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Israel Zelitch

The Connecticut Agricultural Experiment Station, New Haven, Connecticut

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Mechanisms of Carbon Fixation and Associated Physiological Responses

ISRAEL ZELITCH

*The Connecticut Agricultural Experiment Station
New Haven, Connecticut*

I. CONTROL OF STOMATAL APERTURE BY SPECIFIC INHIBITION OF GUARD CELL ACTIVITY

A. Control of Stomatal Opening in Light

Recent work in this laboratory has been directed towards increasing net photosynthesis or decreasing the transpiration rate in higher plants by exercising biochemical control over these processes. In these experiments, the fact that both photosynthesis and transpiration involve diffusion processes that might be regulated by varying appropriate diffusive resistances has been exploited (Gaastra, 1959; Zelitch and Waggoner, 1962a). Since in most instances the pathway of diffusion of water vapor in transpiration is shorter than that of CO₂ uptake in photosynthesis, and both gases must pass through the stomata, it was predicted that closing the stomata in a specific manner would diminish transpiration more than net photosynthesis.

To detect effective biochemical inhibitors of stomatal opening, a leaf disk assay was developed (Zelitch, 1961), and criteria were described for judging whether an inhibitor acts primarily on guard cells (Zelitch and Waggoner, 1962a; Zelitch, 1965a; Waggoner and Zelitch, 1965). Thus we found that the effects of spraying a 10⁻⁴ M solution of phenylmercuric acetate on excised tobacco leaves (Nicotiana tabacum L.) fit these criteria since stomatal widths were decreased and transpiration was inhibited more than photosynthesis (Zelitch and Waggoner, 1962a). When sprayed on leaves, this inhibitor closed stomata and increased efficiency of water use in tobacco plants in a greenhouse (Zelitch & Waggoner, 1962b), sunflower (Helianthus annuus L.) outdoors (Shimshi, 1963a), maize (Zea mays L.) in a chamber (Shimshi,

1963b), cotton (*Gossypium hirsutum* L.) in a greenhouse (Slatyer and Bierhuizen, 1964), and on grass in a growth room and outdoors (Davenport, 1967). However, if an inhibitor of stomatal opening also greatly affected photosynthesis in the mesophyll tissue, then net photosynthesis would likely be decreased as much as transpiration, and probably even more than transpiration. Thus a comparison of the effect of a stomatal inhibitor on these two processes provides a simple test of whether or not the inhibitor acts primarily on the guard cells to cause an increased stomatal diffusive resistance.

Several workers in England have described experiments which led them to conclude that the CO_2 concentration inside leaves in the light largely controls stomatal opening (Meidner and Mansfield, 1965). They suggested that stomata open in the light because CO_2 concentration inside the leaf is decreased by photosynthesis, and that stomata close when the CO_2 concentration increases, as in darkness. Slatyer (1967) in his book on plant-water relations also states that the primary factor controlling stomatal apertures appears to be the CO_2 concentration in the intercellular space.

There is ample experimental evidence which is not in favor of this hypothesis (Zelitch, 1965a, 1967, 1969). An additional troublesome problem would arise if it were in fact necessary to close stomata by inhibiting photosyntheses or increasing respiration in order to raise the internal CO_2 concentration. Raising the CO_2 concentration in the intercellular space would diminish the CO_2 concentration gradient between the leaf interior and the ambient atmosphere, and this would likely decrease net photosynthesis more than transpiration. However, as already indicated, it is possible to close stomata by spraying an inhibitor on leaf surfaces and to decrease net photosynthesis proportionately less than transpiration. This finding raises further doubts about the significance of the intercellular space CO_2 concentration in controlling stomatal apertures in normal air.

B. Effect of Inhibitors of Stomatal Opening on the CO_2 Compensation Point

Several investigators have recently carried out experiments on stomatal control by inhibitors supplied to excised leaves through the petiole (Heath, Mansfield, and Meidner, 1965; Meidner and Mansfield 1966; Mansfield, 1967; Allaway and Mansfield, 1967). They observed that such treatments closed the stomata, but concluded that this occurred largely because photosynthesis in the mesophyll was inhibited. This was inferred because in a closed system the CO_2 compensation point, the steady-state concentration at which there is no net photosynthesis or net respiration, was increased by the inhibitors. However, when such inhibitors are supplied to the epidermis of tobacco leaves where it is more likely to affect primarily the guard cells, rather than through the petiole, where it will first reach the mesophyll, stomata closed with no apparent change in the CO_2 compensation point.

Thus Fig. 10-1 shows the results of epidermal treatment when half of a tobacco leaf was sprayed on both surfaces with 5×10^{-5} M phenyl-

Fig. 10-1—Rate of CO₂ depletion in a closed system containing tobacco with open or closed stomata or maize leaf disks in light. Disks were cut (5 disks, 3.2-cm diameter) from a Havana Seed tobacco leaf that had been sprayed 24 hr earlier in one portion with 5×10^{-5} M phenylmercuric acetate in 0.02% Triton X-100 and in another part with Triton. After the disks were at least one hr in air in light while floated upside down on water at about 25°C, the CO₂ concentration was measured in a closed system with an infrared CO₂ analyzer. Arrows indicate the CO₂ compensation point. Chamber volume, 0.95 liter. Gas flow, 8.5 liters/min. Light intensity from tungsten lamps, 2,000 ft-c. Temperature, 30°C. At the end of the experiments, mean stomatal widths were determined from silicone rubber impressions (Zelitch, 1961).

mercuric acetate in 0.02% Triton X-100, and the other half was sprayed only with Triton. On the next day 3.2-cm diameter disks were cut from each side of the leaf and the disks were floated on water in the light for at least 1 hour in air with the lower epidermis up. The disks were then transferred to a closed chamber, and the CO₂ concentration in the system was measured continuously. Initially net photosynthesis in the control disks was 10.5, and in the disks sprayed with inhibitor it was 7.7 mg CO₂ dm⁻² hr⁻¹. The disks sprayed with inhibitor required a longer time to reach the compensation point from the initial 300 ppm of CO₂, as expected, but the steady-state concentration was about the same in each, 54 ppm in the control and 55 ppm in the disks treated with inhibitor. The stomatal widths at the end of the experiment were much narrower in disks sprayed earlier with phenylmercuric acetate. An additional control showed that untreated disks of hybrid maize had an initial rate of photosynthesis of 17.4 mg CO₂ dm⁻² hr⁻¹, 66% greater than the tobacco control, and a compensation point of only 7 ppm of CO₂ (Moss, 1962; Meidner, 1962).

Bravdo (1968) has described a model, in which stomata play the role of variable resistances to diffusion, that predicts the manner in which the CO₂ concentration should increase from near zero CO₂ to the compensation point if gross photosynthesis, respiration, and the diffusive resistance through the stomata remain constant when leaves are

placed in a closed system in the light. Figure 10-2 shows the manner in which tobacco leaf disks with open and closed stomata increase the CO_2 concentration from near zero to the compensation point. The control disks in this experiment also reached the CO_2 compensation point more quickly and the stomata were more widely open, although the compensation point of 48 ppm was similar to that of leaf disks sprayed earlier with phenylmercuric acetate to close the stomata which was 50 ppm. These data give straight lines with different slopes when plotted by the method of Equation IV in Bravdo (1968), and this further indicates that the rates of CO_2 evolution, stomatal diffusive resistance, and the physical and chemical resistances to CO_2 fixation were constant as the CO_2 concentration increased to the CO_2 compensation point. The stomata were thus functioning as expected as variable resistances to diffusion and the treatment with phenylmercuric acetate did not greatly effect gross photosynthesis or the CO_2 concentration in the leaf interior while the stomata were closed.

Similar results on the closing of stomata without affecting the CO_2 compensation point have also been obtained by applying other inhibitors, such as α -hydroxysulfonates, at appropriate concentrations to the leaf surface. Thus increasing the CO_2 concentration in the intercellular space, as by supplying inhibitors through the petiole, is not a necessary condition for stomatal closure. Finding inhibitors that function still more exclusively on guard cell metabolism should therefore create an increased stomatal resistance and permit increased water use efficiency and exert a beneficial influence on the hydrologic cycle in a more effective manner than has already been accomplished (Waggoner and Bravdo, 1967; Turner and Waggoner, 1968).

II. DIFFERENCES IN PHOTOSYNTHETIC EFFICIENCY AMONG SPECIES

A. The Carboxylation Reactions

As is shown in Fig. 10-3, the rate of net photosynthesis per unit leaf area at high light intensities in normal air is at least twice as great in certain species including maize, sugarcane (Saccharum officinarum L.) and sorghum (Sorghum vulgare Pers.) as in most species such as tobacco and sugar beet (Beta vulgaris L.) (Hesketh, 1963; Hesketh and Moss, 1963). At lower light intensities, the differences in photosynthetic efficiency between such species becomes smaller (Waggoner, Moss, and Hesketh, 1963). It therefore seems important to determine the factors responsible for the increased photosynthetic efficiency shown by these tropical grasses with the view to altering the other species so as to make them equally productive photosynthetically. An obvious consideration is whether the more efficient species fix CO_2 by a biochemically more effective enzymatic carboxylation reaction.

