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PHOTOSYNTHESIS AND BIOSYNTHETIC PATHWAYS TO CHEMICALS

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INTRODUCTION

The storage of chemical energy by green plants using light energy occurs through the conversion of water, carbon dioxide, and minerals to gaseous oxygen and a multitude of chemical compounds which are the substance of the plant itself.

Commonly, carbohydrates are considered to be the major product of photosynthesis, and indeed this is often the case. The pathways of biosyntheses in plants actually diverge, however, after the formation of triose phosphates which are the first carbohydrate products of CO_2 fixation and reduction by the Calvin Cycle in photosynthesis (1).

Triose phosphates may either be converted to starch and other products in the chloroplasts, or may be transported out of the chloroplasts to be used as biosynthetic starting material. In developing green cells, this biosynthesis results in the formation of proteins, lipids and phospholipids, pigments, etc. required to build cell structure and enzymes. As leaves expand and green cells mature, there is a redirection of carbon flow. This results in increased synthesis of sucrose and a few key amino acids which are exported from the leaf to be used in the growing tips, the roots, stem, flowers, and in time, the storage organs and seeds. In those other parts of the plants, a great variety of biosyntheses occur.

The product diversity is immensely increased if one considers the whole plant kingdom. The amounts of these many products, and hence their potential economic value to mankind, vary not only with species, variety and plant part, but also with stage of development, and a myriad of physiological variables including climate, nutrition, solar incidence, disease, etc. These obvious facts are well known to farmers and agronomists; but are perhaps worth emphasizing at a conference such as this one lest we become too much inclined to seek universal solutions to the problems and possibilities of diverse areas. An oil or gas well may produce a somewhat similar product in any part of the world, but the appropriate chemical technology based on plant growth is likely to be very different for humid tropics, semiarid regions, or temperate zone areas with more abundant rainfall. Not only are the economic plant chemicals different in nature and amount, but also the conditions for processing them will vary.

Although the present Conference is devoted to consideration of renewable sources of chemicals, food and energy supplies are closely related to chemical supply, particularly when we are considering plants. If we can derive two or even all three of these basic needs from a plantation system, the overall economics can improve. The constituents of plants range from those that are formed in large amounts but have relatively low economic value per unit weight to those rare chemicals such as pharmaceuticals with very high value

per unit weight. At the low end of the value per unit weight scale is biomass to be burned in stationary power plants. Perhaps comparable in value is biomass for fermentation to produce methane, if ways can be found to carry out this conversion economically. Next in ascending value would be liquid fuel (hydrocarbons or alcohols) which could serve the needs for both transportable fuels and chemical feedstocks. Foods generally have a higher value than these bulk liquid chemicals, but there will no doubt be some specialty chemical feedstocks from plants with higher value yet. Finally there are the valuable pharmaceuticals, dyes, vitamins, etc. from plants. Plant development and selection, economic assessment, etc. should include an evaluation of the total plant constituents. We must look on plants as raw materials much as petroleum chemists would a specific crude oil, with the intent to utilize all possible components to maximum economic advantage. Even for plants with economically desirable and abundant constituents, there are a number of options for separating and utilizing these compounds and for converting them to chemicals useful to mankind.

If we consider all the variables mentioned, species, plant part, environment, range of useful products, utilization, and total economic assessment, we can begin to perceive the magnitude of the technological job to be accomplished. During the next twenty to fifty years we need to replace the world's diminishing supply of petrochemicals with plants as a renewable source of materials. One aspect of this task in particular may require much time. This is the job of developing productive (high yield) plants with desirable characteristics such as disease resistance, ease of planting or propagation, etc. Plant breeding is a slow and arduous process which in its more primitive form has engaged mankind for thousands of years. Even with modern techniques, development of new strains of established agronomic crops can take years. It is hoped that plant development may be accelerated through new methods of plant cell culture, induced mutation and other genetic techniques, cell selection, plant regeneration, and further selection. Such research is now being encouraged in the United States as a means of accelerating improvement in food production. Clearly, this kind of research also should be extended to the development of plants for chemicals and energy.

EFFICIENCY OF SOLAR ENERGY CONVERSION

Overall energy efficiency of biomass production from solar energy in temperate zone regions is commonly in the range of 0.1 to 1%. Since economic factors may depend significantly on the efficiency, it is worthwhile to consider briefly what are the practical limits. Is it worthwhile to consider novel or unusual means for increasing efficiency once we become interested in the whole plant? For many food crops, where the plant goes through a growth cycle to senescence, and where the food organ collected is the seed or root, solar energy conversion efficiency is a factor in productivity, but not necessarily the most important one. If we are to collect leaves, stalks or trunks, etc., however, overall solar energy efficiency leading to greater biomass production becomes more important and may warrant novel undertakings.

