{14}CO_2$				Flushing Out				
Conc. of CO_2	Net Ps. rate	Maximum rate of Incorporation of C.		Conc. of CO_2	Conc. of O_2	Rates of carbon flow from initial changes in ^{14}C		
		Glycine	Serine			Glycine	Serine	$^{14}CO_2$
(vpm)	(μgC.dm.$^{-2}$ min.$^{-1}$)			(vpm)	(%)	(μgC. dm.$^{-2}$ min.$^{-1}$)		
150	33.5	12.5	4.8	0	21	- 16.1	- 2.5	+ 7.8
				150	21	- 12.2	- 9.1	+ 10.2
				1000	21	- 68.5	+25.9	+ 19.6
				0	1	- 35.9	+ 6.8	+ 1.6
				1000	1	- 80.5	+ 38.0	+ 35.5
325	63.2	18.2	4.2	0	21	- 14.9	- 5.4	+ 7.9
				325	21	- 24.9	- 5.3	+ 8.0
				1000	21	- 75.1	+21.3	+24.6
				0	1	- 22.3	+ 4.3	+ 2.5

that the initial rate of ^{14}C decrease in glycine in 1000 vpm CO_2 in all cases gives a greater flow of carbon than the total rate of carbon assimilation. The better estimate for 150 vpm $^{14}CO_2$ is found by the change to 1000 vpm $^{12}CO_2$ in 1% O_2 because here entry of freshly assimilated ^{12}C into the glycollate pathway is less than in 1000 vpm $^{12}CO_2$ in air.

An observation not easily interpreted is that the total amount of $^{14}CO_2$ evolved in 1000 vpm CO_2 during 10 minutes was 3 or 5 fold greater than could be accounted for by the decrease of ^{14}C-glycine if two molecules of glycine are converted to one each of serine and CO_2 (see reaction 5, 5a). The extra $^{14}CO_2$ may have been evolved as a result of an interconversion of glycine and serine as suggested by Tolbert (1973) or by exchange of $^{12}CO_2$ with $^{14}CO_2$ taken into the tissue but not assimilated.

Although less ^{14}C serine was formed than the expected, it has already been pointed out that some serine is being metabolized so the apparent rate of accumulation of ^{14}C in serine will be less than the actual total flow into it. In CO_2-free conditions or with 150 or 325 vpm CO_2 lower rates of carbon flow are calculated from decrease in glycine, because of recycling of $^{14}CO_2$ and ^{14}C serine as already discussed. Recycling of serine through the glycollate pathway may be involved even at 1000 vpm CO_2 or some other photorespiratory mechanism using carbon ultimately from serine and glycine.

4. Effect of exogenously supplied ^{12}C-serine on the further metabolism of labelled photosynthetic intermediates during flushing in 1000 vpm $^{12}CO_2$ in 21% O_2.

An isotopic competition experiment was performed to investigate the mechanism of $^{14}CO_2$ release into 1000 vpm $^{12}CO_2$. Unlabelled serine

was added to the leaf segments and also preincubation was in CO_2-free air to produce a large pool of ^{12}C-serine in the tissue. If $^{14}CO_2$ was partly derived from the further metabolism of serine the additional ^{12}C-serine in the tissue should decrease the rate of evolution of $^{14}CO_2$.

Following preincubation in CO_2-free air for 40 minutes at a light intensity of 2900 - 3000 f.c. the gas stream was changed to 150 vpm $^{14}CO_2$ in 21% O_2. After 15 minutes the atmosphere was again changed to 1000 vpm $^{12}CO_2$ in 21% O_2 and samples were taken after 0, 1, 2, 3, 5 and 10 minutes. $^{14}CO_2$ evolved during these periods was also estimated. During the entire period of the experiment the leaves either stood in water (control) or in a solution of ^{12}C-serine (0.05 M).

The results are presented in Figs. 11a and 11b. Preincubation in CO_2-free air caused a decrease in the amount of glycine and an accumulation of serine (Waidyanatha, 1973). In 150 vpm CO_2 the steady-state pool size of glycine was normally very much greater than that of serine (Fig. 8a). However this situation is not restored even in the control sample during 15 minutes photosynthesis in 150 vpm $^{14}CO_2$ following preincubation in CO_2-free air, for serine was labelled more rapidly than glycine. Added ^{12}C-serine in CO_2-free air increased the pool of serine; in fact both serine and glycine pools were probably increased (Fig. 11 a) since more ^{14}C was found in both, than in the control leaves. This was probably due to recycling of serine carbon through the glycollate pathway especially during the period in 150 vpm $^{14}CO_2$ (Waidyanatha et al., 1974). However, the additional ^{12}C-serine in the tissue did not decrease $^{14}CO_2$ evolution. There was more ^{14}C in glycine in the leaves given ^{12}C-serine than in the controls and although the specific activity should have been lower, the rate of loss of ^{14}C from glycine was faster than in the control leaves. The $^{14}CO_2$ evolved was also in excess of that to be expected on the basis of the decrease of ^{14}C from glycine. The most $^{14}CO_2$ was

Fig. 11 (a & b). The effect of exogenously supplied ^{12}C-serine on the further metabolism of labelled photosynthetic intermediates in 1000 vpm $^{12}CO_2$ and 21% O_2 following preincubation in CO_2-free air and photosynthesis in 150 vpm $^{14}CO_2$ with 21% O_2.

Fig. 11(a)

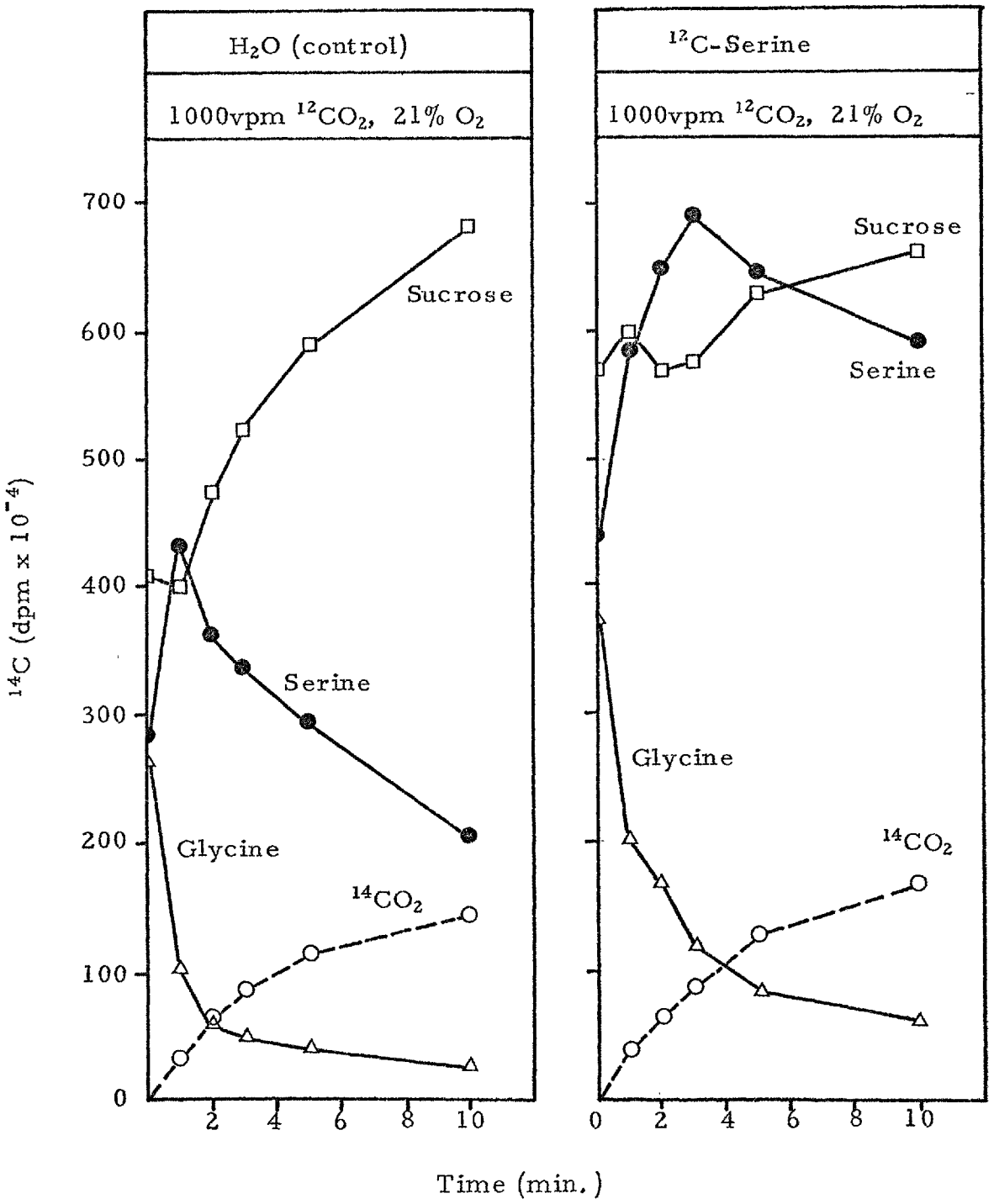
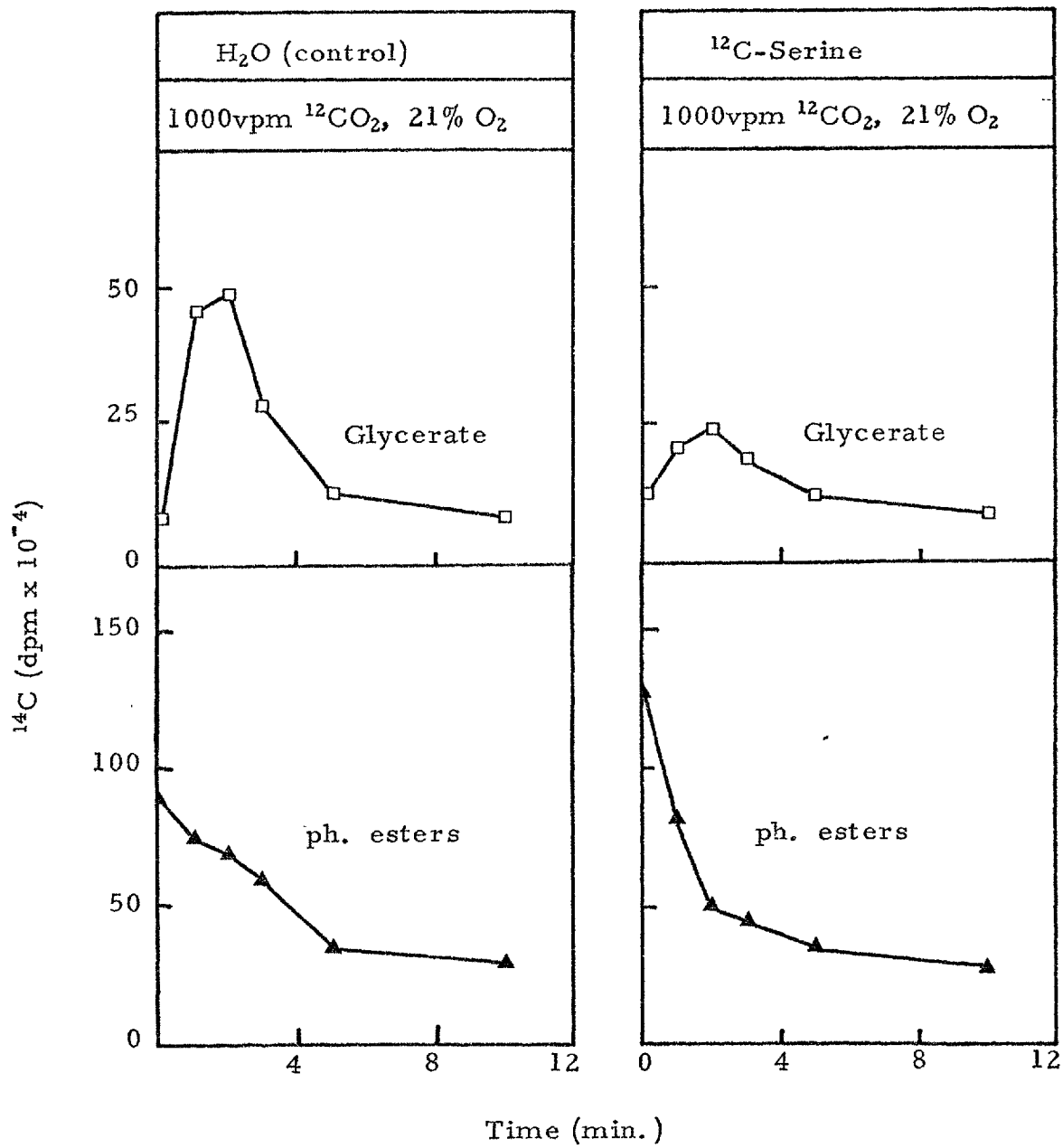


Fig. 11(b)


H2O (control)
1000vpm 12CO2, 21% O2
12C-Serine
1000vpm 12CO2, 21% O2
Glycerate
Glycerate
ph. esters
ph. esters
50
25
0
150
100
50
0
4
8
12
0
4
8
12
14C (dpm x 10-4)
Time (min.)

evolved when the initial pool of glycine was greater but without knowing the pool sizes under these special experimental conditions it is not possible to deduce from the results much about the mechanism by which the $^{14}CO_2$ was produced.

Another striking difference observed in this experiment is in the synthesis of ^{14}C sucrose; it was greater in the absence of added serine than in its presence. This is because the dilution of the pool of serine caused by the added ^{12}C-serine may have resulted in less ^{14}C going to sucrose. This is further evidenced by the lesser incorporation of ^{14}C into glycerate (Fig. 11 b). These results are consistent with formation of sucrose from serine with glycerate as a probable intermediate.

5. Photometabolism of serine-U-^{14}C by detached wheat leaves: the effect of CO_2 and O_2 concentration.

If the apparently excessive production of $^{14}CO_2$ in 1000 vpm $^{12}CO_2$ was arising from recycling of serine through the glycollate pathway then added serine ^{14}C should also be recycled. Production of excessive amounts of $^{14}CO_2$ was more in 1000 vpm CO_2 with 1% O_2 than with 21% O_2, so metabolism of serine in both these conditions was studied.

Illuminated (2900 - 3000 f.c.) leaf segments were preincubated in 325 vpm $^{12}CO_2$ with 21% O_2 for 40 minutes. The leaf segments were then transferred from water to a solution of serine-U-^{14}C (1 μci, 0.4 μmole in 0.05 ml) and illuminated for a further 60 minutes in a stream of 325 vpm $^{12}CO_2$ in 21% O_2, 325 vpm $^{12}CO_2$ 1% O_2, 1000 vpm $^{12}CO_2$ 21% O_2 or 1000 vpm $^{12}CO_2$ 1% O_2. Samples were taken after 7.5, 15, 30, 45 and 60 minutes in these atmospheres and the $^{14}CO_2$ evolved during these periods was also estimated.

Some $^{14}CO_2$ was produced under all conditions tested (Fig. 12 b) but much less in the presence of 1000 vpm CO_2 in 21% O_2 and least of all with 1000 vpm

Fig. 12 (a & b). Photometabolism of exogenously supplied serine-U-^{14}C by detached wheat leaves : the effect of CO_2 and O_2 concentration.

Fig. 12(a)

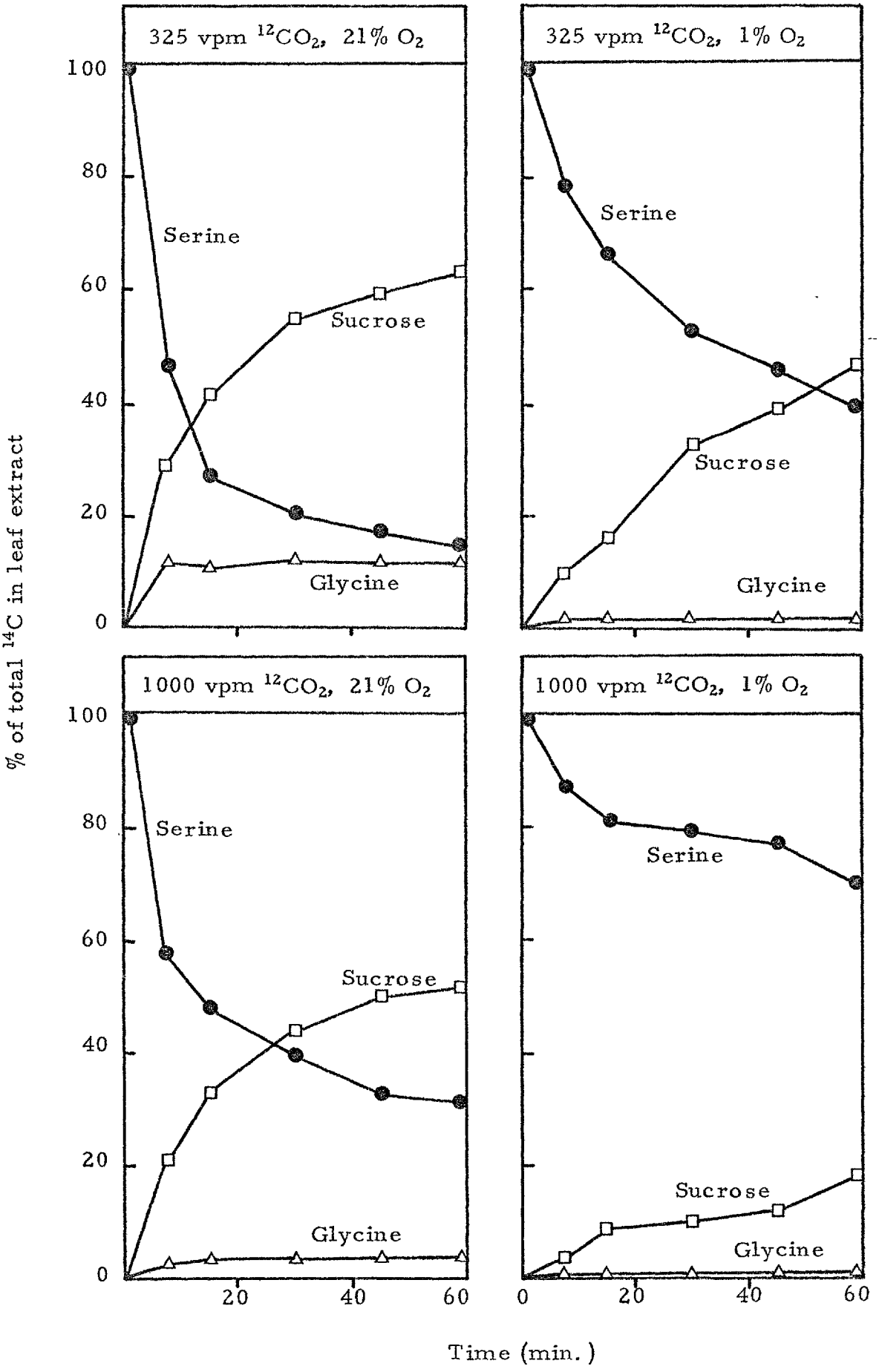


Fig. 12(b)

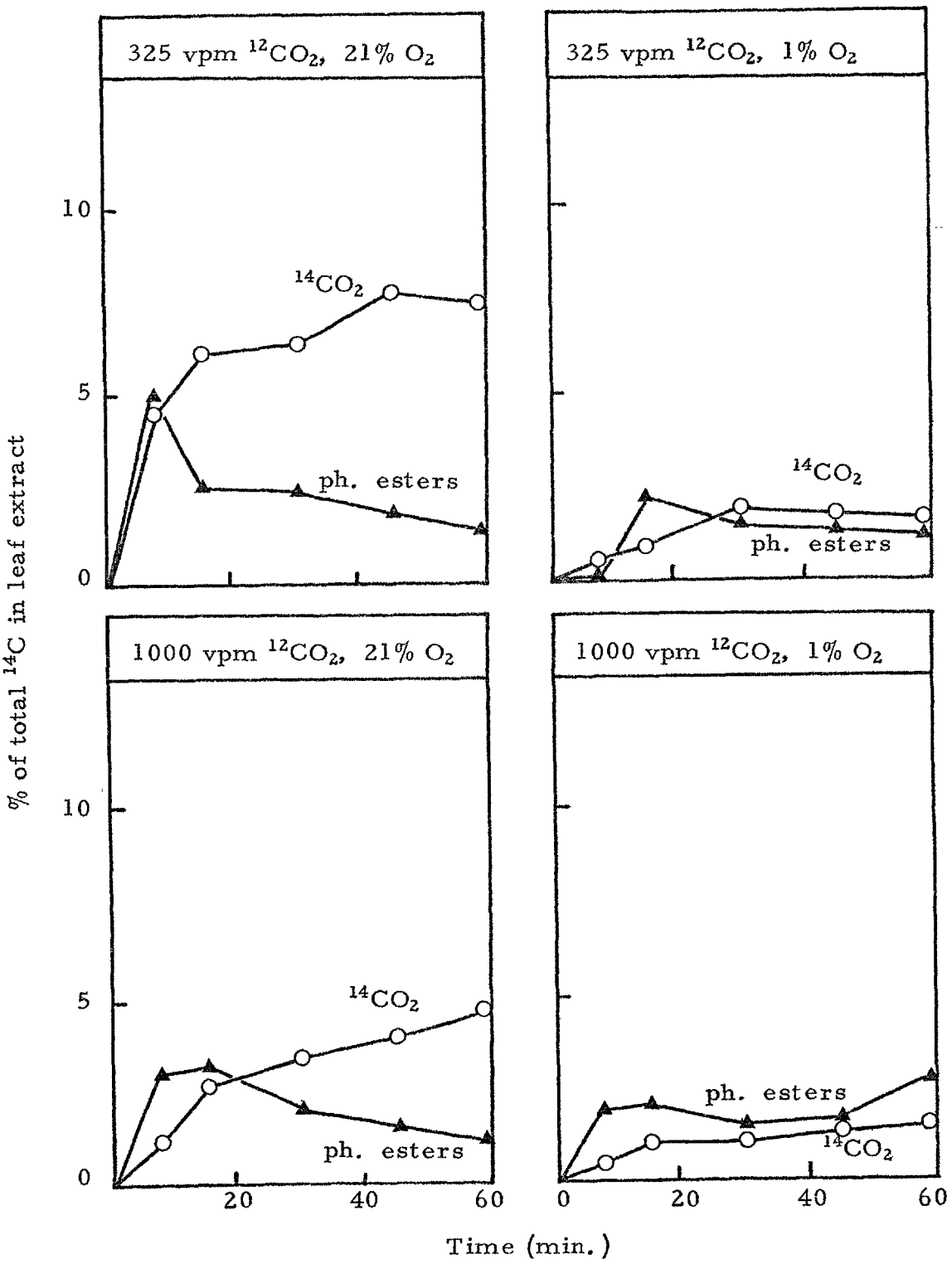

325 vpm $^{12}CO_2$, 21% O_2
325 vpm $^{12}CO_2$, 1% O_2
1000 vpm $^{12}CO_2$, 21% O_2
1000 vpm $^{12}CO_2$, 1% O_2
$^{14}CO_2$
ph. esters
% of total ^{14}C in leaf extract
0
5
10
20
40
60
Time (min.)