In spite of the considerable work in this area, it is not yet known how much CO_2 is fixed by one or another of the carboxylation reactions that are demonstrably present in the chloroplasts of leaves. The brilliant contributions of Calvin, Benson, and their colleagues elucidated the

Fig. 10-2—Rate of CO_2 concentration increase in a closed illuminated chamber containing tobacco with open or closed stomata. Conditions of the experiment were the same as in Fig. 10-1. The CO_2 concentration was depleted to 7 ppm of CO_2 by means of a CO_2 absorbent, and the absorbent was then removed from the system at zero time.

Fig. 10-3—Diagrammatic representation of the effect of light intensity on net photosynthesis at 30°C and 300 ppm CO_2 in air (adapted from Hesketh (1963) and Hesketh and Moss (1963)).

photosynthetic carbon reduction cycle. The main features of this scheme are the carboxylation of ribulose 1,5-diphosphate to produce 3-phospho-D-glyceric acid as the first stable intermediate. The phosphoglyceric acid is then reduced to triose phosphate, and the CO_2 acceptor is regenerated in a cyclic process. In kinetic photosynthetic experiments with barley leaves (*Hordeum vulgare* L.) in which $^{14}\text{CO}_2$ was supplied during progressively shorter periods, phosphoglyceric acid was the first detectable radioactive product and it contained most of its ^{14}C in the carboxyl-carbon atom as was demanded by the functioning of the ribulose diphosphate carboxylase reaction (Bassham, 1964).

However, as was pointed out by Stiller (1962), other carboxylation reactions could fix more carbon and not be detected in kinetic experiments if the early intermediates are unstable during isolation or are undetectable in small amounts. Accordingly, unless it is certain that there is only one carboxylation reaction, it is unsafe on the basis of such kinetic experiments to conclude that the Calvin-Benson cycle accounts for most of the CO_2 fixed by a tissue.

Another troublesome aspect of the Calvin-Benson cycle is that the CO_2 concentration at which the isolated carboxylating enzyme or isolated chloroplasts are half-saturated, (K_m), is considerably higher (Rackett, 1957; Jensen and Bassham, 1966) than the 300 ppm (or about 7×10^{-6} M free CO_2 in solution) which provides half the maximal rate of photosynthesis in intact plant tissue (Hesketh, 1963). The phosphoenolpyruvate carboxylase reaction is another CO_2 -fixing reaction known to occur in higher plants (Bandurski, 1955), and this enzyme is half-saturated at only about 440 ppm CO_2 (Walker, 1962). In this reaction, CO_2 and phosphoenolpyruvate react to produce oxaloacetate as the first product, which may be rapidly converted into malic or aspartic acid. In kinetic $^{14}\text{CO}_2$ experiments with one of the efficient species, sugarcane, the first detectable radioactive products were malic and aspartic acids, which were labeled with ^{14}C it was shown that oxaloacetate also became labeled before phosphoglyceric acid in maize and sorghum as well as in sugarcane leaves (Slack and Hatch, 1967). These results still do not tell us how much carbon is fixed by the phosphoenolpyruvate carboxylase reaction any more than do the kinetic experiments that demonstrated phosphoglyceric acid to be the "first product." They do, however, provide convincing evidence that multiple carboxylation reactions occur in photosynthetic tissues.

When $^{14}\text{CO}_2$ of known specific activity at low concentrations was supplied to tobacco leaf tissue in the light, glycolic acid was produced with a specific activity similar to that of the added $^{14}\text{CO}_2$ and in excess of the specific activity of the carboxyl-carbon atom of phosphoglyceric acid (Zelitch, 1965b). This suggested that glycolic acid was synthesized by still another unknown carboxylation reaction. Recently it was shown, in kinetic $^{14}\text{CO}_2$ experiments carried out on photosynthetic bacteria, that glycolate is labeled with ^{14}C before the phosphate esters, again suggesting that glycolate is the "first product" of photosynthesis in this organism (Anderson and Fuller, 1967).

Doubt is cast on whether the carboxylation reaction need be limiting photosynthetic efficiency since, at high light intensities and saturating CO_2 concentrations, there is little difference in net photosynthesis between leaves of the highly efficient species, such as maize and sugarcane, and those of the less efficient species such as tobacco (Zelitch, 1967b; Goldsworthy, 1968). Moreover, Goldsworthy (1968) showed that if net photosynthesis was measured at low concentrations of oxygen in the ambient atmosphere, there was little difference in the overall K_m , about 300 ppm, between these species. I have confirmed his findings (Fig. 10-4), and will discuss these experiments in more detail in a later section.

Thus although multiple carboxylation reactions probably occur in each tissue, it is still uncertain how much each contributes to the total

Fig. 10-4—Effect of oxygen concentration on the atmosphere on net photosynthesis in tobacco and maize at various CO_2 concentrations. Leaf disks were floated upsidown on water in light for a preliminary period and measurements were made in the system used in Fig. 10-1. Net photosynthesis was measured at about 30°C and 3,000 ft-c. After measurements were made at 1,800 ppm, the CO_2 concentration was lowered with an absorbent to 600 ppm. After several measurements near 600 ppm, the leaf disks were allowed to deplete the CO_2 to the CO_2 compensation point. The system was then flushed with nitrogen (final concentration of oxygen was less than 2%), and the measurements were then repeated. Each point on the curves is the mean of three experiments. The overall K_m is given in parentheses and was determined graphically. (●), tobacco in 20% oxygen; (○), tobacco in 2% oxygen; (x) maize in 20% oxygen; (Δ) maize in 2% oxygen. Only the data from 550 ppm to the compensation point are shown.

CO_2 fixation. Nevertheless, a lower rate of net photosynthesis (compared with maize) in a species does not seem to be attributable to severe limitations in the biochemical mechanism of carboxylation or in the photochemistry. Hence we must seek other causes for these large differences in net photosynthesis between species in normal air. As can be shown in several independent ways, these differences seem to be related to the rate of CO_2 evolution in the light by the process known as photorespiration. Therefore this process must be considered as an important factor in causing a diminished net photosynthesis.

B. Photorespiration and its Measurement

Since CO_2 uptake in bright light in air exceeds dark respiration by 10- to 20-fold, even if photorespiration contributes additional CO_2 inside a leaf, evaluating the gross CO_2 evolution in the light will present difficulties. Any method of measurement of photorespiration will underestimate the CO_2 evolution because some fraction of the respired CO_2 that is evolved will be refixed by the more active photosynthetic system.

Decker (1955, 1959a) observed that, when tobacco leaves were placed in darkness after a period in the light, there was a burst of CO_2

evolution before the dark respiration resumed its lower steady rate. He deduced that the post-illumination burst of CO_2 release is derived from a substrate that was synthesized exclusively in the light, and that, when photosynthesis was interrupted, the substrate continued to be oxidized until its supply was exhausted. In *Mimulus*, he found that photorespiration was about 3.3 times greater than dark respiration (Decker, 1959b). Soybean leaves (*Glycine max* L.) also exhibited such a post-illumination burst, but maize leaves did not (Forrester, Krotkov, and Nelson, 1966a, 1966b).

With use of this assay in tobacco, Decker (1959a) found that photorespiration had the following characteristics: it increased with increasing light intensity in a manner similar in proportion to net photosynthesis; it increased 3.4-fold when the leaf temperature was raised from 17.5°C to 33.5°C, while net photosynthesis changed little; photorespiration differed little when leaves were at the CO_2 compensation point (45 ppm) or in air at 300 ppm of CO_2 .

A different assay of photorespiration was developed (Decker, 1957) based on extrapolation of the curve for net photosynthesis at varying CO_2 concentrations from the CO_2 compensation point to "zero" CO_2 concentration. The intercept was taken to be the photorespiration, and in tobacco as well as in eight other species photorespiration exceeded the dark respiration. Recently it has been shown algebraically (Bravdo, 1968; Samish and Koller, 1968) that the intercept is a function of stomatal diffusive resistance and internal diffusive resistances as well as of photorespiration, and thus evaluation of photorespiration by the extrapolation method clearly underestimates the gross CO_2 evolution by an amount that depends on the size of the other resistances.

Photorespiration has also been estimated by observing the rate of dilution by normal respiratory CO_2 of $^{14}\text{CO}_2$ of known specific activity added to the atmosphere surrounding leaves in light in a closed system (Krotkov, Runeckles, and Thimann, 1958). In spite of the diffusive resistances that would tend to slow the rate of dilution in light, in wheat leaves (*Triticum* sp.), photorespiration was still found to be twice the dark respiration.