Loomis et al. (2) estimated potential productivity of land plants to be 71 g/m² day under illumination of 500 cal/m² day, assuming optimum temperatures and light response and a one-third loss due to respiration. I have made similar assumptions (3), but employed a more optimistic value for the quantum yield.

The first factor limiting solar energy conversion by plants is the efficiency with which plants can convert sunlight by the process of photosynthesis.

A common way of expressing photosynthesis is by the equation: $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{O}_2 + (\text{CH}_2\text{O})$. When (CH_2O) represents 1/6 of a glucose molecule, the Gibbs free energy stored per mole of CO_2 reduced to glucose (ΔG) is about 114 Kcal (4). Actually, free glucose is not an important product of photosynthesis, but glucose moieties are incorporated into starch, so that the equation is a reasonable approximation. If we are considering starch or cellulose as the principal product, however, we must allow for the fact that the molecular weight of the product is not 30, which it would be for CH_2O , but 27, since one H_2O (MW = 18) is removed for each glucose (MW = 180) incorporated into the starch or cellulose chain.

The reduction of a molecule of CO_2 to the level of glucose or sugar phosphates requires the transfer of four electrons from water, liberating an O_2 molecule. Each electron transferred requires two photochemical steps each with a quantum requirement of one photon absorbed per electron transferred, or eight quanta in total. Concurrent with this transfer of four electrons is the production of about three molecules of adenosine triphosphate (ATP), the energy for which is derived from the electron flow. This ATP is sufficient for the reduction of CO_2 to sugar phosphates, although additional ATP may be required for further bioconversions in the green cell. To the extent that such additional ATP is required, it would utilize light energy to drive another process of ATP formation called cyclic photophosphorylation. This cost is not included in the photosynthetic efficiency factor calculated here since such requirements will be combined into the later correction for respiration/biosynthesis.

The reduction of one mole of CO_2 thus requires the absorption of eight "moles" or einsteins of light two einsteins for each of the four equivalents of electrons transferred from water to carbon dioxide.

Green plants can use only light of wave lengths from 400 nm to 700 nm. This photosynthetically active radiation (P.A.R.) constitutes only about 0.43 of the total solar radiation at the earth's surface at a location such as the U.S. Southwest. All of this light is used as if it were 700 nm light, but since the photosynthetically active radiation includes all wavelengths from 400 nm to 700 nm, the energy input is equivalent to that of monochromatic light of about 575 nm wavelength. An einstein of 575 nm light has an energy of 49.74 Kcal. Multiplying by 8, we get 398 Kcal as chemical potential; thus the maximum efficiency of photosynthesis is $114/398 = 0.286$. This would appear to be the maximum possible efficiency of the photosynthetic reaction. Probably the actual efficiency is somewhat less, but measurements of quantum requirements in the range of 8 to 10 einsteins required per O_2 molecule evolved has been reported (5).

The maximum efficiency of 0.286 is for conversion of P.A.R. The efficiency based on total solar radiation incident on the plants with total absorption of P.A.R. is $0.43 \times 0.286 = 0.123$.

The maximum net efficiency, over a 24 hr period, and under field or aquatic conditions, depends on two other factors the amount of incident light actually absorbed in the green tissue, and the cost of energy used in respiration

and biosynthesis. For land plants, it has been estimated that the maximum absorption to be expected from an optimal leaf canopy may be 0.80 (2). This is due to some light being reflected and some reaching the ground or falling on nonphotosynthetic parts of the plant (such as the bark of trees). With aquatic plants such as unicellular algae that are totally immersed, there may be less reflection, and with sufficient density of algae, absorption could be essentially complete in green tissues.

A major loss in stored chemical energy results from respiration which occurs in all tissue not actively photosynthesizing. Respiration occurs in green cells at night or in dim light, and in roots, trunks and other organs. The energy derived from respiration is used for various physiological needs of the plant, transport and translocation, conversion of photosynthate to protein, lipids (including hydrocarbons in some plants), cellulose for structures such as stalks and trunks, and so forth. In the green cells during photosynthesis, some energy from the photosynthetic process itself may be used for such purposes, as mentioned earlier. Like the light absorption factor, the factor for respiration/biosynthesis is extremely variable, depending on the physiological conditions and needs of the plant. It is estimated that in a typical case, respiration and biosynthesis use up to one-third of the energy stored by photosynthesis (27). The factor would thus be 0.67.