Table 4a Radioactivity in glycerate, glycollate and CO_2 as a percentage of serine-U-^{14}C metabolized

Time of uptake (Min.)	325 vpm $^{12}CO_2$, 21% O_2			325 vpm $^{12}CO_2$, 1% O_2			1000 vpm $^{12}CO_2$, 21% O_2			1000 vpm $^{12}CO_2$, 1% O_2		
	Glyc.*	Glycol.⊥	CO_2	Glyc.*	Glycol.⊥	CO_2	Glyc.*	Glycol.⊥	CO_2	Glyc.*	Glycol.⊥	CO_2
7.5	6.4	0.6	9.1	1.7	1.1	2.9	18.9	1.1	3.8	17.7	0.8	4.4
15	3.2	0.8	12.2	6.8	0.7	2.6	12.4	0.8	5.3	14.2	0.9	3.9
30	1.75	0.7	7.0	2.4	0.6	4.7	8.0	0.9	6.7	9.8	0.9	4.5
45	1.08	0.6	10.7	2.6	0.8	3.6	6.4	0.9	6.5	8.0	1.0	4.1
60	1.29	0.6	10.8	1.8	0.7	2.9	4.8	0.9	7.0	5.2	1.2	5.2

* Glycerate ⊥ Glycollate

CO_2 in 1% O_2. So CO_2 enrichment did not stimulate $^{14}CO_2$ release from exogenous serine but did not entirely stop the process. These results are against serine being a significant precursor of $^{14}CO_2$ evolution in atmospheres with a high $^{12}CO_2$ concentration following photosynthesis in $^{14}CO_2$, unless by its metabolism at some very limited site in the cell not readily accessible to exogenous serine because on the basis of the total ^{14}C metabolized, the percentage of $^{14}CO_2$ evolved in 1000 vpm CO_2 with 1% or 21% O_2 was not very different from that evolved in 325 vpm CO_2 with 1% or 21% O_2 (Table 4 a). Therefore the large evolution of $^{14}CO_2$ in 1000 vpm CO_2 following photosynthesis in $^{14}CO_2$ is not likely to be due to increased recycling of serine through the glycollate pathway or to any more direct conversion back to glycine as suggested by Tolbert (1973).

More ^{14}C-serine was metabolized in 325 than in 1000 vpm CO_2 at either O_2 concentrations (1% or 21%) (Fig. 12 a). Also more ^{14}C-serine was metabolized at 21% O_2 than at 1% O_2 with either CO_2 concentrations (325 or 1000 vpm). In 1% or 21% O_2 more ^{14}C-sucrose was formed in 325 than in 1000 vpm CO_2 and similarly more ^{14}C-sucrose was synthesized at 21% O_2 than at 1% O_2 with either CO_2 concentrations. Formation of ^{14}C-sucrose was therefore inversely related to serine metabolism; more sucrose was formed when more serine was metabolized. Fig. 12 a also shows that the highest synthesis of ^{14}C-sucrose took place at air levels of CO_2 and O_2 and the lowest amount at 1000 vpm CO_2 and 1% O_2. These observations are not inconsistent with the pattern of metabolism of ^{14}C-serine formed during photosynthesis in $^{14}CO_2$ (Figs. 8 a and 9a).

The data in Figs. 12a and 12b further illustrate the extent to which serine-^{14}C recycled back into the glycollate pathway through the intermediates of the Calvin cycle. Since it has been shown by Wang and Burris (1963) that glycine is not formed from serine by a reversal of the reaction

converting glycine to serine, the amount of ^{14}C glycine formed during the metabolism of exogenously supplied ^{14}C-serine probably indicated the degree to which the serine carbon was metabolized to glycollate involving the chloroplasts. More ^{14}C-serine was recycled in 325 vpm CO_2 with 1% or 21% O_2 than with 1000 vpm CO_2 at either O_2 concentrations. This may depend upon the size and the degree of saturation of the chloroplastic phosphate ester pools with ^{12}C. When the CO_2 concentration was high these pools contained more ^{12}C which blocked the entry of ^{14}C from serine. The recycling of serine carbon through the glycollate pathway was more inhibited when the O_2 concentration decreased from 21% to 1%. This effect of O_2 is consistent with the known inhibition in the presence of low O_2 of formation of glycollate from RUDP in the chloroplasts.

6. The further metabolism of ^{14}C-labelled compounds by wheat leaves in 325 vpm $^{12}CO_2$ with 21% O_2, 1000 vpm $^{12}CO_2$ with 21% O_2 and 1000 vpm $^{12}CO_2$ with 1% O_2 following steady-state photosynthesis in 150 vpm $^{14}CO_2$ with 1% O_2.

The excessive production of $^{14}CO_2$ in 1000 vpm $^{12}CO_2$ can also be examined by varying the relative proportion of ^{14}C photosynthetic products. The objective was achieved by the use of low O_2 (Figs. 13a and 13b) during exposure to 150 vpm $^{14}CO_2$.

The leaf segments were preincubated in 150 vpm $^{12}CO_2$ with 1% O_2 for 40 minutes at a light intensity of 2900 - 3000 f.c. The gas stream was then changed so as to supply 150 vpm $^{14}CO_2$ in 1% O_2. After 15 minutes photosynthesis in $^{14}CO_2$ the atmosphere was again changed to 325 vpm $^{12}CO_2$ in 21% O_2, 1000 vpm $^{12}CO_2$ in 21% O_2 or 1000 vpm $^{12}CO_2$ in 1% O_2. Samples were taken after 0, 1, 2, 3, 5 and 10 minutes

in these atmospheres and the $^{14}CO_2$ evolved was also estimated. Under all three flushing conditions (Fig. 13a) very little $^{14}CO_2$ was evolved compared to that in 1000 vpm $^{12}CO_2$ with either 1% or 21% O_2 (Figs. 8a, 9a and 10) where there was a rapid decrease in radioactivity in a larger pool of glycine. Also in 1000 vpm $^{12}CO_2$ with 21% O_2 (Fig. 12a) ^{14}C serine was relatively rapidly metabolized but $^{14}CO_2$ evolution was not stimulated compared to the control (Fig. 12b). These data substantiate the previous conclusion that the photorespiratory CO_2 is derived mainly from glycine; most of the $^{14}CO_2$ evolved into 1000 vpm $^{12}CO_2$ (Figs. 8a, 9a and 10) could be accounted for by the decrease of radioactivity from glycine.

Compared to the amounts of ^{14}C incorporated into glycine, serine, sucrose and phosphate esters after steady-state photosynthesis for 15 minutes in 150 vpm $^{14}CO_2$ and 21% O_2 (Figs. 8a and 8b) the decrease from 21 to 1% O_2 increased incorporation of ^{14}C into sucrose by 44% whereas that into glycine, serine and phosphate esters was decreased by 94%, 10% and 45% respectively. The pool sizes of these compounds and particularly that of glycine were extremely sensitive to low O_2. Upon transfer to 325 vpm $^{12}CO_2$ in 21% O_2 radioactivity actually increased slightly in glycine and this is possibly due to a tendency for the pool size of glycine to increase in 325 vpm CO_2 in air compared to that in 150 vpm CO_2 with 1% O_2. Some ^{14}C sucrose was synthesized, particularly during the first 5 minutes, and this could be accounted for mainly by the total decreases of ^{14}C in serine and phosphate esters. The formation of new ^{14}C-sucrose during this whole period (10 minutes) of flushing represented only 13% of the total ^{14}C sucrose synthesized during the previous 15 minutes photosynthesis in $^{14}CO_2$. In 150 vpm $^{14}CO_2$ with 21% O_2 (Fig. 8a) the total increase of ^{14}C sucrose was 42% of that synthesized during photosynthesis in the $^{14}CO_2$. The difference between the two

Fig. 13 (a & b). The effect of 325 vpm $^{12}CO_2$ in 21% O_2, 1000 vpm $^{12}CO_2$ in 21% O_2 and 1000 vpm $^{12}CO_2$ in 1% O_2 on the distribution of radioactivity in glycine, serine, sucrose, glycerate, phosphate esters and CO_2 following steady-state photosynthesis for 15 min. in 150 vpm $^{14}CO_2$ with 1% O_2.

Leaf segments were preincubated in 150 vpm $^{12}CO_2$ with 1% O_2 for 40 min. and the gas stream was then changed to one of similar composition but containing $^{14}CO_2$. After 15 min. photosynthesis in $^{14}CO_2$ the atmosphere was again changed to 325 vpm $^{12}CO_2$ in 21% O_2, 1000 vpm $^{12}CO_2$ in 21% O_2 or 1000 vpm $^{12}CO_2$ in 1% O_2.

Fig. 13 (a)

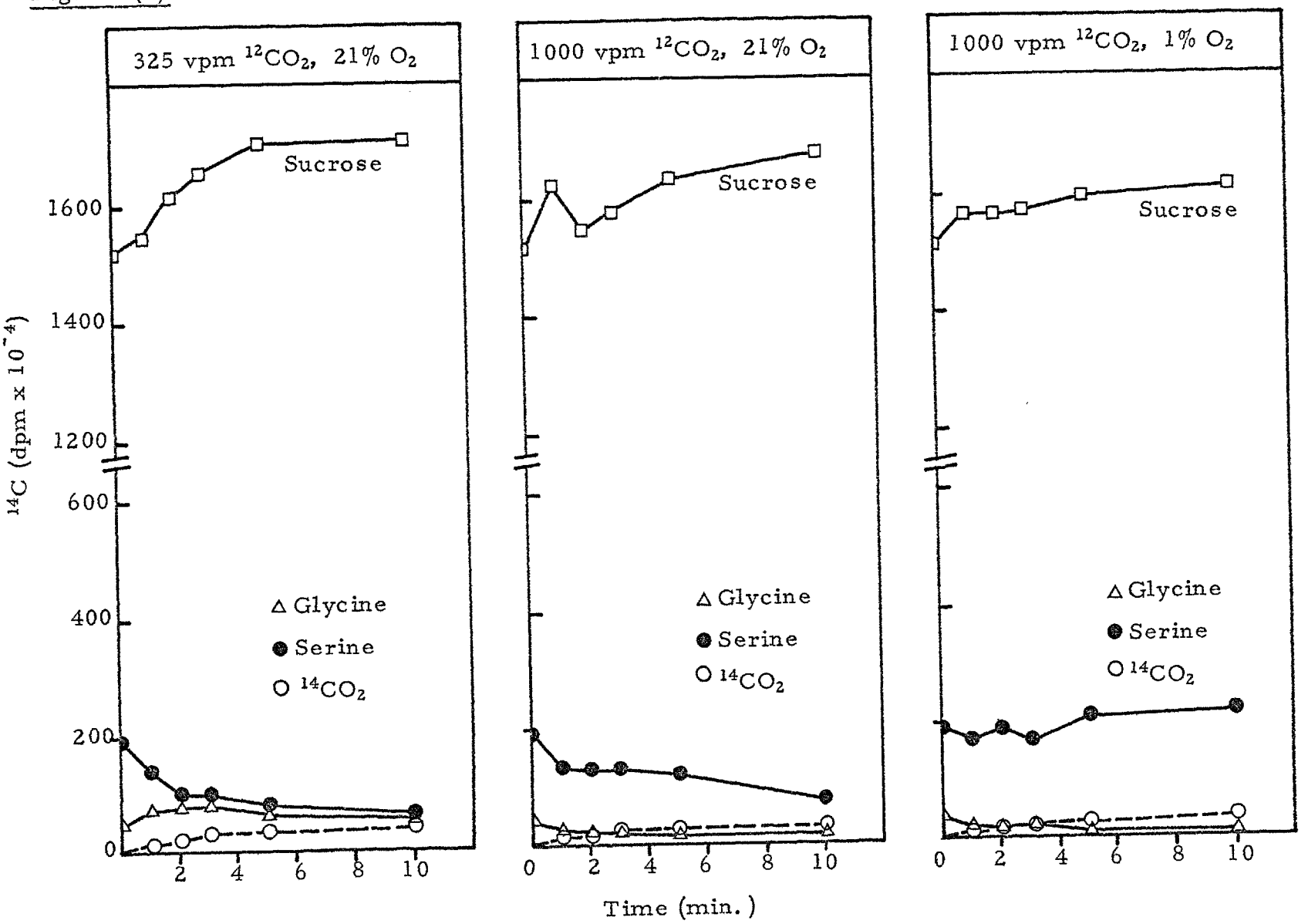
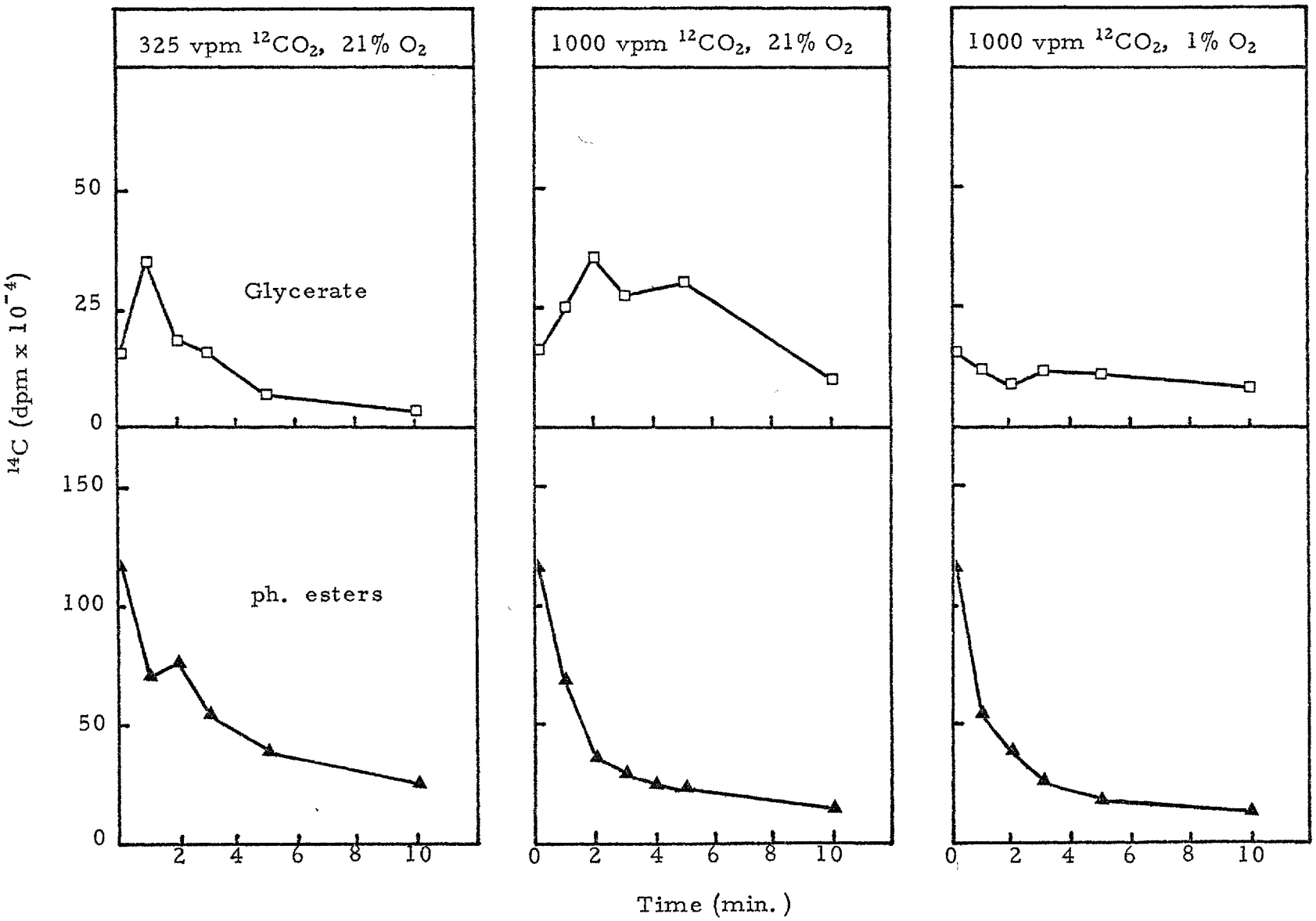

325 vpm $^{12}CO_2$, 21% O_2
1000 vpm $^{12}CO_2$, 21% O_2
1000 vpm $^{12}CO_2$, 1% O_2
Sucrose
Glycine
Serine
$^{14}CO_2$
^{14}C (dpm x 10^{-4})
Time (min.)

Fig. 13(b)


325 vpm $^{12}CO_2$, 21% O_2
1000 vpm $^{12}CO_2$, 21% O_2
1000 vpm $^{12}CO_2$, 1% O_2
Glycerate
ph. esters
^{14}C (dpm x 10^{-4})
50
25
0
150
100
50
0
2
4
6
8
10
Time (min.)

situations (compare Figs. 8a & 8b with 13a and 13b) lies in the amount of ^{14}C present in phosphate esters, glycine and serine which are clearly the source of ^{14}C for the sucrose synthesis. It is not possible from these results to distinguish the important intermediates or their sequence in the pathway between CO_2 assimilated by photosynthesis and sucrose but the pool sizes of the potential intermediates are smaller in 1% compared to 21% O_2 and their turn over must be more rapid.

At a similar CO_2 concentration (1000 vpm) less ^{14}C serine was further metabolized in 1% O_2 compared to in 21% O_2 (Fig. 12a); this is consistent with the previous data (Figs. 8a and 10). The high ratio of ^{14}C-serine to ^{14}C-glycine formed in 150 vpm $^{14}CO_2$ with 1% O_2 (Fig. 13a) compared to the ratio in 150 vpm $^{14}CO_2$ and 21% O_2 (Fig. 8a) is explained by the decreased rate of glycollate synthesis (Bowes et al., 1971; Andrews et al., 1973) and decreased rate of metabolism of serine (Waidyanatha et al., 1974a).

7. The effect of darkness and CO_2-free 21% O_2, CO_2-free 1% O_2 or 1000 vpm $^{12}CO_2$ in 21% O_2 on changes in radioactivity of the compounds formed during steady-state photosyntehsis in 150 vpm $^{14}CO_2$ with 21% O_2.

Leaf segments were illuminated (2900 - 3000 f.c.) in 150 vpm $^{12}CO_2$ in 21% O_2 for 40 minutes. The atmosphere was then changed to one of similar composition but containing $^{14}CO_2$. For some samples, after 15 minutes photosynthesis in $^{14}CO_2$ the gas stream was again changed to CO_2-free 21% O_2, CO_2-free 1% O_2 or 1000 vpm $^{12}CO_2$ in 21% O_2 and at the same time the leaf chambers were darkened by covering with aluminium foil. Samples were taken after 1, 2, 3, 5 and 10 minutes and the $^{14}CO_2$ evolved during these periods was also estimated.

The different atmospheric conditions during flushing out in darkness were chosen as extremes so that any effects of CO_2 or O_2 independent of light and photosynthesis might be detected and considered in the interpretation of other experiments described in this thesis.

The results are illustrated in Figs. 14a and 14b and Table 5. Darkening caused a rapid loss of ^{14}C from glycine, an accumulation of ^{14}C-serine and an evolution of $^{14}CO_2$. Synthesis of new ^{14}C-sucrose was markedly inhibited. In the dark refixation of the photorespired $^{14}CO_2$ was prevented so that most of the $^{14}CO_2$ produced was evolved. Moreover, light is required for further metabolism of serine (Miflin et al., 1966, Waidyanatha et al., 1974) and thus in its absence serine accumulated. Conversion of glycine to serine is however not known to be affected by darkness. In 1000 vpm CO_2, even if some ^{14}C from ^{14}C-serine did reach the chloroplasts and become metabolized to RUDP, formation of glycollate would be suppressed since high CO_2 concentrations are known to inhibit glycollate synthesis (Lee and Whittingham, 1974; Laing et al., 1974). In darkness following a light period, photosynthesis does not cease immediately (Bassham and Calvin, 1957) so some $^{14}CO_2$ produced would be initially refixed. In the presence of 1000 vpm $^{12}CO_2$ such refixation would also be minimized due to greater competition with $^{12}CO_2$ to reach the site of CO_2 assimilation so that little or no ^{14}C in respiratory CO_2 would be recycled. However, loss of ^{14}C from glycine appeared faster in CO_2-free air in darkness than in 1000 vpm CO_2 in air and changes in phosphate esters were small in comparison with changes in glycine, serine and CO_2. In darkness, the rate of evolution of CO_2 from leaves just previously assimilating CO_2 in the light, is faster than the rate of dark respiration and has been called the 'post illumination burst'. It is held that while photosynthesis ceases almost instantaneously photorespiration continues for a short while utilizing the substrate produced

Fig. 14 (a & b). The effect of darkness and different concentrations of CO_2 and O_2 on changes of radioactivity in the compounds formed during steady-state photosynthesis in 150 vpm $^{14}CO_2$.

Fig. 14(a)

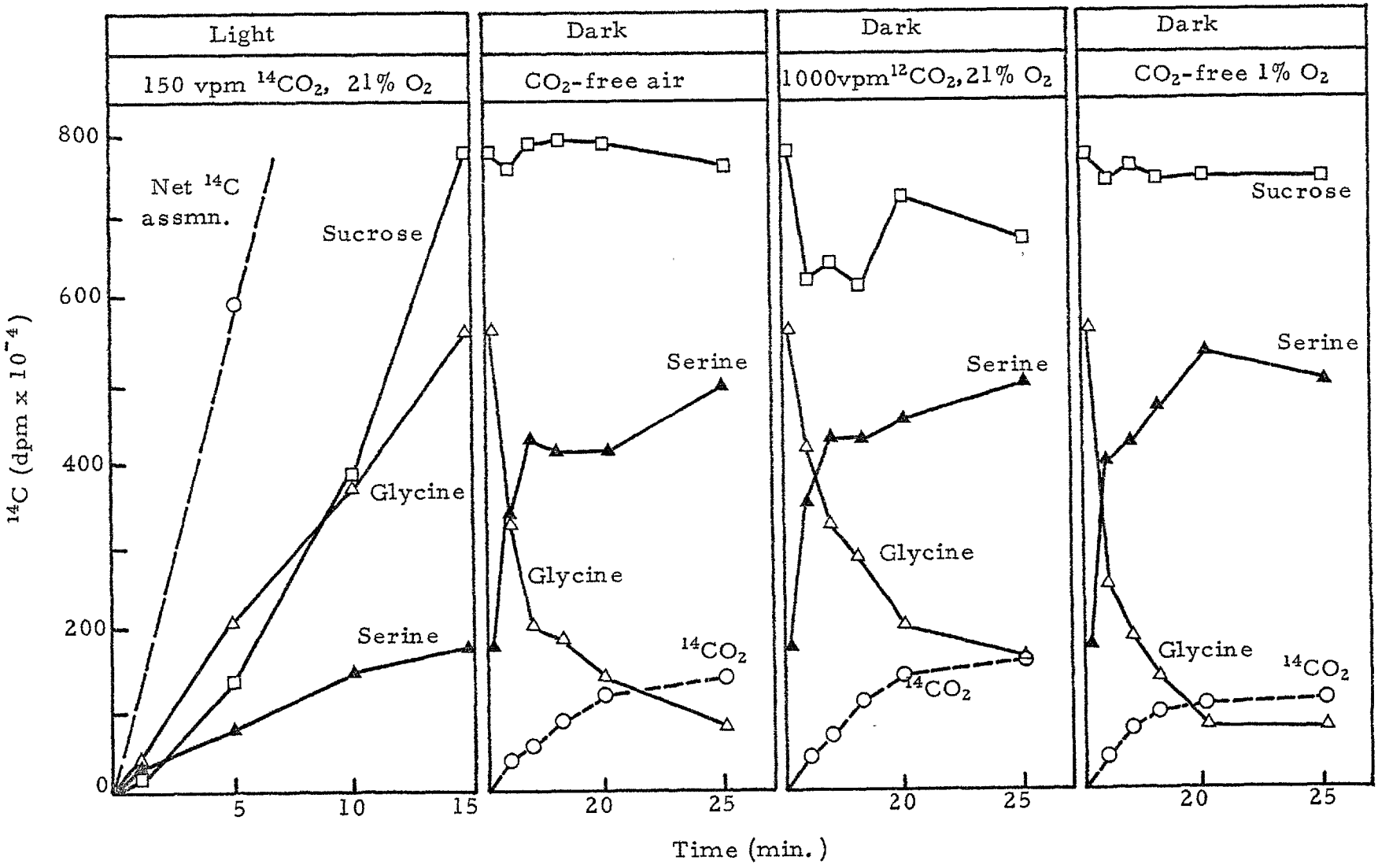

Light
150 vpm $^{14}CO_2$, 21% O_2
Dark
CO_2-free air
Dark
1000vpm $^{12}CO_2$, 21% O_2
Dark
CO_2-free 1% O_2
Net ^{14}C assmn.
Sucrose
Glycine
Serine
$^{14}CO_2$
^{14}C (dpm x 10^{-4})
0
200
400
600
800
5
10
15
20
25
Time (min.)