In CO_2 -free air in the light, the CO_2 flux would be reversed and CO_2 is then released from the leaf at a rate that is a function of the photorespiration as well as internal and stomatal diffusive resistances. This principle was utilized by El-Sharkawy and Hesketh (1965), who showed that CO_2 released in the light in cotton leaves exceeded dark respiration at 35°C, while no CO_2 release was detected in the light in maize. By this method Moss (1966) also found CO_2 released in the light to be greater than dark respiration in five species but again none was released in maize. This technique was modified by first labeling tobacco leaf disks with $^{14}\text{CO}_2$, and then measuring the $^{14}\text{CO}_2$ released in the light in CO_2 -free air (Goldsworthy, 1966). Again photorespiration was found to exceed dark respiration, and further modifications of this method that will be discussed later have provided additional evidence that photorespiration greatly exceeds dark respiration in tobacco leaves but not in maize (Zelitch, 1968).

Thus although all of the methods described undoubtedly underesti-

mate photorespiration by differing amounts, all indicate that photorespiration exceeds dark respiration in many species, but not in maize.

C. The CO₂ Compensation Point

The steady-state CO₂ concentration found when a leaf is placed in light in a closed system is also well correlated inversely with differences in photosynthetic efficiency between species, and this is also likely related to differences in photorespiration. The magnitude of the CO₂ compensation point is determined by the product of the rate of CO₂ evolution inside the leaf times the "resistance" to fixation of CO₂ in the chloroplasts (Bravdo, 1968; Samish and Koller, 1968). Thus a low compensation point could result from a low photorespiration or a low internal "resistance" to CO₂ fixation. The compensation point for maize is less than 10 ppm and for tobacco it is about 50 ppm of CO₂ at 25C, although it will be demonstrated later that the internal "resistance" is not greatly different in these species. Therefore these differences are largely related to differences in photorespiration.

It has long been known that oxygen in excess of 2% in the atmosphere surrounding a leaf decreases net photosynthesis in most species. Part of this effect results from an increase in photorespiration, as may be inferred from the increase in the CO₂ compensation point with increasing oxygen concentration in species like tobacco, wheat, and soybean (Forrester, Krotkov, and Nelson, 1966a; Downton and Tregunna, 1968). On the other hand, even 100% oxygen in the ambient atmosphere did not increase the low CO₂ compensation point in maize leaves (Forrester, Krotkov, and Nelson, 1966b).

In those species with a high CO₂ compensation point, the compensation point also doubles when leaf temperature is increased from 25C to 35C (Zelitch, 1967b). This indicates that photorespiration may even increase more rapidly than gross photosynthesis, and the increase in respiratory CO₂ probably accounts for the lack of increase in net photosynthesis in normal air in tobacco between about 25C and 35C (Decker, 1959a). In maize, net photosynthesis approximately doubles between 25C and 35C (Moss, 1963).

Thus a higher CO₂ compensation point in the less efficient photosynthetic species, and its increase with temperature and increasing outside oxygen concentration all point to an increased photorespiration as the cause of lowered net photosynthesis in species with a high CO₂ compensation point.

D. Net Photosynthesis in Atmospheres Low in Oxygen

If differences in photosynthetic efficiency among species is only little affected by differences in gross photosynthetic rates, but is decreased in the less efficient species because of photorespiration, net photosynthesis should be little different among these species at low oxygen concentrations. Moreover, if the biochemical carboxylation mechanism is not limiting the photosynthetic rate, the overall K_M for

CO₂ concentration in the atmosphere should be similar in such species if photorespiration is largely eliminated.

Goldsworthy (1968) measured net photosynthesis at various CO₂ concentrations at high light intensities in leaf disks of tobacco, maize, and sugarcane with nitrogen in the ambient atmosphere. He found the maximal rates of photosynthesis at saturating concentrations of CO₂ to be about the same in all three species as well as the overall K_m, 300 ppm CO₂. Thus he concluded that the greater photosynthetic efficiency of maize and sugarcane is not the result of a more effective carboxylation system, with a lower K_m in maize than in tobacco.

I have repeated and extended Goldsworthy's experiment (Fig. 10-4). Net photosynthesis was measured in large brightly illuminated leaf disks of tobacco and maize floating upside down on water in a closed system with either 20% oxygen or less than 2% oxygen in the ambient atmosphere. The CO₂ concentration was varied from 1,800 ppm to the CO₂ compensation point. At 1,800 ppm there was little difference in the rate of net photosynthesis between the species in 20% or 2% oxygen; they were all about 22 mg CO₂ dm⁻² hr⁻¹. The overall K_m for maize in both atmospheres and for tobacco in 2% oxygen was about the same and close to 300 ppm of CO₂. The overall K_m for tobacco in air was considerably higher, 488 ppm of CO₂. In a companion experiment, stomatal opening was similar under these photosynthetic conditions in both species. Thus Goldsworthy's (1968) observations were confirmed. In addition, since net photosynthesis in tobacco with low oxygen outside is similar to that in maize at all concentrations of CO₂ (Fig. 10-4), differences in the internal diffusive resistances between these species cannot account for their differences in net photosynthesis in normal air.

Net photosynthesis in soybean leaves was inhibited 35% in air in comparison with an oxygen-free system, while in maize leaves it was not inhibited even in 50% oxygen (Forrester, Krotkov, and Nelson, 1966a, 1966b). Hesketh (1967) compared net photosynthesis in a number of species in air and in an oxygen-free atmosphere. There was no enhancement in an oxygen-free environment in maize and sorghum leaves, but a considerable enhancement occurred in cotton, tobacco, and bean. However, the enhancement did not increase net photosynthesis to the rates observed with maize, although no information was provided about limitations of stomatal diffusion or the hydration of the leaves in the bright light used in these experiments.

E. The Glycolate Oxidase Reaction as a Source of CO₂ in Photorespiration

The data presented already suggest that if one could diminish the photorespiration in species active in this process, one might obtain faster rates of net photosynthesis approaching those of maize. Previously I suggested (Zelitch, 1959, 1964) for several reasons that glycolate is a likely candidate for the primary substrate of photorespiratory CO₂. This metabolite is synthesized in leaves by an unknown mechanism exclusively in the light as an early product of photosynthesis (Zelitch, 1959; Tolbert, 1963). Since it is synthesized in tobacco from ¹⁴CO₂ with

little dilution in specific activity, it must be produced rather directly from CO_2 (Zelitch, 1965b). It is readily synthesized at low CO_2 concentrations in the atmosphere in tobacco (from "zero" to 0.2% CO_2), but its synthesis is inhibited at CO_2 concentrations above 0.2% (Zelitch and Walker, 1964; Zelitch, 1965b). Oxygen is necessary in the atmosphere for rapid synthesis of glycolate (Tolbert, 1963; Zelitch and Walker, 1964), and oxygen is also utilized by an active flavoprotein enzyme, glycolate oxidase, in photosynthetic tissue. This enzyme catalyzes the following reaction in which glycolate is oxidized to CO_2 primarily from the carboxyl-carbon atom:

The glycolate oxidase reaction is effectively inhibited by analogues of glycolate, the α -hydroxysulfonates (Zelitch, 1959, 1965b).

As discussed earlier, changes in the CO_2 compensation point and the post-illumination burst indicate that photorespiration increases greatly with increasing temperature. When glycolate-1- ^{14}C was supplied to tobacco leaf disks, the release of $^{14}\text{CO}_2$ increased about three times faster at 35C compared with 25C than the gross photosynthesis. The relative increase in release of $^{14}\text{CO}_2$ was smaller when either glycolate-2- ^{14}C or acetate-1- ^{14}C were added to the leaf disks. However, in maize leaf disks such effects with glycolate-1- ^{14}C could not be obtained. Thus the carboxyl-carbon atom of glycolate appeared as an important final substrate of photorespiration.

F. Increasing Net Photosynthesis by Inhibition of Glycolate Oxidation

If glycolate oxidation is ultimately the source of the CO_2 evolved in photorespiration, a specific inhibition of this reaction should reveal the magnitude of this process. The inhibition might also cause net photosynthesis to increase to values like those in maize. Experiments to test this hypothesis were carried out on tobacco leaf disks with an α -hydroxysulfonate as inhibitor. Between 25C and 30C net photosynthesis was not adversely affected, although glycolate accumulated rapidly, indicating that the glycolate oxidase reaction was being inhibited *in vivo* (Zelitch, 1965b). When the disks were warmed to 35C, however, the inhibitor made net photosynthesis about 3-fold greater (Zelitch, 1966). In similar experiments with maize, net photosynthesis was not increased by the inhibitor at either temperature. The results were thus consistent with the hypothesis that the CO_2 is evolved in the light in tobacco leaves as a result of glycolate oxidation, and this normally diminishes net photosynthesis in these species especially at higher temperatures when photorespiration accounts for a greater portion of gross photosynthesis.