It may be argued that both the absorption factor and the respiration factor are not true maximum values, since there may be cases where each is exceeded. The product of these two factors, $0.80 \times 0.67 = 0.53$ probably is close to the maximum, since there is some trade-off between the two factors. A C-4 land plant (discussed later) might have all well-illuminated leaves and hence a lower respiration rate but this would mean that its leaf canopy was probably less perfect than required for 0.8 absorption. At the other extreme, in a dense forest, little light may reach the ground, but the respiration in the shade plants may nearly equal photosynthesis. Similarly, an algae pond may absorb nearly all the P.A.R., but the average light intensity for the cells would then be so low as to allow a high rate of respiration. Advocates of algae as the most efficient of photosynthetic plants do not always take this into consideration.

If we combine the photosynthetic efficiency, 0.123, with the product of the absorption and respiration/biosynthesis factors, 0.534, we obtain an overall maximum efficiency for photosynthetic/biosynthetic energy storage by green plants of 0.066.

From the equation and discussion given earlier, the reduction of a mole of CO_2 to the glucose moiety of starch or cellulose stores about 114 Kcal and results in an organic molecular weight of 27. Each Kcal of stored energy thus results in the formation of $27/114 = 0.237$ grams of biomass (dry weight), if the biomass were entirely cellulose and starch. Of course, this is not the actual case, but the assumption provides a reasonable approximation.

Total available solar energy depends not only on latitude, but especially on amount of cloud cover. It is not surprising that the greatest annual incidence of solar energy is found in desert and semiarid regions, rather than in the humid tropics. The greatest amount of photosynthesis is in tropical forests because of the abundance of moisture and year around optimal growing temperatures. A higher intensity of photosynthesis could be

obtained in arid regions, however, if ample water and year around growing temperatures could be provided, and other physiological factors could be optimized.

Calculated maximum biomass production and reported high yields

A typical solar irradiance in arid regions at 35° latitude is the value in the U.S. southwest of 4,610 Kcal/m² day averaged year round for 24 hrs per day. From the foregoing discussion, the upper limit for biomass production can be calculated by multiplying the efficiency, 0.066 times the daily total energy times 0.237. This gives 72 grams/m² day for the U.S. Southwest on an annual basis, equivalent to 117 tons per acre yr, or 263 metric tons per hectare year (Table I).

Since optimal conditions of temperature, light absorption, etc. are never found during all seasons for crops in the temperate zone, it is obvious that reported crop yields will not approach this maximum on an annual yield basis. Nevertheless, it is instructive to compare reported high yields and maximum growth rates with the calculated values. What are the actual rates measured? The figures in parentheses (Table I) are rates during the active growing season, not annual rates. For C-4 plants, these maximum rates range from (138) up to (190) metric tons per hectare per year. The highest (190) is about half the calculated maximum. Similarly, the highest reported annual yield, with sugar cane in Texas, is 112 metric tons per hectare--again about 1/2 the calculated maximum (263) for the U.S. Southwest.

The energy storage efficiency for these reported yields suggest that 3.3% to perhaps 5% as the best we can hope for with land plants in the future. One reason for going above the highest reported yields of total dry material (3.3%) is that we should be able to make some improvements if we can provide for year around growth and frequent harvesting of organic matter, as discussed later. For silviculture in temperate zones, the figure for Eucalyptus of 54 metric tons per hectare-year (1.3%) probably represents a realistic upper limit. For conventional agriculture in temperate zones, the 112 tons per hectare-year (2.8%) for sugar cane is a likely maximum.

C4 METABOLISM AND PHOTORESPIRATION AND EFFECTS OF CO₂ ENRICHMENT

The term C-4 refers to certain plants such as sugar cane that evolved in semi-arid tropical or sub-tropical areas, and which have a special added metabolic pathway (7,8). Some of the intermediate compounds in this pathway are four-carbon acids, hence the term "C-4". Those plants use some of their light energy to drive this extra path, but their overall energy efficiency in air and bright sunlight is higher than for other plants. This is because, by investing energy in the C-4 pathway, the C-4 plants avoid a wasteful process called photorespiration (reviewed by Zelitch, 9) that occurs in other plants (called "C-3" plants) at high light intensities. Photorespiration results in the reoxidation of freshly formed sugar to carbon dioxide. The C-4 plants are more efficient under conditions of high light intensities and temperatures and low CO₂ pressures such as in air (0.03% CO₂) where photorespiration occurs in C-3 plants. At higher levels of CO₂, photorespiration doesn't occur and some C-3 plants become just as efficient as C-4 plants.