Fig. 14(b)

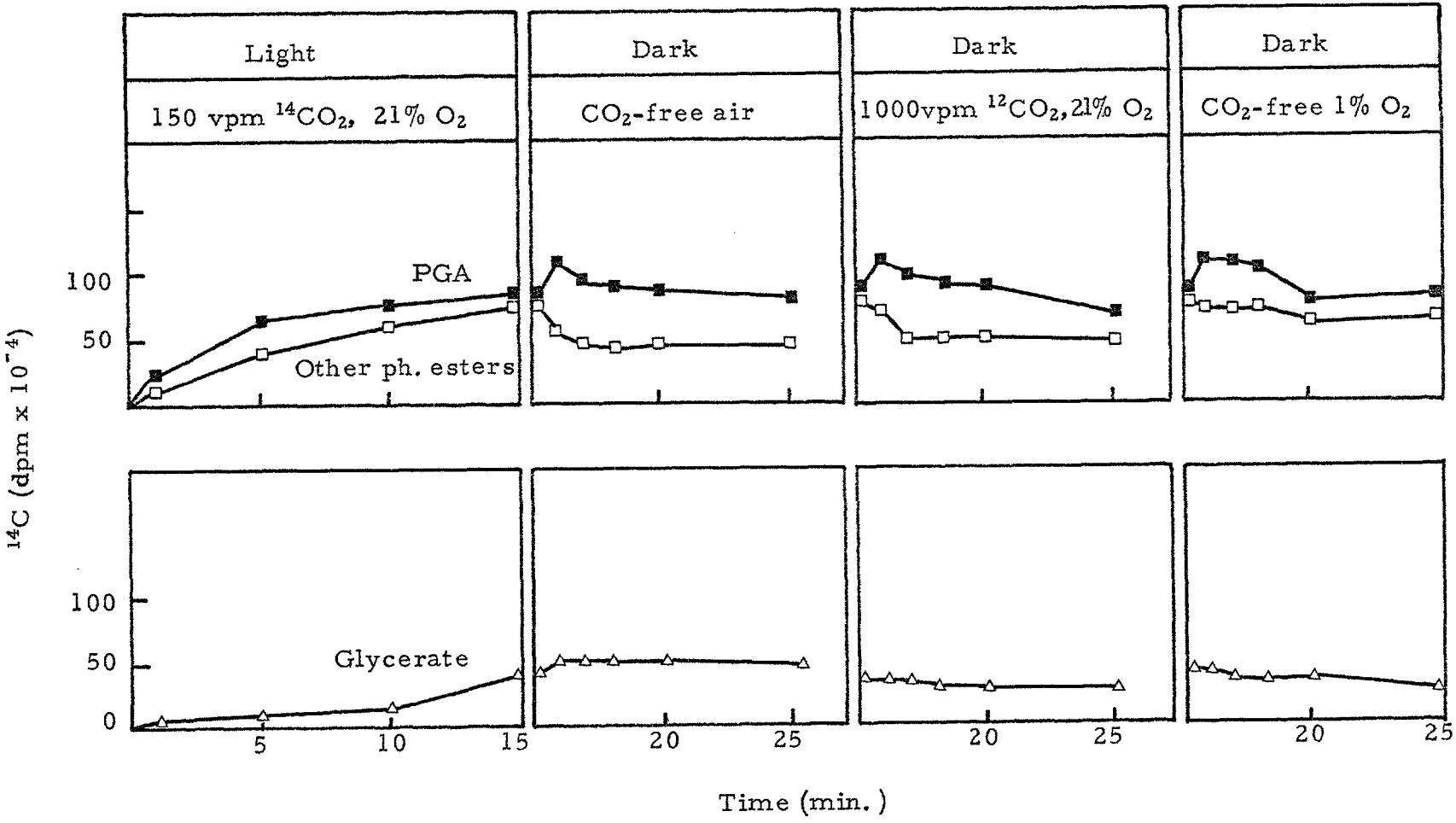

Light
150 vpm $^{14}CO_2$, 21% O_2
Dark
CO_2-free air
Dark
1000vpm $^{12}CO_2$, 21% O_2
Dark
CO_2-free 1% O_2
PGA
Other ph. esters
Glycerate
100
50
0
5
10
15
20
25
20
25
20
25
^{14}C (dpm x 10^{-4})
Time (min.)

Table 5 Changes of radioactivity in glycine, serine and CO_2 during the first minute of darkness under different atmospheric conditions

	CO_2-free air			1000 vpm $^{12}CO_2$ in air			CO_2-free 1% O_2		
	Glycine	Serine	$^{14}CO_2$	Glycine	Serine	$^{14}CO_2$	Glycine	Serine	$^{14}CO_2$
dpm x 10^{-4}	- 240	+ 153	+ 38	- 145	+ 170	+ 43	- 312	+ 217	+ 60
$\mu gC.dm.^{-2}\ min.^{-1}$	- 65.3	+ 41.6	+ 10.3	- 39.4	+ 46.2	+ 11.6	- 84.8	+ 59.0	+ 16.3
Ratio	4.0	2.6	0.6	4.0	4.7	1.2	4.0	2.7	0.7
			3.2			5.9			3.5

+ Increase, - Decrease

(Rate of net photosynthesis, 33.5 $\mu gC.\ dm.^{-2}\ min.^{-1}$)

immediately before darkening and extrapolation of the rate of CO_2 evolution to the instant of darkening has been taken as the rate of photorespiration. In the present experiment $^{14}CO_2$ would be evolved in the post-illumination outburst because the substrates had been nearly saturated with ^{14}C during the previous light period. The only substrate showing a major decrease in ^{14}C in the post-illumination period was glycine. In the data presented, the rate of photorespiration might apparently be equally well calculated from the rate of loss of ^{14}C from glycine or incorporation into serine as from the initial rate of $^{14}CO_2$ evolution. Table 5 shows the rates of carbon flow calculated from the decrease of radioactivity in glycine and the increases in serine and CO_2 during the first minute in darkness. In CO_2-free 21% O_2, for every 4.0 μgC. $dm.^{-2}$ $min.^{-1}$ lost from glycine 2.6 and 0.6 μgC. $dm.^{-2}$ $min.^{-1}$ appeared in serine and CO_2 respectively. In CO_2-free 1% O_2, when the results show that 4.0 μgC. $dm.^{-2}$ $min.^{-1}$ were lost from glycine, 2.7 and 0.7 μgC. $dm.^{-2}$ $min.^{-1}$ appeared in serine and CO_2. In 1000 vpm $^{12}CO_2$ and 21% O_2 the ratio was 4.0, 4.7 and 1.2 μgC. $dm.^{-2}$ $min.^{-1}$ for the loss of carbon from glycine and increase of that in serine and CO_2. It has been shown convincingly *in vitro* studies (Bird *et al.*, 1972) that for every 4 carbon atoms lost from glycine 3 appear in serine and one in CO_2. The changes observed *in vivo* in CO_2-free 21% O_2 and CO_2-free 1% O_2 were reasonably consistent with this stoichiometry but in 1000 vpm CO_2 with 21% O_2 results, this was not so. This discrepancy could be a result of variation between samples since it is difficult to explain the erratic ^{14}C content of sucrose observed during the 1 - 3 minutes. A further experiment on post-illumination changes therefore involved replication of the important measurements.

Meanwhile it should be noted (compare Fig. 14awith Fig. 8a) that the amount of ^{14}C in CO_2 released in the dark into 1000 vpm CO_2 was not

excessive and was very much less than the decrease of ^{14}C in glycine. It seems unlikely therefore that the results in Fig. 8a can be explained in terms of $^{12}CO_2$ from the atmosphere replacing $^{14}CO_2$ in the tissue but not assimilated.

8. Radioactivity changes in glycine, serine and CO_2 during one minute of darkness, following steady-state photosynthesis in $^{14}CO_2$; replication of measurements.

Illuminated (2900 - 3000 f.c.) wheat leaf segments were preincubated in 325 vpm $^{12}CO_2$, 21% O_2 for 40 minutes. The gas stream was then changed and 325 vpm $^{14}CO_2$, 21% O_2 was supplied for a further period of 15 minutes. Ten samples each having 5 leaf segments were killed immediately after feeding $^{14}CO_2$ and another ten samples after the 15 minute photosynthesis with $^{14}CO_2$ were supplied with 325 vpm $^{12}CO_2$ in 21% O_2 for 1 minute in darkness before they were killed and sampled. $^{14}CO_2$ evolved during this period was also estimated.

The results of this experiment are shown in Table 6. Some variation was observed in the rate of photosynthesis and in the amount of ^{14}C incorporated into glycine, serine and CO_2 in individual samples. However the standard errors calculated for the sample means were comparatively small; ranging from 2.4 to 4.4 of the means for glycine, serine and CO_2 (Table 6). In the calculation of changes in glycine, serine and CO_2, changes of ^{14}C in other compounds which were small have been neglected. The changes during the 1 minute in darkness were calculated from the means and show that for every 4.00 μgC lost from glycine 3.15 and 1.06 μgC appeared in serine and CO_2 respectively (Table 6). A chi-square test showed that these values were not significantly different at the 5% level from the expected ratio of 4 : 3 : 1 for the conversion of glycine to serine and CO_2 (see introduction, reaction 5, 5a). It is concluded that the conversion of 2 molecules of glycine to one each of serine and CO_2

Table 6 — The rates of change of carbon in glycine, serine and CO_2 during 1 min. of darkness, following photosynthesis in 325 vpm $^{14}CO_2$

Period of supply (min.) 325 vpm $^{14}CO_2$ (Light)	Period of supply (min.) 325 vpm $^{12}CO_2$ (Dark)	Radioactivity (dpm x 10^{-4})* Glycine	Serine	CO_2
15	0	490.1 ± 14.4	116.4 ± 4.2	0
15	1	355.8 ± 8.4	222.4 ± 6.7	35.9 ± 1.6
Change in 1 min. (dpm x 10^{-4})		- 134.3	+ 106.0	+ 35.9
Equivalent rate of change ($\mu gC.dm^{-2}.min.^{-1}$)		- 36.33	+ 28.66	+ 9.66
Ratio		4.00	3.15	1.06

*Means for 10 samples ± S.E.

is mainly responsible for the photorespiratory CO_2 evolution in vivo.

Measurements of photorespiration by the post-illumination CO_2 outburst assume that this change represents initially a continuation of photorespiration at the steady-state rate. While the change over 1 minute will not strictly provide an initial rate, and will be an underestimate, it was nearest a measure of the initial rate that could be easily obtained with techniques available. The rate of flow of carbon through glycine of 36.3 μgC. dm.$^{-2}$ min.$^{-1}$ (Table 6), equivalent to 8.3 mg. dm.$^{-2}$ h.$^{-1}$ CO_2 assimilated, is to be compared with 43.4 μgC. dm.$^{-2}$ min.$^{-1}$ (9.5 mg CO_2 dm.$^{-2}$ h.$^{-1}$) for the rate of carbon assimilation in 325 vpm CO_2 in this experiment. So even by this underestimate of carbon flow it seems that 84% of carbon assimilated is metabolized by way of glycine.

9. The effect of increasing period of uptake by wheat leaf segments of sodium 2-hydroxy 3-butynoate (SHB) and methyl 2-hydroxy 3-butynoate (MHB) on photosynthesis and incorporation of ^{14}C into various products of photosyntehsis

Wheat leaf segments were illuminated (2900 - 3000 f.c.) for 40, 90, 180 or 270 minutes in a gas stream containing 150 vpm $^{12}CO_2$ in 21% O_2. After these different preincubation periods the gas stream was changed and 150 vpm $^{14}CO_2$ in 21% O_2 was supplied for 15 minutes following which the leaf segments were bathed in boiling ethanol. Throughout the experimental treatments the cut basal ends of the leaf segments stood either in water (control), solutions of sodium hydroxybutynoate (2 x 10^{-4} M) or methyl hydroxybutynoate (2 x 10^{-4} M). Both substances caused increasing inhibition of photosynthesis the longer the leaf samples were supplied with the solutions (Fig. 15a). After 40 minutes SHB caused a 7% inhibition of

Fig. 15 (a & b). The effect of increasing periods of uptake of sodium hydroxy butynoate(SHB) and methyl hydroxy butynoate(MHB) by detached wheat leaves, on net photosynthesis and [^{14}C]-glycollate accumulation in 150 vpm $^{14}CO_2$.

Fig. 15(a) Net photosynthesis

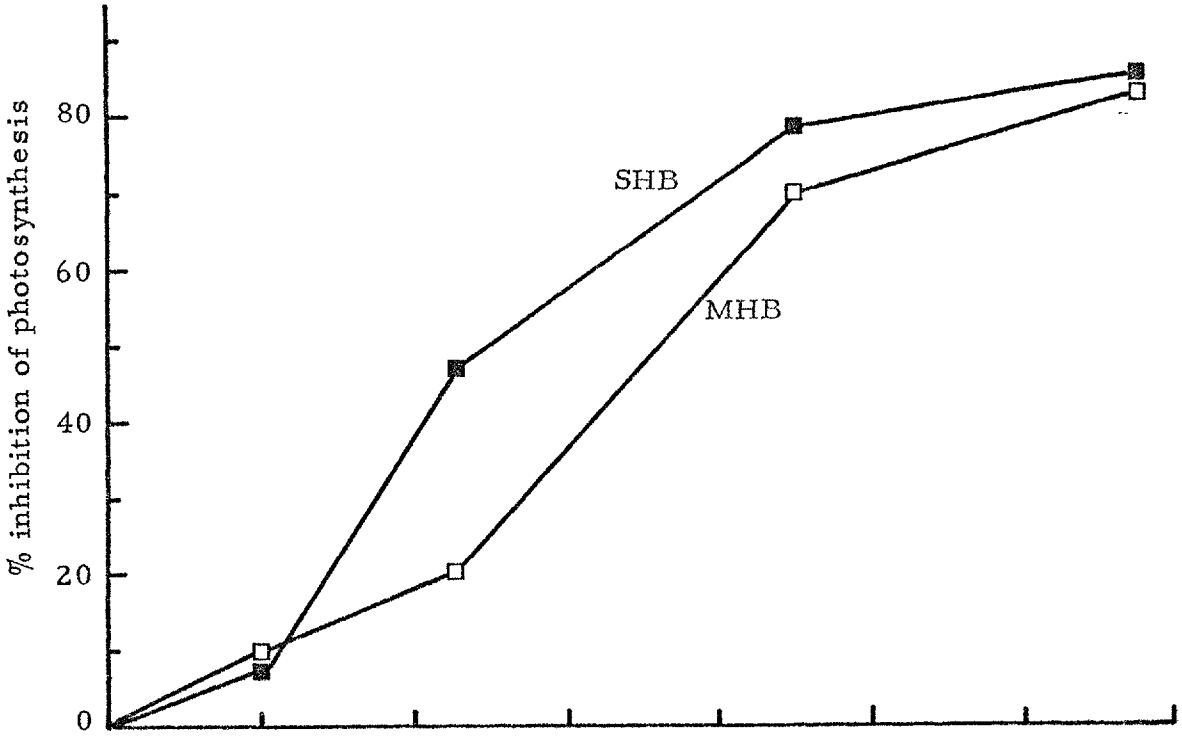


Fig. 15(b) [^{14}C] - Glycollate accumulation

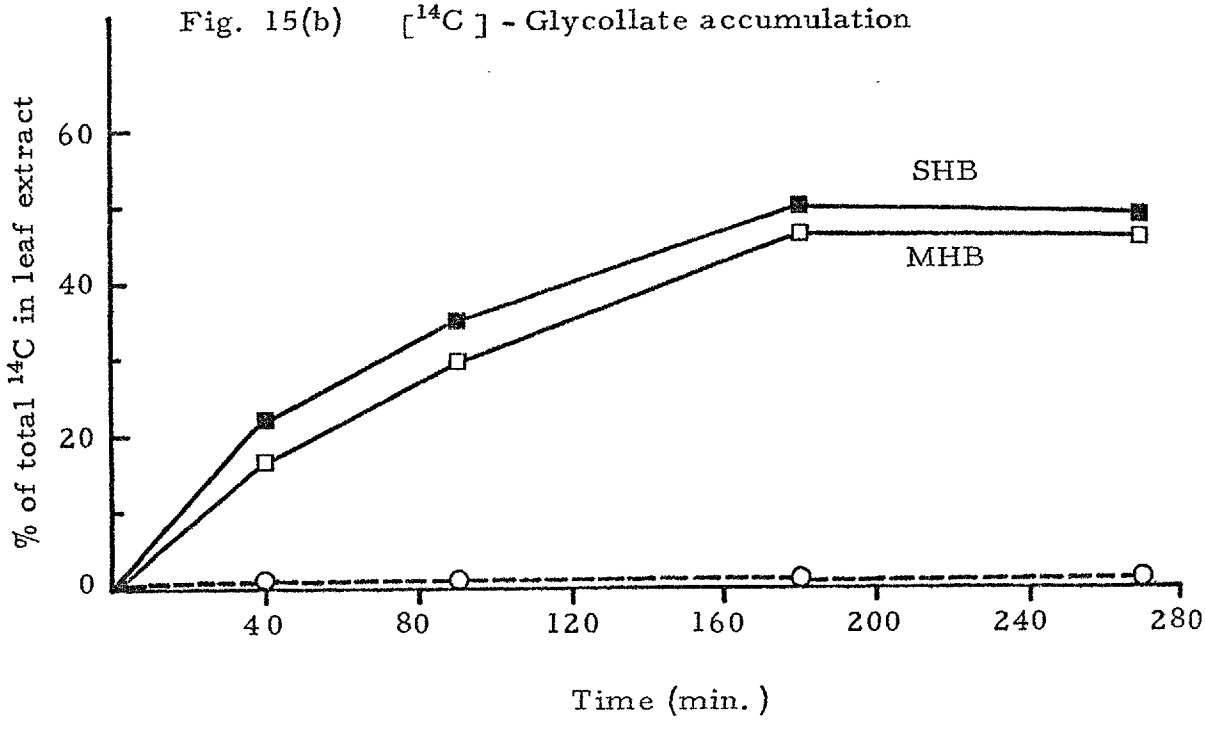
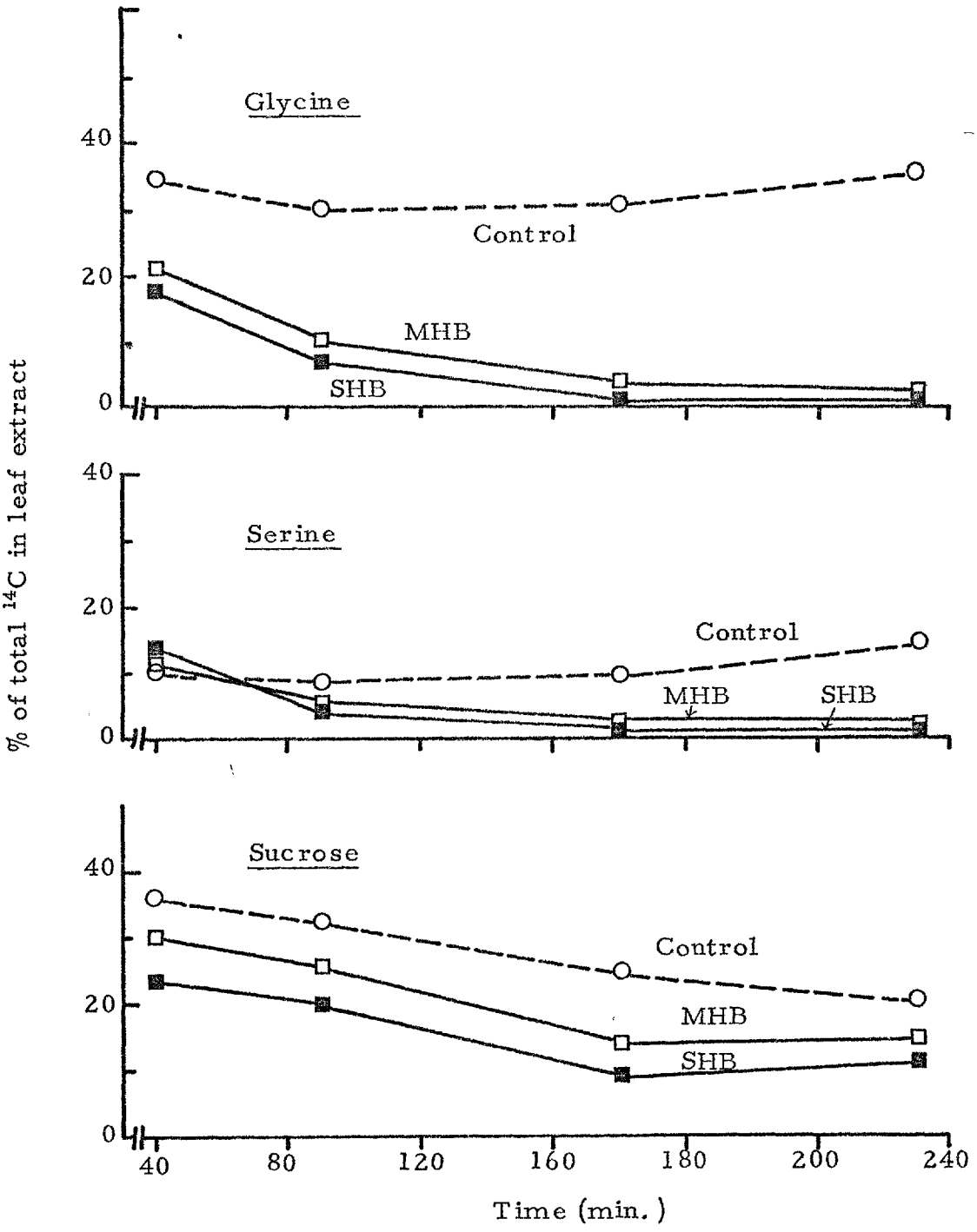


Fig. 15(c). The effect of SHB and MHB on incorporation of ^{14}C into glycine, serine and sucrose during photosynthesis in $^{14}CO_2$.



net photosynthesis whereas inhibition by MHB was about 9%, after 270 minutes the percent inhibition was about 80% for both. After 40 minutes inhibition of net photosynthesis by SHB was less than that caused by MHB but the accumulation of ^{14}C-glycollate was greater with the former than the latter compound (Fig. 15b). The greater accumulation of radioactivity in glycollate resulted in a decreased incorporation of label into glycine, serine and sucrose (Fig. 15c). The amount of ^{14}C in glycine was very much less than that of the control. ^{14}C-sucrose formation (Fig. 15c) was also in accordance with the degree of inhibition of incorporation of ^{14}C into the intermediates of the C-2 pathway; least being formed with SHB. The formation of other compounds such as alanine, glycerate, malate, glutamate, aspartate and sugar phosphates was not greatly affected although aspartate was slightly more radioactive after 40 and 90 minutes uptake of SHB or MHB compared to the control. Since SHB supplied through the cut basal edges of the leaves for 40 minutes brought about a greater accumulation of ^{14}C-labelled glycollate with a lesser inhibition of net photosynthesis compared to MHB, SHB was chosen as the most effective inhibitor of the two.