The data suggested that the internal turnover of CO_2 evolved by photorespiration accounted for 60% of the gross photosynthesis at 35C and 25% of the gross CO_2 uptake at 25C. The data also permitted an

estimate that at 25C in tobacco tissue the CO_2 evolved in the light was about 2.5 times the dark respiration, while at 35C the ratio of light to dark CO_2 evolution was about 6.7 (Table III in Zelitch, 1966).

III. THE RELATION BETWEEN PHOTORESPIRATION AND NET PHOTOSYNTHESIS

A. A Sensitive ^{14}C -Assay of Photorespiration

Because none of the methods of measuring photorespiration, except the post-illumination burst studied by Decker (1955, 1959b), showed such large excesses of photorespiration over dark respiration, I have investigated this process with a more sensitive assay (Zelitch, 1968). Since photorespiration is largely independent of CO_2 concentration up to 300 ppm (Decker, 1959a), I observed the release of $^{14}\text{CO}_2$ in CO_2 -free air from leaf tissue that had previously fixed $^{14}\text{CO}_2$ in the light. Under such circumstances, the CO_2 flux is outward (El-Sharkawy and Hesketh, 1965; Moss, 1966; Goldsworthy, 1966), and the amount released will depend upon the rate of CO_2 evolved inside the leaf and the "resistance" to refixation of evolved CO_2 as well as the diffusive resistance from the intercellular space to the ambient atmosphere (Bravdo, 1968; Samish and Koller, 1968).

Thus in order to release more of the evolved CO_2 in the light and to permit less to be refixed, one should use higher temperatures, rapid flow rates of CO_2 -free air over the leaf tissue, and insure that the stomatal pores are open. In the ^{14}C -assay of photorespiration, leaf disks were exposed to the light for 45 min, and were then allowed to fix a known quantity of $^{14}\text{CO}_2$ in a closed system at 30C to 35C. Then moistened CO_2 -free air was passed rapidly over the leaf disks, and the $^{14}\text{CO}_2$ released was collected and its radioactivity determined. Leaf disks of a standard variety of tobacco, Havana Seed, and those of hybrid maize were compared in the assay, Fig. 10-5. In tobacco there was an approximately linear release of $^{14}\text{CO}_2$ at a rate that was 3 to 5 times greater than the rate of release in darkness. However, in maize, only 2% as

Fig. 10-5—Comparison of $^{14}\text{CO}_2$ released in the light and dark by tobacco and maize leaf disks in the ^{14}C -assay of photorespiration (from Zelitch, 1968).

much $^{14}\text{CO}_2$ was released in the light as from tobacco, although the dark respiration was about the same in these species. I have earlier indicated that it is unlikely that the carboxylation reaction or the photochemistry in tobacco operates at 2% of the rate of maize (Fig. 10-4). Thus I conclude that the difference in efficiency of net photosynthesis between such species is largely caused by the smaller photorespiration in maize, about 2% of that in tobacco.

B. Comparison of Turnover of CO_2 to the Quantity Released in CO_2 -Free Air in the Light

These results suggested the desirability of decreasing the apparently wasteful photorespiration in order to obtain rates of net photosynthesis approaching that of maize. Experiments in this connection will be discussed in the last section. However, first a biochemical method will be described for evaluating the extent of internal refixation of CO_2 in the light in comparison with the amount released to the atmosphere during assays of photorespiration in CO_2 -free air. Thus Lake (1967) and Begg and Jarvis (1968) have clearly recognized this problem and stated that light respiration was at least twice the dark respiration.

When an α -hydroxysulfonate was added to tobacco leaf disks in the photorespiration assay, under conditions that did not close the stomata, the amount of $^{14}\text{CO}_2$ released in the light was strongly inhibited (Fig. 10-6), but not the dark respiration, showing the respiratory CO_2 has different origins in light and darkness. If glycolate is the ultimate source of CO_2 evolved in photorespiration, and this evolution is blocked by the inhibitor, then the ^{14}C present in the glycolate accumulating should be at least equal to the decrease in the quantity of $^{14}\text{CO}_2$ released. However, if more glycolate- ^{14}C accumulated than the decrease of $^{14}\text{CO}_2$ released from the tissue, it would indicate that part of the evolved $^{14}\text{CO}_2$ was refixed and only some fraction of the evolved $^{14}\text{CO}_2$ was normally released to the atmosphere. These assumptions therefore provide a basis for a quantitative evaluation of this fraction of the evolved $^{14}\text{CO}_2$ that is released, x (Zelitch, 1968).

The assumptions in this analysis are shown diagrammatically in

Fig. 10-6— $^{14}\text{CO}_2$ released by tobacco leaf disks in the presence of 10^{-2} M α -hydroxy-2-pyridinemethanesulfonic acid at 35°C (from Zelitch, 1968).

Fig. 10-7—Diagrammatic representation of $^{14}\text{CO}_2$ evolved into the intercellular space and the fraction released to the atmosphere in CO_2 -free air.

Fig. 10-7. If 100 units of $^{14}\text{CO}_2$ were evolved to the intercellular space without inhibitor, and x is the fraction of the $^{14}\text{CO}_2$ that is released to the atmosphere, then $100x$ would be the quantity released and $100(1-x)$ the portion refixed. About 50% of the refixed CO_2 goes into glycolate (Zelitch, 1959, 1965b), and this is represented as $100(1-x)/2$. Since there is negligible accumulation of ^{14}C -glycolate without inhibitor, it is shown in Table 10-1 that the rate of change of ^{14}C -glycolate from endogenous carbon plus that from refixation of $^{14}\text{C}_2$ less the $^{14}\text{CO}_2$ evolved equals zero. With inhibitor (Fig. 10-7), since the inhibitor is used under conditions such that net photosynthesis is not adversely affected, the

Table 10-1—Method of calculating fraction $^{14}\text{CO}_2$ evolved in light that is released to the atmosphere and the fraction that is refixed in the leaf

Rate of change of ¹⁴ C-glycolate		Rate ¹⁴ CO ₂ Evolved	Net change ¹⁴ C-glycolate	Rate ¹⁴ CO ₂ released outside leaf
From endogenous carbon	From refixation of intercellular space ¹⁴ CO ₂			
<u>Without Inhibitor</u>				
$100 - \frac{100(1-x)}{2}$	$+$ $\frac{100(1-x)}{2}$	100	0	100x
<u>With Inhibitor</u>				
$100 - \frac{100(1-x)}{2}$	$+$ $\frac{n(1-x)}{2}$	n	(See below)	nx
Rate change ¹⁴ C-glycolate with inhibitor		$100 - \frac{100(1-x)}{2} + \frac{n(1-x)}{2} - n$		
z = Decrease in ¹⁴ CO ₂ released outside leaf with inhibitor		$= \frac{100x - nx}{2z - 1}$		
Therefore, x, fraction ¹⁴ CO ₂ released outside leaf = $\frac{1}{2z - 1}$				

rate of endogenous synthesis of glycolate is assumed to be unaffected. It follows that if n equals the percentage of $^{14}\text{CO}_2$ evolved in the presence of α -hydroxysulfonate, then $n(1-x)$ is the quantity of evolved $^{14}\text{CO}_2$ that is refixed. The rate of ^{14}C -glycolate accumulation (Table 10-1) with inhibitor would be equal to that arising from unchanged endogenous carbon plus that from refixation of $^{14}\text{CO}_2$, $n(1-x)/2$, less the carbon lost from glycolate oxidation, n . The quotient of the rate of change of ^{14}C -glycolate with inhibitor to the decrease in $^{14}\text{CO}_2$ output with inhibitor, z , can then be represented as shown in Table 10-1. It follows that $x = 1/(2z-1)$.

Therefore if the ratio of the ^{14}C -glycolate accumulated with inhibitor to the decrease in $^{14}\text{CO}_2$ in a given time were 2.0, then $x = 0.33$, indicating that one-third of the CO_2 evolved by photorespiration was released and two-thirds were refixed. Such a result was in fact realized at 35°C (Table 10-2) even though the quantity of ^{14}C -glycolate is somewhat underestimated by the method (Zelitch, 1968), emphasizing how much greater in magnitude photorespiration may be than can be directly revealed even by the sensitive ^{14}C assay. This would be true in spite of a possible overestimation because recently fixed $^{14}\text{CO}_2$ may provide preferred substrates (Zelitch, 1968).