Field grown plants are limited by air levels of CO_2 , and these levels can drop even below the general atmospheric level of 0.032% on a still day in a densely planted field with bright sunshine and otherwise optimal conditions for photosynthesis. Achievement of efficiencies approaching the calculated maxima probably would require higher levels of CO_2 , especially for C-3 plants. There is in fact a two-fold effect on photosynthetic rate in such plants with increased CO_2 since the carboxylation rate increases while photorespiration ceases. The detailed study by Gaastra (10) showed that for sugar beet, turnip, cucumber, spinach, and tomato, increases in photosynthetic rate of two-fold or more could be obtained by increasing the CO_2 pressure from air levels (0.032%) to 0.13%. A more recent study with some other important crops (11) shows similar increases.

CHEMICAL PLANTATIONS

There has been considerable discussion and even some research on "Energy Plantations" in recent years (12). For energy, the emphasis is on rate of biomass production as well as various other economic factors such as land and water cost, environmental impact, cost of planting or propagating, harvesting collection, and processing. For chemical plantations, many considerations may be similar. In fact, as mentioned earlier, the most economic system may be those in which there is an integration of energy, chemicals, and even food production. It is conceivable, for example, that a harvested bushy plant might be pressed to remove juice from which protein would be precipitated (using solar energy), after which the plant material would be extracted for hydrocarbon-like compounds to be processed as chemical feedstocks. Finally, the cellulosic residue could be burned to generate electrical power. Consideration of the possibility for multiple uses of plants (food, energy, chemicals, material) can lead to various novel or unconventional proposals for land use. It appears that good agricultural land with adequate water should be mostly reserved for food production. Of course, conventional agriculture and forestry produce large quantities of materials now wasted, and there has been much discussion of possibilities for collecting and converting this material to useful chemicals. In addition, however, we are coming to realize that plants producing chemicals and fuels do not always require good agricultural land. It is possible to grow chemical and energy crops on semiarid land, in rough or mountainous terrain, in lakes, rivers and even in open oceans (13).

Given the high solar energy received by desert land, it is interesting to consider arid and semiarid regions for chemical/energy plant growth. The obvious limitation is lack of water, but there are at least two possibilities besides the limited one of providing irrigation by bringing water from distant streams or very deep wells. One approach, which I will return to later, is to make use of plants that thrive in those regions.

Another way to take advantage of the high solar incidence and low land cost of the U.S. Southwest would be to employ covered agriculture (3,6,14,15). This rather futuristic proposal is to cover large areas with high greenhouses. The canopies would be made from tough, sun-resistant plastic. The structures might be 1 Km^2 in area and 300 meters high (at maximum extension), perhaps with a capacity to go up and down daily. A requirement would be to maintain growing temperatures year round. Under this canopy might be grown a high-protein forage legume such as alfalfa. It would be harvested periodically

during the year, leaving after each harvest enough of the plant to quickly produce a good leaf canopy. Growth would be maintained all year. The atmosphere would be enriched in CO_2 and neither water vapor nor CO_2 would be allowed to escape, although some CO_2 would diffuse through the plastic canopy.

While there are serious problems to be overcome with this system (economic, engineering, and physiological), there are a number of possible important advantages.

1. With year round growth and CO_2 enrichment (photorespiration eliminated), maximum photo-synthetic efficiency should be possible. At a 5% conversion efficiency, the yield would be 200 metric tons/hectare-year (89 ton/acre-year). The whole plant except for roots would be harvested and used.
2. Enrichment with CO_2 can result in a five-fold increase or more in N_2 fixation in the root nodules of legumes (16). Most or perhaps all of the nitrogen requirements in legumes would be met by N_2 fixation, due to stimulation at these high photosynthetic rates.
3. Alfalfa grown under optimal conditions has as high as 24% protein content based on dry weight. It is feasible and economic to remove a part of this protein as a high value product using the method developed at the Western Regional Research Laboratory of the U.S. Department of Agriculture in Albany, California (17). The residue is a feed for ruminants or a biomass source. Most of the feeding of expensive cereal grains to cattle could be replaced by this alfalfa, and the cereal grains could be sold for human nutrition in the U.S.A. and abroad where there is a rapidly growing market. The protein extract of the alfalfa has a high value as animal feed (poultry, for example). An interesting alternative is to convert part of it to a protein product for human consumption (18). Nutritionally, it is as complete as milk protein (19) and far superior to soy protein. From the 15 metric tons of dry matter removed as juice from the leaves, it might be possible to recover 5 tons of protein.
4. Land with relatively low value at present because of lack of water could be used because of water recycling. With water vapor containment, only a few percent of the present irrigation requirements for desert land would have to be met. This would be an extremely important advantage in some areas of the world.
5. Carbon dioxide could be obtained from flue gasses from fossil fuel power plants, thus decreasing the amount of CO_2 discharged to the atmosphere. Projected increases of CO_2 in the atmosphere when fossil fuels are all burned is a matter of considerable concern with respect to the future temperature and climate of the earth (20). Alternatively, CO_2 from CO_2 gas wells might be used (11).
6. The modular nature of the system would help in the prevention, containment, and elimination of plant diseases.
7. Once the needs for cattle feed are satisfied, additional capacity could supply either fuel for power plants or biomass for conversion to chemicals.