10. <u>The distribution of SHB in leaf segments after 40 minutes uptake, shown by its effects on products of photosynthesis in $^{14}CO_2$.</u>

In experiment 9 it was seen that SHB and MHB caused a 30-fold increase of ^{14}C in glycollate compared to the controls; yet a considerable amount of ^{14}C-label was still incorporated into intermediates of the glycollate pathway. It was necessary to ascertain the extent to which this apparent incomplete inhibition of carbon flow into the glycollate pathway resulted from failure of the inhibitors to reach the distal part of the leaf segments.

Table 7 The effect of distribution after 40 min. uptake of SHB in the basal, middle and apical regions of leaf segments, on the incorporation of radioactivity into products of photosynthesis

Compound	Apical		Middle		Basal	
	H_2O	SHB	H_2O	SHB	H_2O	SHB
Glycollate	2.1 (0.75)	56.5 (18.8)	2.2 (0.7)	56.4 (20.7)	2.5 (0.8)	56.9 (23.5)
Alanine	1.8 (0.5)	2.1 (0.7)	1.4 (0.5)	1.9 (0.7)	1.8 (0.5)	3.0 (1.3)
Glycerate	6.5 (2.2)	8.2 (2.7)	7.1 (2.3)	6.6 (2.4)	6.6 (2.2)	4.5 (1.8)
Glycine	96.1 (31.5)	55.4 (18.5)	85.8 (28.4)	49.2 (18.0)	83.4 (27.6)	37.5 (15.5)
Serine	31.3 (10.2)	40.8 (13.6)	36.4 (12.1)	39.8 (14.6)	38.0 (12.5)	30.3 (12.4)
Sucrose	118.3 (38.8)	88.4 (29.5)	126.5 (42.9)	78.3 (28.7)	123.5 (40.9)	70.1 (29.0)
Malate	7.0 (2.3)	3.5 (1.2)	4.2 (1.4)	2.7 (0.9)	4.7 (1.5)	3.6 (1.4)
Glutamate	4.7 (1.5)	2.1 (0.7)	4.1 (1.5)	1.6 (0.5)	4.1 (1.4)	2.5 (1.0)
Aspartate	3.9 (1.3)	10.9 (3.7)	4.5 (1.4)	8.9 (3.3)	4.4 (1.5)	6.9 (2.8)
Ph. esters	28.1 (9.5)	26.5 (9.4)	24.5 (8.2)	24.8 (8.7)	29.1 (9.5)	31.0 (9.4)
Unknowns	3.4 (1.1)	2.2 (1.0)	3.5 (1.1)	2.4 (0.9)	3.2 (0.9)	2.6 (1.0)
Total in extract	304.6	299.9	300.8	272.5	301.6	241.4
% inhibition of net Ps.		1.53		9.42		19.9

values in parenthesis as % of total ^{14}C in leaf extract

Leaf segments standing either in water (control) or SHB (2×10^{-4}M) were preincubated for 40 minutes at a light intensity of 2900 - 3000 f.c. in a gas stream containing 150 vpm $^{12}CO_2$ in 21% O_2. The atmosphere was then changed to 150 vpm $^{14}CO_2$ in 21% O_2 and after 15 minutes the leaf segments were removed and rapidly cut into apical, middle and basal segments which were killed and analysed separately.

Total ^{14}C assimilated and the distribution of radioactivity in various compounds formed by different portions of the leaf segments are presented in Table 7. In the control leaves the rate of net photosynthesis was similar in all three sections of the leaf segments, but in those treated with SHB net photosynthesis was inhibited by 20% in the basal, 9% in the middle and 1.5% in the apical portion compared to their controls. However SHB caused an equal accumulation of ^{14}C in glycollate in all three parts of the leaf but the basal segments contained least and the apical section the most ^{14}C in glycine, serine and sucrose. Taken together with the observation that photosynthesis was less in the basal region the results are consistent with earlier arrival of the inhibitor in the base section. However the considerable effect on glycollate throughout means that after 40 minutes the inhibitor is adequately distributed through the piece of leaf. With the method of application used SHB clearly gives an incomplete inhibition of glycollate oxidase.

11. Effect of SHB on the incorporation of radioactivity into various compounds during steady-state photosynthesis in 150 vpm $^{14}CO_2$ with 21% O_2 and the changes during subsequent metabolism in atmospheres containing 150 or 1000 vpm $^{12}CO_2$ and 21% O_2 without $^{14}CO_2$.

Leaf segments were preincubated for 40 minutes at a light intensity of 2900 - 3000 f.c. in an air stream containing 150 vpm $^{12}CO_2$ air. After

feeding 150 vpm $^{14}CO_2$ air for 15 minutes the gas stream was changed to 150 vpm $^{12}CO_2$ air or 1000 vpm $^{12}CO_2$ air. $^{14}CO_2$ evolved from these leaves was also estimated. Throughout the experimental period the leaves either stood in water (control) or in SHB (2×10^{-4} M).

Figs. 16a, 16b, 17a and 17b show the major result from this experiment. Without inhibitor the pattern of incorporation of radioactivity into glycine, serine, sucrose and phosphate esters during steady-state photosynthesis in 150 vpm $^{14}CO_2$ and the pattern of their redistribution in 150 vpm $^{12}CO_2$ or 1000 vpm $^{12}CO_2$ is consistent with the observations made previously (Figs. 8a and 8b). Radioactivity decreased rapidly from glycine in 1000 vpm $^{12}CO_2$. ^{14}C-serine metabolism was suppressed in 1000 vpm $^{12}CO_2$ particularly during the first 3 minutes of flushing. More $^{14}CO_2$ was evolved in 1000 than in 150 vpm $^{12}CO_2$ and this cannot be accounted for entirely by the decrease in glycine if only one carbon is expected in CO_2 for each four carbons lost from glycine.

As previously, without inhibitor there was little ^{14}C in glycollate at any stage in the treatments. However with SHB present there was a dramatic accumulation of glycollate during steady-state photosynthesis in $^{14}CO_2$ and less ^{14}C was incorporated into glycine and sucrose. Net photosynthesis was inhibited slightly (2 to 3%) after 40 minutes in the inhibitor. Unlike in the control, labelling of serine continued to increase during the entire 15 minute period in $^{14}CO_2$ and glycollate also was not apparently saturated with ^{14}C in this time. When the gas stream was changed to 150 vpm $^{12}CO_2$ the results show somewhat erratic changes (Fig. 16a) from which it is concluded that ^{14}C in glycollate decreases very slightly and ^{14}C in glycine and serine more definitely (Fig. 16a). With the change to 1000 vpm $^{12}CO_2$ (Fig. 17a) ^{14}C declined rapidly in both glycollate and glycine; ^{14}C in serine increased at first and then declined fairly slowly. More $^{14}CO_2$ was evolved in 1000 vpm $^{12}CO_2$ than

in 150 vpm $^{12}CO_2$ (Fig. 16a); in the former case, the amount might be mostly explained in terms of glycollate being metabolized through glycine to CO_2 and serine; in the latter recycling of ^{14}C from CO_2 and serine may be involved. The results can be readily explained if SHB only partly inhibited flow of ^{14}C from glycollate to glycine; that the rate of decrease of ^{14}C in glycine is much less in the presence of SHB than in its absence, the data are consistent with ^{14}C-glycine being made continuously from the ^{14}C-glycollate during the period in 1000 vpm $^{12}CO_2$.

When SHB was present the increase of radioactivity in sucrose during 10 minutes flushing in 1000 vpm $^{12}CO_2$ was greater than in its absence and glycollate must have been one of the compounds giving rise to the ^{14}C in sucrose. The glycollate was either converted to sucrose through glycine and serine as suggested above or by some other unknown route. It should be pointed out that less $^{14}CO_2$ was evolved, where, in the presence of SHB, the decrease of ^{14}C-glycine was smaller. This point is further illustrated where the period of preincubation in either water or the inhibitor was increased.

The leaf segments were preincubated for 90 minutes before photosynthesis in $^{14}CO_2$ began; but otherwise the treatment procedure was similar to that where the preincubation period was 40 minutes.

The results are illustrated in Figs. 18a, 18b, 19a and 19b. In the control the distribution of radioactivity during photosynthesis in $^{14}CO_2$ and during the subsequent metabolism in 150 or 1000 vpm $^{12}CO_2$ was qualitatively similar to those observed following preincubation for 40 minutes, but there were quantitative differences; for example there was a more pronounced lag in incorporation of ^{14}C into sucrose after preincubation for 90 minutes and less incorporation of ^{14}C into glycine by the end of the 15 minute period in $^{14}CO_2$.

Figs. 16 (a & b), 17 (a & b), 18 (a & b) and 19 (a & b). The effect of SHB on the incorporation of radioactivity into various compounds during steady-state photosynthesis in 150 vpm $^{14}CO_2$ and 21% O_2 and the changes which occurred during subsequent metabolism in atmospheres containing 150 or 1000 vpm $^{12}CO_2$ and 21% O_2 without $^{14}CO_2$.

Figs. 16 (a & b) and 17 (a & b). (40 min. preincubation period)

Glycollate, Glycine, Serine, Sucrose, $^{14}CO_2$, Glycerate and Phosphate esters.

Figs. 18 (a & b) and 19 (a & b). (90 min. preincubation period)

Glycollate, Glycine, Serine, Sucrose, $^{14}CO_2$, Glycerate and Phosphate esters.

Fig. 16(a) (40 min. preincubation)

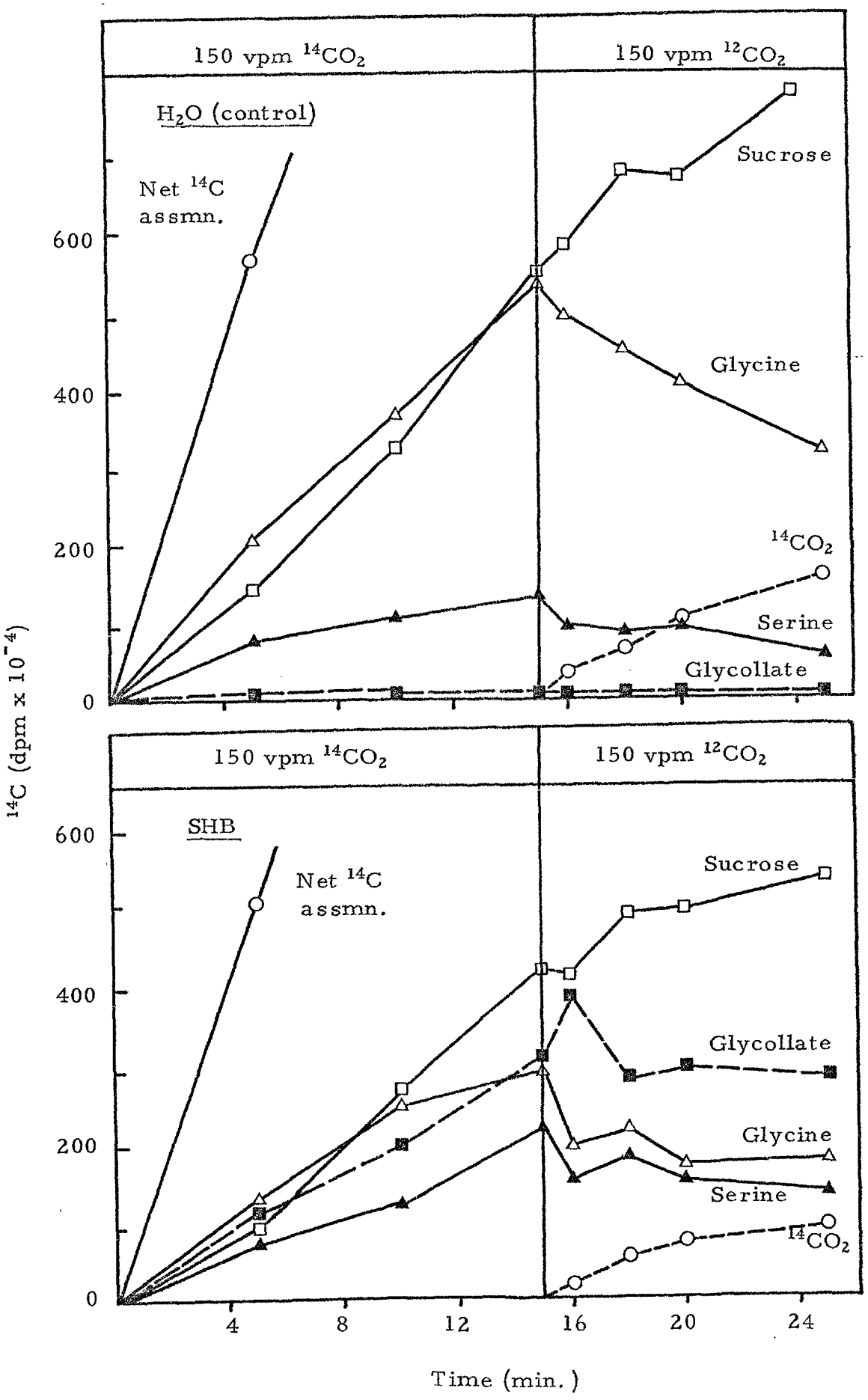


Fig. 17(a) (40 min preincubation)

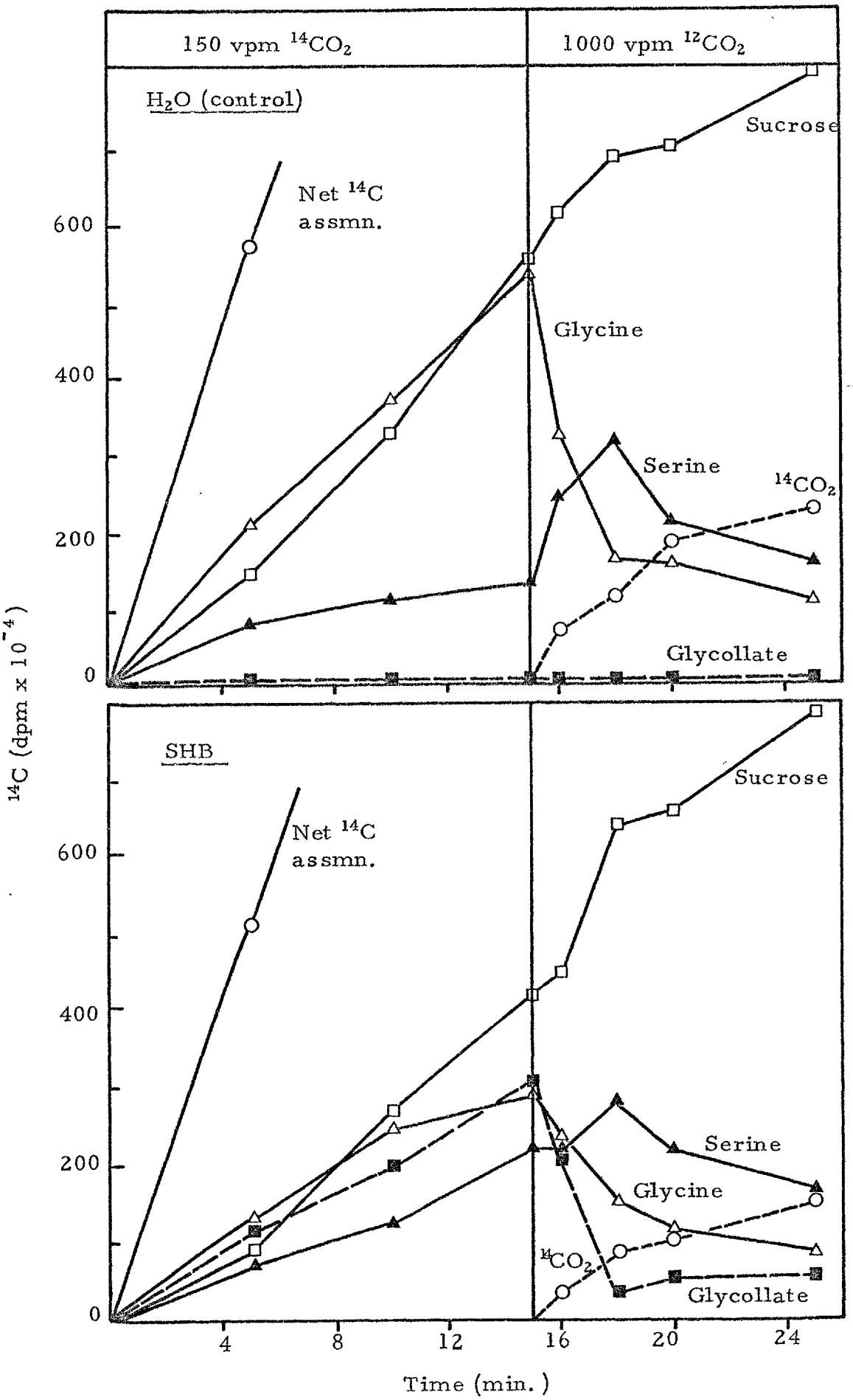


Fig. 16(b) (40 min. preincubation)

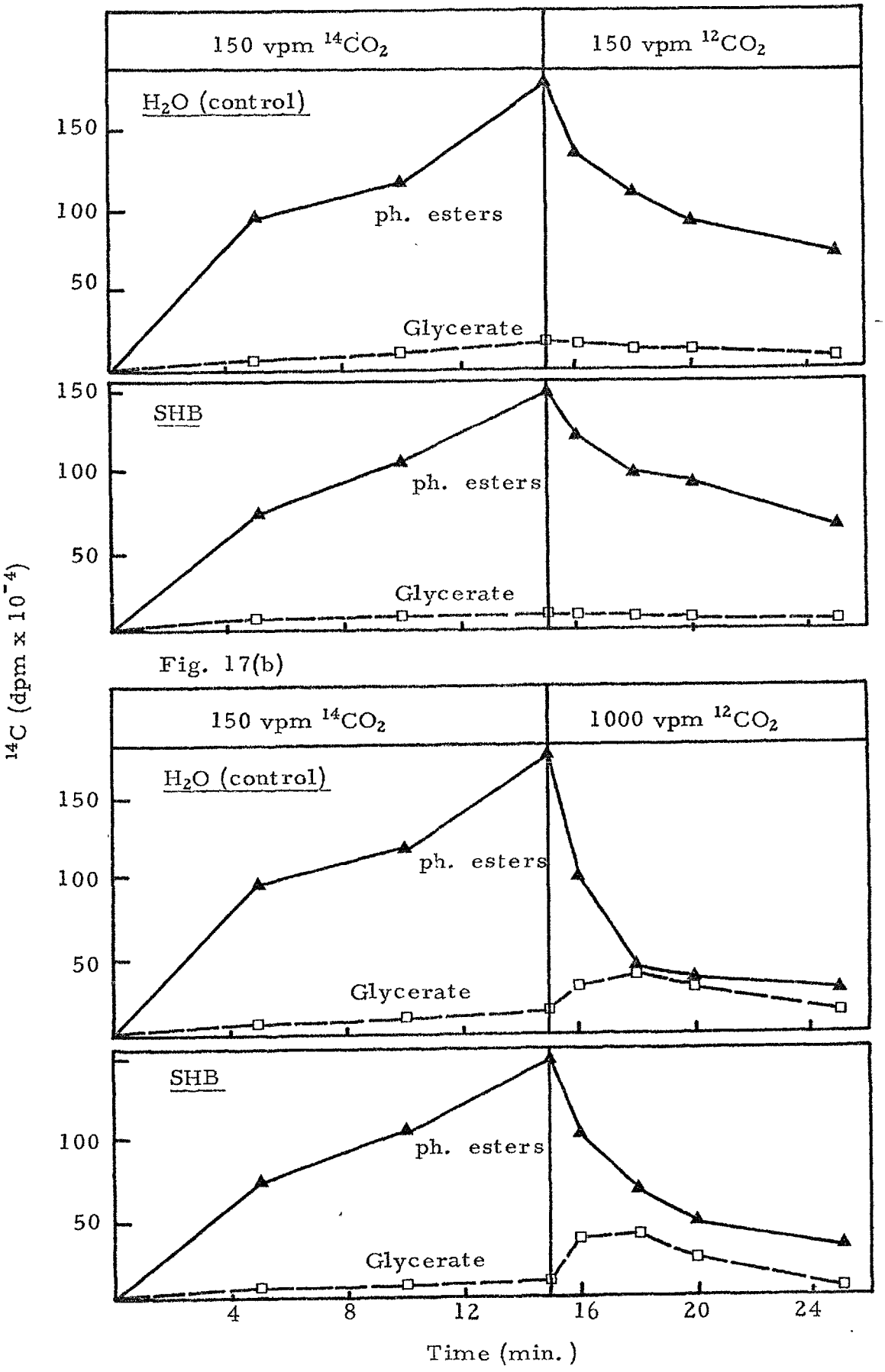


Fig. 17(b)

Fig. 18 (a) (90 min. preincubation)

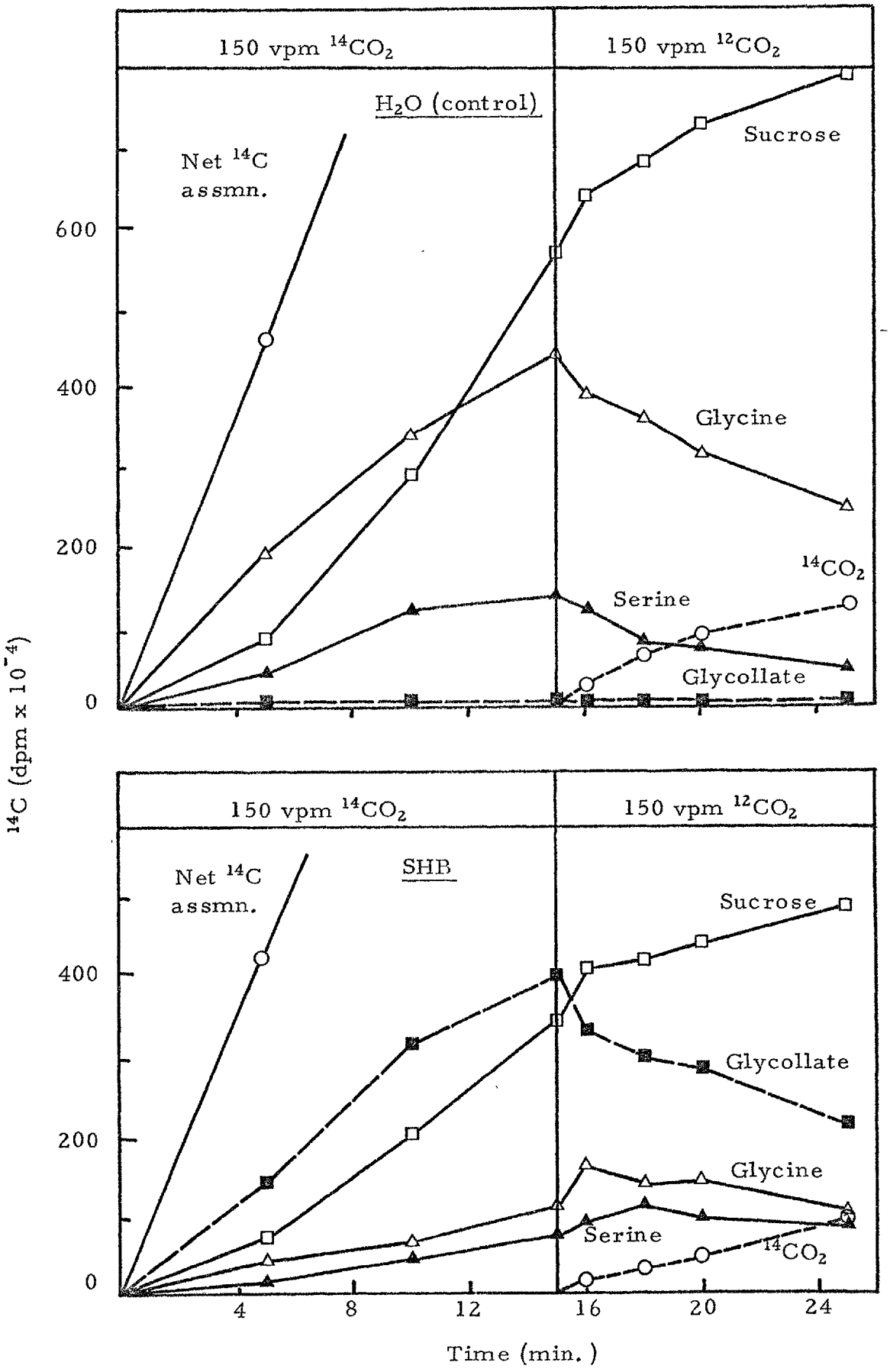


Fig. 19 (a) (90 min. preincubation)