C. Effect of Genetic Control of Photorespiration on Net Photosynthesis

Since rapid CO_2 evolution in the light seemed to diminish net photosynthesis in many species, then a plant with a less active photorespiration might be expected to have a photosynthetic rate approaching that of maize. Such an inhibition of photorespiration and consequent increased photosynthetic efficiency could conceivably be accomplished by means of suitable biochemical inhibitors or perhaps more easily by genetic manipulation. Experiments aimed at testing this hypothesis have been described in great detail (Zelitch and Day, 1968), and can only be summarized here.

Using the ^{14}C assay of photorespiration (Zelitch, 1968; Zelitch and Day, 1968) we searched for leaf material with a lower rate of photorespiration than appeared normal for a given species. A yellow heterozygous mutant of John Williams Broadleaf tobacco (JWB Mutant) and its dark green homozygous recessive sibling (JWB Wild) had been described by Burk and Menser (1964). The ratios of photorespiration to dark respiration were compared in this tobacco as well as in a standard variety, Havana Seed, and in hybrid maize leaves (Table 10-3). Photorespiration was greatest in JWB Mutant (ratios from 3.7 to 6.6 in three experiments with a mean of 4.7), next possibly in Havana Seed (ratios from 3 to 5 in Zelitch, 1968), followed by JWB Wild (ratios from 0.82 to 2.3 in 5 experiments with a mean of 1.6), and least in maize (ratios less than 0.1; Zelitch, 1968). Thus the assay revealed that JWB Wild tobacco has an unusually low photorespiration for tobacco, while its yellow sibling, JWB Mutant, has a high photorespiration rate, perhaps even greater than in Havana Seed.

If the hypothesis is valid that net photosynthesis is diminished because of photorespiration, one would expect from these differences in photorespiration among the three varieties that the net photosynthesis

Table 10-2—Comparison of decrease in $^{14}\text{CO}_2$ and accumulation of ^{14}C -glycolate in presence of α -hydroxy-2-pyridinemethanesulfonic acid in the ^{14}C -assay of photorespiration at 35C (from Zelitch, 1968)

Exp. no.	Fluid	Total $^{14}\text{CO}_2$ fixed	Time in CO_2 -free air	Flow rate CO_2 -free air	$^{14}\text{CO}_2$ released	Decrease $^{14}\text{CO}_2$ released with inhibitor		Increase ^{14}C -glycolate/decrease $^{14}\text{CO}_2$ released	
						count/min	^{14}C -glycolate released	ratio	
1	Water Inhibitor	4.63	6	2.7	42,600	--	2,400	1.3	
		4.63	6	2.7	19,400	23,200	31,900		
2	Water Inhibitor	2.95	15	6.7	108,000	--	7,140	2.0	
		2.95	15	6.7	75,400	32,600	71,200		

Table 10-3—Comparison of $^{14}\text{CO}_2$ released in light and darkness and net photosynthesis in three tobacco varieties and hybrid maize (adapted from Zelitch, 1968; Zelitch and Day, 1968). The light-to-dark ratio was obtained from the ^{14}C -assay of photorespiration. Net photosynthesis was measured in excised leaves in air at about 30C and 300 ppm CO_2 at 1,500 ft-c. The number of determinations of net photosynthesis is given in parentheses next to the standard error of the mean.

Species	Ratio $^{14}\text{CO}_2$ released, Light/dark in photorespiration assay	Net photosynthesis	Comparison net photosynthesis, Havana Seed = 100
	mg CO_2 dm $^{-2}$ hr $^{-1}$		
Tobacco (JWB Mutant)	3.7 to 6.6	10.8 $\pm$ 0.6 (21)	79
Tobacco (Havana Seed)	3 to 5	13.6 $\pm$ 0.5 (17)	100
Tobacco (JWB Wild)	0.82 to 2.3	16.8 $\pm$ 0.4 (22)	124
Maize (Hybrid Penn 602A)	< 0.1	26.5 $\pm$ 0.7 (8)	195

should be clearly superior in JWB Wild to that in JWB Mutant and possibly greater than that in Havana Seed. The results of such measurements are given in Table 10-3. At about 30C and 300 ppm of CO_2 , JWB Wild was 25% more efficient than Havana Seed and JWB Mutant was 21% less efficient than the standard variety. Thus differences in net photosynthesis between species, which had previously been suggested as being caused by the marked differences in their photorespiration, were also found within varieties of a single species that differed in their photorespiration as revealed by the ^{14}C -assay.

Although net photosynthesis in JWB Mutant tobacco was clearly inferior at normal CO_2 concentrations, Schmid and Gaffron (1967) have shown that at 0.45% to 5.0% CO_2 this tissue has a superior net photosynthesis. This efficient photosynthetic system in JWB Mutant is not capable of rapid net photosynthesis because its active photorespiration restricts CO_2 uptake from normal air and hence its growth is also normally severely restricted. Thus blocking photorespiration by genetic manipulation may permit us to increase net photosynthesis greatly. Although the genetic basis for the differences between JWB Mutant and Wild was clearly established by Burk and Menser (1964), these differences may include factors that bear no relation to photorespiration. The genetic aspects of control of photorespiration are still unknown, and further work will be required before we will learn how to control photorespiration in various genetic backgrounds. This genetic and biochemical approach, however, appears to offer hope for a rational means of increasing photosynthetic efficiency.

LITERATURE CITED

- Allaway, W. G., and T. A. Mansfield. 1967. Stomatal responses to changes in carbon dioxide concentration in leaves treated with 3-(4-chlorophenyl)-1, 1-dimethylurea. *New Phytol.* 66:57-63.
- Anderson, L., and R. C. Fuller, 1967. The rapid appearance of glycolate during photosynthesis in *Rhodospirillum rubrum*. *Biochim. Biophys. Acta* 131:198-201.

- Bandurski, R. S. 1955. Further studies on the enzymatic synthesis of oxalacetate from phosphoenolpyruvate and carbon dioxide. *J. Biol. Chem.* 217:137-150.
- Bassham, J. A. 1964. Kinetic studies of the photosynthetic carbon reduction cycle. *Ann. Rev. Plant Physiol.* 15:101-120.
- Begg, J. E., and P. G. Jarvis. 1968. Photosynthesis in Townsville lucerne (*Stylosanthes Humilis* H.B.K.). *Agr. Meteorol.* 5:91-109.
- Bravdo, B.-A. 1968. Decrease in net photosynthesis caused by respiration. *Plant Physiol.* 43:479-483.
- Burk, L. G., and H. A. Menser. 1964. A dominant aurea mutation in tobacco. *Tobacco Sci.* 8:101-104.
- Davenport, D. C. 1967. Effects of chemical antitranspirants on transpiration and growth of grass. *J. Exp. Bot.* 18:332-347.
- Decker, J. P. 1955. A rapid, postillumination deceleration of respiration in green leaves. *Plant Physiol.* 30:82-84.
- Decker, J. P. 1957. Further evidence of increased carbon dioxide production accompanying photosynthesis. *J. Solar Energy Sci. and Eng.* 1:30-33.
- Decker, J. P. 1959a. Comparative responses of carbon dioxide outburst and uptake in tobacco. *Plant Physiol.* 34:100-102.
- Decker, J. P. 1959b. Some effects of temperature and carbon dioxide concentration on photosynthesis of *Mimulus*. *Plant Physiol.* 34:103-106.
- Downton, W. J. S., and E. B. Tregunna. 1968. Photorespiration and glycolate metabolism: A re-examination and correlation of some previous studies. *Plant Physiol.* 43:923-929.
- El-Sharkawy, M., and J. Hesketh. 1965. Photosynthesis among species in relation to characteristics of leaf anatomy and CO₂ diffusion resistances. *Crop Sci.* 5:517-521.
- Forrester, M. L., G. Krotkov, and C. D. Nelson. 1966a. Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. I. Soybean. *Plant Physiol.* 41:422-427.
- Forrester, M. L., G. Krotkov, and C. D. Nelson. 1966b. Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. II. Corn and other monocotyledons. *Plant Physiol.* 41:428-431.
- Gaastra, P. 1959. Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. Mededel. Landbouwhogeschool Wageningen 59:1-68.
- Goldsworthy, A. 1966. Experiments on the origin of CO₂ released by tobacco leaf segments in the light. *Phytochemistry* 5:1013-1019.
- Goldsworthy, A. 1968. Comparison of the kinetics of photosynthetic carbon dioxide fixation in maize, sugar cane and tobacco, and its relation to photorespiration. *Nature* 217:849.
- Heath, O. V. S., T. A. Mansfield, and H. Meidner. 1965. Light-induced stomatal opening and the postulated role of glycolic acid. *Nature* 207:960-962.
- Hesketh, J. D. 1963. Limitations to photosynthesis responsible for differences among species. *Crop Sci.* 3:493-496.
- Hesketh, J. 1967. Enhancement of photosynthetic CO₂ assimilation in the absence of oxygen, as dependent upon species and temperature. *Planta* 76:371-374.
- Hesketh, J. D., and D. N. Moss. 1963. Variation in the response of photosynthesis to light. *Crop Sci.* 3:107-110.
- Jensen, R. G., and J. A. Bassham. 1966. Photosynthesis by isolated chloroplasts. *Proc. Nat. Acad. Sci. U.S.* 56:1095-1101.
- Kortschak, H. P., C. E. Hartt, and G. O. Burr. 1965. Carbon dioxide fixation in sugarcane leaves. *Plant Physiol.* 40:209-213.
- Krotkov, G., V. C. Runeckles, and K. V. Thimann. 1958. Effect of light on the CO absorption and evolution by Kalanchoe, wheat, and pea leaves. *Plant Physiol.* 33:289-292.