Although originally proposed for energy and protein production, it is worth considering the residue not only as a source of fuel for power plants, but also as a chemical feedstock. Since the plastic canopy would have to be replaced,

it is considered necessary to regenerate the plastic from some of the biomass residue. One possibility is to convert some of the cellulosic residue to glucose. This cellulose is already in a more finely divided state than in wood and perhaps is associated with less lignin. Fermentation of glucose to ethanol and CO_2 (which would be returned to the greenhouse) would be followed by conversion to ethylene and polyethylene. If such a process were set up to regenerate plastic for the canopy, perhaps it would be economic to convert all of the cellulosic residue to chemicals. The residue also contains xylans which could perhaps be converted to furfural (21).

Such a C.E.A. scheme would take many years of development before becoming economic. There are many problems of plant physiology, chemical and mechanical engineering, agronomy, etc. to solve. Research is needed in the next few years to determine the response and productivity of a variety of plants including alfalfa to CO_2 enrichment, high year round growing temperatures, repeated harvesting, etc. Even if we do not build large scale C.E.A., it would be worthwhile to study the food/chemical potential of continuously harvested leafy plants. If large scale C.E.A. were developed, it would, of course, not need to be limited to forage crops, but could be used for whatever is most advantageous economically. A likely scenario would be the production of a valuable food substance, with the remaining biomass to be used for chemicals, material and energy. The relevance to the objectives of this conference lies in the huge expected yields. If two hundred tons dry weight per hectare could be collected per year there are obvious advantages for industrial use as compared with conventional biomass production requiring collection over an area ten-times greater for the same amount of material. In cold climates, heat from fossil-fueled power plants may be used to maintain year round growing temperatures. Turning to less "futuristic" plans, there are many opportunities to utilize plant growth in diverse environments for the production of renewable resources.

In semiarid regions there are opportunities to increase the utilization of native plants and other species which can be grown in such areas. Although the growth rate of desert plants is generally slow because of the adaptive mechanisms for their environment, such plants often contain relatively high proportions of hydrocarbons and other energy rich compounds. One notable example is the guayule plant which is being grown especially in Mexico as a source of rubber. Other desert plants being considered include jojoba, mesquite, and yucca, as discussed by Dr. Campos-Lopez at this Conference. Calvin (22), and Nielsen, et al. (23) have proposed and are investigating the potential of various species of *Euphorbia* as sources of hydrocarbon-like compounds. Analysis of chemicals extracted from several species, but especially *Euphorbia lathrus*, grown in test plots, shows about 10% by dry weight of hydrocarbon-like materials. In the case of *E. lathrus*, the steroids constituted the largest part of this fraction, but there were also significant amounts of other isoprenoid materials, and triglycerides.

The heat of combustion of this mixture is about 9.54 Kcal/g, essentially the same as the heat of combustion of terpeneol. This may be compared with 4.18 Kcal/g for starch, 11.5 Kcal/g for hydrocarbons and a range of 4.6-5.0 Kcal/g for totally dry wood from trees (corrected for the 7-13% water in most "dry" wood). Such figures illustrate the point that carbohydrates (sugar, starch, cellulose) contain carbon that is one half reduced (redox state = 0) compared with CO_2 (+4) whereas hydrocarbons are fully reduced (-4). These facts lead

to a conclusion that for some purposes it is desirable to allow plants to convert carbohydrates to hydrocarbon-like materials, even though the conversion will result in a decrease in biomass so that the overall efficiency of solar energy conversion will not be improved. In fact, the conversion will cost energy in the plant but the price may be less than making the conversion after the plant is harvested as by converting cellulose to glucose to ethanol. The humid tropics, including the jungles of South America, Africa, and Asia offer a great diversity of large trees and other plants with large numbers of potentially useful chemical compounds. The production of rubber in Hevea is an important industry and great advances have been made in the last several decades in increasing productivity. There are other trees in the humid tropics which produce considerable amounts of essential oils and resins and there may be many more chemicals from such trees that could be economically collected. An area of research deserving intense effort is the thorough exploration of the conversion-efficiencies, chemical composition, and possibilities for non-destructive collection of chemicals from trees in the humid tropics. Among such trees are legumes capable of N_2 fixation. This ability may prove very important to maintenance of good yields of chemicals in wet climates where applied fixed nitrogen could be easily lost by rain runoff.