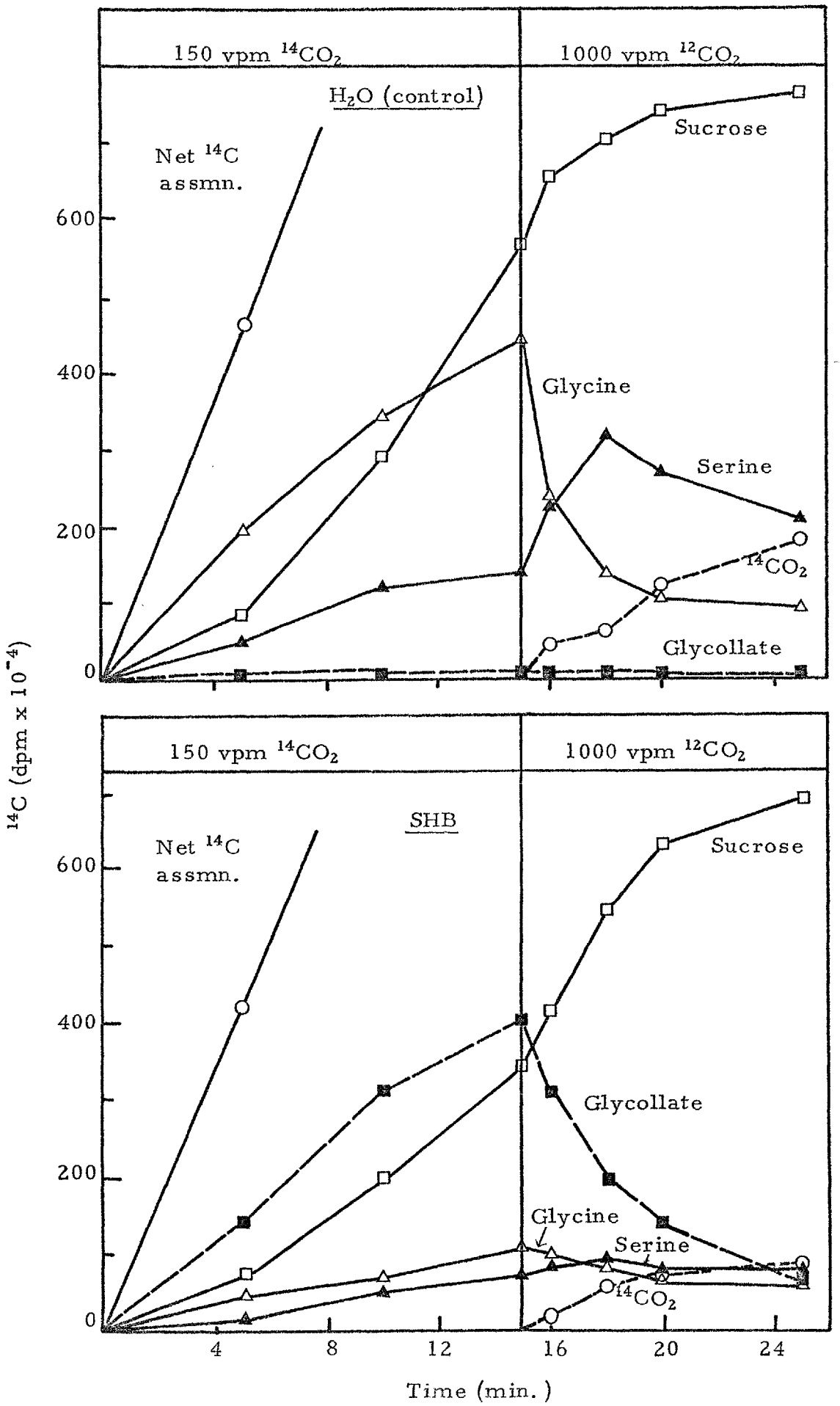


Fig. 18 (b). (90 min. preincubation)

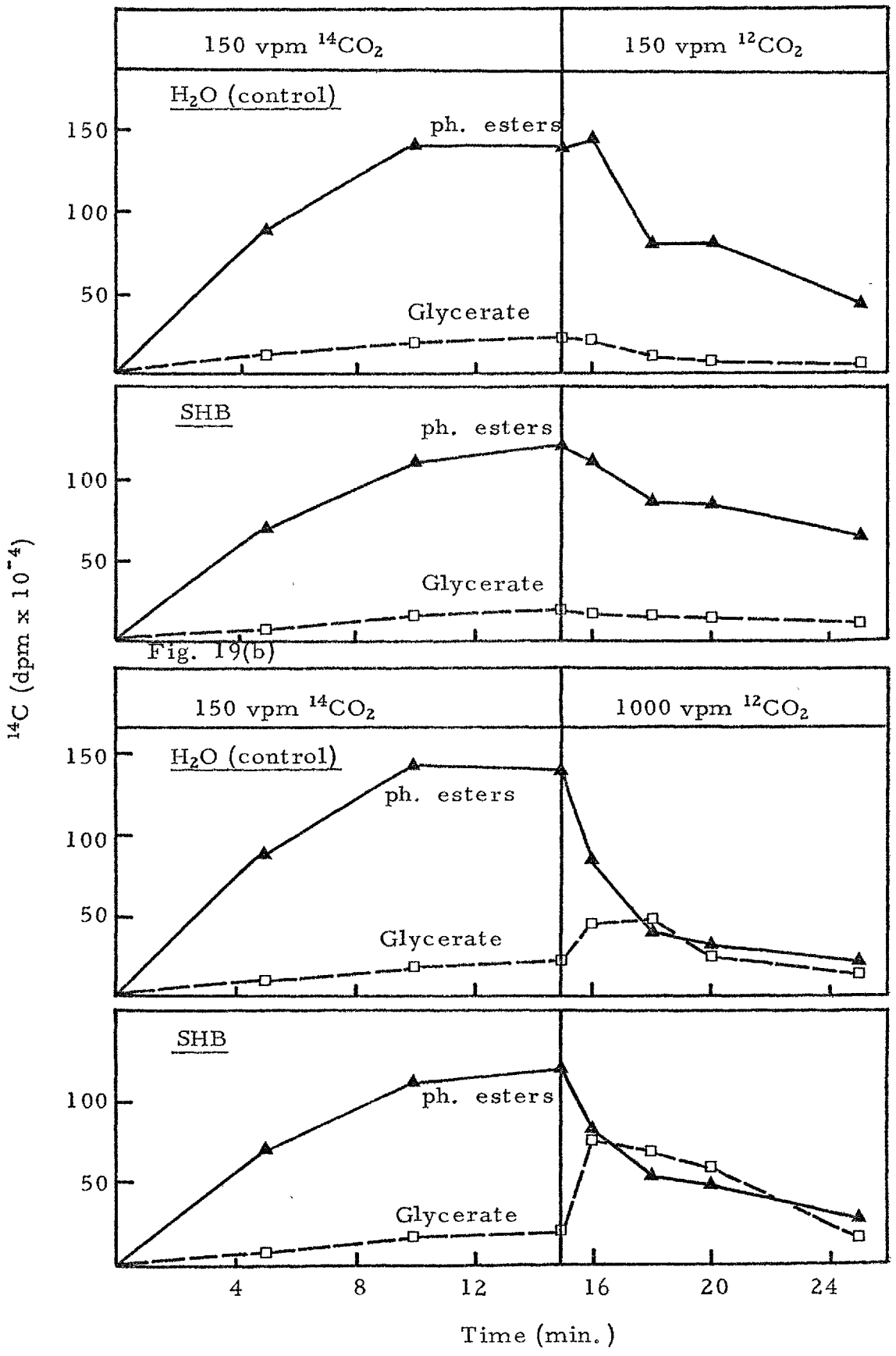


Fig. 19(b)

In the presence of SHB more marked differences were observed following 90 minutes compared to 40 minutes preincubation; photosynthesis was inhibited 15 - 20% and after 15 minutes uptake of $^{14}CO_2$ the most radioactivity was found in glycollate whereas when the preincubation period was 40 minutes most radioactivity was found in sucrose. Furthermore, there was 50% less ^{14}C in glycine in leaves given SHB for 90 minutes compared to 40 minutes, and during metabolism in 150 vpm $^{12}CO_2$ (Fig. 18a) radioactivity in glycine declined only very slowly even after the first minute. As following uptake of SHB for 40 minutes, the slow decrease of ^{14}C-glycine may be explained by a continuous supply of ^{14}C from glycollate which in this case nearly balances flow of ^{14}C out of glycine. Another factor may be that the pool of glycine does not approach saturation with ^{14}C in 15 minutes because of the large pool of unlabelled glycollate between it and $^{14}CO_2$ caused by the prolonged exposure to the inhibitor. In 150 vpm $^{12}CO_2$ a considerable amount of recycling of $^{14}CO_2$ and ^{14}C-serine for photorespiration takes place as evidenced by the slower decrease of radioactivity from glycollate (Fig. 18a) compared to that in 1000 vpm $^{12}CO_2$ (Fig. 19a); the rate of decrease of ^{14}C in glycollate in 1000 vpm $^{12}CO_2$ being taken as the rate unaffected by recycling, for the reasons pointed out earlier.

In SHB treated leaves during further metabolism in 1000 vpm $^{12}CO_2$ the predominant decrease of radioactivity was in glycollate (Fig. 19a). Less $^{14}CO_2$ was evolved than in the control but more ^{14}C-sucrose was formed. Nevertheless, the decrease of radioactivity from glycine was very small compared to the control. As before this could be explained in terms of the conversion of the ^{14}C-glycollate into glycine. However, ^{14}C is converted so rapidly from glycollate to sucrose that ^{14}C-sucrose is formed faster than during the previous period of steady-state photosynthesis. Because of the increased availability of $^{12}CO_2$ to the

chloroplasts an acceleration of the incorporation of ^{14}C into sucrose seems unlikely to have involved metabolism of carbon from glycollate by any pathway involving a pool of an intermediate involved in the chloroplast in photosynthetic assimilation. The sensible interpretation is that under these conditions sucrose is being made from glycollate by a pathway taking place entirely in the cytoplasm. Because ^{14}C-serine, in the presence of SHB, did not increase or decrease as rapidly as in the control and less $^{14}CO_2$ was evolved it is not certain how much of the ^{14}C-glycollate was converted to sucrose by the pathway in which glycine and serine were the intermediates. An experiment involving both INH, which inhibits the conversion of glycine to serine, and SHB may solve this problem.

12. The effect of different concentrations of potassium glycidate on photosynthesis and incorporation of radioactivity into various compounds

Illuminated (2900 - 3000 f.c.) wheat leaf segments were preincubated in a stream of air containing 150 vpm $^{12}CO_2$ for 40 minutes. The air stream was then changed and 150 vpm $^{14}CO_2$ in 21% O_2 was supplied for a further 3 minutes. Throughout the experiment the cut basal ends of the leaves were immersed in water (control) or in solutions of potassium glycidate of different strengths (0.02 mM, 0.2 mM 2.0 mM and 20 mM).

Fig. 20a shows that the rate of net photosynthesis was not affected at the three lower concentrations of the inhibitor (0.02, 02, 2.0 mM) but was drastically decreased at 20 mM. When the inhibitor concentration was high (2.0 and 20 mM) more ^{14}C-phosphate esters accumulated and less ^{14}C-glycine (Fig. 20b). The percentage radioactivity in serine also decreased but that in sucrose increased. At higher concentrations of the inhibitor, also more ^{14}C accumulated in glycollate suggesting that

Fig. 20 (a & b). The effect of different concentrations of potassium glycidate on photosynthesis and incorporation of radioactivity into various compounds.

Fig. 20(a) Net Photosynthesis

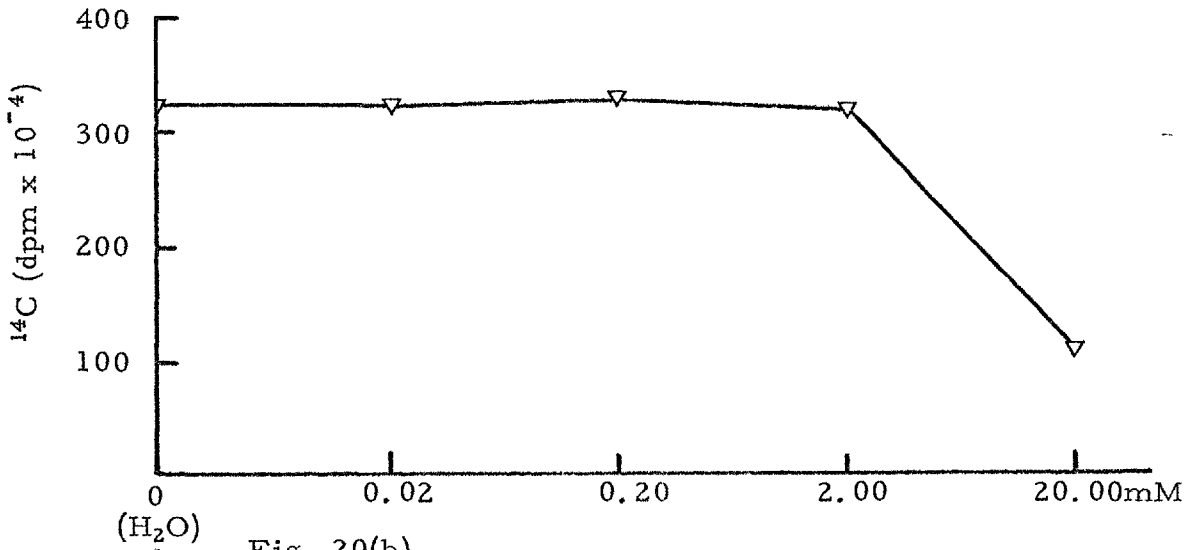
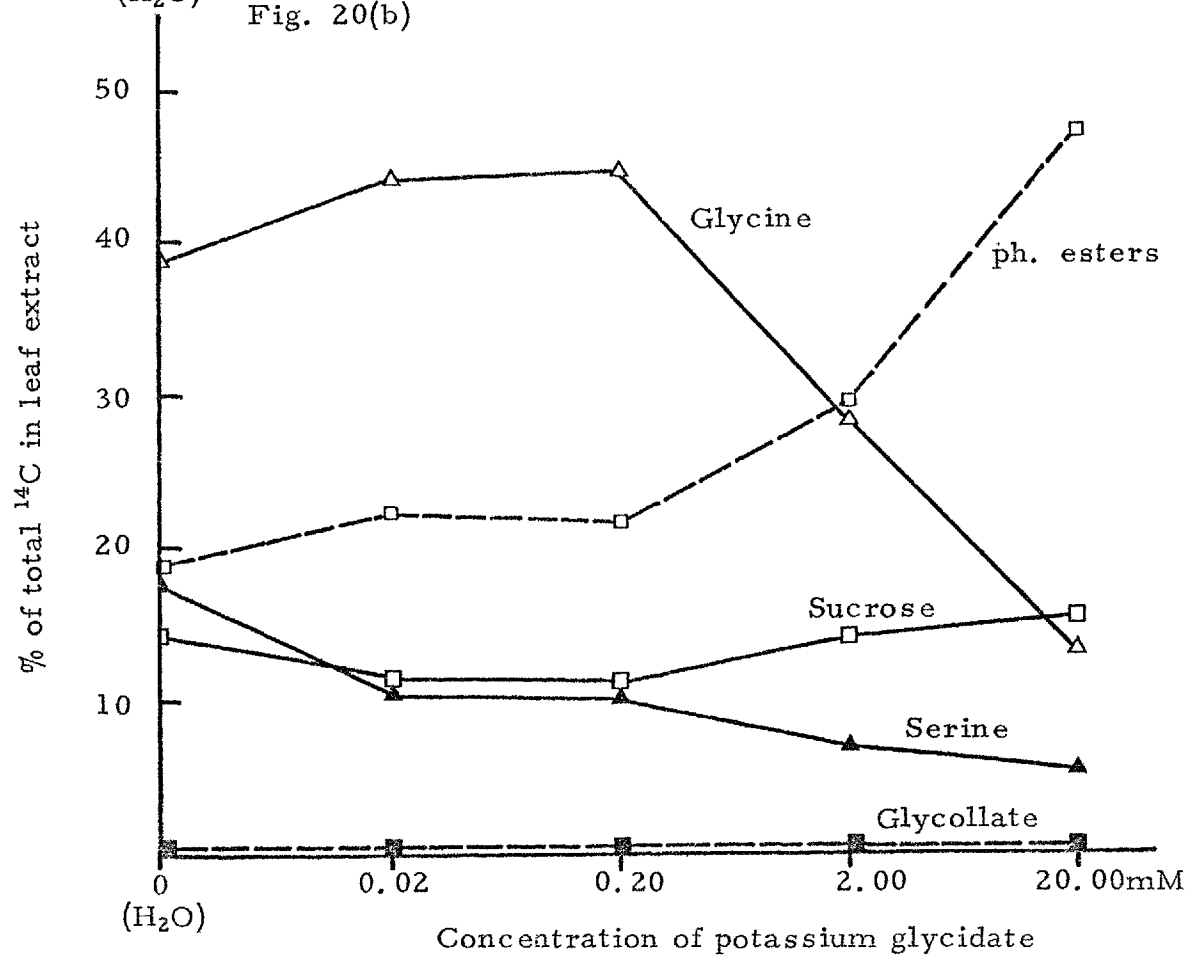


Fig. 20(b)



glycollate oxidase might be inhibited. These results differ from those of Zelitch (1974) who reported that glycollate synthesis was inhibited by 20 mM potassium glycidate in tobacco leaves and photosynthesis stimulated. If glycollate synthesis were inhibited phosphoglycollate might be expected to accumulate. The position of phosphoglycollate on the developed 2-dimensional chromatograms was determined and no ^{14}C compound was found in this position in any of the extracts from leaves treated with potassium glycidate but ^{14}C accumulated slightly in glycine at lower concentrations and considerably in all phosphate esters except PGA at the higher concentrations of the inhibitor and sucrose synthesis was stimulated. Therefore the results are more consistent with inhibition of phosphoglycollate formation possibly through an effect on RUDP oxygenase activity. If this is so then it is not surprising that RUDP carboxylase activity is also affected because the same protein probably catalyses both the oxygenation and the carboxylation. Although net photosynthesis was decreased by 20 mM potassium glycidate this concentration was selected for further experiments since it did decrease incorporation of radioactivity into glycine and serine.

13. The effect of potassium glycidate on photosynthesis and incorporation of ^{14}C into various compounds by wheat leaf segments at 28°C

At 20°C (experiment 12) potassium glycidate at 4 different concentrations (0.02, 02, 2.0 and 20.0 mM) did not significantly stimulate photosynthesis by wheat leaves (Fig. 20a). However it was with tobacco leaves at 28°C that Zelitch (1974) had shown that photorespiration was inhibited by about 40% and net photosynthesis was stimulated by some 40%, following treatment with 20 mM potassium glycidate. An experiment with wheat leaves was therefore performed at 28°C rather than at 20°C.

Fig. 21 (a & b). The effect of potassium glycidate on photosynthesis and incorporation of ^{14}C into various compounds by wheat leaf segments at 28°C.

Fig. 21 (a) Net photosynthesis.

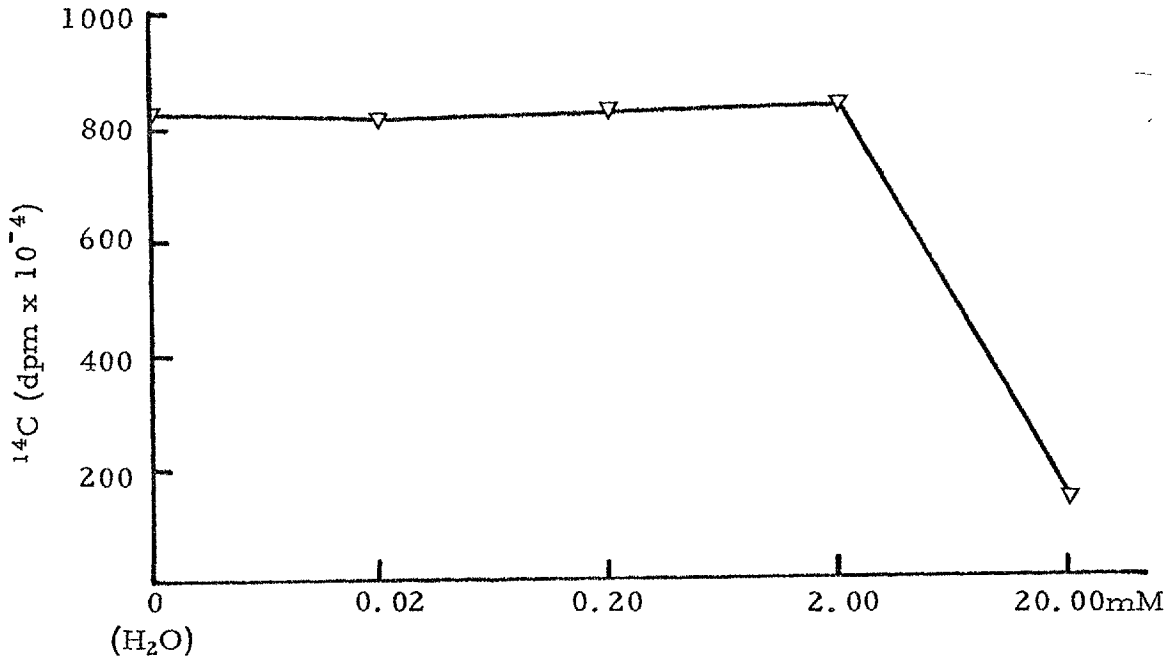
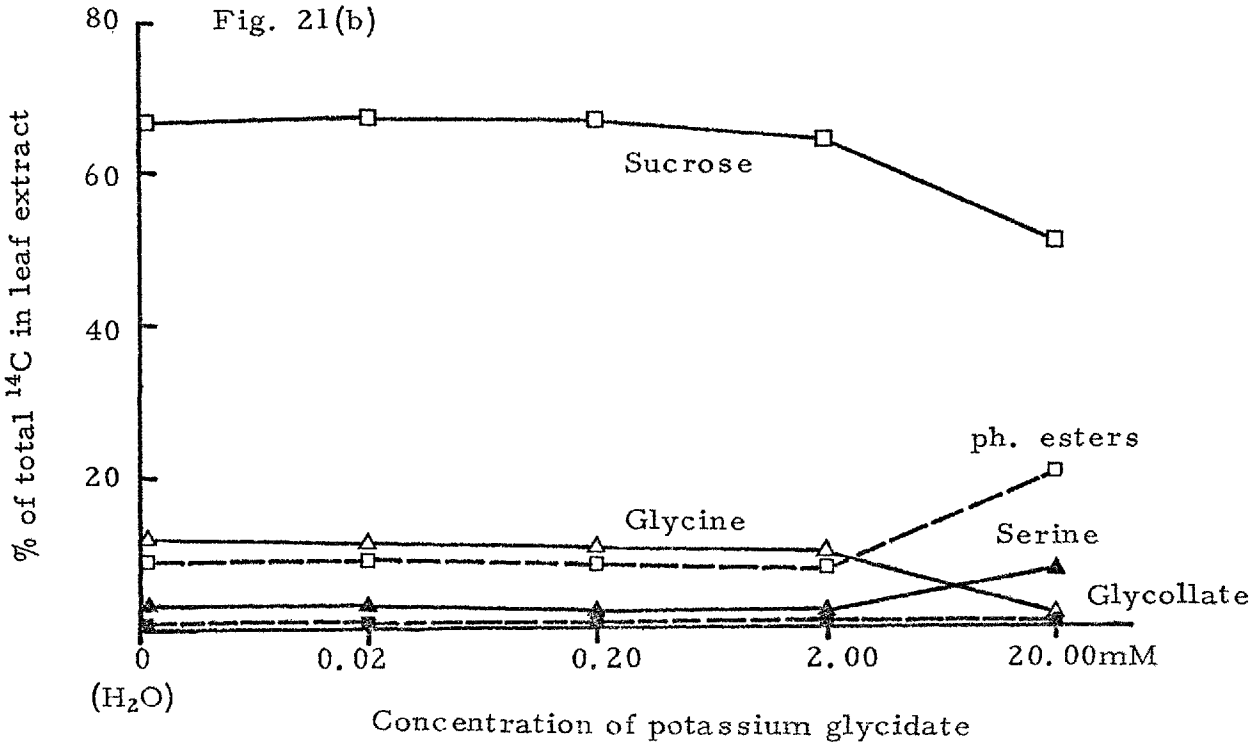


Fig. 21(b)



Illuminated (2900 - 3000 f.c.) wheat leaf segments were preincubated in a stream of gas containing 150 vpm $^{12}CO_2$ in 21% O_2, for 40 minutes. The gas stream was then changed and 150 vpm $^{14}CO_2$ (1 μci/μmole) in 21% O_2 was supplied for a further period of 15 minutes. The temperature was maintained at 28° ± 0.5°C throughout the experiment.