- Lake, J. V. 1967. Respiration of leaves during photosynthesis. I. Estimates from an electrical analogue. *Aust. J. Biol. Sci.* 20:487-493.
- Mansfield, T. A. 1967. Stomatal behavior following treatment with auxin-like substances and phenylmercuric acetate. *New Phytol.* 66:325-330.
- Meidner, H. 1962. The minimum intercellular-space CO_2 -concentration (Γ) of maize leaves and its influence on stomatal movements. *J. Exp. Bot.* 13:284-293.
- Meidner, H., and T. A. Mansfield. 1965. Stomatal responses to illumination. *Biol. Rev.* 40:483-509.
- Meidner, H., and T. A. Mansfield. 1966. Rates of photosynthesis and respiration in relation to stomatal movements in leaves treated with α -hydroxysulfonate and glycolate. *J. Exp. Bot.* 17:502-509.
- Moss, D. N. 1962. The limiting carbon dioxide concentration for photosynthesis. *Nature* 193:587.
- Moss, D. N. 1963. The effect of environment on gas exchange of leaves. In *Stomata and water relations in plants*. Conn. Agr. Exp. Sta. (New Haven) Bull. 664:86-101.
- Moss, D. N. 1966. Respiration of leaves in light and darkness. *Crop Sci.* 6:351-354.
- Racker, E. 1957. The reductive pentose phosphate cycle. I. Phosphoribulokinase and ribulose diphosphate carboxylase. *Arch. Biochem. Biophys.* 69:300-310.
- Samish, Y., and D. Koller. 1968. Estimation of photorespiration of green plants and of their mesophyll resistance to CO_2 uptake. *Ann. Bot.* 32:687-694.
- Schmid, E. H. and H. Gaffron. 1967. Light metabolism and chloroplast structure in chlorophyll-deficient tobacco mutants. *J. Gen. Physiol.* 50:563-582.
- Shimshi, D. 1963a. Effect of chemical closure of stomata on transpiration in varied soil and atmospheric environments. *Plant Physiol.* 38:709-712.
- Shimshi, D. 1963b. Effect of soil moisture and phenylmercuric acetate upon stomatal aperture, transpiration, and photosynthesis. *Plant Physiol.* 38:713-721.
- Slack, C. R., and M. D. Hatch. 1967. Comparative studies on the activity of carboxylases and other enzymes in relation to the new pathway of photosynthetic carbon dioxide fixation in tropical grasses. *Biochem. J.* 103:660-665.
- Slatyer, R. O. 1967. Plant-water relationships. p. 249-253. Academic Press, London and New York.
- Slatyer, R. O., and J. F. Bierhuizen. 1964. The influence of several transpiration suppressants on transpiration, photosynthesis, and water-use efficiency of cotton leaves. *Aust. J. Biol. Sci.* 17:131-146.
- Stiller, M. 1962. The path of carbon in photosynthesis. *Ann. Rev. Plant Physiol.* 13:151-171.
- Tolbert, N.E. 1963. Glycolate pathway, p. 648-662. In *Photosynthetic mechanisms of green plants*. Nat. Acad. Sci.-NRC, Washington, D. C.
- Turner, N. C., and P. E. Waggoner. 1968. Effects of changing stomatal widths in a red pine forest on soil water content, leaf water potential, bole diameter and growth. *Plant Physiol.* 43:973-978.
- Waggoner, P. E., and B.-A. Bravdo. 1967. Stomata and the hydrologic cycle. *Proc. Nat. Acad. Sci. U. S.* 57:1096-1102.
- Waggoner, P. E., D. N. Moss, and J. D. Hesketh. 1963. Radiation in the plant environment and photosynthesis. *Agron. J.* 55:36-39.
- Waggoner, P. E., and I. Zelitch. 1965. Transpiration and the stomata of leaves. *Science* 150:1413-1420.
- Walker, D. A. 1962. Pyruvate carboxylation and plant metabolism. *Biol. Rev.* 37:215-256.
- Zelitch, I. 1959. The relationship of glycolic acid to respiration and photosynthesis in tobacco leaves. *J. Biol. Chem.* 234:3077-3081.

- Zelitch, I. 1961. Biochemical control of stomatal opening in leaves. *Proc. Nat. Acad. Sci. U.S.* 47:1423-1433.
- Zelitch, I. 1964. Organic acids and respiration in photosynthetic tissues. *Ann. Rev. Plant Physiol.* 15:121-142.
- Zelitch, I. 1965a. Environmental and biochemical control of stomatal movement in leaves. *Biol. Rev.* 40:463-482.
- Zelitch, I. 1965b. The relation of glycolic acid synthesis to the primary photosynthetic carboxylation reaction in leaves. *J. Biol. Chem.* 240:1869-1876.
- Zelitch, I. 1966. Increased rate of net photosynthetic carbon dioxide uptake caused by the inhibition of glycolate oxidase. *Plant Physiol.* 41:1623-1631.
- Zelitch, I. 1967a. Control of leaf stomata - Their role in transpiration and photosynthesis. *Amer. Sci.* 55:472-486.
- Zelitch, I. 1967b. Water and CO₂ transport in the photosynthetic process, p. 231-248. In A. San Pietro, F. A. Greer, and T. J. Army (ed.) *Harvesting the Sun: Photosynthesis in plant life*. Academic Press, New York and London.
- Zelitch, I. 1968. Investigations on photorespiration with a sensitive ¹⁴C-assay. *Plant Physiol.* 43:1829-1837.
- Zelitch, I. 1969. Stomatal control. *Ann. Rev. Plant Physiol.* 20: In press.
- Zelitch, I., and P. R. Day. 1968. Variation in photorespiration. The effect of genetic differences in photorespiration on net photosynthesis in tobacco. *Plant Physiol.* 43:1838-1844.
- Zelitch, I., and P. E. Waggoner. 1962a. Effect of chemical control of stomata on transpiration and photosynthesis. *Proc. Nat. Acad. Sci. U.S.* 48:1101-1108.
- Zelitch, I. and P. E. Waggoner. 1962b. Effect of chemical control of stomata on transpiration and photosynthesis of intact plants. *Proc. Nat. Acad. Sci. U.S.* 48:1297-1299.
- Zelitch, I., and D. A. Walker. 1964. The role of glycolic acid metabolism in opening of leaf stomata. *Plant Physiol.* 39:856-862.

10... DISCUSSION

DOV KOLLER

*The Hebrew University
Jerusalem, Israel*

Dr. Zelitch has shown that, for comparable conditions, the "efficiency of water use" could be improved by partial stomatal closure, because diffusion of water-vapor is dependent on stomatal resistance to a greater extent than diffusion of CO₂. However, any stomatal closure will reduce CO₂ uptake and thus also reduce photosynthesis. Dr. Zelitch was also quite justified in indicating the futility of using photosynthetic inhibitors, or promoters of photorespiration to increase CO₂ concentration in the mesophyll, thereby inducing stomatal closure. It is worth bearing in mind that, for crops grown under glass or plastic, both efficiency and overall CO₂ fixation have been improved by manipulating

mesophyll CO_2 through judicious increase of ambient CO_2 (C_x). Thus, if

- P = net-photosynthetic flux of CO_2
- R'_w = diffusive resistance to CO_2 over the same pathway taken by transpiration vapor
- R_c = total "resistance" to photosynthetic CO_2 uptake from the ambient atmosphere
- r_m = residual "internal," or "mesophyll" resistance to CO_2 uptake, i.e., $r_m = R_c - R'_w$
- C_m = intercellular CO_2 concentration at the mesophyll evaporating surfaces.
- C_i = intracellular CO_2 concentration ($=0$, after carboxylating its acceptor)

then

$$P = (C_x - C_i)/R_c = (C_x - C_m)/R'_w = (C_m - C_i)/r_m \quad (1)$$

from which, taking $C_i = 0$,

$$C_x/R_c = (C_x - C_m)/R'_w = C_m/r_m \quad (2)$$

$$C_m/C_x = r_m/R_c = r_m/(R'_w + r_m). \quad (3)$$

Thus, doubling C_x will double the gradient, so long as the increase in C_m does not increase R'_w (by inducing stomatal closure) and r_m (by overloading the acceptor sites for CO_2). The increase in P will not be as large if R'_w increases. If, however, r_m increases, the degree to which P may increase would depend on the magnitude of the increase in C_m relative to the increase in r_m [equation (1)].