Much more could be said about the possibility for various types of chemical plantations, but these subjects are covered by others, and I wish now to turn to another important subject: the allocation of photosynthate to biosynthesis.

CONVERSION OF PHOTOSYNTHATE TO SECONDARY PRODUCTS

As indicated in the INTRODUCTION, photosynthetic fixation and reduction of CO_2 is completed with the formation of the triose phosphate, glyceraldehyde-3-phosphate (Gal3P) (Figure 1). In the normal operation of the Reductive Pentose Phosphate Cycle (Calvin Cycle), each complete turn of the cycle requires the fixation of three molecules of CO_2 to 3 molecules of the pentose phosphate, ribulose-1,5-diphosphate (RuDP), resulting in the formation of 6 molecules of 3-phosphoglycerate (PGA), each of which is reduced by ATP and NADPH to give Gal3P. Of the 6 triose phosphate molecules produced, 5 are required to regenerate the pentose phosphate molecules. Each of these is then converted with ATP to RuDP, thus completing the cycle.

The remaining triose phosphate molecule (formed from 3 CO_2 , 9 ATP, and 6 NADPH molecules) is available for biosynthesis. In order to maintain a store of reduced carbon, the chloroplasts convert a part of the excess triose phosphate molecules to starch which is stored in the chloroplasts. Also, some triose phosphate is reduced to glycerol phosphate which is used in subsequent synthesis of phospholipid for chloroplast membranes.

Most of the net triose phosphate is exported to the cytosol (21-24). There is a specific inorganic phosphate (P_i) triose phosphate translocator for this process (22-24). It appears that increased P_i in the cytosol may stimulate increased triose phosphate export at the expense of starch formation. At the same time, the P_i entering the chloroplast in exchange for triose phosphate can inhibit starch formation (25,26).

How P_i level in the cytosol is regulated is not known but its regulation may be of considerable importance to control the export of photosynthate. One possibility could be through control of formation of P_i from inorganic pyrophosphate (PP_i), which is formed as a by-product during sucrose biosynthesis from glucose phosphate and UTP.

A balance of reaction rates is maintained within the chloroplasts to insure that triose phosphate in the chloroplasts remain within physiological levels required for continued operation of the RPP Cycle. The rate limiting steps in the RPP cycle are the carboxylation of RuDP and the diphosphatase-mediated reactions converting fructose-1,6-diphosphate (FDP) and sedoheptulose-1,7-diphosphate (SDP) to their respective monophosphates, F6P and S7P (3). In the light, triose phosphates are nearly in equilibrium with PGA, from which they are formed, and with FDP and SDP, to which they are converted. Triose phosphates, PGA, FDP and SDP thus constitute a pool of carbon compounds. This pool increases or decreases with the relative rates of carboxylase and diphosphatase reactions. As triose phosphates are withdrawn, and their steady-state concentrations in the chloroplasts decrease, it appears likely that the rates of FDPase and SDPase-mediated steps decrease relative to carboxylation rates, thus tending to keep the triose phosphate concentrations from dropping still lower.

A further export of carbon from chloroplasts occurs in the form of glycolate (Figure 2). This compound is believed by many but not all workers to be derived mainly from phosphoglycolate which is formed by the oxygenase activity of the RuDP carboxylase/oxygenase (27-30). The reaction with O_2 instead of CO_2 results in the formation of one molecule of phosphoglycolate and one of PGA (28-30). A specific phosphatase converts phosphoglycolate to glycolate. Formation of glycolate is believed to be less in C-4 plants, since CO_2 in the bundle-sheath chloroplasts (where RuDP carboxylase is located) is maintained at a higher level due to operation of the C-4 shuttle for CO_2 . Finally, it should be mentioned that PGA also may be exported from the chloroplasts, especially under conditions of low light or where for other reasons levels of ATP and NADPH inside the chloroplasts are inadequate to maintain high rates of reduction to triose phosphates. Probably this PGA export is dependent on PGA concentration in the chloroplasts, as well as cytosol P_i level.