The results are illustrated in Figs. 21a and 21 b. The curve for net photosynthesis shows that the response to glycidate is similar to that at 20°C. Although losses of assimilated CO_2 due to photorespiration are expected to be higher at 28°C than at 20°C there was no stimulation of net photosynthesis caused by potassium glycidate and in contrast to Zelitch's results with tobacco leaf discs, there was a drastic inhibition of photosynthesis when the concentration of the inhibitor was 20 mM. Radioactivity accumulated slightly in phosphate esters and with increase of inhibitor concentration decreased in glycine, indicating a decreased flow of carbon from phosphate esters into the C-2 pathway but no other significant effect was observed; at 28°C the proportion of ^{14}C in sucrose decreased with increased concentration of inhibitor.

14. Effect of potassium glycidate on the incorporation of radioactivity into various compounds during steady-state photosynthesis in 150 vpm $^{14}CO_2$, 21% O_2 and the changes of ^{14}C during subsequent metabolism in 150 vpm $^{12}CO_2$ with 21% O_2.

Segments of wheat leaf were preincubated in 150 vpm $^{12}CO_2$, 21% O_2 for 40 minutes at a light intensity of 2900 - 3000 f.c. The gas stream was then changed so as to supply 150 vpm $^{14}CO_2$ in 21% O_2; after 15 minutes the atmosphere was changed to 150 vpm $^{12}CO_2$ in 21% O_2. The ^{14}C in photosynthetic products and in the $^{14}CO_2$ evolved was estimated. Throughout

Fig. 22 (a & b). Effect of potassium glycidate on the incorporation of radioactivity into various compounds during steady-state photosynthesis in 150 vpm $^{14}CO_2$ and the changes of ^{14}C during subsequent metabolism in 150 vpm $^{12}CO_2$.

Fig. 22(a)

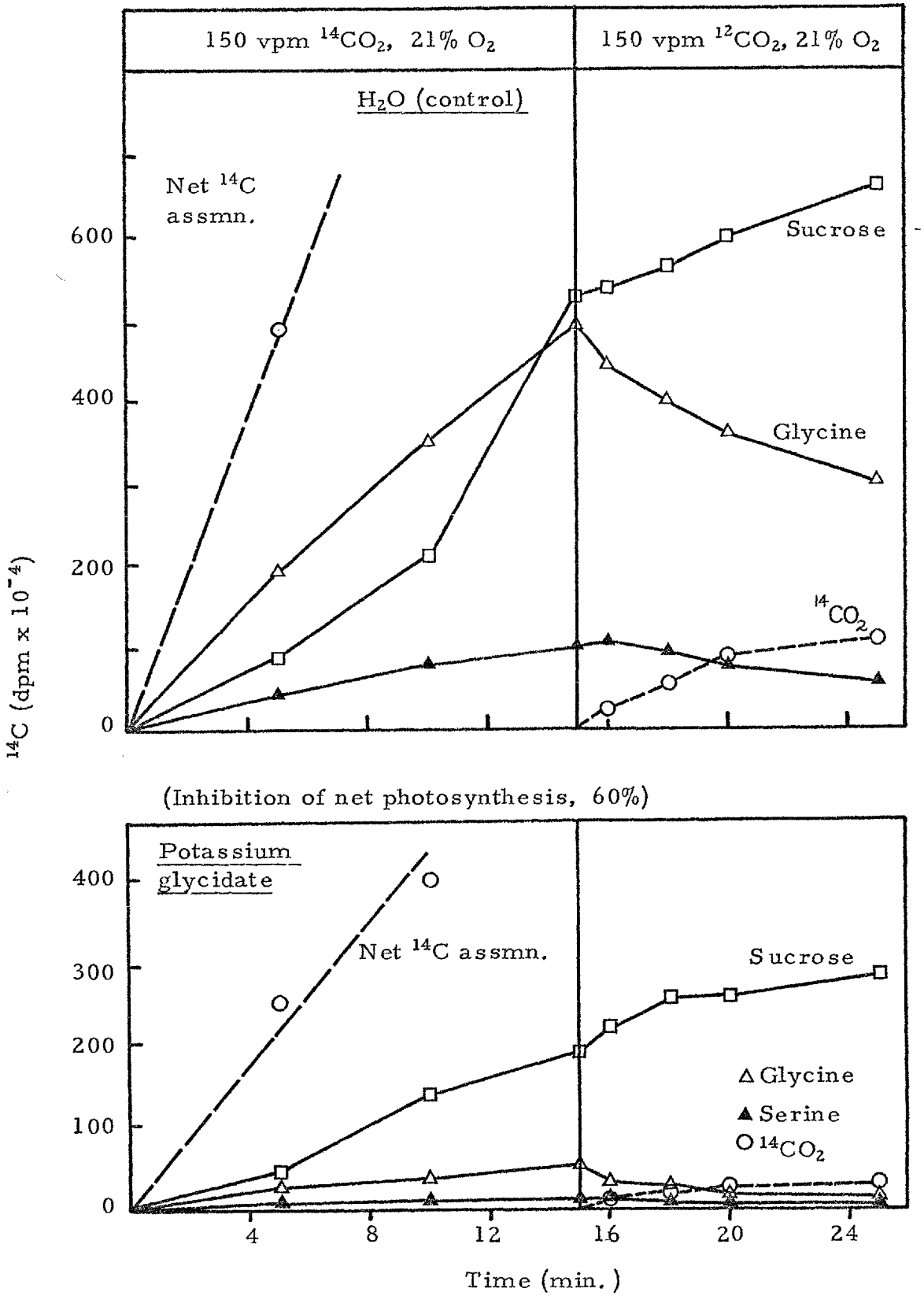
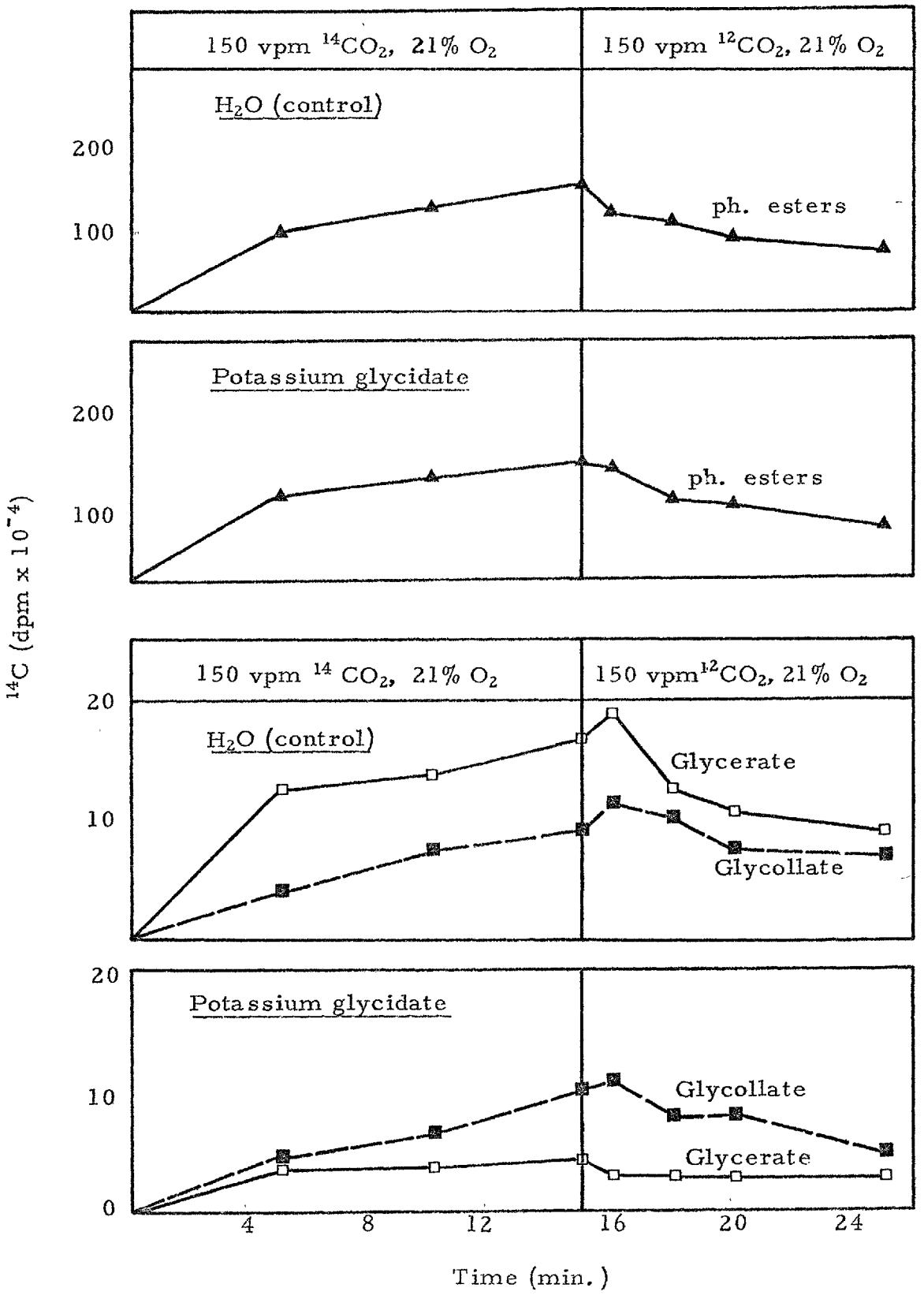


Fig. 22 (b)


150 vpm $^{14}CO_2$, 21% O_2
150 vpm $^{12}CO_2$, 21% O_2
H_2O (control)
ph. esters
Potassium glycidate
ph. esters
150 vpm $^{14}CO_2$, 21% O_2
150 vpm $^{12}CO_2$, 21% O_2
H_2O (control)
Glycerate
Glycollate
Potassium glycidate
Glycollate
Glycerate
^{14}C (dpm x 10^{-4})
Time (min.)

the experiment the cut basal edges of the leaf segments were dipped in water (control) or in a solution of potassium glycidate (2×10^{-2} M) and the temperature was 20°C.

Figs. 22a and 22b illustrate the results of this experiment. Potassium glycidate inhibited net photosynthesis to the extent of 60% (Fig. 22a). Incorporation of radioactivity into glycine and serine was decreased by 88 and 89% respectively. This suggests that the decreased incorporation of the ^{14}C-label into glycine and serine was not only due to decreased photosynthetic rate but to inhibition of the flow of carbon into the glycollate pathway. During flushing in 150 vpm $^{12}CO_2$ radioactivity from glycine and serine decreased; some $^{14}CO_2$ was evolved and new ^{14}C-sucrose was synthesized. It is clear that potassium glycidate did not completely inhibit metabolism of carbon through the glycollate pathway.

15. Effect of SHB and potassium glycidate on the rate of net photosynthesis and distribution of ^{14}C among the products of steady-state photosynthesis in 1000 vpm $^{14}CO_2$ with 21% O_2 —

Wheat leaf segments were preincubated in 1000 vpm $^{12}CO_2$, 21% O_2 for 40 minutes at a light intensity of 2900 - 3000 f.c. The gas stream was then changed to one of similar composition but containing $^{14}CO_2$ (Specific activity, 0.5 μci/μmole). Samples were taken after photosynthesis in $^{14}CO_2$ for 5, 10 and 15 minutes.

The distribution of radioactivity in glycollate, glycine, serine, sucrose and sugar phosphates are illustrated in Figs. 23a and 23b. During steady-state photosynthesis in 1000 vpm $^{14}CO_2$ the amount of ^{14}C in glycine is smaller than in serine in contrast to the situation following 15 minutes photosynthesis in 150 or 325 vpm $^{14}CO_2$; in 80 vpm $^{14}CO_2$, label in the two compounds was almost equal. SHB caused some accumulation of radioactive glycollate, but this was less as a proportion of the total

Fig. 23 (a & b). The effect of SHB and potassium glycidate on the rate of net photosynthesis and distribution of ^{14}C among the products of steady-state photosynthesis in 1000 vpm $^{14}CO_2$.

Fig. 23 (a)

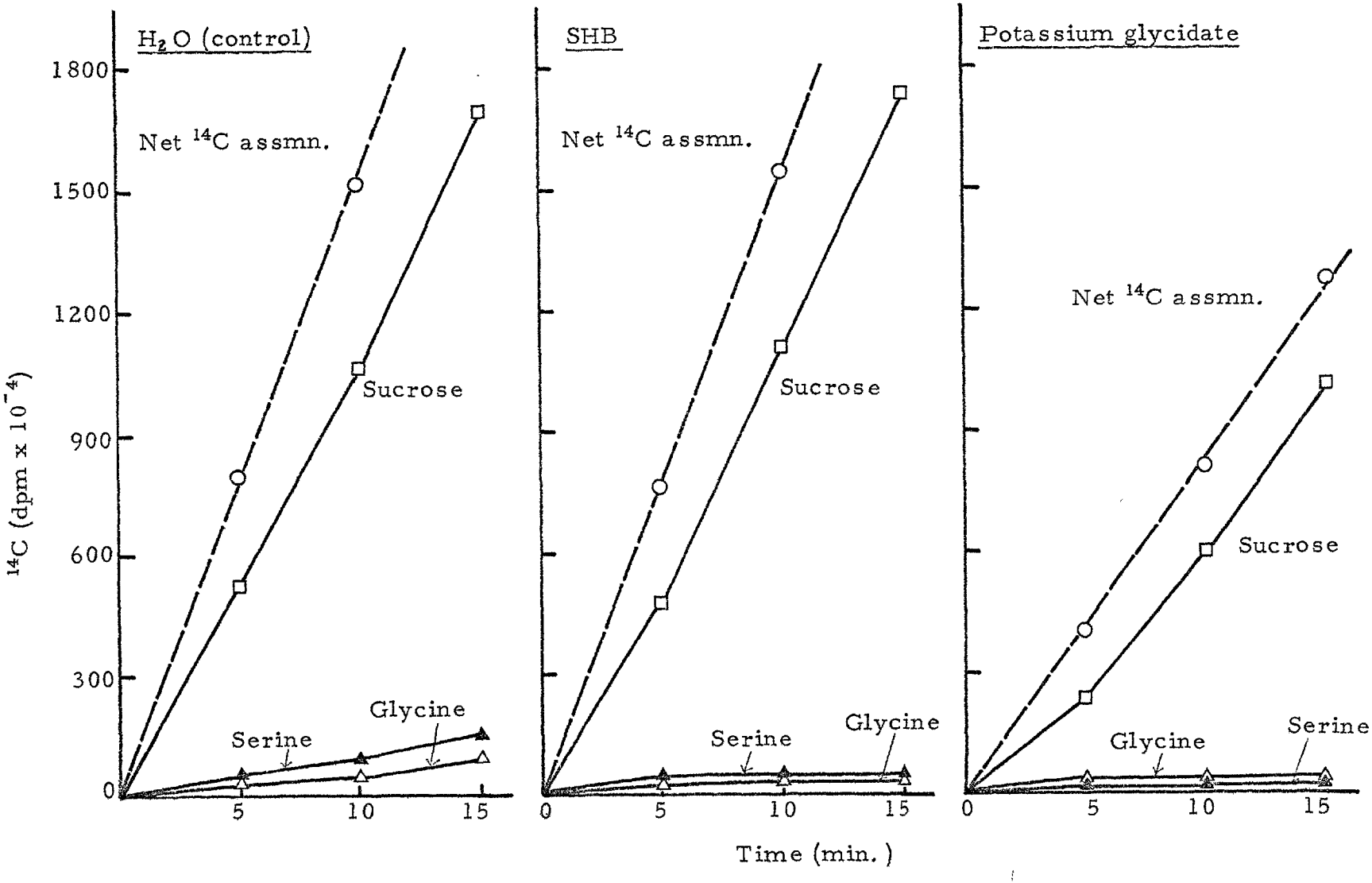

H₂O (control)
SHB
Potassium glycidate
Net ¹⁴C assmn.
Sucrose
Glycine
Serine
¹⁴C (dpm x 10⁻⁴)
1800
1500
1200
900
600
300
0
5
10
15
Time (min.)

Fig. 23 (b.1). Glycollate

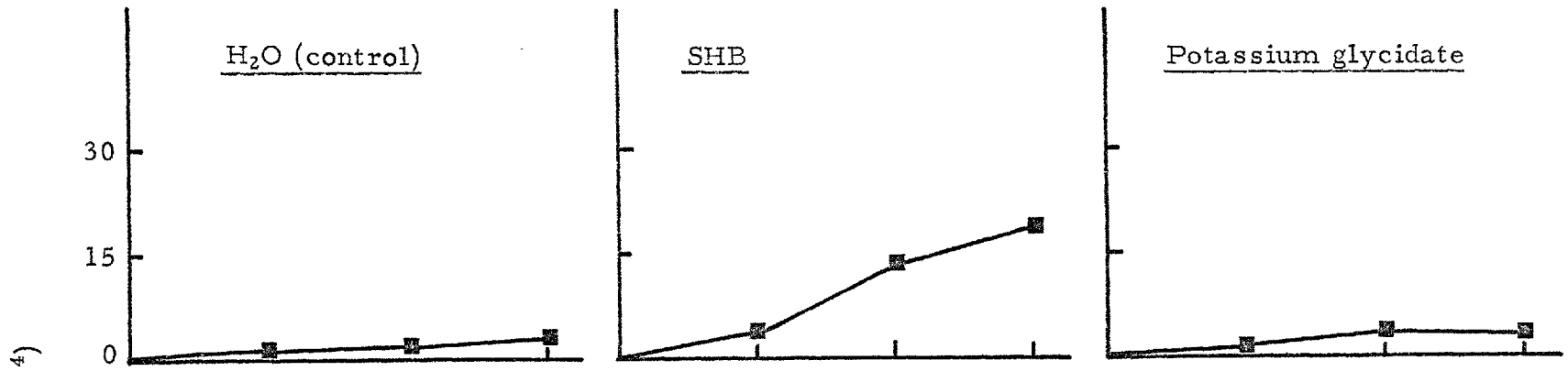
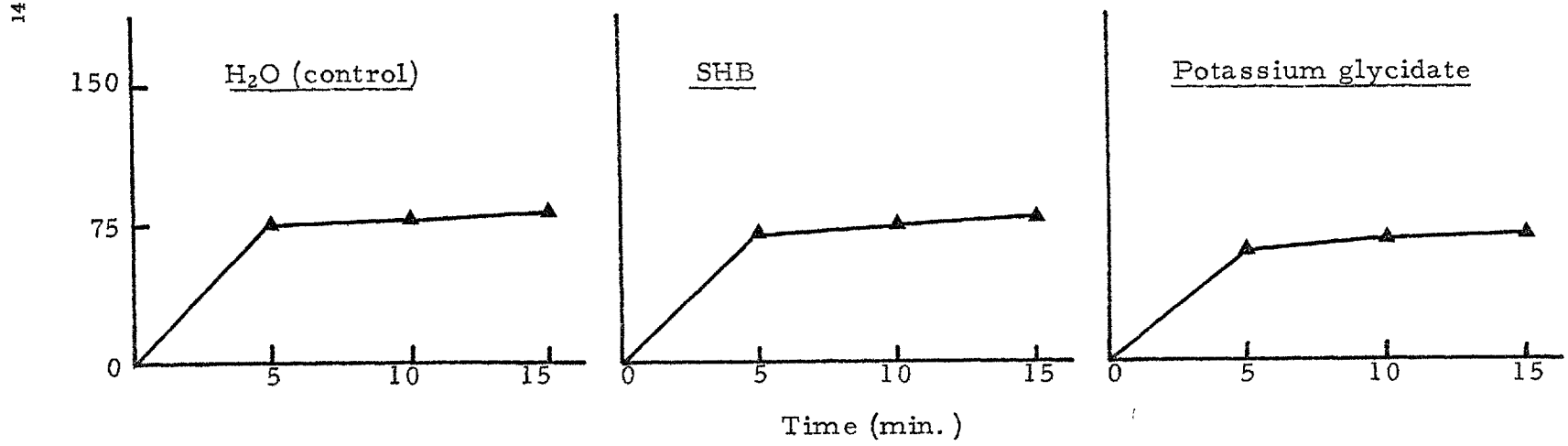


Fig. 23 (b.2). Phosphate esters



^{14}C (dpm x 10^{-4})

^{14}C assimilated than was observed after 15 minutes photosynthesis in 150 vpm $^{14}CO_2$. This observation is consistent with there being a decreased flow of carbon through the glycollate pathway at high CO_2 concentrations (Lee and Whittingham, 1974). SHB however, did not affect the rate of net photosynthesis; with only 150 vpm CO_2 (Fig. 15a) a slight inhibition was found. This difference may indicate that the glycollate pathway is less important in photosynthesis with 1000 vpm CO_2.

Potassium glycidate in 1000 vpm CO_2 as in 150 vpm CO_2, inhibited photosynthesis (by 43%). The total ^{14}C in phosphate esters and glycollate was decreased less than ^{14}C in sucrose, glycine and serine; this is consistent with the previous conclusions that glycidate inhibits conversion of phosphate esters to phosphoglycollate with some inhibition of glycollate oxidation and inhibition of RUDP carboxylase.

16. The effect of INH, SHB and potassium glycidate on photometabolism of D-glyceric acid-U-^{14}C by detached wheat leaves in 325 vpm CO_2 air.

Leaf segments were preincubated in 325 vpm CO_2 air for 40 minutes, at a light intensity of 3000 - 3100 f.c. while the basal cut edges were dipping in water (control), INH (20 mg/ml), SHB (2×10^{-4} M) or potassium glycidate (2×10^{-2} M). The leaf bases were then transferred into a solution of D-glycerate-U-^{14}C (1.5 µci, 0.5 µmoles in 0.05 ml). Segments were illuminated in the same atmosphere for a further 45 minutes before extraction and analysis. Only three leaf segments per sample were employed in this experiment.