My second comment relates to the CO_2 compensation point, Γ . The value of this parameter is (i) it is easily obtained, (ii) it is the point at which photorespiratory output and photosynthetic input of CO_2 are exactly equal, and (iii) it is the state at which $C_x = C_m$. However, it is not an independent plant characteristic, but the product of two such characteristics, namely photorespiration and r_m , and any attempt to use it as a unique indicator of photorespiration, or of photosynthetic efficiency, may be entirely misleading. Thus, increase in Γ may result from inhibition of photosynthesis alone, or from enhancement of photorespiration alone (Fig. 10D-1). In both cases, however, stomatal closure associated with increase in Γ may be attributable to increased C_m . By the same reasoning, the fact that PMA treatment (phenylmercuric acetate) caused stomatal closure, without affecting Γ , does not constitute proof that PMA was affecting the stomata directly, not through changing C_m . If PMA were to affect stomatal closure without affecting photorespiration (L), R_c would increase, and so would Γ . The very lack of effect of PMA on Γ seems to indicate that PMA may be inhibiting the same apparatus which fixes CO_2 photosynthetically and releases CO_2 in photorespiration (Fig. 10D-2), so that at Γ , $P - \Delta P = L - \Delta L$.

I would also like to question the significance of the conclusion drawn

Fig. 10D-1—The CO₂ compensation point may change solely as a result of changes in photorespiration, L , (left), or of changes in resistance to CO₂ uptake R_C , (right). In the former, R_C is constant, in the latter, L is constant.

Fig. 10D-2—PMA may affect the CO₂ compensation point, Γ , by affecting photorespiration, L , (dashed line), without acting on photosynthetic efficiency (slope). Γ may remain unchanged, though photosynthetic efficiency and hence also photorespiration, are affected by PMA (solid line).

from Goldsworthy's K_m value being nearly equal for tobacco, sugarcane, and maize. (For references, see chapter by I. Zelitch.) Goldsworthy's (1968) data show that maximal photosynthesis was far from equal (tobacco > sugarcane > maize), probably resulting from nonsaturation with light of the tropical grasses. Furthermore, the apparent " K_m " has very little in common with the K_m value of enzyme kinetics, which characterizes the interaction between a single enzyme and its substrate. Photosynthetic rates, on the other hand, depend on several reactions, controlled by different environmental factors, and to a different degree in different

Fig. 10D-3—Effects of light intensity (sunflowers). Estimate of photorespiration (the extrapolated intercept with the ordinate) is greater when P is plotted as a function of CO_2 at the evaporating surfaces of the mesophyll, C_m , than in the external atmosphere, C_x (after Samish & Koller, 1968).

species. Therefore, the fact that the " K_m " values were nearly the same is an insufficient indication to equality in the "carboxylation efficiency."

My last comment is on the effects of oxygen tension on net photosynthesis. Dr. Zelitch attributes the inhibitory effects of increased $[\text{O}_2]$ on P largely to enhancement of photorespiration. One of the best examples of these effects is given by Forrester, Krotkov, and Nelson (1966a), showing P of soybeans as a function of C_x (their Fig. 7). From equation (1) above it is clear that $C_m = C_x - P \cdot R_w'$, so that when P is plotted as a function of C_m , not C_x , the lines become steeper, and the intercept with the ordinate (L) becomes more negative (i.e., estimate of photorespiration increases) (Fig. 10D-3). Samish and Koller (1968) have shown that photorespiration estimated within the mesophyll (L_i) is greater than that estimated outside the leaf (L_x) by a factor of $1 + R_w'/r_m$. Thus, when R_w' is constant, L_i/L_x will increase as r_m becomes smaller. Therefore, relating P to C_m , instead of C_x , would increase the difference in slopes and decrease the difference in intercepts. Effects of oxygen on photosynthesis therefore appear to be concerned more with carboxylation efficiency (or r_m , to be exact) than with photorespiration. It turns out that the linear P/C_x relationships at all oxygen concentrations, as provided by Forrester, Krotkov and Nelson (1966a), can be extrapolated to a mutual point of intersection (Fig. 10D-4). Assuming that R_w' was constant, as well as unaffected by oxygen concentration (these data were not provided), it can be shown (Appendix 1) that transformation of the results to the P/C_m relationship will cause the lines to converge on the ordinate (i.e., where $C_m = 0$), if

Fig. 10D-4—Effects of oxygen concentration, when P is plotted as a function of CO_2 in the ambient atmosphere. (Adapted from Forrester, Krotkov & Nelson, 1966a. Dashed lines extrapolated.)

$$\begin{aligned} R'_W &= G \cdot (R_2 - R_1) / (\Gamma_2 - \Gamma_1) = G \cdot (R_3 - R_2) / (\Gamma_3 - \Gamma_2) = G \cdot (R_4 - R_3) / (\Gamma_4 - \Gamma_3) \\ &= G \cdot (r_2 - r_1) / (\Gamma_2 - \Gamma_1) = \dots \text{etc.} \end{aligned} \quad (4)$$

where G is the projection on the abscissa of the original point of intersection, R and r are abbreviated symbols for R_c and r_m , respectively, and the numerical suffixes refer to the appropriate lines. The derived intersection on the ordinate will be the estimate of intercellular photorespiration, which is equal at all oxygen concentrations. (This does not rule out the possibility that very low oxygen concentrations may indeed inhibit photorespiration, as they also inhibit dark respiration.) If indeed increased oxygen concentration inhibits net photosynthesis largely, if not uniquely, by increasing "resistance" to carboxylation, it may play the role of an end-product inhibitor of an initial and essential reaction in photosynthesis.

APPENDIX 1 (FIG. 10D-4)

Let:

R and r represent R_c and r_m , respectively ($R = r + R'_W$)

L_X and L_i represent rates of photorespiration, measured externally and inside the mesophyll intercellular spaces, respectively.

Suffixes 1 and 2 designate lines 1 and 2, respectively.

Then:

$$L_{X1} = \Gamma_1 / R_1 = \Gamma_1 / (r_1 + R'_W); \quad L_{X2} = \Gamma_2 / R_2 = \Gamma_2 / (r_2 + R'_W). \quad (1)$$

According to Samish and Koller (1968)

$$L_i = L_X \cdot (1 + R'_W / r) = L_X \cdot R / r. \quad (2)$$

Substituting L_x values from equation (1) we obtain

$$L_{i1} = (\Gamma_1/R_1) \cdot (R_1/r_1) = \Gamma_1/r_1; L_{i2} = (\Gamma_2/R_2) \cdot (R_2/r_2) = \Gamma_2/r_2 \quad (3)$$

If $L_{i1} = L_{i2}$, then

$$\Gamma_1 \cdot r_2 = \Gamma_2 \cdot r_1. \quad (4)$$

From Fig. 10D-4:

$$(\Gamma_1 + G)/R_1 = (\Gamma_1 + G)/(r_1 + R'_W) = (\Gamma_2 + G)/R_2 = (\Gamma_2 + G)/(R'_W + r_2). \quad (5)$$

Solving to obtain R'_W

$$\begin{aligned} (r_2 + R'_W) \cdot (\Gamma_1 + G) &= (r_1 + R'_W) \cdot (\Gamma_2 + G) \\ r_2 \cdot (\Gamma_1 + G) - r_1 \cdot (\Gamma_2 + G) &= R'_W \cdot (\Gamma_2 - \Gamma_1) \\ G \cdot (r_2 - r_1) + \Gamma_1 \cdot r_2 - \Gamma_2 \cdot r_1 &= R'_W \cdot (\Gamma_2 - \Gamma_1). \end{aligned} \quad (6)$$

Substituting equation (4) in equation (6)

$$G \cdot (r_2 - r_1) = R'_W \cdot (\Gamma_2 - \Gamma_1) \quad (7)$$

$$\text{or } R'_W = G \cdot (r_2 - r_1) / (\Gamma_2 - \Gamma_1) = G \cdot (R_2 - R_1) / (\Gamma_2 - \Gamma_1). \quad (8)$$