Once triose phosphate leaves the chloroplasts (Figure 2) alternative fates are possible. It can be oxidized to PGA, producing ATP and NADH for biosynthesis and respiration in the cell. PGA can be converted to phosphoenolpyruvate (PEPA) which then can undergo two alternative rate-limiting and regulated reactions. One reaction produces pyruvate and ATP. The other reaction, a carboxylation, produces oxalacetic acid. Together, these reactions feed carbon into the biosynthetic and respiratory reactions of the mitochondria, leading to production of ATP and of amino acids for protein synthesis. Acetyl coenzyme A formation from pyruvate also provides building blocks for fatty acid synthesis and isoprenoid synthesis leading to carotenoids, isoprenes, sterols, etc. Presumably part of the acetyl CoA, ketoacids, and some amino acids formed in mitochondria and cytoplasm are used in the chloroplasts for biosynthesis of pigments, enzyme membranes, etc. The principal alternative fate for triose phosphate exported from chloroplasts is conversion to sucrose, which is then translocated from the cell and the leaf to other parts of the plant. Relatively little is known so far about the mechanisms of regulation of the flow of carbon from photosynthesis to these important alternative biosynthetic pathways. The physiological control of the switch should be related ultimately to genetic programming of the immature leaf cell to become a mature cell, and to regulation by plant hormones. Some indication of the mechanism of metabolic control can be found from studies on the fate of photosynthetically incorporated $^{14}CO_2$ into secondary products.

For example, $1mM NH_4^+$ administered to *Chlorella* (32), spinach leaf discs (33), and recently isolated leaf cells from poppy (34), caused pronounced stimulation of the flow of carbon from photosynthesis into the tricarboxylic acid cycle. This shift resulted from activation of pyruvate kinase (converting PEPA to pyruvate) and PEPA carboxylase (forming oxalacetic acid). This increased flow is

at the expense of sucrose formation. In the dark in *Chlorella*, catabolism of sucrose in the cells is accelerated concomitant with increased flow of carbon into amino acids (32). The level of free NH_4^+ ions in the cytosol is probably very low, and it is not yet known what mechanism operates in the shift in carbon metabolism seen on addition of NH_4^+ outside the cells. In *Chlorella*, a decrease in the steady-state level of ATP in the dark was observed upon addition of NH_4^+ .

The change in biosynthesis accompanying NH_4^+ is indicative of a profound difference in the fate of photosynthate, namely growth of cells vs. export of carbon and energy. Reduced carbon export may be viewed as a more differentiated (specialized) function of the cell. Possibly, one form of control operates via uncoupling of phosphorylation, and perhaps can also uncouple oxidative phosphorylation, thus mimicing physiological regulation. Like the control of P_i level, these possibilities merit further research since they bear on the key question of control of allocation of photosynthate. Besides export of sucrose from the green cells, export of key amino acids such as asparagine and glutamine are of importance to subsequent biosynthesis. Also, it is possible that other low-molecular weight compounds essential to subsequent biosynthesis may be translocated from green cells to other parts of the plants.

Control of metabolism in the green cells is only the first stage in biosynthesis in plants. Many of the compounds that are of the greatest interest to chemists as potential feedstocks are formed in stems, seeds and other plant organs. Such biosynthesis has no light energy as a driving force, but is dependent on sucrose and other organic compounds from the green cells. Time does not allow for any extended discussion of the possibilities of regulation which occur in the transport of photosynthate and its subsequent conversion to important products. This biosynthesis in non-photosynthetic part of the plants may well be as important as photosynthesis and biosynthesis in the leaves. The biosynthetic pathways are generally known, though there are some significant uncertainties. Regulatory mechanisms are much less understood. Especially intriguing are the mechanisms regulating genetic expression. These determine whether a cell will become a carbohydrate storage organ, a latex-producing vascular cell, an oil-storing seed, etc. The field of single cell plant tissue culture and the study of effects of plant hormones on differentiation may prove of great value here. From a more practical standpoint, cell culture, mutation, selection, and plant generation may offer opportunities for altering the programming of the plant in beneficial ways. Such basic research can provide the foundation for systematic manipulation of plant biosynthesis to produce better yields of the renewable resources we need.

SUMMARY

Green plants offer a great variety of opportunities for development of renewable resources. Some principles to be kept in mind in developing use of plants include the following:

1. We should seek optimum use of all parts of the plant as chemicals, fuel, food and materials.
2. Opportunities are very diverse due to range of plants, multitude of products, physiological variables and needs of regions. Research and development should not be unduly restricted.