The products of photometabolism of the exogenously supplied ^{14}C-glycerate are shown in Table 8. ^{14}C-glycerate was metabolized chiefly to sucrose, glycine and serine. The amount of added glycerate that was metabolized was decreased compared to the control by the presence

Table 8 The effect of INH, SHB and potassium glycidate on photometabolism of D-Glyceric acid-U-^{14}C by detached wheat leaves in 325 vpm CO_2 in air

	Percentage of ^{14}C metabolized			
Compound	Control	INH	SHB	pot. glycidate
Glycollate	0.98	9.61	4.89	3.08
Alanine	0.19	1.02	0.79	0.34
Glycine	9.12	23.45	7.29	2.14
Serine	2.34	10.34	5.51	7.92
Sucrose	77.49	44.93	72.58	70.83
Malate	4.52	4.51	3.85	5.08
Aspartate	1.99	0.69	2.46	3.74
Ph. esters	2.81	4.39	2.55	5.90
Origin	0.32	0.61	0.19	0.71
Total ^{14}C in leaf extract (dpm x 10^{-4})	72.06	61.59	73.45	79.23
% not metabolized (Glycerate)	16.50	75.65	16.11	47.90

of INH and potassium glycidate. Less of the ^{14}C-glycerate that was metabolized appeared in sucrose in the presence of INH than with the other treatments and control. Thus metabolism of glycerate into sucrose was inhibited by INH. The results with INH are consistent with those of Reimer (1970) who observed that INH inhibited the metabolism of glycerate. However, recycling of the ^{14}C from glycerate through the glycollate pathway may not be severely affected because, of the ^{14}C metabolized, a greater percentage was found in glycollate, glycine, and serine than in the control; incorporation into sucrose was more inhibited than recycling. This suggests inhibition by INH of a reaction in the pathway between sugar phosphates and sucrose. It must however be pointed out that the accumulation of glycollate and particularly glycine is also a result of decreased metabolism of glycine in the presence of INH. It has been shown (Bird et al., 1972) that the conversion of glycine to serine is coupled to phosphorylation of ADP to ATP and Waidyanatha et al. (1974) have suggested this ATP may be necessary for synthesis of sucrose from glycerate in the cytoplasm. In the presence of INH the conversion of glycine to serine is inhibited and therefore the supply of ATP would also be prevented. The present finding of an inhibition of sucrose synthesis by INH is therefore not inconsistent with the hypothesis that carbon metabolism through the glycollate pathway leading to sucrose synthesis is sustained to a great extent by energy conserved during operation of the pathway and that steps from glycerate can take place in the cytoplasm.

Like INH, potassium glycidate inhibited metabolism of glycerate; this may have been as a competitive inhibitor because this substance is an analogue of glyceric acid and can be hydrolysed to glyceric acid. Glycidate did not decrease the proportion of ^{14}C-glycerate metabolized that was converted to sucrose but inhibited recycling of ^{14}C through the

glycollate pathway, possibly by inhibiting phosphoglycollate formation. The ^{14}C-content of glycine was very much less than that in the control and ^{14}C accumulated in phosphate esters although incorporation into serine was increased. Generally therefore results with potassium glycidate, unlike those with INH do not support a role for the C-2 pathway in supporting ATP formation for sucrose synthesis.

SHB did not inhibit glycerate metabolism but incorporation of ^{14}C into sucrose and glycine decreased slightly and into glycollate increased. The results are consistent with a competitive type of inhibition of glycollate oxidase by SHB which increased the pool size of glycollate and caused a corresponding decrease in glycine and sucrose.

17. Changes in total pool size and relative specific activity of glycine and serine during steady-state photosynthesis in 150 vpm $^{14}CO_2$ and subsequently in 150 and 1000 vpm $^{12}CO_2$ in 21% O_2 in the light or in CO_2-free air in the dark

Total amounts of glycine and serine determined by paper chromatography and colorimetric analysis (see Methods and Materials) are shown in Figs. 24a and 24b. The corresponding ^{14}C data are shown in Fig. 8a. Those during CO_2-free air in the dark relate to the ^{14}C data in Fig. 14a.

The results confirm the existence of a steady-state during photosynthesis in 150 vpm $^{14}CO_2$ and subsequently in 150 vpm $^{12}CO_2$ since the total amounts of glycine and serine in the leaf segments remained almost constant. However when the leaves were transferred to 1000 vpm $^{12}CO_2$ in the light or to CO_2-free air in the dark there was a striking decrease in the size of the glycine pool and an increase in serine pool. From the data on pool sizes (Figs. 24a and 24b) the relative specific activity of glycine and serine were calculated as μci per μg.atom of carbon

expressed as a percentage of the specific activity (μci/μg.atom of carbon) of $^{14}CO_2$ employed. The values show that the main metabolically active pools of glycine and serine represent only half the total of these compounds in the leaves. It has been already shown that some active pools of glycine and serine may be saturated in much less than 15 minutes photosynthesis in $^{14}CO_2$; these new results confirm the existence of further almost inactive pools. Therefore it is accepted, contrary to the findings of Atkins et al. (1971) for sunflower leaves, that there are multiple pools of glycine and serine (see also Miflin et al., 1966; Oaks and Bidwell, 1970).

The rate of decrease of relative specific radioactivity of glycine was slowest in 150 vpm $^{12}CO_2$, faster in the dark in CO_2-free air and fastest in 1000 vpm CO_2. However in 1000 vpm $^{12}CO_2$ and in darkness in CO_2-free air there were large decreases in total pool size. These results are in accordance with those of Mahon et al. (1974) who showed that in sunflower leaf discs the relative specific activity of glycine decreased much faster at 967 vpm CO_2 than at 115 vpm CO_2. It was argued previously that high CO_2 and darkness decreased incorporation of further carbon into glycine. Darkness also prevented further metabolism of serine. In darkness the data show that glycine falls from the steady-state value of 5.32 μmoles/g fresh weight to 1.55 μmoles. This means a change to much less than half in the amount of glycine whereas the labelling suggest that only one half the glycine was in an active pool. A similar situation appears to exist for the flush out in 1000 vpm CO_2; the pool size decreases to slightly less than half when all the ^{14}C should have been lost, were half the total glycine in the tissue was in a metabolically active pool initially saturated with ^{14}C, but some 20% of the ^{14}C remained. Either the glycine pool was not saturated with ^{14}C initially or the new conditions (1000 vpm CO_2 or darkness) caused the inactive pools to become active.

Fig. 24 (a & b). Changes in total pool size and relative specific activity of glycine and serine during steady-state photosynthesis in 150 vpm $^{14}CO_2$ and subsequently in 150 and 1000 vpm $^{12}CO_2$ in 21% O_2 in the light or in CO_2-free air in the dark.

These data correspond to ^{14}C changes in Figs. 8(a) and 14(a).

Fig. 24 (a). Total pool size of glycine and serine

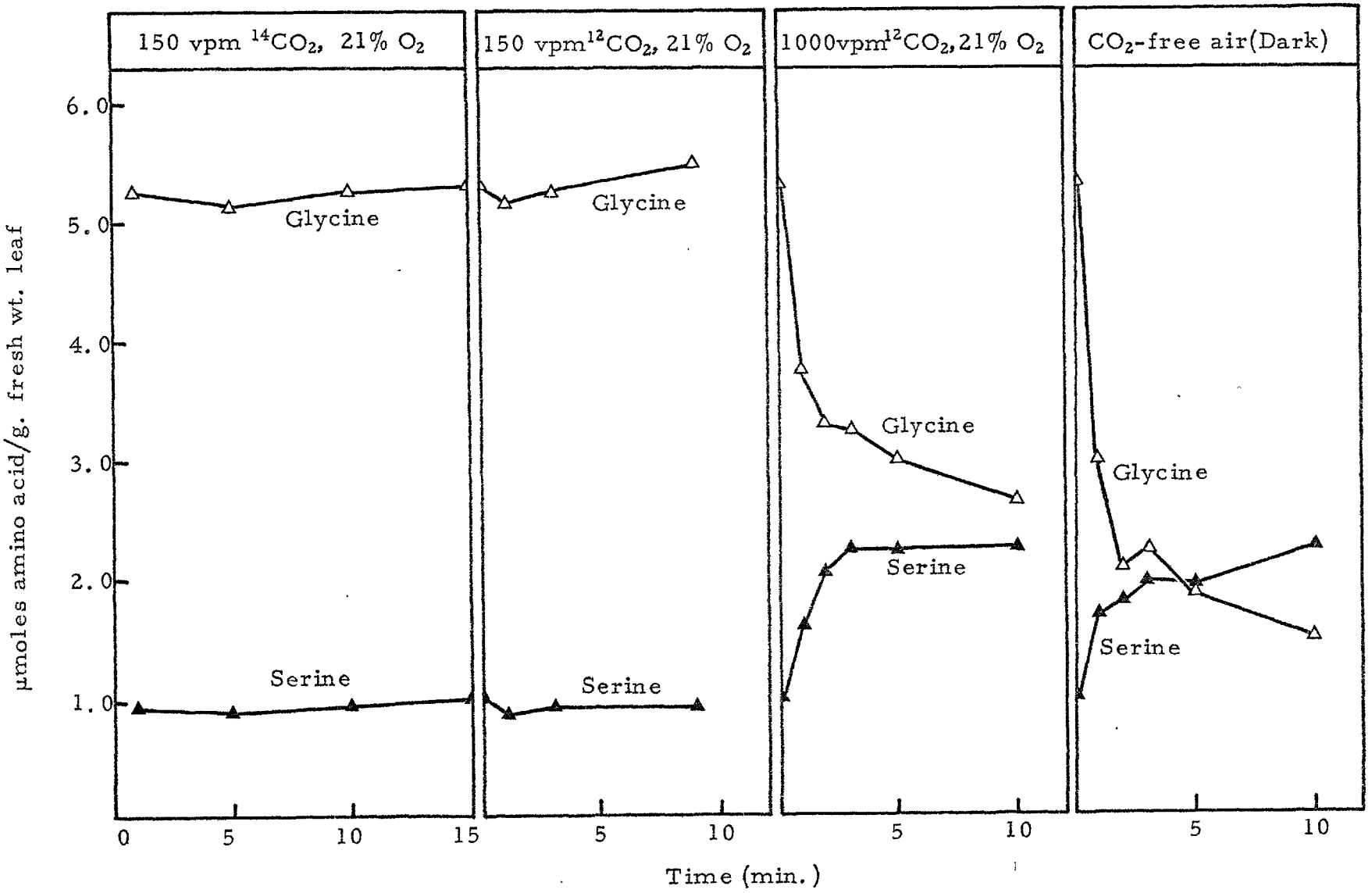


Fig. 24 (b). Relative specific activity of glycine and serine

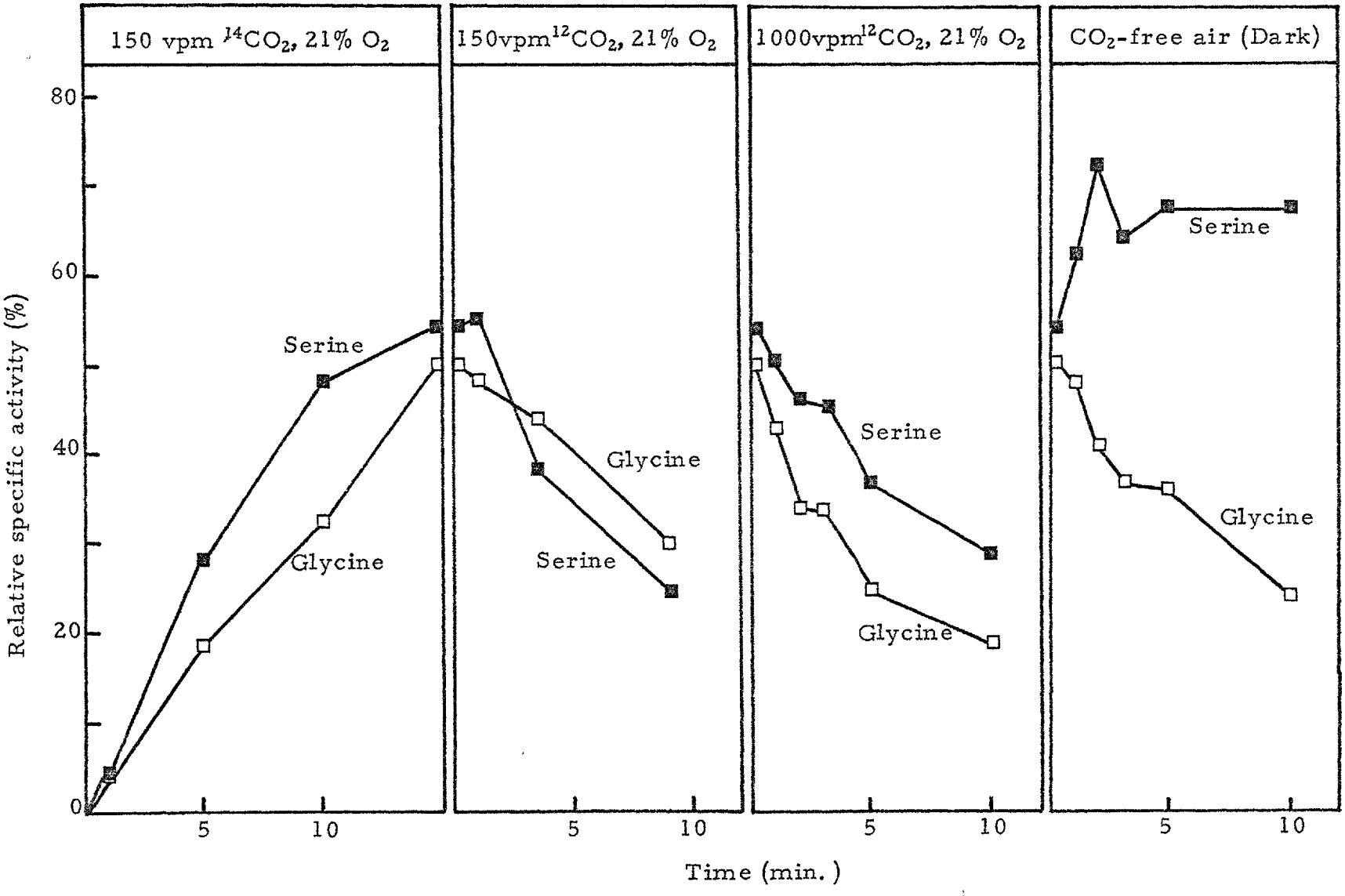


Table 9 Changes in relative specific activity of glycine and serine during flushing in darkness in CO_2-free air

Time (min.) in darkness in CO_2-free air	1	2	3	5	10
* Relative specific activity of glycine lost (%)	52.5	52.6	60.3	60.5	58.3
* Relative specific activity of serine gained (%)	64.0	86.0	67.2	75.2	60.5

(* Calculated from changes in ^{14}C and total carbon during 0-1, 0-2, 0-3, 0-5 and 0-10 min. flushing.)

Table 10 The rates of carbon flow calculated from the initial changes in total pool size of glycine and serine in atmospheres containing 1000 vpm CO_2 in the light or CO_2-free air in the dark following steady-state photosynthesis in 150 vpm $^{14}CO_2$

	Changes during the first minute of flushing			
	Glycine	Serine	Glycine	Serine
	(μmoles amino acid/g.fr.wt.)		(μgC.dm.$^{-2}$ min^{-1})	
1000 vpm CO_2 in air (light)	- 1.6	+ 0.7	- 93.0	+ 63.9
CO_2 - free air (Dark)	- 2.3	+ 0.8	- 136.2	+ 72.0

- Decrease, + Increase

(Rate of steady-state carbon assimilation in 150 vpm $^{14}CO_2$ = 33.5 μmC.dm.$^{-2}$min^{-1}

The apparent relative specific activity of the glycine lost can be calculated from the loss of ^{14}C and loss of carbon. The values obtained were 66.3 and 76.8, for losses from 0 to 1 and 0 to 2 minutes respectively in 1000 vpm CO_2. The corresponding relative specific activities of serine formed for these times were 40.8 and 37.0, suggesting synthesis of serine from an unlabelled source as well as from the labelled glycine. (The relative specific activity values for serine during times between 0 to 3, 0 to 5 and 0 to 10 were very small because the total pool of serine remained more or less constant while the radioactivity decreased). This source can be partly the relatively unlabelled pool of glycine and partly ^{12}C coming from newly assimilated CO_2.

In the dark (Table 9) it is noticeable that the calculated specific activity of serine formed was higher than the specific activity of the glycine lost through the inaccurate nature of the calculation means that this difference may not be very significant. Rather than the ^{14}C being in a pool encompassing only 50% of total glycine in the tissue it appears that the glycine for serine synthesis was being used from a uniform pool that was only half saturated. Either the apparent saturation at 15 minutes must be caused by another phenomenon or upon change to darkness the tissue glycine behaves as though it were a single pool due to increased permeability of some membrane by which it was partitioned. A phenomenon leading to false assumption about saturation could be deduced from the data of Ludwig and Canvin (1971) who showed from the specific activity of the $^{14}CO_2$ evolved that in 149 vpm $^{14}CO_2$ the photorespiratory substrates were not saturated even after 60 minutes of steady-state photosynthesis. This was explained as due to the use by the leaf of an unlabelled storage product for synthesis of some of the intermediates of the photorespiratory pathway at low ambient concentrations of CO_2. The full explanation of

the data would demand further careful analysis of pool sizes and specific activities. However if we accept the pools are not saturated with $^{14}CO_2$ in 15 minutes or that membrane permeability changes increase the effective pool sizes, the rate of carbon flow via glycollate pathway calculated from the other data in this thesis (compare Table 4 and Table 10) must be underestimated. Furthermore, the flow of unlabelled carbon into serine in 1000 vpm CO_2 may suggest either an alternative to glycine as precursor of serine under these conditions or that flow through the glycollate pathway from $^{12}CO_2$ was not insignificant under these conditions. Fig. 23a shows, indeed, that ^{14}C does enter both glycine and serine during steady-state photosynthesis in 1000 vpm $^{14}CO_2$ but at rates which cannot be estimated from the data.

DISCUSSION

The results described for young wheat leaves suggest three main conclusions: (1) that during photosynthesis in air with 150 or 325 vpm CO_2 the amount of carbon metabolized by the glycollate pathway is as much, or more than the amount assimilated, (2) that the main source of CO_2 in photorespiration is the conversion of glycine to serine and (3) that in air containing low partial pressures of CO_2 (80, 150 or 325 vpm) sucrose is mainly made from intermediates of the glycollate pathway. These conclusions are based on the results obtained from changes in the ^{14}C content of products of photosynthesis and photorespiration both during steady-state assimilation of $^{14}CO_2$ and following sudden changes in the experimental conditions. Other evidence was obtained by supplying leaves with inhibitors, especially INH, ^{14}C-glycerate or ^{14}C-serine.

Carbon flow through the glycollate pathway

The proportion of carbon fixed during photosynthesis that is metabolized by the glycollate pathway was investigated. The rates of increase of ^{14}C in glycine and serine during steady-state photosynthesis (Table 4) were less than 37% of the net photosynthetic rate. But these rates provide only minimum estimates of the rate of carbon flow into the glycollate pathway. Contrary to the findings of Atkins et al. (1971) glycine and serine appear to exist in more than one pool as shown by the measurements of the total amounts of glycine and serine in wheat leaves compared to the ^{14}C present after 15 minutes photosynthesis from $^{14}CO_2$. The calculated specific activities suggest that only half the total pool was labelled (Fig. 24a) and yet the active metabolic pools appeared

to be almost saturated by this time (see Fig. 1b and Table 2). The existence of such multiple pools of amino acids has been previously postulated by Smith et al. (1961), Hellebust and Bidwell (1963), Miflin et al. (1966) and Oaks and Bidwell (1970). An alternative method of measuring the carbon flow through the glycollate pathway is to determine the efflux of ^{14}C from glycine or serine immediately following transfer from $^{14}CO_2$ to $^{12}CO_2$. Under such conditions, provided that the active pools were saturated with ^{14}C initial changes in ^{14}C in intermediates should involve carbon having the specific activity of the $^{14}CO_2$ supplied. Calculations made on this basis (Table 4) show that after 15 minutes photosynthesis in 150 vpm or 325 vpm $^{14}CO_2$ the rates of loss of ^{14}C from glycine in 1000 vpm $^{12}CO_2$ and 21% O_2 were 68.5 and 75.1 μg C. $dm.^{-2}$ $min.^{-1}$ respectively. The rates observed in CO_2-free 1% O_2 or 21% O_2 or in 150 or 325 vpm CO_2 in air were from a fifth to one half the rates in 1000 vpm CO_2. This is because in these atmospheres there is considerable refixation of ^{14}C from photorespired $^{14}CO_2$ that will be partly recycled, together with some ^{14}C from serine, to form more ^{14}C glycollate and glycine and so obscure the true rate of loss of radioactivity from glycine. In the presence of high partial pressures of $^{12}CO_2$ (1000 vpm) refixation of the $^{14}CO_2$ is decreased as shown by the greater evolution of $^{14}CO_2$ in 1000 vpm $^{12}CO_2$ with 1% O_2 (Fig. 10) compared to that in CO_2-free 1% O_2 (Fig. 8a). Further, recycling of ^{14}C from serine through the chloroplastic pools will also be lessened because the increased uptake of $^{12}CO_2$ competes strongly for the available ATP and NAD(P)H. The chloroplastic phosphate ester pools from which glycollate is made would be quickly saturated with ^{12}C (Bassham and Kirk, 1962) and if glycollate formation is inhibited at high CO_2 concentrations (Lee and Whittingham, 1974) little or no ^{12}C or ^{14}C would flow

into glycollate. Therefore these initial rates of decrease of ^{14}C from glycine (68.5 and 75.1 µgC. dm.$^{-2}$ min.$^{-1}$) are probably better estimates of the rate of carbon flow through glycine during photosynthesis in 150 and 325 vpm CO_2 than changes of ^{14}C during the steady-rate or in CO_2-free 1% or 21% O_2. These rates of flow of carbon through glycine (68.5 and 75.1 µg C. dm.$^{-2}$ min.$^{-1}$) exceed the rate of carbon assimilation in 150 and 325 vpm CO_2 (33.5 and 63.2 µg C. dm.$^{-2}$ min.$^{-1}$ respectively).

Further evidence in support of a rapid flow of carbon through glycine is provided by experiments in which leaves were transferred to darkness following photosynthesis in $^{14}CO_2$. In darkness there is little flow of carbon into the glycollate pathway, even under CO_2-free conditions, and flow of ^{14}C from the saturated pool of glycine should be closely related to the rate of carbon flow in the period of steady-state photosynthesis. The calculated rate of loss of carbon from glycine following steady-state photosynthesis in 150 vpm $^{14}CO_2$ (Table 5) was rapid (65.3 and 84.8 µg C. dm.$^{-2}$ min.$^{-1}$ in CO_2-free air and CO_2-free 1% O_2 respectively) and exceeded the rate of net carbon assimilation (34.5 µg C. dm.$^{-2}$ min.$^{-1}$). Following steady-state photosynthesis in 325 vpm $^{14}CO_2$ (Table 6) the rate of loss of carbon from glycine (36.3 µg C. dm.$^{-2}$ min.$^{-1}$) in 325 vpm $^{12}CO_2$ in darkness was also close to the rate of assimilation of $^{14}CO_2$ (43.4 µg C. dm.$^{-2}$ min.$^{-1}$).

Calculation from the total amounts of glycine in the leaves (Table 10) show that the rate of loss of carbon from glycine (93.0 µg C. dm.$^{-2}$min.$^{-1}$) in 1000 vpm CO_2 following steady-state photosynthesis in 150 vpm $^{14}CO_2$ was in excess of the value calculated on the basis of loss of radioactivity from glycine (68.5 µg C. dm.$^{-2}$ min.$^{-1}$). The rates of loss of carbon in CO_2-free air in the dark calculated from decrease in total amount and radioactivity in glycine were respectively 136.2 and 65.3 µg C. dm.$^{-2}$min.$^{-1}$.