10...DISCUSSION

T. A. MANSFIELD

*University of Lancaster
Lancaster, England*

Dr. Zelitch raised the question of the control of stomatal aperture by the CO_2 concentration inside the leaf. The fact that increases in CO_2 concentration cause stomatal closure cannot reasonably be disputed in view of the vast amount of evidence available. Freudenberger (1940), Heath (1948), Scarth and Shaw (1951), Kuiper (1961) and Stalfelt (1967) are a few of the workers who have observed that stomatal aperture is influenced by changes in CO_2 concentration in the physiological range, that is, around 300 ppm. Their observations were made on a variety of species and between them they used all the major techniques for observing stomatal aperture. I have recalculated some of the data of Heath and Russell (1954) to show how a change in CO_2 concentration affects stomatal

Fig. 10D-1—Dependence of stomatal opening on CO₂ concentration in 'Charter' wheat (*Triticum* sp.). Light intensity 8,000 lux. The cube-root of the viscous conductance was used for the ordinate to indicate the effect of CO₂ on the diffusive conductance of the stomata. Recalculated data of Heath and Russell (1954).

diffusive conductance in light (Fig. 10-D-1). It is data like these that have led to the view that internal changes in CO₂ concentration contribute to changes in stomatal aperture. The relevant concentration is presumably that inside the guard cells and if a photosynthetic inhibitor like phenylmercuric acetate is sprayed on to the leaf surface it is not surprising that the stomata close. In my opinion phenylmercuric acetate is a successful antitranspirant because it is taken up by the guard cells but is not translocated to the mesophyll, and it therefore has the desired effect of causing stomatal closure without impairing the photosynthetic activities of the major part of the leaf.

In spite of the evidence presented by Dr. Zelitch I remain unconvinced that maize possesses a low CO₂ compensation point merely because it lacks photorespiration. I think that the answer is more likely to lie in the efficiency of photosynthetic carboxylation, and I am concerned about the quality of the evidence that is quoted relating to the efficiency of carboxylation in maize, and comparing it with that in plants having a high CO₂ compensation point. In particular I consider that experiments of the type performed by Goldsworthy (1968) are open to serious objections. Determining K_m values *in vivo* is always likely to be hazardous because there is the possibility of interference from other systems, and in this case there is likely to have been interference from the stomata in addition to intracellular factors. In Goldsworthy's experiments the stomata constituted a resistance between the supply of substrate (CO₂ in the ambient atmosphere) and the reaction sites. At the start of the determinations the material was exposed to conditions likely to cause stomatal closure, namely, a high CO₂ concentration (6,000 ppm) and anaerobic conditions. Thus there was almost certainly a considerable resistance to diffusion in the path from the main source of substrate to the carboxylation centers, and the CO₂ concentration at the latter must have been much lower than in the atmosphere. The quoted values of K_m

are the CO_2 concentrations in the atmosphere and they depend, therefore, not only on the efficiency of carboxylation, but also on the magnitude of the diffusion resistance. Stomata are, however, a variable resistance, and if they opened during the determinations (as is likely since the CO_2 concentration was decreasing), the kinetics of uptake are further complicated. If the same diffusion resistance occurred at given CO_2 concentrations for both maize and tobacco the errors would cancel out in a comparison between the two species. However, it is most unlikely that the diffusion resistances are the same since the stomata are different in size, number, distribution, and physiology (maize stomata are more sensitive to CO_2 than those of most species).

The view that carboxylation is not more efficient in maize is thus based on very flimsy evidence. There is, however, good evidence from in vitro studies that phosphoenolpyruvate carboxylase, which is now thought to be responsible for photosynthetic carboxylation in maize, has a higher affinity for CO_2 than ribulosediphosphate carboxylase. In some succulents, where it has long been known that PEP carboxylase is responsible for dark CO_2 fixation, Wilkins (1959) found no CO_2 output into a stream of CO_2 -free air in the dark. This is equivalent to a CO_2 compensation point of zero.

ADDITIONAL LITERATURE CITED

- Freudenberger, H. 1940. Die Reaktion der Schliesszellen auf Kohlensäure-und Sauerstoff-Entzug. *Protoplasma* 35:15-54.
- Heath, O.V.S. 1948. Control of stomatal movement by a reduction in the normal carbon dioxide content of the air. *Nature, Lond.* 161:179-81.
- Heath, O.V.S., and J. Russell. 1954. An investigation of the light responses of wheat stomata with the attempted elimination of control by the mesophyll. Part 2. Interactions with external carbon dioxide, and general discussion. *J. Exp. Bot.* 5:269-92.
- Kuiper, P.J.C. 1961. The effects of factors on the transpiration of leaves, with special reference to the stomatal light response. *Meded. Landb Hoogeschool Wageningen* 61:1-49.
- Scarth, G. W., and M. Shaw. 1951. Stomatal movement and photosynthesis in Pelargonium. I. Effects of light and carbon dioxide. *Plant Physiol.* 26:207-25.
- Stalfelt, M. G. 1967. The components of the CO_2 -induced stomatal movements. *Physiol. Plant.* 20:634-42.
- Wilkins, M. B. 1959. An endogenous rhythm in the rate of carbon dioxide output of Bryophyllum. *J. Exp. Bot.* 10:377-90.

ERRATUM---PHYSIOLOGICAL ASPECTS OF CROP YIELD

For Chapter 10--Discussion, by Dov Koller, pages 226-231

After this book had gone to the press, the author of the Discussion for Chapter 10, p. 226-231 showed¹ that the generally used relationship $R_C = R'_W + r_m$ is inapplicable in photorespiring plants. This necessitated the following changes to be made in the text, as well as replacing the APPENDIX: -

page 227, line 8: substitute 'intracellular' for 'residual "internal" '.

line 9: delete 'i. e. $r_m = R_C - R'_W$ '.

line 14: should read 'then, if photorespiration is negligible'

page 230, equation (4) should read

$$\begin{aligned} R'_W &= G \cdot (r_2 - r_1) / (\Gamma_2 - \Gamma_1) = G \cdot (r_3 - r_2) / (\Gamma_3 - \Gamma_2) \\ &= G \cdot (r_4 - r_3) / (\Gamma_4 - \Gamma_3) = \text{etc.} \end{aligned}$$

The APPENDIX on pages 230-231 should be replaced with the following:

APPENDIX 1 (FIG. 10D-4)

Let:

R and r represent R_C and r_m , respectively; L_X and L_I represent rates of photorespiration, measured externally and inside the mesophyll intercellular spaces, respectively; suffixes 1 and 2 designate lines 1 and 2, respectively.

Then: $L_{X1} = \Gamma_1 / R_1$; $L_{X2} = \Gamma_2 / R_2$ (1)

$$(L_{X1} / L_{X2}) \cdot (\Gamma_2 / \Gamma_1) = R_2 / R_1 \quad (2)$$

$$L_{I1} = L_{I2} = \Gamma_1 / r_1 = \Gamma_2 / r_2 \quad (3)$$

$$\Gamma_1 / \Gamma_2 = r_1 / r_2 \text{ and } r_1 \cdot \Gamma_2 = r_2 \cdot \Gamma_1 \quad (4)$$

According to Samish and Koller (1968)

$$L_{I1} = L_{I2} = L_{X1} \cdot (1 + R'_W / r_1) = L_{X2} \cdot (1 + R'_W / r_2) \quad (5)$$

¹ Koller, D. 1969. Characteristics of the photosynthetic apparatus derived from its response to natural complexes of environmental factors. Proc. IBP/PP Technical Meeting "Productivity of Photosynthetic Systems, Models and Methods". Trebon, Czechoslovakia. (I. Setlik, editor). In press.

Rearranging equation (5) and substituting from equation (2)

$$\begin{aligned} (r_2 + R'_W)/(r_1 + R'_W) &= (L_{X1}/L_{X2}) \cdot (r_2/r_1) \\ &= (L_{X1}/L_{X2}) \cdot (\Gamma_2/\Gamma_1) = R_2/R_1 \end{aligned} \quad (6)$$

$$R'_W \cdot (R_2 - R_1) = r_2 \cdot R_1 - r_1 \cdot R_2 \quad (7)$$

Dividing by R_2

$$R'_W \cdot (1 - R_1/R_2) = r_2 \cdot (R_1/R_2) - r_1 \quad (8)$$

From Fig. 10D-4

$$(\Gamma_1 + G)/R_1 = (\Gamma_2 + G)/R_2 \quad (9)$$

$$R_1/R_2 = (\Gamma_1 + G)/(\Gamma_2 + G) \quad (10)$$

Substituting equation (10) and (4) in equation (8)

$$R'_W [1 - (\Gamma_1 + G)/(\Gamma_2 + G)] = r_2 \cdot (\Gamma_1 + G)/(\Gamma_2 + G) - r_1 \quad (11)$$

From which

$$R'_W \cdot (\Gamma_2 - \Gamma_1) = G \cdot (r_2 - r_1) \quad (12)$$

$$R'_W = G \cdot (r_2 - r_1)/(\Gamma_2 - \Gamma_1) \quad (13)$$