3. Efficient solar energy conversion to biomass production is important economically, especially when utilization of the total plant is contemplated.
4. Although the efficiency for most agricultural and forestry crops is rather low, practical efficiency of solar energy conversion to biomass energy storage up to 5% may be possible, and novel systems such as controlled environment agriculture for achieving such efficiencies are worthy of consideration for research and development.
5. Exploitation of plants for chemicals should not compete too much with food production for good agricultural land. There are opportunities to utilize different environments such as semiarid regions and humid tropics, where plants rich in hydrocarbon-like chemicals can be grown.
6. Regulation of the allocation of photosynthate to biosynthesis begins in the green cell where reduced carbon can be used either for new cell growth or can be exported to other parts of the plant as sucrose. Some clues as to the mechanisms of such regulation are being obtained.

Research recommendations include the following:

- A. Survey of plants in all regions and analysis of yield and chemical content of promising species.
- B. Economic assessment based on the total plant biomass of those and needs of people in each region.
- C. Plant development through both environmental plant breeding and through cell culture.
- D. Evaluation and research of special systems for increasing plant productivity.
- E. Research on mechanisms of regulation of biosynthesis in plants, and application of knowledge gained to plant development.
- F. Chemical research engineering and development to make increased economic use of all plant components.

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TABLE 1. Maximum photosynthetic productivity and measured maximum yields in selected plants

	Assumed Radiation Kcal/cm ² yr	gm/m ² day	metric ton/ hectare yr.	eff. %
Theoretical max. (Table 2)				
High Solar Desert ann.	200	86	313	6.6
U.S. Average ann.	144	61	224	6.6
U.S. Southwest ave. ann.	168	72	263	6.6
U.S. Southwest, summer	247	106	387	6.6
Maximum Measured				
C-4 Plants				
Sugar cane	247	38	(138)*	2.4
Napier grass	247	39	(139)	2.4
Sudan grass (Sorghum)	247	51	(186)	3.2
Corn (Zea mays)	247	52	(190)	3.2
C-3 Plants				
Sugar beets	247	31	(113)	1.9
Alfalfa	247	23	(84)	1.4
Chlorella	247	28	(102)	1.7
Annual Yield				
C-4 Plants				
Sugar cane	168	31	112	2.8
Sudan grass (Sorghum)	168	10	36	0.9
Corn (Zea mays)	168	4	13	0.4
C-3 Plants				
Alfalfa	168	8	29	0.7
Eucalyptus	168	15	54	1.3
Sugar beet	168	9	33	0.8
Algae	168	24	87	2.2

*Parentheses indicate maximum rates. Since these are not sustained over a whole year, they are much higher than annual yields.

XBL 7611-9660

Fig. 1
J.A. Bassham

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Fig. 2
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FIGURE LEGENDS

Figure 1. The reductive Pentose Phosphate Cycle of Photosynthesis.

This RPP cycle (Calvin Cycle) occurs in all green plant cells and in blue-green algae. Some "tropical grass" plants and other selected species have an additional, preliminary cycle (C-4 cycle), in which CO_2 is first fixed in mesophyll cells by carboxylation of phosphoenolpyruvate (PEPA); the resulting C-4 acid is translocated into the chloroplasts of bundle sheath cells containing the enzymes of the RPP cycle. There the C-4 acids are oxidatively decarboxylated giving CO_2 to be refixed by the RPP cycle, and C-3 acids which are shuttled back to the outer, mesophyll cells for conversion to PEPA.

Abbreviations: RuDP, ribulose-1,5-diphosphate; PGA, 3-phosphoglycerate; DPGA, phosphoryl-3-phosphoglycerate; GA13P, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; FDP, fructose-1,6-diphosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; TPP, thiamine pyrophosphate; F5P, erythrose-4-phosphate; SDP, sedoheptulose-7-phosphate; Xu5P, Xylulose-5-phosphate; R5P, ribose-5-phosphate; Ru5P, ribulose-5-phosphate.

Figure 2. Metabolic pathways among subcellular organelles in green cells.

Reduced carbon from photosynthesis in chloroplasts is exported as triose phosphate, PGA and glycolate. These metabolites are subsequently converted to other substances in the cytoplasm, peroxisomes and mitochondria.

Abbreviations: PEPA, phosphoenol-pyruvic acid; OAA, oxalacetic acid; ASP, aspartic acid; ASN, asparagine; GLY, glycine; SER, serine; GLU, glutamic acid; GLN, glutamine; THFA-C, tetrahydrofolic acid bound to a one-carbon moiety; GlP, glucose-1-phosphate.

For other abbreviations, see Fig. 1.

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