Furthermore, in CO_2-free air in the dark and in 1000 vpm CO_2 (Fig. 24a) the data show that the total amounts of glycine in the leaf samples decreased to approximately half that present during the preceding steady-state in 150 vpm $^{14}CO_2$. If these decreases represented the decreases of carbon from the active pools only, then all the ^{14}C should have been lost; in fact some 20 to 25% (Fig. 24b) remained even after 10 minutes flushing in the above atmospheres. The explanation of the differences would follow if the active pool was not saturated with ^{14}C after 15 minutes exposure to $^{14}CO_2$; or alternatively unlabelled glycine from the previously inactive pool is metabolized in the presence of high partial pressures of CO_2 and in CO_2-free air in darkness. If the active pool of glycine is not saturated during 15 minutes then the above rates calculated from the decreases of radioactivity in glycine would be underestimates of the true rate of carbon flow through glycine. If all the carbon fixed during photosynthesis is metabolized through glycine with one in every four carbons released as CO_2 (Kisaki and Tolbert, 1970; Bird et al., 1972) the flow of carbon through serine should be approximately 75% of the rate of photosynthesis. In fact Atkins et al. (1971) and Mahon et al. (1974) showed that in sunflower leaf discs the flow through serine was about 75% of the rate of photosynthesis. The general conclusion is that all the carbon assimilated at normal levels of CO_2 may be metabolized through the glycollate pathway.

Source of photorespiratory CO_2 –

The results from use of INH (Fig. 7a) and from steady-state $^{14}CO_2$ and $^{12}CO_2$ assimilation (Figs. 6a and 7a) provide evidence (as discussed later) that glycine is a precursor of photorespired CO_2. However, such data do not establish the extent to which the conversion of glycine to serine

is responsible for the CO_2 evolved during photorespiration *in vivo*. Upon darkening the leaves it is assumed that the rates of metabolism of glycine and evolution of CO_2 continue as in the previous steady-state but that synthesis of new glycollate and refixation of respired CO_2 cease almost instantaneously and further metabolism of serine which is light dependent, is slow. The stoichiometry of conversion of glycine to serine and CO_2 was calculated and for every four carbon atoms leaving glycine in one minute of darkness approximately three appeared in serine and one in CO_2 (Table 6). This is consistent with the known stoichiometry *in vitro* (Kisaki and Tolbert, 1970; Bird *et al.*, 1972) for the conversion of two molecules of glycine to one each of serine and CO_2.

It should be pointed out that the leaves used in the present experiments were young and were grown in relatively low light intensities. With such material light saturated photosynthesis is expected to be slow so measurements of photorespiratory rates, which are known to be correlated with rates of photosynthesis, will also be slow. In making comparisons with measurements made by others, rates of photorespiration may conveniently be expressed relative to the rate of photosynthesis. The rates of CO_2 release calculated for wheat leaves in Table 11 are of a similar order to rates calculated (Table 12) by others for various leaves (Zelitch, 1975). The use of higher concentrations of CO_2 in the flushing gas lead to significantly higher estimates of the rate of CO_2 evolution which because of decreased refixation should more nearly represent total photorespiratory metabolism in the leaves. The measured rates of CO_2 production (Table 11) are sufficient to account for all the carbon assimilated from air with 325 vpm CO_2 being metabolized by way of glycine and serine. Under special circumstances, such as with 150 vpm CO_2 in the atmosphere, the data show that more carbon is metabolized by the glycollate pathway than is

Table 11 — Photorespiratory rates calculated from measurements of $^{14}CO_2$ evolved at 20°C, from wheat leaves allowed to photosynthesize for 15 min. in 150 or 325 vpm $^{14}CO_2$, into atmospheres containing different concentrations of CO_2.

CO_2 conc. of feeding gas mixture (vpm)	Rate of true PS. (mg CO_2. dm.$^{-2}$ h^{-1})	CO_2 conc. of flushing gas mixture (vpm)	Rate of photorespiration (mg CO_2.dm.$^{-2}$ h^{-1})	Photorespiration as % of true PS.	Ref.
150	8.9	150	1.9	21.3	Fig. 8 a
	9.2	150	2.2	22.9	16 a
	7.6	150	1.6	21.0	18 a
	7.0	150	1.4	20.0	22 a
	8.7	0	1.7	19.5	8 a
	11.8	1000	4.8	40.7	8 a
	8.8	1000	2.8	31.8	16 a
	10.1	1000	4.2	41.5	22 a
325	15.8	0	2.0	12.7	9 a
	15.9	325	2.1	13.2	9 a
	18.1	1000	4.2	23.2	9 a
	11.5	325	2.1	18.3	Table 6

Table 12 Comparison of photorespiratory rates in soybean, tobacco, sugar beet and sunflower assayed by different methods during photosynthesis in normal air.

(After Zelitch, 1975)

Plant	Temp.	Rate of true PS.* (mg CO_2 dm^{-2} h^{-1})	Rate of L_R† (mg CO_2 dm^{-2} h^{-1})	L_R † as % of true PS*	Method of estimation	Reference
Soybean	26	51	16	31	CO_2 release into CO_2-free air	Samish et al. (1972)
	30	25	8	29	" " "	Hofstra & Hesketh (1969)
	25	19	8	43	Post-illumination CO_2 - outburst	Bulley & Tregunna (1971)
Tobacco	26	25	8	31	Post-illumination CO_2 - outburst	Decker (1959)
	34	26	10	49	" "	" "
Sugar beet	25	37	12	31	CO_2 release into CO_2-free air	Terry & Ulrich (1973)
	25	36	10	38	" " "	Hofstra & Hesketh (1969)
Sunflower	25	35	8	21	" " "	Ludwig & Canvin (1971)
	25	40	15	37	^{14}C dilution mtd.	Bravdo & Canvin (1971)

* Photosynthesis † Photorespiration

assimilated by photosynthesis; that is the rate of CO_2 production is more than 25% of the rate of true photosynthesis. This carbon for glycollate metabolism may be partly provided by recycling of carbon in the glycollate pathway, partly by newly assimilated carbon and probably, partly by carbon from storage products. Alternatively, some of the CO_2 evolved may be derived from a reaction other than conversion of glycine to serine. Figs. 8a and 9a (see 1000 vpm $^{12}CO_2$ in 21% O_2) show that more $^{14}CO_2$ was produced than could be accounted for by the conversion of glycine to serine and CO_2, and $^{14}CO_2$ was being produced (Fig. 10) when there was no longer a decrease of ^{14}C in glycine. However, at this stage there was decrease of ^{14}C in serine which largely accounted for the extra CO_2 produced. The extra CO_2 may be formed from ^{14}C-serine by some mechanism not involving glycine that is stimulated by high CO_2 in the atmosphere; indeed Kent, Pinkerton and Strobel (1974) showed in Vicia faba that in the presence of 10,000 vpm CO_2 serine was a precursor of CO_2 formed, by operation of the Krebs tricarboxylic acid cycle during photosynthesis.

In order to investigate the source of the extra $^{14}CO_2$ in wheat leaves serine-U-^{14}C was supplied to wheat leaf segments in atmospheres containing various amounts of CO_2 and O_2. During a 60 minute period the evolved CO_2 contained 7.8, 1.5, 5.2 and 1.8 dpm x 10^{-4} ^{14}C in 325 vpm $^{12}CO_2$ in air, 325 vpm $^{12}CO_2$ in 1% O_2, 1000 vpm $^{12}CO_2$ in air and 1000 vpm $^{12}CO_2$ in 1% O_2 respectively (Fig. 12b). This represented 10.8, 2.9, 7.6 and 5.2% (Table 4a) of the total ^{14}C-serine metabolized in these atmospheres. Therefore, the high CO_2 partial pressures decreased but did not stop $^{14}CO_2$ production from ^{14}C-serine. Furthermore, ^{14}C was incorporated into glycerate, glycollate (Table 4a) and glycine (Fig. 12a). It is possible that when the external CO_2 concentration is suddenly increased, ^{14}C-serine formed during a preceding period of

photosynthesis in $^{14}CO_2$ may be metabolized, producing $^{14}CO_2$ at some limited site not so readily accessible to ^{14}C-serine supplied.

Wang and Waygood (1962) showed that when ^{14}C-glycine was supplied to excised wheat leaves together with unlabelled serine, synthesis of ^{14}C sugars was decreased. They concluded that serine was an intermediate between glycine and sucrose. Therefore, unlabelled serine was supplied to leaves photosynthesizing in 150 vpm $^{14}CO_2$ and the atmosphere then changed to one with 1000 vpm $^{12}CO_2$ (Fig. 11a). Radioactivity decreased rapidly in glycine but that in serine increased much more than in control leaves not supplied with unlabelled serine. The ^{12}C-serine also decreased ^{14}C-sucrose formation so the flow of ^{14}C from serine to sucrose was suppressed due to the dilution of the serine pool with ^{12}C. This was further evidenced by the decreased formation of ^{14}C-glycerate (Fig. 11b); glycerate is known to be an intermediate between serine and sucrose (Wang and Waygood, 1962; Tolbert, 1973; Zelitch, 1971). In spite of this decreased metabolism of ^{14}C-serine the amount of $^{14}CO_2$ evolved was not significantly less from leaves supplied with ^{12}C-serine compared to the controls. This result is not consistent with the extra CO_2 evolved being derived from serine.

On the other hand during steady-state photosynthesis in 150 vpm $^{14}CO_2$ with 1% O_2 (Fig. 13a) compared to in 21% O_2 (Fig. 8a) very little ^{14}C was incorporated into glycine although the amount incorporated into serine was hardly affected. When the leaves were transferred to 1000 vpm CO_2 in 1% or 21% O_2 or 325 vpm CO_2 in 21% O_2 (Fig. 13a) only a small amount of $^{14}CO_2$ was evolved. The conclusion here was that a supply of carbon to glycine is important for CO_2 evolution.

While the evidence is not entirely consistent, the extra $^{14}CO_2$ evolved into atmospheres with $1000^{vpm}_{\wedge}\,^{12}CO_2$ is probably accounted for by recycling

of carbon from serine by way of the glycollate pathway and the observations are not inconsistent with photorespiratory CO_2 coming mainly from the conversion of glycine to serine.

Source of carbon for sucrose synthesis

An experiment using INH (Fig. 7a) confirmed the findings of Kisaki and Tolbert (1970) and Goldsworthy (1966) rather than those of Zelitch (1972). Photosynthesis in 150 vpm $^{14}CO_2$ was inhibited 50% (Table 3) but synthesis of serine was decreased more than synthesis of glycine and both CO_2 evolution and sucrose synthesis were strongly inhibited. The contrary results described by Zelitch could be explained by his failure to introduce sufficient inhibitor in some of the treatments. Thus, INH apparently inhibited the formation of serine from glycine-1-^{14}C but under similar conditions formation of serine from glycollate-1-^{14}C, for which there is good evidence that glycine is an intermediate (Wang and Waygood, 1962), was unaffected.

A major effect of INH was clearly inhibition (Figs. 6a and 7a) of the conversion of glycine to serine (see also Pritchard et al., 1962; Whittingham et al., 1963; Miflin et al., 1966) and it seems reasonable to conclude that in addition to CO_2 evolution sucrose synthesis was also dependent on this reaction.

In the absence of INH, the formation of ^{14}C-sucrose and $^{14}CO_2$, after the $^{14}CO_2$ supply was withdrawn (Figs. 6a, 6b, 7a and 7b), is of such a magnitude that it can only be balanced by decreases of ^{14}C in glycine, serine and phosphate esters. It will be observed that ^{14}C accumulation in sucrose (Figs. 5a and 6a) continued for a short time at the same rate after the change from $^{14}CO_2$ to $^{12}CO_2$; during this time the ^{14}C could only come from glycine and phosphate esters. This suggests

that glycine and serine are intermediates in a major pathway synthesizing sucrose. The initial lag in ^{14}C incorporation into sucrose (see Figs. 8a, 9a, 16a, 18a and 22a) is also most readily explained if glycine and serine are intermediates in sucrose synthesis.

Exogenous ^{14}C-serine was metabolized mainly to sucrose, glycine, phosphate esters and CO_2 (Figs. 12a and 12b). Less serine was metabolized in 1% compared to 21% O_2. Waidyanatha et al. (1974 b) suggested that metabolism of serine to sucrose might involve only enzymes in the cytoplasm and be dependent on ATP produced by oxidative phosphorylation. This would explain the effects of low oxygen. Indeed, INH inhibited the formation of ^{14}C-sucrose from the exogenously supplied ^{14}C-glycerate and decreased the amount metabolized (Table 8). This result is consistent with that of Reimer (1970). However, in the present investigation the incorporation of radioactivity into glycollate, glycine and serine was not significantly inhibited by INH suggesting that metabolism of the glycerate ^{14}C through the chloroplast phosphate ester pools to glycollate had not been prevented. Heber et al. (1974) reported that glycerate can enter intact chloroplasts and be metabolized to intermediates of the Calvin cycle and glycollate. Conversion to sucrose was however strongly inhibited. Possibly there is a mechanism for metabolism of glycerate to sucrose that does not involve the chloroplast pools of phosphate esters but is inhibited by INH. Alternatively INH may inhibit both the phosphorylation of glycerate in the chloroplast and also some late step in synthesis of sucrose from phosphate esters as well as conversion of glycine to serine and transaminations (Youatt, 1955). A further possibility is that INH inhibits the flow of carbon through the glycollate pathway causing accumulation of ^{14}C in the intermediates and its effects on sucrose synthesis are only because it inhibits glycerate kinase.

Sodium hydroxy butynoate (SHB), an inhibitor of glycollate oxidase (Jewess et al., 1974) caused a remarkable accumulation of ^{14}C-glycollate (Fig. 15b) but did not significantly decrease the rate of production of $^{14}CO_2$ from substrate previously labelled by photosynthesis in $^{14}CO_2$ (Fig. 16a). After longer periods (90 minutes) of uptake (Fig. 19a) SHB inhibited incorporation of ^{14}C into glycine during photosynthesis in $^{14}CO_2$, by about 75% and the subsequent loss of radioactivity from glycine in 1000 vpm $^{12}CO_2$ (Fig. 19a) was not sufficient to account for the rate of formation of ^{14}C-sucrose and $[^{14}C]CO_2$ during this period. The only compound losing radioactivity fast enough to account for the increases in sucrose and CO_2 was glycollate (see Figs. 19a and 19b). Accordingly, from these data one might propose a pathway for glycollate metabolism to sucrose with evolution of CO_2 not involving glycine and serine. However, Kerr (personal communication) has pointed out that hydroxy butynoate is not a simple competitive inhibitor of glycollate oxidase but competes with glycollate for a site on the enzyme and irreversibly destroys the enzyme activity, probably by forming an adduct with the FMN co-factor (Jewess et al., 1974). Therefore the build-up of glycollate results from a decreased activity of glycollate oxidase; further decrease of the enzyme is prevented by competition from the increased concentration of glycollate. It appears that the amount of active enzyme remaining, when saturated with glycollate, must support a flow of carbon to glycine similar to that occurring in the absence of the inhibitor. Normally leaves in the light contain an excess of glycollate oxidase (Zelitch, 1959; Osmund, 1969) and the active pool of glycollic acid is very small. The rate of ^{14}C loss from glycine in Fig. 19a is probably obscured by a continuous supply of ^{14}C from ^{14}C-glycollate, accumulated during the previous period of photosynthesis in the presence of the inhibitor. An experiment involving both SHB and INH should

demonstrate whether or not this glycollate carbon is metabolized to sucrose via glycine.

Inhibitors in photorespiration

Since CO_2 evolution is a consequence of carbon metabolism through glycollate, the glycollate pathway has often been regarded as wasteful. According to Andrews et al. (1973) the pathway begins with an unavoidable oxidation reaction forming phosphoglycollate. Goldsworthy (1969) has suggested that the glycollate pathway evolved to metabolize the resulting glycollate, thus recovering the carbon and avoiding toxic effects. So far the only effective means of decreasing the flow of carbon through the glycollate pathway without producing toxic effects on the plant has been to decrease the O_2/CO_2 ratio in the air surrounding the plant either by decreasing the O_2 concentration or increasing the CO_2 concentration. Björkman et al. (1966) and Björkman and Gauhl (1969) demonstrated that mimulus plants (C_3) grow faster in artificial atmospheres containing low concentrations of O_2 (4%). Bishop and Whittingham (1968) showed that the rate of photosynthesis of a tomato plant grown and assimilating in air enriched to 1000 vpm CO_2 is three times that of a plant grown and assimilating in air. Also Lee and Whittingham (1974) showed that the rate of photosynthesis in tomato leaves was seven times greater when photosynthesizing in atmospheres containing 950 than 100 vpm CO_2. These increases in photosynthetic rates were attributed partly to inhibition of photorespiration by low O_2 or higher CO_2 concentrations. It is impossible to control O_2 and CO_2 concentrations in this way under field situations but it may be possible to inhibit the glycollate pathway by the use of chemicals. INH inhibited the conversion of glycine to serine

causing a reduction in CO_2 evolution in the light (Fig. 7a) but photosynthesis was also inhibited rather than stimulated. SHB caused an accumulation of glycollate in wheat leaves but photosynthesis was not stimulated (Fig. 15a and 15b). In this case although glycollate metabolism was inhibited photorespiratory CO_2 evolution was little affected. Similar results were obtained by Jewess et al. (1974) who reported that in pea leaf discs this inhibitor caused a 99% inhibition of glycollate oxidase activity, but no detectable effect on the rate of photorespiration, and there was no apparent stimulation of photosynthesis (Kerr, personal communication).

Zelitch (1966) blocked the oxidation of glycollate with α-HPMS in leaves and found, that net photosynthetic CO_2 assimilation was increased 3-fold at 35°C. At 25°C α-HPMS inhibited $^{14}CO_2$ uptake by about 50%. Recently, Zelitch (1974) reported that potassium glycidate inhibited the formation of glycollate in tobacco leaf discs by 40 to 50%; decreased the release of CO_2 by about 40% and, of special significance, it increased net photosynthesis by 40 to 50%. These experiments were performed at 28°C. In wheat leaves (Figs. 20a and 20b) potassium glycidate inhibited incorporation of ^{14}C into glycine and serine, but, instead of stimulation, photosynthesis was inhibited by more than 50% at both 20 and 28°C (Figs. 20a and 21a).

It is therefore clear that inhibition of photorespiration and carbon metabolism through the glycollate pathway, at normal levels of CO_2, O_2 and temperature, does not necessarily lead to stimulation of photosynthesis. Furthermore, Keys et al. (1974) have made measurements in crop situations, of rates of net photosynthesis per unit leaf area for maize which were less than for wheat, although the photorespiratory rate by wheat was ten times greater than by maize.

Bird et al. (1972) showed that the conversion of glycine to serine is associated with ATP formation in mitochondria so the glycollate pathway cannot be regarded as a wholly wasteful process. More must be discovered about the biochemical reactions involved, and what the photorespiratory pathway achieves, before its true significance can be recognized. A highly specific and potent inhibitor would be a most useful experimental tool to decide whether, or under what conditions, photorespiration might be an undesirable metabolic pathway in green plants.

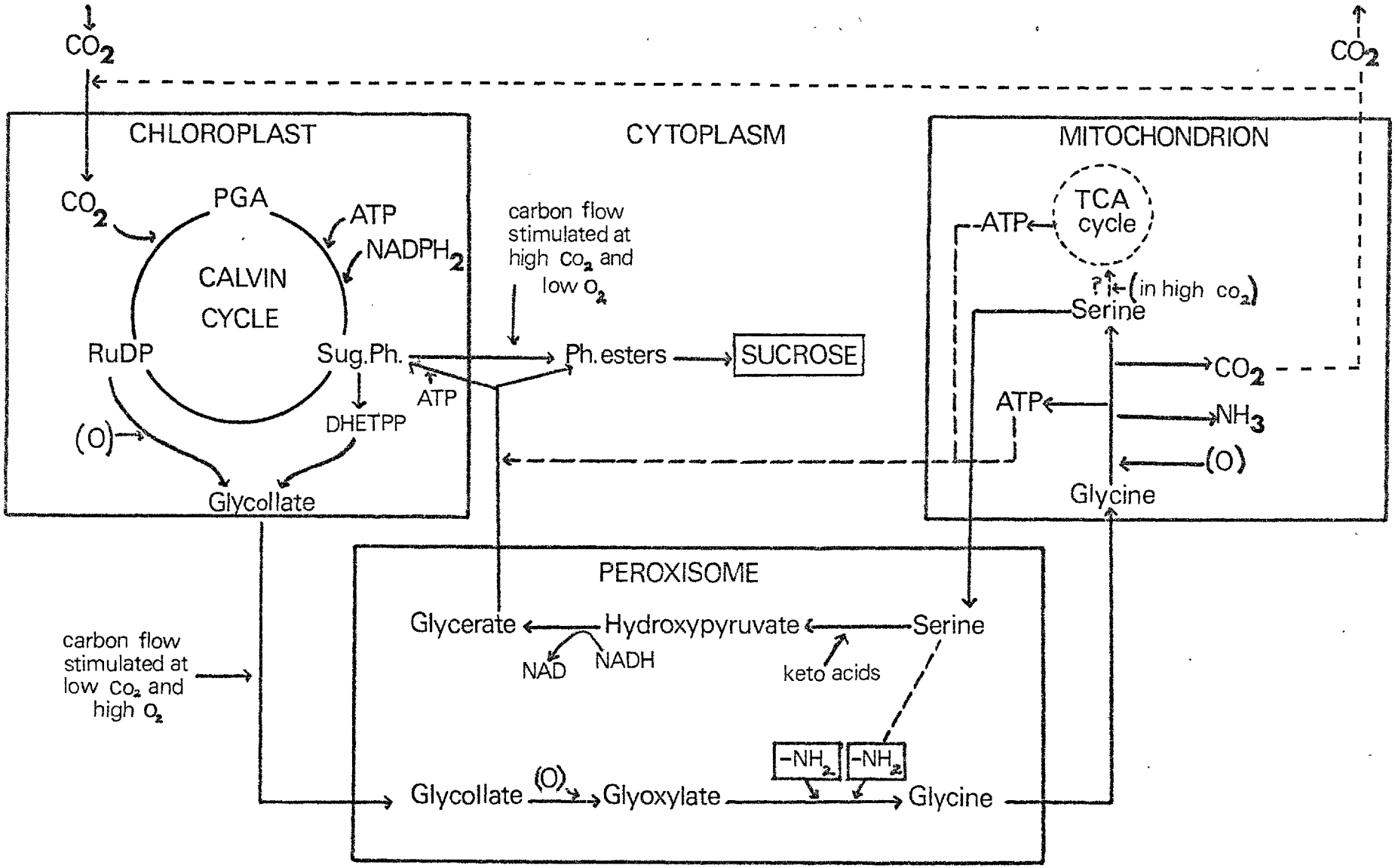


Fig. **25** The proposed pathway of photorespiratory metabolism

Acknowledgements

I wish to express my sincere thanks and gratitude to Professor C.P. Whittingham, who supervised this work and made many valuable suggestions and Professor A.J. Rutter of Imperial College who acted as my Director of studies.

I am greatly indebted to Dr. A.J. Keys for his guidance and keen interest in this work. His willingness to help at all times is greatly appreciated. I thank also I. Pearman and R.W. Soffe for help in growing plants, A.T. Young for help in setting up the $^{14}CO_2$ feeding apparatus and A.C. Kendall, D.C. McIlroy, Mrs. J.C. Turner and Dr. U.P. de S. Waidyanatha for helping in many ways. I am grateful to Mrs. P. Evans, Mrs. G. Lawlor and Mrs. A.J. Webb for help in the preparation of, and to Mrs. H. Cairns for typing, this thesis.

I wish to express my appreciation of the U.K. Government and the British Council for the award of a scholarship under the Technical Co-operation scheme of the Colombo-plan. The research was pursued whilst on overseas study leave from the University of Sri Lanka.

Finally, I thank my wife, Rohini, for her help and encouragement during the course of this work.

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